Soil Biology

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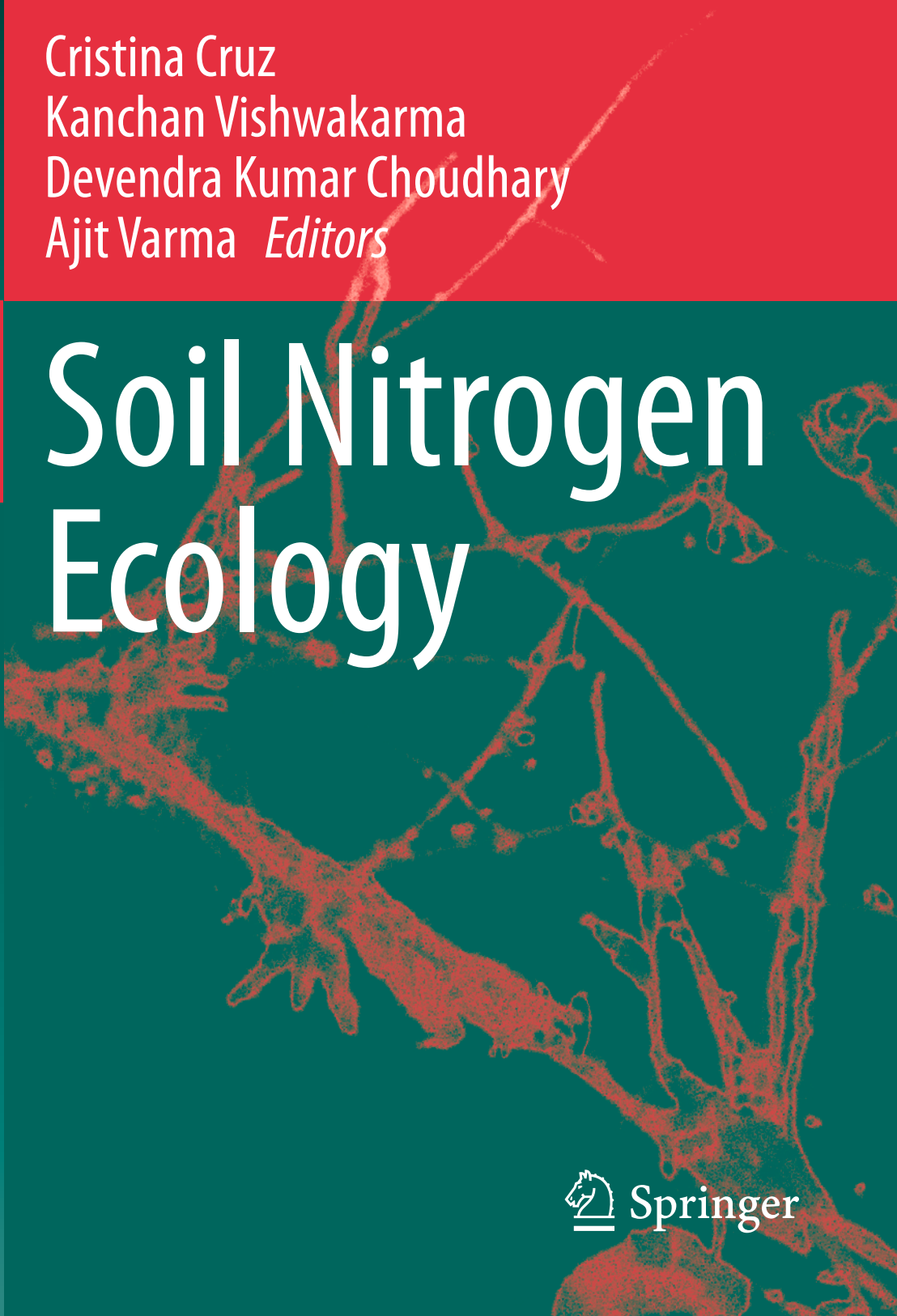
Soil Nitrogen Ecology



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Preface

Nitrogen (N) is one of the essential macronutrients which help in plant development and also act as a limiting factor for plant growth. It represents about 2% of the total plant dry matter that enters the food chain. Nevertheless, plants cannot directly access dinitrogen gas, which makes up about 78% of the atmosphere. Plants absorb the available nitrogen in the soil through their roots in the form of ammonium and nitrates. The limited bioavailability of nitrogen and the dependence of crop growth on this element have spawned a massive N-based fertilizer industry worldwide. However, the utilization of N-based fertilizers has led to many ecological problems. Biological nitrogen fixation has come up as an ecological means to employ nitrogen to the plants. Although plant growth promoting microbes are great nitrogen fixer but their selected host range and some limiting factors such as nodule formation put research forward to search for other nitrogen fixer microbe and till now so many bacteria have been reported with such activity. Hence, a variety of soil microbes with diversified niches, functions, and phylogenetics such as nitrifying and denitrifying microbes, nitrogen fixers, and ammonia oxidizers have displayed their involvement in nitrogen cycling for enhancing soil nutrients and plant growth. For efficient availability of nitrogen, symbiotic association is needed among bacteria, fungi, endophytes, and plant host. Several studies have been conducted to characterize and employ these microbes for ecological benefits. Such microbes can be characterized by molecular and nonmolecular based methods. Continued research with N-fixer microbial colonization and biofilm formation by these bacterial genera also holds the potential for developing biofertilizer and biocontrol agents that may be self-perpetuating within the colonizing host plants. Although so many N-fixers are found with the potential of N-fixation, applications of N-fixer microbes will significantly reduce the use of chemical fertilizers and pesticides which will be essential for achieving sustainable crop yield in agriculture.

With this background, the book “Soil Nitrogen Ecology” will cover different and significant aspects of nitrogen in soil and its ecology. The objectives of the book will be to know the distribution and occurrence of N in soil, tools used to characterize the nitrogen fining microbes, to understand the process of biological nitrogen fixation, to

know the importance of soil microflora and fauna and their role in N-fixation, to elucidate the importance of plant growth promoting microbes, arbuscular mycorrhizae, and endophytes in N-fixation, perspectives of metagenomics and metatranscriptomics in N-ecology, and deployment of microbe–plant signals in N-ecology and related aspects. The overall significance of the book will be to give the readers a whole coverage of nitrogen processes happening in soil and plants, biological nitrogen fixation, and research studies to depict the importance of microbes in nitrogenous processes. In this book, the global coverage is made by the editors and the authors with respect to the objectives. Highly qualified authors from various countries have given cutting-edge scientific contributions in broad areas of soil nitrogen ecology. In addition, this book will be suitable for the audiences like students, researchers, academicians, agronomists and crop scientists, biochemists, plant physiologists, plant breeders, biotechnological/biofertilizer companies, policymakers, national plan and policy formulating organizations, and central and state agricultural ministries and boards.

This volume contains 27 independent chapters including several protocol chapters and contribution was made by 85 authors from all over the world. These chapters were sent to many experts to seek their opinion. The changes suggested by the referees were incorporated.

The editors are thankful to Dr. Sabine Schwarz for helpful suggestions. The authors are also thankful to Dr. Andrew Sehlit-Burger, the project coordinator for achieving this volume in record time.

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Nitrogen Physiology and Ecology

Chapter 1
Physiology and Distribution of Nitrogen in Soils

Mohiraa Shafreen, Kanchan Vishwakarma, Neeraj Shrivastava, and Nitin Kumar

Abstract One of the important element for plant growth and which signifies soil health is Nitrogen. Soil is majorly bound with inorganic (NO_2-, NO_3^-, non-exchangeable (mineral-fixed) NH_4^+, exchangeable NH_4^+, nitrous oxide (N_2O) and dinitrogen gas (N_2)) and organic forms (amino acids, amino sugars and nucleic acid bases) of Nitrogen. Soil N amount depends upon N_2 fixation through microorganisms, losses from leaching and crop removal, volatilization and Emission of N_2O and N_2. Initially NH_+ conversion is processed through ammonification followed by the conversion of nitrite to nitrate through nitrification process. Further, NH_+ and NO_3^- are utilised by microorganisms and plants by process immobilization and assimilation, respectively. In the end, the N cycle is completed with denitrification process i.e. N releases back to atmosphere. Soil N is vast and not only related to transformation and distribution of inorganic and organic N but also its interaction with biosphere and atmosphere. The following chapter gives an overview of soil N by keeping in mind to keep a detailed view of its distribution and physiology.

Keywords Soil Nitrogen · Distribution · Physiology · Nitrogen Cycle · Nitrification · Leaching

1.1 Introduction

Next to Oxygen and Carbon, the most abundant element found in dry matter of plant is Nitrogen (N). Being the key component of plant's chlorophyll, nucleic acids and amino acids, N is acquired by plants in huge amounts from the soil compared to other elements (Kumar et al. 2020). For all animals including humans the dietary N

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Table 1.1 Amount of N in the different spheres of the earth

Sphere	N (million metric tons)
Atmosphere	3.86×10^9
Biosphere	2.8×10^5
Hydrosphere	2.3×10
Lithosphere	1.636×10^{11}
–Organic compounds at sea bottom	5.4×10^5
–Coal	1.0×10^5
–Earth's core	1.3×10^8
–Terrestrial soils	
(a) NH_4^+ (clay fixed)	2.0×10^4
(b) organic matter	2.2×10^5
–Fossil N (sediments)	$3.5–5.5 \times 10^8$
–Igneous rocks	
(a) of the mantle	1.62×10^{11}
(b) of the crust	1.0×10^9

(protein) is provided by plant N. Through the process of deposition, the earth receives the main forms of N (NH3, NO_3^- and $NH4^+$) from the atmosphere. Deposition of organic N in the form of organic nitrates, particulate N (dust, pollen, bacteria) and amine aerosols makes up ca. 30% of N deposited in the atmosphere. Deposition of N in soils of forests is comparatively higher than deposition in many other ecosystems, also deposition depends upon altitude of the place as it is more in soils from higher altitude than lower areas (McNeill and Unkovich 2007). In soil, Nitrogen exists in various forms based on its different oxidative states (Robertson and Groffman 2007). Nitrogen is a very mobile element as it circulates between living organisms, the soil and the atmosphere (Mengel et al. 2001). Specially in atmosphere it is very stable in the form of N_2 and hence found predominantly at 79.08% of all gases in atmosphere. Amount of N vary in the different spheres of the earth (Table 1.1) and every acre above the earth's surface contains about 35,000 tons of N which present as elemental nitrogen that is not available to higher plants, in other words this form cannot be used by higher plants. In soil N is present in four major forms (a) ammonium ions (NH_4^+) which are held by organic matter and clay minerals, (b) mineral N (NO_2^-, NO_3^-, NH_4^+), (c) microorganisms and clay minerals, (d) organic matter fungi, plant material and humus (Cameron et al. 2013).

Symbiotic fixation, nonsymbiotic fixation, fertilizer additions, organic matter and rainfall are the processes that work on supplying available nitrogen to the soil (Scarsbrook 1965). About 2000–6000 kg N/ha of organic matter is present on arable soil's surface layer. The amount of organic matter in soil shows its history and composition, generally higher in soils from grassland and forest as it contains clay which stabilizes organic matter (Powlson 1993). The top 15 cm of soil contains 0.1 and 0.6% nitrogen (N) and based on the soil type the amount varies between 2000 and 12,000 $kgNha^{-1}$ (Cameron et al. 2013).

Incubation method is often used to evaluate the availability of N in soil which is based on the amount of inorganic N released when soil is incubated at 30°C under moisture conditions of field (Matsumoto et al. 2000). Many factors such as temperature, soil type, wind, pH and precipitation can fluctuate the availability of soil N (Masclaux-Daubresse et al. 2010).

In soil N exists in many different forms and transforms easily from one state to another. The path that allows N to flow through the soil system's in and out is called the "nitrogen cycle" (Lamb et al. 2014). In ecosystems the cycling of N is divided into an internal and external N cycle. Those processes that remove or add N from ecosystem comes under external N cycle. External N cycle include dry and wet N deposition, dinitrogen (N_2) fixation, N fertilization, runoff erosion, N leaching, ammonia volatilization and denitrification. The processes that work on converting N from one chemical form to another or to mobilize N between ecosystem pools comes under internal N cycle. Processes involved in internal N cycle are root turnover, mineralization of N (the process that converts organic Nitrogen to inorganic Nitrogen), plant assimilation of Nitrogen, return of Nitrogen to soil in plant litterfall, nitrification (NO_2^-, NO_3^- production from NH_4^+ or organic Nitrogen), N immobilization by microbes are processes (Hart et al. 1994). In soil the most important N transformations occurs viz., immobilization, aminization, nitrogen fixation, ammonification, denitrification, nitrification leaching and volatilization (Yeasmin et al. 2012).

1.2 Inorganic and Organic Forms of Nitrogen

1.2.1 Inorganic N Compounds in Soils

About six Inorganic N compounds have been detected in soil which includes NO_2^-, NO_3^-, non-exchangeable (mineral-fixed) NH_4^+, exchangeable NH_4^+, nitrous oxide (N_2O) and dinitrogen gas (N_2). Other biological or chemical intermediates, such as nitrogen dioxide (NO_2), nitric oxide (NO), hyponitrous acid (HON = NOH), hyponitrous acid (HON = NOH), hyponitrous acid (HON = NOH) and azide (N_3^-) are also formed in the soil environment (Young and Aldag 1982).

1.2.1.1 Nitrate

In nitrification process, the final product is Nitrate formed after oxidization of NO_2^-. In soils *Nitrobacter* (the genus of chemoautotrophs) oxidizes NO_2^- to NO_3^-. Nitrate production in soil is greatly related to the levels of NH_4^+ in soil, which in turn are a function of the amounts of soil organic N, mineralization rates of soil organic matter, manures or crop residues or other wastes (Bronson 2008).

In most agricultural soils, the NO_3^- concentration is in millimolar range of about 1–5 mM. Though NH_4^+ is readily available in some soils than NO_3^-, plant root take

up N mostly as NO_3^-. This is because the concentration of NO_3^- is higher than NH_4^+ or NO_2^- in soils and also due to an overall negative charge possessed by the soil makes it move freely within the root solution. NO_3^- is readily available to the roots of plant as it has high diffusion coefficient (ca. 1×10^{-10} m^2 $s^{-1)}$ which also has a consequence that it gets easily lost through leaching from root zone. About 30% of soil inorganic N is lost per growing season through leaching which accounts for the high losses of soil inorganic N. In soil, the diffusion coefficients of nitrate are determined by charge, ion size, viscosity of water, soil moisture, temperature the soil buffer capacity and tortuosity. Being an anion nitrate moves with seepage, surface runoff and subsurface flow into lakes, streams, estuaries and groundwater (Bronson 2008).

1.2.1.2 Nitrite

In a two-step process of nitrification, Nitrite is the intermediate product of NH_4^+ to NO_3^-. It is also known to be a small source for available nitrogen (Scarsbrook 1965). In soils NH_4^+ is oxidized to NO_3^-. The chemoautotrophic bacteria of the genus *Nitrosospira, Nitrosococcus* or *Nitrosomonas* oxidize NH_4^+ to NO_3^-. Adequate moisture and aeration is required for nitrification to take place in soil. The process is optimal at pH from 6.6 to 8.0 and at temperature of 30–35°C. Nitrite does not accumulate normally above the required level in soil and if its level is excess, then it would be toxic to plant roots (Bronson 2008).

Soil solution always contains nitrite. Laboratory and field experiments have revealed that in large amounts of nitrites gets accumulated alkaline soils which might get held up in the soil for long periods. High pH and large quantities of NH_4^+ will cause nitrite accumulation. Oxidation of nitrite to nitrate is inhibited by NH_4^+ (Scarsbrook 1965).

1.2.1.3 Ammonium

In soil solution NH_4^+ ion is found as a part of exchange complex and in positions restricting its exchangeability (often called "fixed" or "non-exchangeable"). Only small amount of ammonium is found in soil as it is in dynamic equilibrium with the non-exchangeable and exchangeable NH_4^+. Ammonification releases NH_4^+ in an area where plant roots are permeated to absorbs NH_4^+ immediately and hence not letting microorganisms to utilize the released NH_4^+ (Scarsbrook 1965).

Ammonium is a cation and hence it is attracted to soil particles which are negatively charged. In soil NH_4^+ is held as an exchangeable cation and therefore it does not leach easily. In aerobic (oxygen-rich) soils NH_4^+ is relatively unstable (Walworth 2013). The relative concentration of NH_3 and NH_4^+ is determined by measuring the pH of the soil solution. Production of ammonia is favoured when the pH condition is high (Cameron et al. 2013).

Concentrations of ammonium in agricultural soils normally are in the range of 20–200 μM. NH_4^+ is immobile in soil and is not easily lost through leaching. Moreover agricultural, human and industrial activities (pollution) have resulted in NH_4^+ accumulation. This makes NH_4^+ as the predominant N form with an average concentration of 2 mM in some soils of forest up to 20 mM in agricultural soils. Such high concentrations are may be due to anion/cation imbalance, problems with pH balance and/or efflux of the ion which causes energy drain. The diffusion coefficients for NH_4^+ and NO_3^- are determined by charge, ion size, viscosity of water, soil moisture, soil buffer capacity, temperature and tortuosity (Miller and Cramer 2005).

Fixation of ammonium in soils is widely differed depending on the clay fraction. No fixation occurs when the clay fraction is kaolinitic. Soils where micaceous minerals and vermiculites predominate, NH_4^+ fixation occurs in the range from 1 to 6 me. NH_4^+ per 100 g soil (Scarsbrook 1965).

1.2.1.4 Exchangeable NH_4^+

The most important form of available N for plant is exchangeable NH_4^+. Exchangeable NH_4^+ is abundantly found in flooded soil. In non-flooded soils, both tropical and temperate, extractable levels of NH_4^+ are less than NO_3^-. This is due to biological oxidation of NH_4^+ to NO_3^- or rapid nitrification. In soil, their sources include mineralization of soil organic matter and plant residues and ammoniacal N fertilizers. Slurries or fresh manures contain considerable concentrations of exchangeable NH_4^+ (Bronson 2008). Also, Biological immobilization processes requires exchangeable NH_4^+ (Schulten and Schnitzer 1997).

1.2.1.5 Non-Exchangeable NH_4^+

Non-exchangeable form of Ammonium fixation is defined as immobilization of NH_4^+ ions by the organic or mineral part of the soil in a manner so that they cannot be exchanged by usual cation exchange method. Non-exchangeable NH_4^+ or intercalary NH_4^+ are those NH_4^+ which are fixed by clay (Scherer 1993). Ammonium is fixed by clay minerals in non-exchangeable form at high amounts which are slowly released into the soil thereby increasing soil N availability. Vermiculite, montmorillonite and illite are some of the interlayer regions of clay minerals where the most fixation occurs (Cavalli et al. 2015). It is thought that the biological availability is low to those non-exchangeable ammoniums fixed in the lattice of clay minerals (Rosswall 1982).

1.2.1.6 Dinitrogen Gas (N_2) and Nitrous Oxide (N_2O)

The most abundant nitrogen gas in atmosphere as well as in soil is N_2. Nitrification process emits Nitrogen oxides [NO*x*, nitrogen dioxide (NO_2) nitric oxide (NO)] and

Nitrous oxide (N_2O) to the atmosphere which are produced in the top soil (Bronson 2008). Microbial role of N_2 fixation has a great role in biosphere nitrogen cycle. Biological N_2 fixation contributes 83% and the fertilizers contribute about 14% of total N fixed (Weaver and Danso 1994). As O2 availability decreases the N_2:N_2O ratio increases and the ratio decreases when there is high NO_3 availability (Jackson et al. 2008). Two biogeochemical pathways that emit N_2O from soil are denitrification and nitrification. Oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) is carried out by Nitrifying microbes, but during intermediary steps some N is lost as nitric oxide (NO) and N_2O (Del Grosso et al. 2000).

1.2.2 Organic N Compounds in Soil

Studies based on hydrolysis with hot acid revealed various proportions of organic N in soil. Organic N exists in various forms such as amino acids (20–50%), amino sugars (3–10%) and nucleic acid bases such as pyrimidine and purine derivatives (1%) (Sowden et al. 1977).

About 90% of total N in soils accounts for organic N derived from microbial decomposition of animal and plant remains. In majority of terrestrial ecosystems, the major pathway involved in the supply of N and energy to the soil is from litter originating from both below- and above ground plant parts. Organic matter in surface soil contains nearly 0.4% dry weight of nitrogen (Greenfield 1972).

Organic N compounds in soil consist of 20–40% of proteins, 5–10% of amino sugars and $<1\%$ of pyrimidine and purine derivatives and also a complex of few unidentified compounds formed by different reactions in soil such as quinone polymerization with N compounds, reaction of lignin with NH_4^+ and condensation of amines and sugars. All these N fractions undergo various transformation processes (Hofman and Van Cleemput 2004). Soluble N is separated into different classes based on the recognition for protein hydrosylates, i.e. (1) basic (diamino)-N, (2) ammonia and amide nitrogen, (3) humin-N and (4) nonbasic (monoamino)-N (Bremner 1949).

Grassland and forests usually have high amounts of Soil Organic Nitrogen (SON). Arable soils have high proportion of amino compounds. The range of hydrolysable SON in arable soils is between 23 and 55% (Murphy et al. 2000). A large proportion, about 20–25% of soil N from surface soil recovered is in the form of NH_3 which is another feature about fractionation of soil N. "Amide N" of proteins is the name given to this N form but recently it was found that only a small proportion of NH_3 could be derived amides of amino acids glutamine and asparagine. A part of NH_3 is released from partially destructed amino sugars and also from indigenously fixed NH_4^+. Hydrolytic breakdown of certain amino acids also gives NH_3. Some amino acids like tryptophan is completely lost during hydrolysis but other like threonine and serine are destroyed partially (Stevenson 1983).

1.2.2.1 Amino Acids in Soils

Amino acids in soil occur as proteins and peptides which are made up amino acid residues covalently joined through amide linkage. Free amino acids also exist in soil but in very small proportion which decomposed readily by microorganisms and thus have a very short life span in soil. When mineral surfaces get absorbed with free amino acids stabilization of free amino acids could be achieved. Sorption of free amino acids to humic colloids or its retention in micropores or small voids of soil aggregates can also stabilize free amino acids (Deng and Dixon 2002).

Sources of amino acid N in soil are from plant debris, microflora and its products of decomposition and metabolites (Pollock et al. 1977). Different soils have different composition of amino acids. The proportion of the amino acid N from histidine, lysine, ornithine and arginine in some soils is over one-third where as in other soils it is less than one-tenth. Its concentration in soil solution is as low as $<2\mu g\ g\ m^{-1}$ soil but these are sevenfold higher in the rhizosohere due to plant root exudation. Soil humic acids when extracted contain half of the total N as amino acid N. In response to cultivation, the order of individual amino acid abundance in Spodosols HA is aspartic acid > glutamic acid > glycine > valine > lysine > arginine > phenylalanine > methionine (Ding et al. 2001; Friedel and Scheller 2002).

Microbes degrade amino acids very rapidly. But the rapid degradation does not occur when amino acids and peptides are bound to humic acids (Szajdak and Österberg 1996). Long-term cultivation and cropping systems alters the nature of fraction of amino acids in soil both quantitatively and qualitatively. Soil N content can be increased by involving legumes to cropping systems. There has been a marked decrease in the content of amino acid N in soil due to cultivation. Composition of peptide amino acid corresponding to size fractions differed between uncultivated and cultivated soils of same series (Senwo and Tabatabai 1998).

1.2.2.2 Amino Sugars in Soils

About 5–10% of the total soil N accounts for N-containing carbohydrates otherwise called the "amino sugars". In soil, the amino sugar which is most abundant is D-glucosamine (Glc) and the second most is D-galactosamine. Other amino sugars such as D-manosamine, D-fucosamine and Muramic acid (Mur) have been observed in soil as well. Structural components of mucilages or muco-polysaccharides in soil make up amino sugars. Amino sugars can also combine with muco-proteins, muco-peptides, chitin and antibiotics. Amino sugars in soil are supposed to be originated from microbes (Salton 1965; Coelho et al. 1997; Dai et al. 2002; Deng and Dixon 2002; Niggemann and Schubert 2006; Haynes 2012). Amino sugars in soil are contributed by soil microorganisms. Bacterial cell wall's murein layers contain Mur-N. About 3–16% of amino sugar content is contributed by Mur-N and hence it has been used as a biomarker for soil bacteria. The sources for GlcN in soil are

higher fungi's cell walls and chitin is the main component in these cell walls (Joergensen 2018).

1.2.2.3 Nucleic Acid Bases in Soils

Nucleic acid and their products of decomposition becomes a portion of soil organic matter in two ways. One is by introducing the nucleic acids of dead plant matter and its decay in the soil and the other is from nitrogenous compounds of microorganisms in soil. Proteolytic enzymes like trypsin and pepsin does not split the pyrimidine derivatives of the nucleic acids and its other products of decomposition but the cleavage can be done by the nuclease enzyme called Erepsin. Microorganisms also undergo a similar decomposition. From this it is clear that the decomposition of nucleic acid product occurs constantly in soils as plants contain nucleoprotein. The purine bases hypoxanthine, guanine, xanthine and adenine present in both animal and plant tissues and are also derived from nucleoprotein break down (Schreiner and Shorey 1910; Adams et al. 1954). Less than 1% of the soil organic N comes from the purine and pyrimidine bases in nucleic acids of majorly from bacteria and small fraction from plants. All of the RNA and DNA bases (adenine, guanine, cytosine, thymine, uracil and 5-methylcytosine) are identified in the soil organic matter hydrolysates (Deng and Dixon 2002).

1.3 Nitrogen Distribution

The top 6 inches of soil contain about 2000–4000 pounds of N per acre in the soils of sub-humid and humid areas of the USA. Despite this huge amount not more than 1% of total N would be available at any time. Above every acre of earth's surface nearly 35,000 tons of N is present. This N is present in the form of elemental nitrogen. Significantly the availability of N in cold soils is reduced. At high temperatures the rate of ammonification has increased notably in SOM. In tropical soils the available form of N is often accumulated under a mulch during dry season. In 1945 Gilbert found that the nitrate content in mulch soils was double the amount than in bare soils. When moisture content is high under the mulch seems to support mineralization of organic N to a great extent. Due to leaching nitrate content under mulch lower than the nitrate content in bare soils during rainy periods. The infiltration rate of mulched soils is greater than bare soils which allow the soluble nitrogen to leach out (Scarsbrook 1965).

Trees contribute about 13% of the total N in ecosystem, the soils of boreal forests have large N pools. In many studies soil N pools varied largely. In Scandinavian forests, dominant tree species and soil texture seemed to influence the N stocks and due to this different soil textures had different amounts of N. It was observed that coarse-textured soils had 480 g N m^{-2}, 680 g N m^{-2} of N in fine-textured soils whereas in calcareous soils it was 1120 g N m^{-2} and sites that are dominated by

beech and oak had 680 g Nm^{-2} whereas pine dominated sites had 230 g N m^{-2}, respectively (Marty et al. 2017).

Riparian zone ecotones, waterside (including mires and reservoirs) ecotones, surface water/ground water ecotones, fountain head water ecotones are the four inland/land water ecotones that are distinguished based on landscape functions. Inland/land water ecotones are able to retain ground water and surface water to some extent and most of the materials are removed from land sources. The amount of N retained in waterside and natural wetlands ecotones depend on the biological and hydraulic characteristics that range between 10 and 90% (Bai et al. 2004).

The first account of global soil nitrogen, i.e. 95 Pg was reported by Post et al. (1985) by reviewing organic soil nitrogen and carbon global database. Batjes and Dijkshoorn (1999) later reported 133–140 Pg of soil organic nitrogen by checking data from a global soil emission inventory. Data of 2840 soil profiles were used by Tian et al. (2016) in China to determine the soil N storage. It was estimated that to a depth of 100 cm about 8.3 Pg of N is stored in soil. A comparatively contrast value of 7.4 Pg was estimated by Yang et al. (2007) using the data from 3283 soil profiles.

A large amount of N in soil exist in combined form which is bound to mineral material and organic matter, but per acre only few pounds mineral N exists in available forms (as exchangeable ammonium and as nitrates) (Nie et al. 2017). It is stipulated that N in the atmosphere and in soil came because of outgassing of the earth's crust. Rayleigh (1939) have reported that there is a surprisingly constant N in igneous rocks, i.e. 0.04 cc/g. In the atmosphere, nitrogen's total mass is found to be 38.648 $\times$ 1020 g, whereas in ancient sedimentary rocks it was found to be 4.0 $\times$ 1020 g. Under natural conditions, N has been added to the soils through various mechanisms, but so far, the mechanism of the biological agents to the fix elemental N and the acquisition of nitrate and ammonia in rain water has been considered as important processes in N cycle (Stevenson 1965). On the other hand, the oceans cause nearly 400 Tg fixation of N per annum. N relocation is also done by crops: in one metric ton dry mass of wheat (straw and seed) 26–28 kgN is present whereas in rice, sugarcane and maize it is 16–17 kgN, 7 kg N, 9–11 kgN, respectively (compiled by Kennedy et al. 2004). Mineralization of this organic N occurs after harvest and thus returns back to the cycle (Valentine et al. 2010).

Subsoil arid zones contain nearly 104 kg N per hectare which is about 3–16% N globally. In desert soils, the long-term leaching has led to accumulation of nitrate in the subsoil region. In desert ecosystems, the natural sources of nitrogen include NH_4^+ and NO_3^- in eolian deposition, precipitation of nitrate salts and also in N fixing organisms. The maximum concentrations of NO_3^-N in the subsoil of the vegetation communities where nutrient is limited may exceed 2000 mg $liter^{-1}$. Evidently, not all NO_3^-N is consumed in the soil zone, once NO_3^- gets concentrated there, it returns back to the atmosphere via upward movement of water as vapour through plants. The downward movement of water is restricted below the subsoil reservoir, it enables accumulation of NO_3^- for many years. The subsoils of desert are low in microbial populations, organic matter, water content and the pH ranges between neutral to basic, all of which promotes the stability of NO_3^- and thus inhibits denitrification (Walvoord et al. 2003).

N losses and gains within the soil area are controlled by uneven distribution of microbial and plant activities. General trends are observable even with the large variability. For example, Lowest NO_3^-−N amounts are observed in the semiarid woodland (pinyon-juniper) regions of northern New Mexico, suggesting an environmental condition where the accumulation of NO_3^- is appreciable. A moderately more rainfall is received by nearby woodland (ponderosa pine) where only a little or sometimes no NO_3^-−N is accumulated in the subsoil. In deserts, the soil zone base is continuously established with hydraulic sinks is the main factor for the contrasting behaviour of NO_3^-−N in humid and arid soils. Comparisons of soil inventories to subsoil inventories for semiarid-to-arid soil inventories worldwide indicate that the magnitude of subsoil N inventories is similar to total inventories of soil N. These comparisons led to a conclusion that NO_3^-−N in the subsoil area accounts a major quantity of the total N in the vadose zone (ground surface to water table) region in the non-riparian arid territories. In Mojave Desert, Sonoran Desert and in the High Plains region the ratio of subsoil NO_3^-−N to total vadose zone N were found to be 44–92%, 41–81%, 41–62%, respectively. In the Chihuahuan Desert around 4–20% of subsoil NO_3^-−N accounts for total N in vadose region. In the 3×10^9 ha of Earth's arid shrublands and warm deserts, out of total bioavailable N, subsoil NO_3^-N accounts for about 3–15 Pg. This indicates a total global estimate of 21 and 95 Pg in desert soil and in all soils (Walvoord et al. 2003).

Leaching of NO_3^-−N contributes an appreciable fraction of deposition of atmospheric N over large areas. Below a depth of 1 m a large quantity of NO_3^-N is present, which demonstrates that the soil zone does not consumes the entire available N (NO_3^-−N) as well as not all is returned back to the atmosphere. Recently studies have shown that nutrients and water is not taken up by desert plants simultaneously. In addition, the available N present on the soil surface maybe be solely enough for some species. Subsoil NO_3^- reservoirs have effects on groundwater quality. The U.S. Environmental Protection Agency established that maximum contaminant level of NO_3^-−N should not exceed 10 mg liter^{-1}. If exceeds, it might lead to non-Hodgkin's lymphoma, methaemoglobinaemia and miscarriages. In the 1970s, investigations reported a large quantity of subsoil NO_3^-−N in central Nebraska and southern California and thus could not be used for human or agricultural activities. Similarly, the groundwater of Las Vegas Valley contained high levels of NO_3^-−N that ruled out septic systems, livestock and fertilizer as a source of pollution. Recently studies indicated that mobilization of subsoil NO_3^- to groundwater occurs readily due to conversion of desert land to irrigation. Climate change, construction of dam or changes in vegetation also mobilize subsoil NO_3^- (Walvoord et al. 2003).

27 soils from Wales and England were used to assess the available N by growing perennial ryegrass in pots for more than 6 months under standard environmental conditions. Using multiple regression relationships between the distribution and availability of soil N were examined (Warren and Whitehead 1988).

Due to agricultural activities, every year the creation of reactive nitrogen (Nr) tends to increase continuously. From the year 1865 to 1995, food and energy production increased leading to increased Nr creation. During 1860s the amount of Nr was ~15 Tg N whereas due to increased activities this value elevated to 156 Tg N

in 1995. This change was enormous but it did not stop there Nr further increased to 187 Tg N $yr.^{-1}$ in the year 2005 due to increased meat (207–260 million tons) and cereal production (1897–2270 million tons).

In some agricultural systems, biological nitrogen fixation was induced by cultivation. In 1995 cultivation induced biological nitrogen fixation (C-BNF) was 31.5 Tg N and due to increased meat and cereal production, C-BNF was raised to 40 Tg N in 2005. On a global scale, the distribution of Nr is dominated by atmospheric transport followed by its deposition. In 1860, it was estimated that NH_3 and NOx emits 34 Tg N $yr.^{-1}$ of Nr and gets deposited to the surface of the earth as other N forms. This value got raised to100 Tg N $yr.^{-1}$ in 1995 and by 2050 it is expected to be 200 Tg N $yr.^{-1}$ (Galloway et al. 2008).

N status in the ecosystem plays a major in magnitude and the response timing of N addition to N losses in soils. Loss of N is less in forests having limited N (N-oxide emissions or NO_3^- leaching) due to intense competition between microbes and plants. N loss is accelerated when N in the ecosystem reaches the saturation point. In the watersheds of Bear Brook, West Bear (7.3 kg N hm^{-2} $year^{-1}$) had stream export 24 times higher than that of East Bear (0.3 kg N hm^{-2} $year^{-1}$) (Zhu et al. 2015). During early 1991 at Gardsjon, Sweden an experiment was conducted to examine the N saturation risk by adding N (41 kg N hm^{-2} $year^{-1}$) and due to runoff, it resulted in increase in loss of input N from 0 to 10% (Moldan and Wright 2011).

When compared with temperate forests, the sub-tropical and tropical forests tend to have high amounts of biologically available N and N turnover rate is also faster in these forests due to their highly weathered soils (Zhu et al. 2015). Rock derived nutrients or P seem to limit NPP in tropical forests. There is a possibility that elevated levels of N deposition causes the N to be lost immediately at higher rates. Hall and Matson (2003) conducted an experiment in a forest having limited-N by adding N for the first time and indicated that emissions of N-oxide were negligible and after that the amount of N added was increased significantly followed by 11-year N fertilization. A very contrast result was observed as emissions of N-oxide were huge during the first as well as the long-term fertilization in P-limited and NP-limited forests. It was then suggested by Corre et al. (2010) that mineral N retention and production influences the response of N loss in soil to high input of N. A research conducted in the lowland forest of Panama (non-N-limited) showed that in the first year of N addition (125 kg N hm^{-2} $year^{-1}$) there was no effect on gross rates of N-oxide emissions as well as mineral N production. NO_3^- leaching and N-oxide emissions increased significantly after continuous addition of N for the following 9 years. The control plots of N-oxide emissions had values of 0.01 mg N L^{-1}, 70 μg Nm^{-2} day^{-1}, 448 μg Nm^{-2} day^{-1} for NO_3^-, N_2O, NO_2-N whereas after 9 years of continuous N addition, plot values increased notably as 0.93 mg N L^{-1}, 196 μg N m^{-2} day^{-1}, 1498 μg N m^{-2} day^{-1} for NO_3^-, N_2O, NO_2-N, respectively. In contrast, the N-limited montane forest had increased NO_3^- leaching and N-oxide emissions in the first year of N addition itself. The control plots had 0.03 mg N L^{-1}, 28 μg N m^{-2} day^{-1}, 326 μg Nm^{-2} day^{-1}, whereas in N addition plots it was 0.14 mg N L^{-1}, 66 μg N m^{-2} day^{-1}, 658 μg N m^{-2} day^{-1}. After

1.5 years of N addition it has been observed that a significant increase in N leaching in monsoon from broadleaf and pine forests (Fang et al. 2006).

1.4 Nitrogen Cycle

N cycle is one of the complex biogeochemical cycles on earth, where it transforms into various forms with the help of specialized microorganisms (Fig. 1.1). N cycle involves a reservoir of its different forms in oceans, atmosphere, sediments, soils and the crust. The main environmental and evolutionary significance of N cycle is to maintain the availability of fixed N to the biota. Most of the earth's atmosphere constitutes free N_2 but it is only accessible to N_2 fixing organisms and not to all kinds of prokaryotes, where it is reduced to ammonium. Other prokaryotes and some eukaryotes require fixed N (also called combined or reactive nitrogen) in forms of organic N, ammonium or nitrate for assimilation.

The balance between the fixation of atmospheric N and recycling the fixed nitrogen to dinitrogen gas controls the fixed nitrogen's availability. In terrestrial environments, now more nitrogen is fixed through industrial production of fertilizers (140 $TgNyear^{-1}$) than the amount of N produced through natural sources (110 $TgNyear^{-1}$). Human activities also contribute in fixing atmospheric N through legume cultivation which forms symbioses with nitrogen-fixing bacteria (Thamdrup 2012).

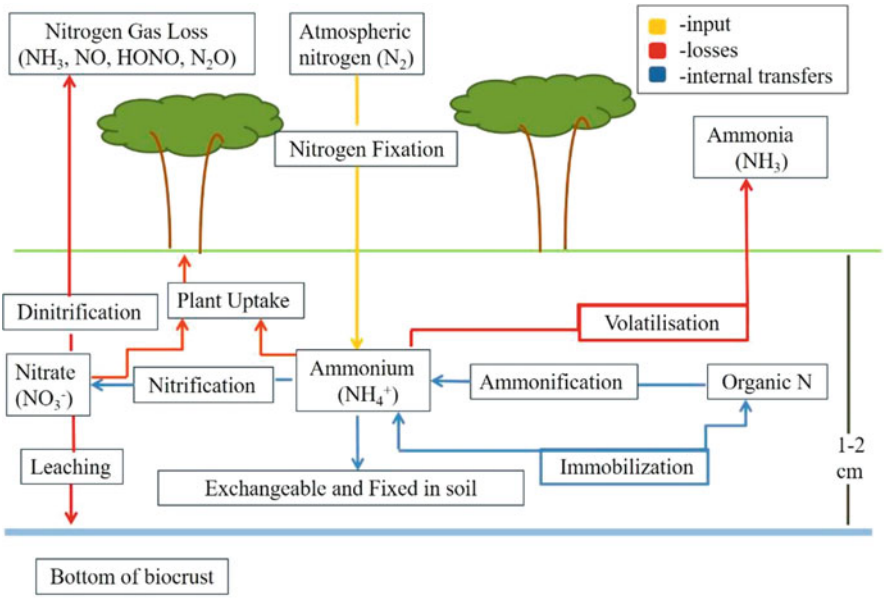

Fig. 1.1 Nitrogen cycle

N cycle in soil is very essential in determining the rate of N cycle in the ecosystem. Most of the soil N is present in dead organic matter, in an insoluble form which is then converted to DON (dissolved organic N). In order to overcome C limitation, DON is broken down by microbes from which C skeleton is used for their growth and hence ammonium is secreted into the soil. This process is known as ammonification or nitrogen mineralization. A part of NH_4^+ undergoes microbial immobilization or the clay minerals absorb it or even assimilated by plants, whereas the other part is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-) by nitrifiers. This conversion of NH_4^+ to NO_3^- is termed as nitrification. A possible acidification of soil can happen due to the production of H+ cation from nitrification. NO_3^- is can be easily lost from ecosystem by N oxide emissions or by leaching (Zhu et al. 2015).

Through $_{15}N$ isotope tracing experiments a close relationship between nitrification and mineralization was shown. In denitrification process nitrite or nitrate is reduced to N_2 or N_2O by denitrifying bacteria. Ecosystem N budget is balanced through denitrification. In general, natural forest ecosystems have less losses of N through denitrification. In N-limited forests, 1 kg N hm^{-2} $year^{-1}$ or even less rates of denitrification have been reported and even in tropical forests which are rich N, N_2O emissions are as less as 2 kg N hm^{-2} $year^{-1}$. Long-term deposition of excess N in forest ecosystems has expected to increase NO and N_2O emissions to atmosphere where the rates of denitrification and nitrification are accelerated (Zhu et al. 2015).

In most ecosystems, the microbial biomass helps in moving the N from soil to plant and again back to soil. During this coarse time N undergoes various transformations (nitrogen cycle) and occurs in different valence states ranging from −3 (in NH_4^+) to +5 (in NO_3^-). This variation in valence state is mainly due to the environmental conditions and is mediated by biological means. N is distributed readily by atmospheric and hydrologic transport process.

Atmospheric N_2 gas has 0 valency is converted to various oxides of N by lightning and finally nitrate of valency +5 is formed. This nitrate gets deposited on soil which is then used by plants for growth. Ammonium (NH_4^+) (valence −3) is also formed from N_2 gas by biological N fixation which is a more important process than lightning. In plants, this ammonium plays a major role in many biochemical reactions. Decomposition of plant residues result in conversion of organic N compounds to various forms starting from NH_4^+ (ammonification) and to NO_3^- (nitrification). Various oxides of N are formed from NO_3^- under anaerobic conditions from which N_2 gas is produced as an end product of denitrification and goes back to the atmosphere thereby closing the N cycle (Hofman and Van Cleemput 2004).

Transformation of N and its loss from soil is all interconnected by the role of biotic and abiotic factors of soil. Abiotic factors like porosity, pH and temperature control N leaching and nitrification (Van Groenigen et al. 2015).

Four microbiological processes are involved in the nitrogen cycle: N fixation, N mineralization (decay), denitrification and nitrification which are the important nutrient cycles in the ecosystem (Hayatsu et al. 2008).

Biological molecules getting incorporated with N is a basic requirement for an active biosphere. This happens through nitrogen fixation where archaeal and bacterial domains of the prokaryotes convert N_2 to NH_4^+.Some eukaryotes (termites and

legumes) also take part in this process only when they are in symbioses with nitrogen-fixing organisms.

Reduction of N_2 needs lot of energy as to attain the activation energy which is required for breaking N≡N bond, a catalyst is therefore required. This is done by the enzyme complex nitrogenase, where for every molecule of fixed N_2, ~16 molecules of ATP are hydrolysed. Being an α2β2 tetramer, the two α subunits of nitrogenase catalyses the reduction of the ATP dependant reaction, where N_2 is reduced to NH3. $MoFe_7S_9$ metal cluster is available in each subunit from which electrons are donated to N_2. When an organism dies, it releases NH_4^+, which is returned to the environment. The fate of released the NH_4^+ depends on the presence or absence of oxygen. When oxygen is present, specific groups of archaea and bacteria oxidizes of NH_4^+ to NO_3^-.

In the process of nitrification, organisms containing the enzymes ammonium monooxygenase, hydroxylamine oxidoreductase, nitrite oxidoreductase, oxidize NH_4^+ to NO_3^-. Inorganic C is fixed by the microbes in the absence of light by using protons and electrons derived from the oxidation of nitrite and ammonium (chemoautotrophy) (Canfield et al. 2010). Many important abiotic and biological processes take place under the nitrogen cycle involving various compounds in solid, liquid and gaseous phases (Jaffe 2000).

1.4.1 Nitrogen Fixation

N fixation is a process of converting N_2 into various forms of reactive N, which occurs via lightning, industrial or biological processes. The conversion of dinitrogen gas (N_2) to NH_4^+ is achieved only through the process of nitrogen fixation. Lightning is the first and foremost main process involved in this conversion, followed by nitrogen fixation by industrial and biological means and combustion of fossil fuels. Recent estimation has indicated that per year around 160 million tonnes of fixed N is deposited on terrestrial ecosystems, 40 tonnes from BNF, 22 tonnes from fossil fuel combustion for energy generation and 10 tonnes or less from lightning (McNeill and Unkovich 2007; Kox and Jetten 2015).

1.4.1.1 Biological Nitrogen Fixation

N fixing microbes categorizes in various groups such as legume microbes, non-nodulated non-legume microbes, free-living microbes, plant associated algae and blue-green algae (Table 1.2). About 90 genera of diazotrophs own the nitrogenase enzyme complex that reduces N_2 to $2NH_3$ and this process of conversion of N_2 to $2NH_3$ with the help of specialized microorganisms is known as Biological N Fixation (BNF). Some of these organisms live closely associated with plants which benefit the plants and some as free-living entities and yet some other also form complex symbiotic relationship with plants where the bacteria fixed N_2 is taken up

Table 1.2 Average rates for microbial nitrogen fixation per year

System or organism	Amount of N_2 fixed in kg/ha per year
Legumes	
Lupines (*Lupinus sp.*)	150–169
Clover (*Trifolium hybridum L.*)	104–160
Soybeans (*Glycine max L.Merr.*)	57–94
Cowpeas (*Vigna, Phaseolus, Lespedeza*)	84
Alfalfa (*Medicago sativa L.*)	128–600
Nodulated non-legumes	
Ceanothus	60
Hippophae	2–179
Alnus	40–300
Coriaria	150
Plant-algal associations	
Lichens	39–84
Gunnera	12–21
Azollas	313
Microorganism (free-living)	
Clostridium pasteurianum	0.1–0.5
Azotobacter	0.3
Blue-green algae	25

by the plants in exchange for energy source (carbon). Such symbiotic relationship is formed by some wide range microorganisms ranging from cyanobacteria with *Azolla*, cycads and lichens, these form symbioses within root nodules on perennial and annual legumes and are collectively called "rhizobia" and symbioses are also formed in the nodules of root of non-legumes such as *Alnus* and *Casuarina* by the actinomycete *Frankia*. N_2 fixers also establish symbioses with animals like termites and ruminants. Symbioses formed by *Azolla* and legume have a great economic importance in agriculture (McNeill and Unkovich 2007).

A elementary stoichiometry equation for BNF is shown below, energy expenditure is reflected by consumption of ATP and the amount varies for every type. For example, to fix per gram of N, ca. 20 to >100 g of C is required for diazotrophs that are free living, and 3–7 g of C for crop legume and >8 g of C for other terrestrial symbiotic systems.

$$N_2 + 16ATP + 8e– + 8H+ \rightarrow 2NH_3 + H2 + 16ADP + 6Pi$$

N_2 fixed quantity vary depending on the system involved in fixing N_2, in general more amounts of N_2 is fixed by plant's symbiotic systems than by associative and free-living N_2 1fixers. BNF is supressed by NO_3^- and NH_4^+ in soils. The establishment of BNF and symbiosis is strongly reduced. In soils BNF can be supressed by both NO_3^- and NH_4^+. Therefore, whenever there is enough mineral nitrogen to cope up with plant N demand, BNF and symbiosis both will be greatly reduced (McNeill and Unkovich 2007).

SNF (symbiotic nitrogen fixation) systems of few non-legume associations like *Anabaena azolla* and *Azoll*: grasses and sugarcane, endophytic bacteria, free-living diazotrophic bacteria in soil (such as *Acetobacter*, *Azotobacter*, *Anabaena*, *Nostoc, Clostridium* and *Azospirillum*), *Frankia* (actinorhizal bacteria) and the plant–fern–cyanobacterium tripartite symbiosis of rice has important roles and huge impact on crop production. Yearly, non-legume symbioses contribute 23 Tg of biologically fixed N (Halvin et al. 2005). In agricultural fields, the input of usable N is mainly contributed by legumes as it forms symbioses with rhizobial bacteria. In 2005, through SNF 26 Tg, 40 Tg, 121Tg of N is fixed in natural ecosystems, crop systems and by the Haber–Bosch process. While it has shown that in uncultivated grazing zones the SNF ranges from 50 to 70 Tg N annually (Conley et al. 2009).

1.4.1.2 Industrial Nitrogen Fixation

One of the greatest advances of the twentieth century is the development of industrial N_2 fixation through Haber–Bosch process (Smil 1997). Although it is a cost-effective process, this process demands more energy. In Haber–Bosch process, N_2 fixation process occurs by generating H_2 from water and natural gas and the atmospheric N_2 combines with H_2 at a temperature of 600°C and $\leq$106 kPa and with the presence of catalyst NH_3 is produced.

Presently, 90 million tonnes of nitrogen fertilizers are being added to agricultural fields every year. In several countries, the addition of N fertilizers to agricultural fields are even exceeding the projected contributions from BNF (Xing and Zhu 2002). The forefronts in N fertiliser utilization are India, Europe, North America and China (IFA 2005). Cereals such as maize, rice, barley, wheat and sorghum are the main crops which get these fertilizers in high proportion. Other than crops, N is significantly used in vegetables and fruits in USA and China, grasslands in Europe, and cotton and sugar cane in India. Urea is the cheapest and highest N fertilizer produced by industries, it is also easy to handle and transport, and therefore holds 75% of entire fertilizer N produced. Among other important N fertilizers Ammonium nitrate holds 16%, ammonium sulphate holds 5% and calcium ammonium nitrate holds 4% of entire fertilizer N produced (McNeill and Unkovich 2007).

1.4.2 Nitrogen Mineralization

N mineralization is a process of converting organic N to inorganic N (NH_4^+-N and NO_3^--N) aided by soil animals and microbes. N mineralization is influenced by many factors like organic matter content (Table 1.3), soil type, total N availability, ATP content, microbial N content, microbial respiration, water soluble N, pH, C:N ratio, soil moisture, temperature, dryness, lignin content, litter cellulose content, plant/soil interactions and inorganic nutrients supply (Bengtsson et al. 2003). In soil, the form of nitrogen is in organic form which gets liberated to NH_4^+-N through this

Table 1.3 Amount of N mineralized between 0 and 30 cm of the topsoil based on early inputs of organic material and field history

Type of land	Yearly input of organic matter	Amount of N mineralized (kg N ha^{-1} day^{-1})
Agricultural land	High	1.1–1.3
Agricultural land	Moderate	0.9–1.1
Agricultural land	Low	0.5–0.7
Grassland		2.1–5

process. In vegetation covered soil the concentration of NH_4^+−N is very low as 5 mg kg^{-1}. Its low concentration is not an indicator of low rates of mineralization, it indicates either plant uptake or rapid nitrification. N is required for growth of microorganisms, and for mineralization or immobilization of N by microbes the process depends on the C:N ratio of the substrate compared to the decomposer organisms. The substrate is utilized for both energy production and synthesis of new biomass (Jansson and Persson 1982; Rosswall 1982; Yeasmin et al. 2012).

In soil N cycle, N mineralization is recognized as the centre point which controls the availability of N to the plants (Schimel and Bennett 2004). Factors influencing the N mineralization's magnitude vary with depth. Studies have confirmed that when the depth is ≥1 m, N mineralization decreases and concluded with many in situ and in vitro approaches that when the depth increases N mineralization decreases rapidly (Dessureault-Rompré et al. 2016).

When there is a large amount of N than the required N in decomposing material, inorganic N is released with net N mineralization and if the decomposing organic material has the exact amount of required N for the microbial biomass, it results in no net N mineralization. If only small amount of N is present than the required N, then immobilization of more inorganic N from the soil should occur for the completion of decomposition process (Cabrera et al. 2005). The proteolytic degradation of soil peptides and proteins is usually related to be the rate-limiting step in N mineralization (Lipson and Näsholm 2001).

1.4.3 Immobilization

N immobilization occurs through both abiotic and biotic processes. NH_4^+−N is immobilized in both non-exchangeable (fixed) as well as exchangeable forms by clays. NH_4^+−N in exchangeable form and the liquid phase's ions are in dynamic equilibrium and hence undergo biological immobilization. Microorganisms perform rapid immobilization of inorganic soil N when a high C:N ratio substrate is added. Plant roots are high competitors for NH_4^+−N than nitrifying bacteria that oxidize NH_4^+−N to NO_2^-−N and NO_3^-−N (Rosswall 1982).

It is essential to note that immobilization and mineralization occurs at the same time within small portions of soil. Protein-rich and N-rich piece of soil organic matter is consumed by a group of microbes whereas another group 100 μm away consuming high C but low amounts of N from detritus and the process of immobilization is performed by this group whereas mineralization is done by the first group or the immobilization of the same N might be occurring which is mineralized by the first group. Gross N mineralization is the production of total soluble N by microorganisms, and gross N immobilization is the consumption of total soluble N and the balance between the two is Net N mineralization. When gross mineralization surpasses gross immobilization, inorganic N in the soil is increases and called as net mineralization. While in vice versa condition, inorganic N in the soil get decreases and hence called as net immobilization (Robertson and Groffman 2007). Immobilization and mineralization are performed by wide range of microorganisms—anaerobes, aerobes, bacteria and fungi. Soil fauna also has important role in immobilization and mineralization processes. The quality and quantity of detrital inputs in soils are the important factors controlling the patterns and rates of immobilization and mineralization. When there is favourable temperature and moisture, microbial activity at high rates are led by organic matter which result in high rates of immobilization and mineralization. As a general thumb rule immobilization is stimulated in material when the ratio of C:N >25:1 and when C:N ratio <25:1 mineralization is stimulated. Highly decomposed substances are the only exception to this rule as the C:N ratio is very low, and only complex forms of C remains which is resistant to decomposition and mineralization (Robertson and Groffman 2007).

1.4.4 Nitrification

In the process of nitrification, reduced forms of nitrogen are oxidized to nitrate with the help of autotrophic nitrite oxidizers, autotrophic ammonia oxidizers and heterotrophic nitrifiers (Fig. 1.2). Under aerobic conditions, based on the capacity to oxidize ammonia to nitrite, autotrophic nitrite and ammonia oxidizers are characterized. The reduced forms of N in soil are mainly from decomposition and excretion of organic nitrogen derived from plants and animals and from ammonia-based fertilizers. Ammonium is required by plants and many microorganisms for growth while nitrite is assimilated by others. Although ammonia has the tendency to bind with soil particles, the conversion of ammonia to nitrite results in losses of soil N through dentrification where it is converted to gaseous forms and leaching. Soil acidification is also caused by nitrification thereby increasing the toxic metals mobilization mainly in poorly buffered and heavily fertilized soils. In addition, many greenhouse gases are produced by nitrifiers (Prosser 2005). The formation of nitrite is initiated by one group of ammonium oxidizers and once nitrite is formed, the second group oxidizes nitrite to nitrate thereby completing the nitrification process (Haynes 2012).

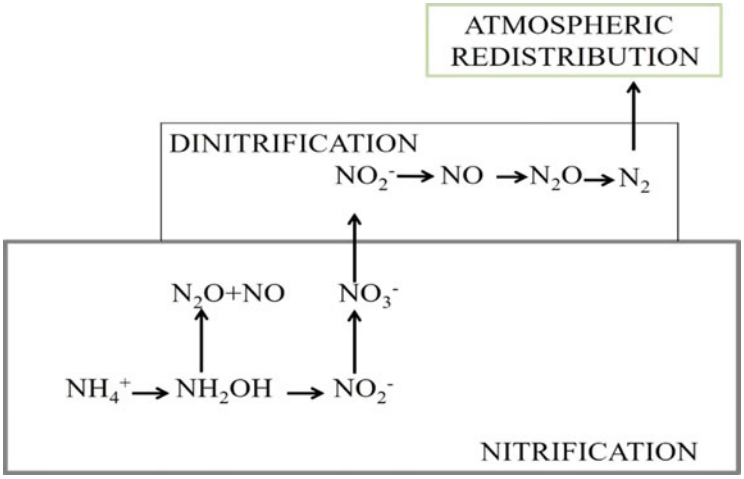

Fig. 1.2 Process flow chart of nitrification and denitrification

1.4.4.1 Diversity of Nitrifying Bacteria in Soil

The classifications of AOB are done based on the phylogenetic relationships of gene sequences of 16S rRNA. The three genera of AOB are *Nitrosococcus* (-γ-proteobacteria), *Nitrosospira* (β-proteobacteria) and *Nitrosomonas* (-β-proteobacteria). Further genera *Nitrosospira* and *Nitrosomonas* are divided into 7 clusters (*Nitrosomonas*, clusters 5–7; *Nitrosospira* clusters 1–4), Clusters 2, 3 and 4 of *Nitrosospira* spp. were found to be dominant in soils than other groups. The population of nitrifying bacteria in soil is increased on addition of NH_4^+ fertilizer. Soil pH is a limiting factor for the occurrence of nitrification in soils. For nitrifying bacteria, the optimum pH is 7–9 (Hayatsu et al. 2008).

In soils both nitrification and denitrification occur simultaneously since the most soils contain anaerobic as well as anaerobic zones. They are considered to be coupled processes as nitrite does not persist for a long time in soil. The autotrophic oxidation of nitrite to nitrate occurs at a faster rate in well-aerated soils that are unfertilized. This rate is even faster than the formation of nitrite from ammonium and hence the concentration of nitrite in soils is low (<1 μg g–1). There is a group of nitrifying organisms in soils which are called autotrophic ammonia oxidisers. The role of these organisms is to oxidize ammonium to nitrite and followed by reduction of nitrite to nitrous oxide and dinitrogen gas. This process is called nitrifier denitrification (McNeill and Unkovich 2007).

The supply of ammonium is the most important factor that regulates the nitrification process in most soils. The uptake of ammonium and immobilization of N by plants or heterotrophs will be high in soils where the rate of decomposition and mineralization of N is low and thus.

the rate of nitrification will also be low. Ecosystem disturbances like tillage, clear-cutting, fertilization, waste disposal and deposition of atmospheric N has increased

soil NH_4^+ availability and has accelerated the nitrification process in soils. This nitrification gets accelerated only when the supply of NH_4^+ exceeds the demand of heterotrophs and plant implying the fact that nitrifiers are poor competitors (Robertson and Groffman 2007).

1.5 Influence of C/N Ratio

Models predicting N retention and turnover in soils showed that C/N ratio is the important factor in controlling the rates of immobilization, mineralization and nitrification. Soil C/N ratio is used to predict the variations in N immobilization and mineralization is based on the fact that heterotrophic bacteria inhabit the soil which has high C/N ratio than the C/N ratio that the bacteria contains. Rate of N immobilization is used to characterize the soil, rapid immobilization means the soil has high C/N ratio whereas slow N immobilization occurs in the soils where there is low C/N ratio (Bengtsson et al. 2003).

Mineralization is dominated over immobilization in soils and residues where the C/N ratio is very small and the available forms of N are used in microbial processes or absorbed by plants. When N fertilizer is applied on the soil surface which contains straw with high C/N ratio increases the rate of immobilization of applied N fertilizer thereby decreasing N_2O emissions and denitrifying reactions. When the soil contains straw with low C/N ratio or no straw, N immobilization does not occur and there will be more N available for denitrification and nitrification process and also high amounts of N_2O will be emitted (Scarsbrook 1965; Piñeiro et al. 2010; Signor and Cerri 2013).

1.6 Losses of Nitrogen from Soil

N losses from plant/soil system not only reduce plant yield and soil fertility but also have great impacts on the environment. Emissions of ammonia into the atmosphere cause acid rain and also indirectly represent a source of greenhouse gas emissions. Eutrophication is caused when nitrate is leached into lake and rivers resulting in the growth of algae and aquatic weeds in large amounts affecting the fish populations and the quality of water. Drinking of nitrate contaminated water pose health risks. In horticultural and agricultural systems losses of mineral N occur through ammonia volatilisation, denitrification and leaching (Cameron et al. 2013).

1.6.1 Ammonia Volatilisation

Ammonia is present in the soil in various forms as free NH_4^+ ion or absorbed physically to the particles of soil or organic matter. Volatilisation of ammonia occurs when there is free ammonia near the surface and the rate of volatilisation increases with temperature and pH. Plants acts as both a sink as well as a source for ammonia. Emissions depend on various factors like N content, plant age and leaf NH_3 compensation point. A proportion of ammonia hydrolyses to ammonium from the excretion of faeces and urine by grazing animals which then deprotonates to ammonia. Although total loss of NH_3 is related to a wide range of management and environmental factors, NH_3 volatilization from animal wastes occur more rapidly (hours–days). A large proportion of N is lost from urea and ammonium fertilizers due to ammonia volatilisation. Out of total applied N, in rainfed agricultural ecosystems the losses might reach 50% whereas in flooded systems the loss might reach up to 80%. As for volatilisation of NH_3 to occur, NH_3 has to be in contact with soil surface, if N fertilizers are applied through drilling, injection or incorporation, the losses can be reduced. Annual global output of NH_3 emissions from animal excreta during the application of fertilizers contribute around 57% and have indicated as the most important source of NH_3 in the atmosphere. (McNeill and Unkovich 2007; Ahmed et al. 2010; Haynes 2012; Rochette et al. 2013; Kun et al. 2014).

1.6.2 Emission of N_2O and N_2

In soils, the transformations of N are mainly dominated by water regime. Irrigated soils are used for production of rice which gets flooded for different lengths of time. Upland soils also go through alternate dry and wet cycles. It has been found that nitrous oxide causes ozone layer depletion in the stratosphere. 10% of ozone layer is decreased when the concentration of N_2O is doubled. There is a concern that soil fertilization might lead to a considerable increase in concentrations of N_2O in the troposphere region and thereby resulting in partial destruction of ozone layer in the stratospheric region and also causing greenhouse effect (Bremner et al. 1980).

In soils, the transformations of Nr (reactive N) generate most of the N_2O. Reactive N (Nr) is defined as inorganic and organic bound compounds of N except N_2. Once Nr (mineral or organic) is entered, there exist a numerous processes in soil that lead to the formation of N_2O.

Dissimilatory nitrate reduction, denitrification and nitrification are the processes that are considered as the main contributors for emissions of N_2O. N_2O is used by both autotrophic and heterotrophic bacteria for respiration which is further reduced to N_2 (Cayuela et al. 2014).

Table 1.4 Environmental factors and management practices influencing emissions N_2O derived from fertilizers

Environmental factors	Management practices
Ph	Fertilizer type
Temperature	Application technique
Soil moisture content	Application rate
Precipitation	Timing of application
Microorganisms	Use of other chemicals
Porosity	Irrigation
Organic C content	Tillage practices
Freeze and thaw cycle	Crop type
Oxygen availability	Residual C & N from fertilizer and crops

In biological denitrification either one or both of the oxides of N (NO_2^- and NO_3^-) are reduced to gaseous oxides (N_2O, NO) which further get reduced to N_2 by itself.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Fungi and heterotrophic bacteria are the dominant organisms to carry out denitrification process. These organisms utilize oxides of N as terminal electron acceptors and organic form of C as electron donors where oxygen availability is restricted. Most of the bacteria that carry out denitrification are chemoheterotrophic, use nitrate to obtain energy where nitrate acts as the primary electron acceptor. There are a few autotrophic bacteria as well in which energy is obtained from oxidation of inorganic compounds by nitrate. It has been shown that NO_3^- respiration can be done by bacteria through aerobic denitrification and for managed as well natural ecosystem it can be highly significant. Farming management processes increases availability of soil nitrate and hence can cause high denitrification rates in agroecosystems where management increases soil nitrate availability, further it can be worsened by irrigation. These losses can be 0.1–18% of N in urine and dung on grazed pastures and nearly 7% of applied N fertilizers. Though, these data taken from studies from temperate regions and in tropical and sub-tropical regions may be the losses will be high (McNeill and Unkovich 2007). Soil characteristics and environmental conditions combinedly influence the emissions of N_2O (Table 1.4). Soil acts as both a source as well as a sink for nitrous oxide (Cayuela et al. 2014).

Since N_2O formation is aided by microbial processes, the factors like pH, temperature and rainfall that affect the microorganism's growth also have an influence on N_2O production. In addition, fertilizer rate, soil type, tillage practice, availability of carbon, oxygen concentration, vegetation, use of chemicals, land use practices, irrigation practices also affect the production of N_2O. Fertilizer application causes immediate emission of large amount of nitrous oxide which may last up to 6 weeks. 0.6–1.7 ng N m^{-2} s^{-1} is the range of flux for N_2O in non-agricultural soils and this range of emission got increased two- to seven-fold when soils were deposited with N. After fertilizer application the rate of N_2O emission fluctuates and falls to a low value, and this fall in emission rate is not dependant on the quantity of

fertilizer applied. A large amount of N_2O were emit via fertilizers containing anhydrous ammonia. The largest emissions occurred when anhydrous ammonia was used and the smallest emissions occurred when nitrogen solutions were applied. Legumes fixed atmospheric N can be denitrified and nitrified in a similar way as fertilizer N and hence being a source of N_2O. *Rhizobia*, living symbiotically in the nodules of root also produce nitrous oxide through denitrification. Forest area being converted to pasture and crop production also has a great effect on nitrous oxide emission (Freney 1997).

1.6.3 Nitrate Leaching

Since most of the soil particles are negatively charged, NO_3^- being an anion is not retained in the soil and can be easily leached into the groundwater. The water quality that passes through soil profile and NO_3^- concentration in soil at the time of water passage are the two main determinants of nitrate leaching. Harvest, fire cultivation, grazing, fallowing are few of the many ecosystem disturbances that tend to increase the NO_3^- leaching in both agricultural and natural systems. This is mainly due to NO_3^- accumulation in soils due to the intrinsically linked processes (mineralization and N uptake by plants) getting uncoupled along with increased drainage due to hydrologic cycle imbalance. Soils that are light textured and shallow rooted crops tend to loose considerable amount of nitrate under conditions like irrigation or high rainfall (Peralta and Stockle 2002). Overall, leaching is high on soils that are under irrigation and also on sandy soils and it is much larger in frequently disturbed agricultural ecosystems where high amounts of animal manures, legumes and fertilizers are applied.

A conclusion in a recent review on temperate agroecosystems was made that forests tend to loose least amount of NO_3^- and systems with intensive vegetable production loose high amount of NO_3^-. A recent review of temperate agroecosystems concluded that, in general, the potential for NO_3^-—leaching was least in forests, increasing in the order cut grasslands < grazed pastures < arable cropping < ploughed pastures, and was highest for intensive vegetable production system. The scale of organic N losses via leaching in managed and natural terrestrial ecosystems is still unknown. Few studies have measured dissolved organic N and water-extractable organic N in forest and agricultural soil and it was around 0.1–5 mg l^{-1} and 10–30 kg ha^{-1}, respectively. (McNeill and Unkovich 2007; Padilla et al. 2018).

On a global level, nitrate leaching and N_2O emission induced by fertilizer are estimated to be 19 and 8%, respectively (Kim et al. 2015). From root zone to soil thickness with respect to ground water, all determine the risk of pollution of the aquifers. There are great chances of Nitrate leaching into groundwater from fractured soil on shallow soils. Also, various studies have shown nitrate leaching into ground water through agricultural soil in karst regions. Soils contain considerable amounts

of nitrate, when evaporation is exceeded by rainfall and nitrate taken by crop is small, leaching of nitrate is large from arable soils (Podgornik and Pintar 2007).

To get high crop yields, fertilizer N is added which eventually increase leaching losses. Nitrate leaching on soils that are light textured is large when high rates of fertilizer are combined with heavy regimes of irrigation (Haynes 2012). Not only soil fertility is lost through leaching of nitrate into water, it also poses a threat to human health. A risk of methaemoglobinaemia can be created when drinking water supplies contain nitrate and is also linked to heart disease and cancer. Reports have shown that half of the European population lives in high nitrate containing (~5.6 mg $NO_3^-N\ L^{-1}$) ground water areas and about 20% lives in areas with nitrate concentrations surpassing the recommended level (11.3 mg $NO_3^-N\ L^{-1}$) and estimated that in French Brittany the nitrate concentration is higher than 11.3mgN L^{-1} in 80% of surface water (Cameron et al. 2013).

Variations in distributions of rainfall and patterns of evapotranspiration from season to season and from year to year affect the pattern of leaching. The extent and pattern of leaching is mainly determined by amount and intensity of rainfall. Soil physical properties also influence leaching, as the water storage capacity and hydraulic conductivity are directly related to the structure and texture of the soil, sandy soils that are poorly constructed tend to lose more NO_3^- than clay soils that are coarsely structured. Wet clay soils have high rates of denitrification which further reduce the leaching loss. Lysimeter, borehole, catchment and column studies have shown that rate of nitrate leaching is fast in coarse-textured soils than fine-textured soils.

Leaching is also affected by soil organic N. The process of nitrification and mineralization of soil organic N in agricultural soils also causes NO_3^- leaching. However, mineralization might get stimulated due to addition of fertilizer and hence leaches the native soil NO_3^-. This means that because of mineralization-immobilization turnover, immobilization of fertilizer N occurs and mineralization of soil organic N also occurs at the same time and getting possibly lost through leaching.

Application of organic waste can increase leaching. Spreading large amount of animal wastes as slurries or as solids on agricultural land than the required amount can cause NO_3^- leaching.

The determination of optimum rate is hard as mineralization of organically combined N must occur before it is taken by plant or being subjected to leaching. Thus, the rate of mineralization is the key factor to estimate optimum rate of application. As crop growth is increased by irrigation the amount of N uptake is also increased. Leaching losses can be reduced when optimum amount of water is supplied for crop growth. Many cases have been reported that irrigation increases NO_3^- leaching due to the passage of excess amount of water through the root zone of the crop. The quantity of the leached NO_3^- is greatly affected by the irrigation method. The nutrients are directly removed below the furrow in case of furrow irrigation, whereas with sprinkler and flood irrigation, the volume of soil leached is very high that facilitates a uniform downward movement of nitrate (Haynes 2012).

1.7 Conclusions

Nitrogen covers major portion of gases in air and one of the most important elements in soil. The global N content is majorly regulated by what is happening in soil, as most of the N in soil has various varieties and tend to go in lots of transformations generally in organic forms. All processes involved in nitrogen cycle act individually but affected by other processes happening sequentially. Apart from environmental N cycle, there is a separate cycle for N in soil known as internal "N cycle". Continuous transformation of N occurs through organic and inorganic forms by mineralization-immobilization processes. Soils N content changes greatly, and many factors such as temperature, soil type, wind, pH and precipitation fluctuates its availability. In uncultivated lands, the soil N availability is administered through soil-forming factors. But during cultivation the soil N level declines and reaches to new equilibrium level. Though lot of studies have already been done for estimating N distribution and its physiology in environment, but still more studies are needed in the current situation of global warming. In future, various processes of nitrogen cycle like nitrification, denitrification, immobilization, mineralization, etc. are needed to be analysed and the soil N content should be monitored for effective sustainable agricultural practices. The area of soil N loss due to denitrification and leaching also need attention and studies should focus on how to manipulate N cycle to reduces N losses and affirm that the plants will get adequate amount of available N. Not only we need to research more about environment friendly fertilizers but also should focus on the use of technology which ensure effective release of the N equivalent to plant N uptake.

References

Adams AP, Bartholomew WV, Clark FE (1954) Measurement of nucleic acid components in soil. Soil Sci Soc Am J 18:40–46

Ahmed OH, Yap CB, Muhamad AN (2010) Minimizing ammonia loss from urea through mixing with zeolite and acid sulphate soil. Int J Phys Sci 5:2198–2202

Bai J, Deng W, Zhu Y, Wang Q (2004) Spatial variability of nitrogen in soils from land/inland water ecotones. Commun Soil Sci Plant Anal 35:735–749

Batjes N, Dijkshoorn J (1999) Carbon and nitrogen stocks in the soils of the Amazon region. Geoderma 89:273–286

Bengtsson G, Bengtson P, Månsson KF (2003) Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biol Biochem 35:143–154

Bremner JM (1949) Studies on soil organic matter: part I. the chemical nature of soil organic nitrogen. J Agric Sci 39:183–193

Bremner JM, Robbins SG, Blackmer AM (1980) Seasonal variability in emission of nitrous oxide from soil. Geophys Res Lett 7:641–644

Bronson KF (2008) Forms of inorganic nitrogen in soil. In: Schepers JS, Raun WR (eds) Nitrogen in agricultural systems, agronomy monograph 49. ASA, CSSA, and SSSA, Madison, WI, pp 31–56

Cabrera ML, Kissel DE, Vigil MF (2005) Nitrogen mineralization from organic residues. J Environ Qual 34:75–79
Cameron KC, Di HJ, Moir JL (2013) Nitrogen losses from the soil/plant system: a review. Ann Appl Biol 162:145–173
Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of Earth's nitrogen cycle. Science 330:192–196
Cavalli D, Consolati G, Marino P, Bechini L (2015) Measurement and simulation of soluble, exchangeable, and non-exchangeable ammonium in three soils. Geoderma 259:116–125
Cayuela ML, Van Zwieten L, Singh BP, Jeffery S, Roig A, Sánchez-Monedero MA (2014) Biochar's role in mitigating soil nitrous oxide emissions: a review and meta-analysis. Agric Ecosyst Environ 191:5–16
Coelho RRR, Sacramento DR, Linhares LF (1997) Amino sugars in fungal melanins and soil humic acids. Eur J Soil Sci 48:425–429
Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009) Controlling eutrophication: nitrogen and phosphorus. Science 323:1014–1015
Corre MD, Veldkamp E, Arnold J, Wright SJ (2010) Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. Ecology 91:1715–1729
Dai XY, Ping CL, Hines ME, Zhang XD, Zech W (2002) Amino sugars in arctic soils. Commun Soil Sci Plant Anal 33:789–805
Del Grosso SJ, Parton WJ, Mosier AR, Ojima DS, Kulmala AE, Phongpan S (2000) General model for N_2O and N2 gas emissions from soils due to dentrification. Global Biogeochem Cycles 14:1045–1060
Deng Y, Dixon JB (2002) Soil organic matter and organic-mineral interactions. In: Dixon JB, Schulze DG (eds) Soil mineralogy with environmental applications, vol 7. SSSA, Madison, WI, pp 69–107
Dessureault-Rompré J, Zebarth BJ, Burton DL, Grant CA (2016) Depth distribution of mineralizable nitrogen pools in contrasting soils in a semi-arid climate. Can J Soil Sci 96:1–11
Ding G, Mao J, Xing B (2001) Characteristics of amino acids in soil humic substances. Commun Soil Sci Plant Anal 32:1991–2005
Fang YT, Zhu WX, Mo JM, Zhou GY, Gundersen P (2006) Dynamics of soil inorganic nitrogen and their responses to nitrogen additions in three subtropical forests, south China, J Environ Sci China 18:752–759
Freney JR (1997) Emission of nitrous oxide from soils used for agriculture. Nutr Cycl Agroecosyst 49:1–6
Friedel JK, Scheller E (2002) Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. Soil Biol Biochem 34:315–325
Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–892
Greenfield LG (1972) The nature of the organic nitrogen of soils. Plant Soil 36:191–198
Hall SJ, Matson PA (2003) Nutrient status of tropical rain forests influences soil. N Dynamics after N additions Ecol Monogr 73:107–129
Havlin JL, Beaton JD, Tisdale SL et al (2005) Soil fertility and fertilizers: an introduction to nutrient management, 7th edn. Pearson Prentice Hall, Upper Saddle River, NJ, p 515
Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties 5:985–1018
Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45
Haynes RJRJ (2012) Mineral nitrogen in the plant-soil system. Elsevier, Amsterdam, The Netherlands
Hofman G, Van Cleemput O (2004) Soil and plant nitrogen. International Fertilizer Industry Association, Paris

IFA (2005) Production and international trade. https://www.fertilizer.org/IFA
Jackson LE, Burger M, Cavagnaro TR (2008) Roots, nitrogen transformations, and ecosystem services. Annu Rev Plant Biol 59:341–363
Jaffe DA (2000) The nitrogen cycle. In: Jacobson MC, Charlson RJ, Rodhe H, Orians GH (eds) Earth system science. Academic Press, San Diego, CA, pp 322–342
Jansson SL, Persson J (1982) Mineralization and immobilization of soil nitrogen. Nitrogen in agricultural soils 22:229–252
Joergensen RG (2018) Amino sugars as specific indices for fungal and bacterial residues in soil. Biol Fertil Soils 54:559–568
Kennedy IR, Choudhury ATMA, Kecskés ML (2004) Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Biol Biochem 36:1229–1244
Kim Y, Seo Y, Kraus D, Klatt S, Haas E, Tenhunen J, Kiese R (2015) Estimation and mitigation of N_2O emission and nitrate leaching from intensive crop cultivation in the Haean catchment, South Korea. Sci Total Environ 529:40–53
Kox MA, Jetten MS (2015) The nitrogen cycle. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer, Cham, pp 205–214
Kumar N, Srivastava P, Vishwakarma K, Kumar R, Kuppala H, Maheshwari SK, Vats S (2020) The rhizobium–plant symbiosis: state of the art. In: Varma A, Tripathi S, Prasad R (eds) Plant microbe symbiosis. Springer, Cham, pp 1–20
Kun HAN, Zhou CJ, Wang LQ (2014) Reducing ammonia volatilization from maize fields with separation of nitrogen fertilizer and water in an alternating furrow irrigation system. J Integr Agric 13:1099–1112
Lamb JA, Fernandez FG, Kaiser DE (2014) Understanding nitrogen in soils. University of Minnesota Extension (Revised) 1–5
Lipson D, Näsholm T (2001) The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128:305–316
Marty C, Houle D, Gagnon C, Courchesne F (2017) The relationships of soil total nitrogen concentrations, pools and C:N ratios with climate, vegetation types and nitrate deposition in temperate and boreal forests of eastern Canada. Catena 152:163–172
Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann Bot 105:1141–1157
Matsumoto S, Ae N, Yamagata M (2000) The status and origin of available nitrogen in soils. Soil Sci Plant Nutr 46:139–149
McNeill A, Unkovich M (2007) The nitrogen cycle in terrestrial ecosystems. In: Marschner P, Rengel Z (eds) Nutrient cycling in terrestrial ecosystems. Springer, Berlin, pp 37–64
Mengel K, Kirkby EA, Kosegarten H, Appel T (2001) Nitrogen. In: Mengel K, Kirkby EA (eds) Principles of plant nutrition. Springer, Dordrecht, pp 397–434
Miller AJ, Cramer MD (2005) Root nitrogen acquisition and assimilation. In: Lambers H, Colmer TD (eds) Root physiology: from gene to function. Springer, Dordrecht, pp 1–36
Moldan F, Wright RF (2011) Nitrogen leaching and acidification during 19 years of NH4NO3 additions to a coniferous-forested catchment at Gårdsjön, Sweden (NITREX). Environ Pollut 159:431–440
Murphy DV, Macdonald AJ, Stockdale EA, Goulding KWT, Fortune S, Gaunt JL, Wilmer WS (2000) Soluble organic nitrogen in agricultural soils. Biol Fertil Soils 30:374–387
Nie X, Xiong F, Yang L, Li C, Zhou G (2017) Soil nitrogen storage, distribution, and associated controlling factors in the northeast Tibetan plateau shrublands. Forests 8:416
Niggemann J, Schubert CJ (2006) Sources and fate of amino sugars in coastal Peruvian sediments. Geochim Cosmochim Acta 70:2229–2237
Padilla FM, Gallardo M, Manzano-Agugliaro F (2018) Global trends in nitrate leaching research in the 1960–2017 period. Sci Total Environ 643:400–413

Peralta JM, Stockle CO (2002) Dynamics of nitrate leaching under irrigated potato rotation in Washington state: a long-term simulation study. Agric Ecosyst Environ 88:23–34
Piñeiro G, Paruelo JM, Oesterheld M, Jobbágy EG (2010) Pathways of grazing effects on soil organic carbon and nitrogen. Rangel Ecol Manag 63:109–119
Podgornik M, Pintar M (2007) Causes of nitrate leaching from agriculture land in Slovenia. Acta Agric Slov 89:207–220
Pollock GE, Cheng CN, Cronin SE (1977) Determination of the D and L isomers of some protein amino acids present in soils. Anal Chem 49:2–7
Post WM, Pastor J, Zinke PJ, Stangenberger AG (1985) Global patterns of soil nitrogen storage. Nature 317:613–616
Powlson DS (1993) Understanding the soil nitrogen cycle. Soil Use Manag 9:86–93
Prosser JI (2005) Nitrogen in soils: nitrification. In: Hillel D (ed) Encyclopedia of soils in the environment. Elsevier, University of Aberdeen, Aberdeen, pp 31–39
Rayleigh L (1939) Nitrogen, argon and neon in the Earth's crust with applications to cosmology. Proc R Soc Lond A Math Phys Sci 170:451–464
Robertson GP, Groffman PM (2007) Nitrogen transformations. In: Paul EA (ed) Soil microbiology, ecology and biochemistry. Academic Press, Burlington, MA, pp 341–364
Rochette P, Angers DA, Chantigny MH, Gasser MO, MacDonald JD, Pelster DE, Bertrand N (2013) Ammonia volatilization and nitrogen retention: how deep to incorporate urea? J Environ Qual 42:1635–1642
Rosswall T (1982) Microbiological regulation of the biogeochemical nitrogen cycle. Plant Soil 67:15–34
Salton MRJ (1965) Chemistry and function of amino sugars and derivatives. Annu Rev Biochem 34:143–174
Scarsbrook CE (1965) Nitrogen availability. Soil Nitrogen 10:481–502
Scherer HW (1993) Dynamics and availability of the non-exchangeable NH4-N—a review. Eur J Agron 2:149–160
Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602
Schreiner O, Shorey EC (1910) Pyrimidine derivatives and purine bases in soils. J Biol Chem 8:385–393
Schulten HR, Schnitzer M (1997) The chemistry of soil organic nitrogen: a review. Biol Fertil Soils 26:1–15
Senwo ZN, Tabatabai MA (1998) Amino acid composition of soil organic matter. Biol Fertil Soils 26:235–242
Signor D, Cerri CEP (2013) Nitrous oxide emissions in agricultural soils: a review. Pesqui Agropecu Trop 43:322–338
Smil V (1997) Cycles of life: civilization and the biosphere. Scientific American Library Series, No 63, New York
Sowden FJ, Chen Y, Schnitzer M (1977) The nitrogen distribution in soils formed under widely differing climatic conditions. Geochim Cosmochim Acta 41:1524–1526
Stevenson FJ (1965) Origin and distribution of nitrogen in soil. Soil Nitrogen 10:1–42
Stevenson FJ (1983) Nitrogen—organic forms. In: AL P et al (eds) Methods of soil analysis: part 2 chemical and microbiological properties, vol 9. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI, pp 625–641
Szajdak L, Österberg R (1996) Amino acids present in humic acids from soils under different cultivations. Environ Int 22(3):331–334
Thamdrup B (2012) New pathways and processes in the global nitrogen cycle. Annu Rev Ecol Evol Syst 43:407–428
Tian D, Niu S, Pan Q, Ren T, Chen S, Bai Y, Han X, Whitehead D (2016) Nonlinear responses of ecosystem carbon fluxes and water-use efficiency to nitrogen addition in Inner Mongolia grassland. Funct Ecol 30:490–499

Valentine AJ, Benedito VA, Kang Y (2010) Legume nitrogen fixation and soil abiotic stress: from physiology to genomics and beyond. Annu Rev Plant Biol 42:207–248

Van Groenigen J, Huygens D, Boeckx P, Kuyper TW, Lubbers IM, Rütting TP, Groffman PM (2015) The soil N cycle: new insights and key challenges. Soil 1:235–256

Walvoord MA, Phillips FM, Stonestrom DA, Evans RD, Hartsough PC, Newman BD, Striegl RG (2003) A reservoir of nitrate beneath desert soils. Science 302:1021–1024

Walworth J (2013) Nitrogen in soil and the environment. College Of Agriculture and Life Sciences Cooperative Extension. The University of Arizona Cooperative Extension, United States of America

Warren GP, Whitehead DC (1988) Available soil nitrogen in relation to fractions of soil nitrogen and other soil properties. Plant Soil 112:155–165

Weaver RW, Danso SK (1994) Dinitrogen fixation. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) Methods of soil analysis: part 2 microbiological and biochemical properties, vol 5. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI, pp 1019–1045

Xing GX, Zhu ZL (2002) Regional nitrogen budgets for China and its major watersheds. Biogeochemistry 57–58:405–427

Yang Y, Ma WAM, Fang J (2007) Storage, patterns and controls of soil nitrogen in China. Pedosphere 17:776–785

Yeasmin S, Islam AM, Islam AA (2012) Nitrogen fractionation and its mineralization in paddy soils: a review. J Agric Sci Technol 8:775–793

Young JL, Aldag RW (1982) Inorganic forms of nitrogen in soil. In: Stevenson FJ (ed) Nitrogen in agricultural soils, vol 22. American Society of Agronomy, Madison, WI, pp 43–66

Zhu X, Zhang W, Chen H, Mo J (2015) Impacts of nitrogen deposition on soil nitrogen cycle in forest ecosystems: a review. Acta Ecol Sin 35:35–43

Chapter 2
Ecophysiology of Nitrogen in Symbiotic Relationships of Plants and Microorganisms

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Abstract Nitrogen (N) is one of the essential elements for life on Earth and is mainly distributed in an inert form in the atmosphere, so it cannot be used by plants. Therefore, the N found in the atmosphere must undergo several transformations to be fixed in the soil and then used by plants. With the presence of N in several organic molecules, the acquisition of this element by plants has an impact on the growth and development of several agricultural crops. Understanding the metabolic mechanisms related to N and the methods of uptake, transport and use of this nutrient by plants have always been a topic of interest in several types of research. N also influences the soil microbiota, because microorganisms participate in the N cycle, especially in relation to the process of biological application of N. Thus, with the discovery of N-fixing microorganisms, it was possible to explore the impact that the microbiota would have on the plant's N metabolism. In this context, we have gathered in this chapter, information about the ecophysiology of N in plants and how some fungi and bacteria are able to influence the metabolism of this element. We also explain a little how the association between plants and endophytic bacteria occurs, since this symbiotic relationship is very important to make N available to the plants.

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2.1 Introduction

Widely distributed in the atmosphere in an inert form as atmospheric gas (N_2) and found in a lower concentration in the soil, nitrogen (N) is considered an important element for life (Frossard et al. 2009). For N to be available in the soil and used by plants, it needs to undergo specific transformations in order to be fixed or mineralized and transformed into the form of ammonium (NH_4) and nitrate (NO_3), both of which are absorbable by plant organisms, and for this reason, N is considered one of the most limiting nutrients for plant growth (Wang et al. 2019).

Due to the importance of this element in the structuring of plant organisms, since it is found in several essential structures, such as amino acids, genetic material and photosynthetic pigments, the methods to increase N availability in the soil are of great relevance to the ecosystem, like the fixation processes, in which atmospheric N_2 is transformed into NH_4 and NO_3 (Taiz et al. 2017). The availability of N in the soil results, mainly, from the action of microorganisms, through a process called biological N fixation, usually performed by diazotrophic bacteria, which can be free-living or associated with plants (Sylvia et al. 2005). Other organisms of the soil microbiota that help plants through an endosymbiont relationship are fungi (Bonfante et al. 2019). These microorganisms increase the mobilization of N and its uptake by plants, as they expand the contact surface of the roots and facilitate the mobilization of NO_3 and NH_4 in the soil (Tao et al. 2019).

With the action of microorganisms in the rhizosphere, the plant is able to absorb a greater amount of N, causing the presence of beneficial fungi and bacteria in the plant's roots to directly influence plant growth (Tao et al. 2019). With the higher availability of N, plants can use this nutrient efficiently, maintaining their physiology in optimal conditions and investing in the production of amino acids, chlorophyll and plant material (Hallik et al. 2009). Some endosymbiont microorganisms are also capable of increasing the production of secondary metabolites from the plant, removing toxic compounds from the soil, protecting the plant against herbivory, increasing the plant resistance levels to toxic organic or inorganic compounds, increasing the response to water stress and influencing enzyme activity (Mouradi et al. 2016; Staudinger et al. 2016; El-Serafy and El-Sheshtawy 2020; Harindintwali et al. 2020; Liu et al. 2020a, b; Jack et al. 2019).

Therefore, when considering that microorganisms influence the physiology of N in the plant, as well as its mobilization in the soil, we will address in this chapter the ecophysiology of symbiotic relationships between plants and microorganisms in order to understand their influence and the impact that can be observed in the uptake, transformation and mobilization of N, in addition to understanding the establishment of the N-fixing root nodule.

2.2 Ecophysiology of Nitrogen in Plants

In natural ecosystems, the sources of N are the atmosphere and the organic matter present in the soil (Guignard et al. 2017). In the atmosphere, the abundance of N is not directly related to its availability for plants due to high chemical stability, multiple transformations and interactions with other important biogeochemical elements (Craine et al. 2015; Taiz et al. 2017). However, there are plants that manage to establish a symbiotic association with N_2-fixing bacteria (NFB), a process capable of supplying more than 90% of the necessary N for plant growth and development (Valarini and Godoy 1994; Mahmud et al. 2020). The N in the soil originates from the N_2 fixation and from the decomposition of organic matter, whereas the mineralization process is mediated by microorganisms that produce NO_3 and NH_4 (Taiz et al. 2017).

For this, the metabolism of N comprises the processes of uptake, assimilation and transport of the nutrient. These processes depend on several factors such as the availability of N in the soil, location and regulation of the enzymes involved, as well as the energy from photosynthetic processes (Masclaux-Daubresse et al. 2010). This element has a direct effect on the distribution of photoassimilates between the vegetative and reproductive part, promoting changes in the physiological and morphological apparatus of the plant (Han et al. 2017), being related to gas exchange, architecture of roots, nutrient uptake and cell development, as well as being present in the molecular composition of ATP, NADH, NADPH, DNA, RNA and proteins (Masclaux-Daubresse et al. 2010; Guo et al. 2019).

According to Williams and Miller (2001), plants absorb N from the environment in several forms, including NO_3, NH_4, urea and amino acids, but they have a greater affinity for NO_3, since this ion is the end product of the microbiological use of ammoniacal N. Both NO_3 and NH_4 are rapidly absorbed by the roots of the plants, but the uptake of one form in relation to another is influenced by pH changes in the medium and also by the plant itself (Jiang et al. 2015).

To maintain the internal electrical neutrality in the cytoplasm when NH_4 is absorbed by cell, a proton (H^+) is extruded into the apoplast, thus reducing the pH of the medium, but when NO_3^- is absorbed, a hydroxyl (OH^-) or a (bicarbonate) HCO_3 is released, causing an increase in the medium pH. This action is possibly associated with the competitive effects of H^+ and OH^- on the NH_4^+ and NO_3^- absorption process, respectively (Lea and Morot-Gaudry 2001; Zhu et al. 2011). In electrophysiological studies, together with the pH-dependent balance between the uncharged NH_3 and the charged NH_4^+ forms, it is suggested that the ion is predominant in all physiological conditions and is the dominant species in the controlled transport of the membrane, since they provide the driving force for active influx and efflux of ions and metabolites across the plasma membrane (Ludewig et al. 2007).

After being absorbed by the roots, N is transported to the aerial part of the plant by the xylem vessels, via transpiratory current, but the way that N is transported depends on the form it was absorbed, assimilated (incorporated into organic

compounds) in the root tissues and transported as amino acids, by means of low and/or high affinity transporters (Tegeder and Masclaux-Daubresse 2018).

The transport of N to the shoot depends on the plant potential for reducing NO_3 to NH_4. Once transported, the assimilation of NH_4 into amino acids is attributed to an enzymatic action involving glutamine synthetase, glutamine oxoglutarate aminotransferase and asparagine synthetase (Masclaux-Daubresse et al. 2010). Due to its high mobility within the plant, N is easily redistributed via phloem, in the form of amino acids. However, when the supply of this element is insufficient due to a nutritional deficiency, the N of the older leaves is gradually remobilized to the younger organs and leaves, coinciding with the reduction of the chlorophyll content (Maillard et al. 2015). It is important to highlight that the reduction of NO_3^- has a higher energy cost and NH_4 can become toxic to the plant, needing to be quickly assimilated into organic compounds, so most plants preferentially absorb N in the nitric form (Turpin et al. 1988; Chaillou et al. 1994).

All inorganic N is first reduced to NH_4^+ before being incorporated into organic forms. NO_3 is reduced to NH_4 by the action of two main enzymes, nitrate reductase (NR), located in the cell cytoplasm, which can use NADH or NADPH as a H^+ donor, and the nitrite reductase (NiR), located within the chloroplasts (leaves) and plastids (roots), that uses reduced ferredoxin as an electron donor. NR catalyses the reduction of two electrons for the conversion of NO_3 to nitrite (NO_2), then NiR transforms NO_2 into NH_4 through a reduction of six electrons and NH_4 is converted to glutamine, which is essential for the synthesis of amino acids via glutamine synthetase (GS) and glutamate synthetase (GOGAT) (Wickert et al. 2007; Taiz et al. 2017). Thus, the regulation of N is influenced by different metabolic factors, aiming to adjust the assimilation of NO_3 to the needs of the plant.

The N assimilation is dependent on the availability of electron-accepting molecules such as glutamate and energy molecules such as NADH, ATP and ferredoxin, supplied by photosystems in light condition. As a consequence of these needs, the uptake of N has a strong link with carbohydrate metabolism, especially with photosynthesis, since the N content can determine the amount of enzymes, chlorophyll, NADPH and ATP available for the metabolism of the photosynthetic process (Nunes-Nesi et al. 2010). Therefore, this shows that N activity has a direct effect on the CO_2 assimilation of plants (Correia et al. 2005; Bassi et al. 2018).

2.3 Nitrogen and the Symbiotic Relationships of Plants and Fungi

2.3.1 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are microorganisms capable of promoting symbiotic associations with plant roots, occurring in more than 80% of plant species and characterized by the presence of intraradicular hyphae, arbuscules,

extraradicular mycelium and spores (Peterson et al. 2004; Bonneau et al. 2013). This symbiosis is considered a cosmopolitan association and is recognized as an important and integral part of natural ecosystems of the world (Gadkar et al. 2001), providing the stimulus for plant growth and a perfect morphological and physiological integration, resulting from nutritional improvements, mainly the increased uptake of phosphorus (P) and N (Cozzolino et al. 2013).

AMF can transfer inorganic N (NO_3^- or NH_4^+) to their host plant, but this nutrient is transferred, predominantly, in the form of NH_4^+. This transfer involves a metabolic route, in which N is moved by AMF from the soil to the host, consisting of metabolic processes known to operate in fungi (the assimilation of inorganic N), together with a new variant of the urea cycle, in which the anabolic and catabolic parts are separated by the long-distance translocation of arginine. This assimilation of N into arginine allows it to be moved in a concentrated and non-toxic manner, making the transfer of N to the host plant with minimal loss of carbon to the fungus (Govindarajulu et al. 2005; Vergara et al. 2018).

These microorganisms, when inoculated in legumes, promote an increased capacity of N_2 fixation (Veresoglou et al. 2012). According to Datta et al. (2019), AMF together with NFB play an important role as symbionts of plants, due to their ability to solubilize and absorb nutrients, obtain N from the atmosphere and still protect the plant from abiotic and biotic stresses. Also according to these authors, in leguminous plants there are several similarities in the symbiosis of NFB and AMF, mainly from an ecophysiological point of view, involving the search for macro and microelements of the mycorrhizal roots, essential for the functioning of N_2 fixation and the symbiosis capacity of NFB to obtain large amounts of N that influence the supply of photoassimilates to AMF.

It is worth noting that the participation of AMF in the N cycle can be variable, helping to overcome stresses in plants and influence the N cycle in the soil-plant system (Hodge and Fitter 2010). For Veresoglou et al. (2012), plants colonized with AMF have an increased root volume, which stimulates plant growth. Therefore, the N cycling processes can occur in a larger volume of the soil, as there is an increase, induced by the AMF, in the root size and surface areas, thus stimulating the N cycling process. Another benefit associated with AMF is that plants colonized by these fungi can reduce leaching of N, as they promote improvements in water relations, which include greater stomatal conductance and faster drying of the soil (Augé 2001).

Symbiosis with AMF can also have an indirect effect on the acquisition of N via N_2 fixation. This effect is associated with a strong positive correlation of P supply to plants, linked to the increase between the total P content of the plant and the percentage of N fixed, clearly documenting the importance of P nutrition in the plant for the efficiency of NFB. Such fact was observed by Püschel et al. (2017) working with *Medicago truncatula* and *Medicago sativa* inoculated with *Rhizophagus irregularis* 'PH5', in which the symbiosis improved the uptake of P in plants and considerably stimulated the efficiency of NFB under low P availability.

However, even when the symbiotic microorganisms act in the mineralization of organic to inorganic forms of both N and P, these interactions are not entirely

symmetrical, because when affecting N fixation, the supply of P affects the amount of N in ecosystems, whereas additional phosphatases enzymes affect the rate of cycling, but not the amount of P in ecosystems. Despite this, it is known that the P cycle is closely linked to the mycorrhizal fungi, since they release extracellular phosphatases, thus having a compensatory effect for the incorporation of these elements (Vitousek et al. 2010; Spohn and Kuzyakov 2013; Guignard et al. 2017). According to these findings, Javaid (2010) reported that there is generally a synergistic interaction between NFB and AMF, resulting in better root nodulation, nutrient uptake, higher photosynthetic rate and plant yield compared to symbiotic plants, with any single organism.

Thus, AMF can obtain substantial amounts of N from the soil, as well as from the decomposition of organic materials and, consequently, enhancing their fitness. So, the large biomass and high N demand of AMF represent a global set of N equivalent in magnitude to the fine roots of the plants (Hodge and Fitter 2010), thus showing its importance in the uptake and fixing of N.

2.3.2 Ectomycorrhizal Fungi

Ectomycorrhizal (ECM) fungi, unlike AMF, are symbiotic microorganisms characterized by developing their hyphae intensively around the root, forming a structure called the mantle, and only between the cells of the cortex, forming the Hartig net (Peterson et al. 2004). These microorganisms are found mainly in forest areas, preferably in association with woody plants, increasing the uptake of N and other nutrients from the soil to the plant, in exchange for photoassimilates (Datta et al. 2019).

The ecological stability of the mycorrhizal association depends on its competitiveness in relation to a non-mycorrhizal plant, which in turn depends on the N availability from the soil (Franklin et al. 2014). ECM symbiosis has a much higher efficiency in the uptake of specific N compared to non-mycorrhizal roots, especially in situations of low availability of N (Smith and Read 2008).

In forests with limited N, trees depend on the decomposition activity of their ECM symbionts to access N from the soil (Wang et al. 2020). In this process, ECM fungi produce extracellular lignocellulolytic enzymes that depolymerize the soil's organic matter, releasing N and transferring this nutrient to the host plant (Pellitier and Zak 2018). Even though ectomycorrhizae produce less lignocellulolytic enzymes than their saprotrophic ancestors (Martin et al. 2016), they still play an important role in releasing the N of mineral-associated proteins in forest trees, thus influencing the biogeochemical cycles of forest ecosystems.

Paul et al. (2007) observed that inoculation with the ECM fungus *Suillus tomentosus* in *Pinus contorta* resulted in an increased nitrogenase activity, which can be an important contribution to the necessary N nutrition in *P. contorta*. Also, it has been demonstrated that inoculation with multiple mycorrhizal symbionts has a great impact on NFB, and the use of mycorrhizal diversity can promote the

efficiency of N-fixing symbiosis for legume tree species (Diagne et al. 2013). Thus, either alone or together with NFB, ECM fungi are extremely important in the acquisition of N for plants, since these microorganisms play an important role in the dynamics of ecosystems, especially in forest environments.

2.4 Nitrogen and the Symbiotic Relationships of Plants and Diazotrophic Bacteria

Leguminous plants have great importance in the nutrition of human beings and animals because they are rich in nutrients, fibres and low in fat (Kaufman et al. 1997; Ponte et al. 2008; Kazydub et al. 2020; Sá et al. 2020; Zhang et al. 2020). In addition, in agriculture, they stand out for not requiring a large external supplementation of N, since they are able to supply their biological need for N almost independently (Kakraliya et al. 2018). For this reason, leguminous plants have become a target of several studies, which have shown that these plants have an endosymbiont relationship with diazotrophic N-fixing bacteria, that supply much of the N necessary for plant metabolism (Silveira et al. 2016; Santos et al. 2019).

Diazotrophic bacteria were identified from anatomical studies of leguminous plants roots in the seventeenth century (Beijerinck 1888; Hellriegel and Wilfarth 1888). Currently, more than 19.000 species of N-fixing diazotrophic bacteria are known and characterized as sources of nitrogen accumulation (Azani et al. 2017; Huisman and Geurts 2020). Although being free-living, these bacteria are capable of forming an endosymbiosis with plants, known as rhizobia, characteristic for developing small nodules in the root tissue of their host, in which bacteria grow and exchange nutrients with plants (Huisman and Geurts 2020). However, the association of NFB with plants occurs only in favourable conditions to the formation of the symbiotic relationship, in which plants provide photoassimilates for the microorganism, in exchange for the N_2 fixed in the form of NH_4 (Beijerinck 1888).

Several studies have characterized and described the genera and families of endosymbiont bacteria capable of fixing N (Burrill and Hansen 1917; Bruijn et al. 1995, Beatty and Good 2011; Mus et al. 2016). It has also been determined that the biological fixation of N is not limited to the symbiotic relationship between plants and rhizobia, since other microorganisms are also capable of transforming atmospheric N into ammonia (NH_3), like protists of the genus *Frankia*, either free-living or in symbiosis (Vessey 2003; Pawlowski and Newton 2008; Sellstedt and Richau 2013).

Bacteria of the genus *Azospirillum* are an example of free-living microorganisms capable of fixing N and forming beneficial association with plants. These bacteria do not have high specificity for their host plant and have the ability to promote N fixation without the need for N depletion in the soil to start the symbiosis process, characteristics that differentiate these bacteria from the rhizobia group, being called symbiotic endophytic bacteria (Okon and Kapulnik 1986; Bianco and Defez 2011;

Tewari and Arora 2013). The biological N fixation performed by symbiotic endophytic bacteria occurs without being organized into a specific structure, such as nodules. These bacteria are dispersed in the intercellular spaces of plant tissues, where the N fixation process occurs. Bacteria of the genus *Burkholderia*, *Delftia* and *Herbaspirillum* are also some examples of this type of symbiosis (Bhattacharjee et al. 2008; Govindarajan et al. 2008; Morel et al. 2013; Ejaz et al. 2020; Romero-Gutiérrez et al. 2020).

Considering all microorganisms present in the soil with the ability to fix N and capable of forming an endosymbiotic relationship with plants, rhizobia stand out mainly for the large amount of N_2 fixed and supplied to the plant, in addition to other promoted benefits (Hansen et al. 2017; Wang et al. 2019). Thus, it is crucial to better understand the impacts of rhizobia and the formation of nodules on plant roots (Sharma et al. 2017).

2.4.1 Introducing the Rhizobia Group

The name rhizobia refer to the order Rhizobiales, being a collective of 84 genera divided into seven families of diazotrophic bacteria, capable of fixing atmospheric nitrogen in the soil in symbiosis with host plants (Gueddes et al. 2020). Some of these genera are *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Burkholderia*, *Cupriavidus*, *Devosia*, *Mesorhizobium*, *Methylobacterium*, *Ochrobactrum*, *Photorhizobium*, *Phyllobacterium*, *Ralstonia*, *Sinorhizobium* and the most well-known genus *Rhizobia* (Epstein and Bloom 2006; Marschner and Marschner 2012; Gueddes et al. 2020).

Host plants that form endosymbiosis with rhizobia are known as the N-fixing clade, belonging mainly to the *Fabaceae* family and other plant orders such as *Fabales*, *Curcubitales*, *Fagales* and *Rosales* (Gueddes et al. 2020). However, it is important to highlight that, although these families are able to establish a symbiotic relationship with different species of NFB, not all of them have the same effectiveness in relation to the nodular formation at the root, which varies according to each species and the genes involved in the endosymbiotic relationship (Azani et al. 2017; Huisman and Geurts 2020).

It is believed that this symbiosis may have arisen due to a common ancestor of plants that had the ability to incorporate bacterial cells into their plant tissues, since all families in the nitrogen fixation group belong to the same evolutionary clade (Parniske 2018). Also, the discovery of the genus *Frankia*, which are microorganisms capable of efficiently fixing atmospheric N in its free life form, led researchers to hypothesize that this genus was the ancestor of N-fixing endosymbiont microorganisms (van Velzen et al. 2018; van Velzen et al. 2019). These microorganisms are able to protect the complex of the enzyme responsible for fixing N against oxidation, which is a fundamental mechanism for fixing N at its maximum capacity (Sellstedt and Richau 2013).

To better understand the formation of root nodules, as well as the biological reason that led to the formation of this connection between plants and NFB, it is necessary to understand the basic mechanism of transformation of N_2 into NO_3 and NH_4 (Huisman and Geurts 2020). Biological N_2 fixation occurs through the action of the nitrogenase enzyme, which is able to break the triple bond that connects the two gaseous nitrogen atoms. However, in order to be activated, the enzyme requires an environment with little oxygen (O_2) and large amounts of energy, in the form of ATP (Sprent and Raven 1985; Becana and Rodriguez-Barrueco 1989).

Among all existing metabolisms, aerobic metabolism is the most efficient in generating energy (Yang et al. 2020), so the production of highly energetic molecules requires large amounts of oxygen. Antagonistically, to function at its maximum capacity the enzyme nitrogenase cannot be oxidized, which occurs in the presence of O_2 (Misra 1999; Staal et al. 2007; Allen et al. 2019), thus the presence of aerobic metabolism becomes a disadvantage for living beings that have this enzyme to fix atmospheric N_2 (Weiss et al. 2016). With the emergence of the symbiotic relationship between plants and diazotrophic bacteria, the full potential of the nitrogenase enzyme can be reached, allowing biological N fixation to be maximized by bacteria (Boivin and Lepetit 2020). Therefore, plants provide energetic compounds, as well as an environment favourable to enzymatic activity, while bacteria have in their metabolism the enzyme nitrogenase and provides nitrogenous compounds to plants (Dixon and Kahn 2004; Marschner and Marschner 2012; Mbengue et al. 2020).

Not all plants are capable of forming this type of symbiosis, being necessary that these organisms have several characteristics, such as the presence of nodulation genes (Nod) that allow the formation of nodules, which are spaces in the roots favourable to bacteria to grow, develop and fix N, in addition to being able to secrete molecules that act as a communication bridge between the plant and free-living bacteria in the soil (Heidstra and Bisseling 1996; Mbengue et al. 2020). It is important to note that the symbiotic relationship between plants and rhizobia is not mandatory (Marschner and Marschner 2012; Boivin and Lepetit 2020) since plants are able to germinate and develop independently, as well as bacteria are able to live and fix N freely in the soil (Marschner and Marschner 2012). However, depending on the ecosystem, the absence of an endosymbiotic relationship can trigger negative consequences to the development of plants, such as leaf chlorosis, smaller growth and less biomass (Epstein and Bloom 2006).

2.4.2 Establishment of the Symbiotic Plant–Rhizobia Relationship

Symbiosis is ecologically described as a beneficial coexistence between two organisms that live in the same place, therefore the establishment of an exchange of benefits between the two living beings. In the terrestrial ecosystem, several of

these relationships have been established between plant organisms and the microbiota, such as the relationship formed between plants and rhizobia (Hodge and Fitter 2012; Regus et al. 2017; Porter and Sachs 2020). However, the formation of rhizobia-plant symbiosis does not occur spontaneously, since this symbiosis is not considered mandatory (Tewari and Arora 2013).

In unfavourable situations, where the availability of readily absorbable N is low, plants release exudates that act as chemical signals in order to communicate with the microorganisms present in the soil (Coskun et al. 2017). These signals are carried out by chemical signalling molecules composed of betaines and isoflavonoids, which are recognized by microorganisms capable of symbiotically relating to these plants. These microorganisms perceive the signals and release their own in response, which are compounds of lipochitooligosaccharide, also known as nodulation factors (NF) (Epstein and Bloom 2006; Oldroyd et al. 2011; Coskun et al. 2017). These NF, in turn, activates calcium oscillations in the nuclear region and several transcription factors that regulate NF-induced gene expression, resulting in the initiation of bacterial colonization and nodule organogenesis (Oldroyd et al. 2011).

Thus, for the establishment of symbiosis and the formation of an active and N-fixing nodule, two distinct and simultaneous steps are required: infection by symbiotic diazotrophic bacteria and the organogenesis of the nodular structure (Oldroyd and Downie 2008; Oldroyd et al. 2011). In the stage of infection by diazotrophic bacteria, the free-living rhizobia are organized into an infection line, which is formed by the junction of several vesicles secreted by the Golgi bacterial cell complex, then organize themselves into a tubular shape and will subsequently penetrate the root cells (Epstein and Bloom 2006; Mergaert 2020; Venado et al. 2020).

The nodular organogenesis process takes place after the signalling and release of the NF by the rhizobia, as well as the activation of the plant Nod genes. Then, a cascade of hormonal signalling occurs inside the plant, which modifies the levels of auxin and cytokinins in the root apical meristem (Hirsch et al. 1989; Cooper and Long 1994; Plet et al. 2011; Gueddes et al. 2020). The NF release inhibitors that affect auxin transporters in the same location, decreasing the hormone concentration in the meristem (Hirsch et al. 1989). In response, there is an increased amount of cytokinin due to the auxin-cytokinin ratio of plants (Gamas et al. 2017), stimulating the appearance of lateral roots, which is important for rhizobia colonization and to improve the uptake of N by the plant (Miri et al. 2016; Gu et al. 2018).

The rhizobia entry occurs through the root hairs and later there is a release of different NF that stimulate the curvature of the root structure, so the root hair is aligned with the bacterial cells. Then, another NF signalling occurs and the cell wall of the plants is degraded, allowing the bacteria to enter the cortical cells of the root. After the entry and colonization of the first cortical cell, the bacteria and root cells are transformed, generating their own area within the root for the formation of the nodule, which is the structure where the exchange of molecules between the rhizobia and the plant cells occurs (Ridge and Rolfe 1985; Lazarowitz and Bisseling 1997; Epstein and Bloom 2006; Mergaert 2020; Venado et al. 2020).

In the nodule, the cells of the diazotrophic bacteria multiply, modifying their cell wall, differentiating into bacterioids and forming the symbiosis with the necessary conditions for the activity of the nitrogenase enzyme (Poole and Allaway 2000; Huisman and Geurts 2020). This enzyme is able to convert N_2 to NH_3 by consuming ATP and releasing hydrogen (H_2), then NH_3 is delivered to α-ketoglutarate/glutamate to form glutamate/glutamine and is further transmitted to other amino acids and N-containing compounds in N metabolism of the host plant (Wang et al. 2019). The structure and activity of the nodule have a defined useful life, because with a decreased production of NH_3, plants and rhizobia coordinate a programmed cell death through hormonal chemical signals, that act to initiate the senescence of the nodule, protecting the plant from a possible drain and ensuring the preservation of the relationship for the establishment of a new colonization later (Kazmierczak et al. 2020).

2.4.3 Importance of the Plant-Rhizobia Symbiosis Formation

A major part of the N_2 fixed in the soil is obtained by the action of the symbiotic relationship between leguminous plants and rhizobia, which generates approximately 18×10^9 kg of N per year (Sylvia et al. 2005; Herridge et al. 2008; Wang et al. 2019). In addition, this symbiotic association has an indirect impact on the ecosystem, altering the dynamics of the relationships between plants in relation to N uptake in the soil, because there will be less competition between a plant with symbiosis and a plant without symbiosis, allowing both to develop better and receive the amount of N necessary for their metabolism. This happens because the direct receiver of the NH_4 produced by diazotrophic bacteria in symbiosis is the host plant, but the N fixed by free-living diazotrophic bacteria is available in the soil and can also be used by uninoculated plants, so both plants, in symbiosis or not, can acquire higher levels of N from soil (Hodge and Fitter 2012; Liu et al. 2020a, b).

Rhizobia also stimulate plant growth and productivity through the production of phytohormones, nutrient mineralization, protection against pathogens and other benefits (Gopalakrishnan et al. 2015; Gopalakrishnan et al. 2017; Silveira et al. 2016; Jack et al. 2019). Thus, several studies seek to demonstrate the benefits of using these microorganisms as biofertilizers, in order to reduce the use of synthetic nitrogen fertilizers, which make agricultural production more expensive and are a major source of pollution, negatively impacting the environment through eutrophication of water bodies and reduced soil fertility (Tetteh 2015; Ríos-Ruiz et al. 2020). However, although already used in some cultures, further studies are needed to better understand the affinity of plant species capable of symbiotically interacting to one or more species of rhizobia, aiming to optimize the use of these microorganisms as biofertilizers for agricultural production (Wang et al. 2019).

The importance of the plant–rhizobia relationship is wide and diversified, with great ecological and physiological effects on the terrestrial ecosystem, helping the environment and improving the quality of life of plants, animals and microorganisms

as a whole (Tetteh 2015; Regus et al. 2017; Wang et al. 2019; Porter and Sachs 2020; Ríos-Ruiz et al. 2020).

2.5 Final Considerations

N is very important for plants, being able to influence several enzymes and even affecting photosynthesis, since it is present in the main organic molecules, such as chlorophyll, ATP, NAPH and several amino acids. As a result, different biological mechanisms have been evolving to increase the uptake of nitrate and ammonium, which are the forms of N used by the plant and are also present in the soil in less concentration.

One of these mechanisms is the establishment of beneficial symbiotic relationships with soil microorganisms such as fungi and bacteria. Depending on the individuals present in these relationships, as well as their mechanism of action, symbiotic relationships impact N ecology in different ways. In this context, arbuscular mycorrhizae are able to transfer inorganic N from the soil to the plant, while ectomycorrhizae are able to increase the availability of N in the soil surrounding the host plant, thus providing the ecosystem with mobile forms of N through the decomposition of organic matter.

In the symbiotic relationship between plants and diazotrophic bacteria, the acquisition of N by the plant occurs by the action of the enzyme nitrogenase present in the protist metabolism, fixing N directly from the atmosphere. Thus, symbiosis between the plant and the diazotrophic bacteria is considered one of the main beneficial associations with a better quantitative result in the acquisition of N.

The different types of symbiotic associations influence the nitrogen cycle at different levels, since the acquisition and mobilization of the element in the soil–plant relationships differ. Therefore, for a better understanding of this influence, several studies aim to understand the communication and establishment of symbiotic relationships, as well as the impact of this symbiosis between the two beings involved. However, future studies are still needed to discover more efficient ways to apply the benefits of symbiotic relationships in agricultural crops, reducing the need to apply external sources of N such as chemical fertilizers, which in excess can act as toxic compounds, decreasing the natural fertility of the soil and polluting large bodies of water.

References

Allen JF, Thake B, Martin WF (2019) Nitrogenase inhibition limited oxygenation of earth's proterozoic atmosphere. Trends Plant Sci 24:1022–1031

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Azani N, Babineau M, Bailey CD, Banks H, Barbosa AR et al (2017) A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny: the legume phylogeny working group (LPWG). Taxon 66:44–77

Baldani VLD, Oliveria E, Balota E, Baldani JI, Kirchhof G, Döbereiner J (1997) Burkholderia brasilensis sp. nov., uma nova espécie de bactéria diazotrófica endofítica. An Acad Bras Ciênc 69:116

Bassi D, Menossi M, Mattiello L (2018) Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. Sci Rep 8(1):2327

Beatty PH, Good AG (2011) Future prospects for cereals that fix nitrogen. Science 333:416–417

Becana M, Rodriguez-Barrueco C (1989) Protective mechanisms of nitrogenase against oxygen excess and partially-reduced oxygen intermediates. Physiol Plant 75:429–438

Beijerinck MW (1888) Rhizobial systematic. Bot Zeit 46:796–804

Bhattacharjee R, Singh A, Mukhopadhyay S (2008) Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. Appl Microbiol Biotechnol 80:199–209

Bianco C, Defez R (2011) Soil bacteria support and protect plants against abiotic stresses. In: Shanker A (ed) Abiotic stress in plants-mechanisms and adaptations. Intech, Rijeka

Boivin S, Lepetit M (2020) Partner preference in the legume-rhizobia symbiosis and impact on legume inoculation strategies. Adv Bot Res 94:323–348

Bonfante P, Venice F, Lanfranco L (2019) The mycobiota: fungi take their place between plants and bacteria. Curr Opin Microbiol 49:8–25

Bonneau L, Huguet S, Wipf D, Pauly N, Truong HN (2013) Glomeromycotan mycorrhizal fungi from tropical Australia. III measuring diversity in natural and disturbed habitats. Plant Soil 370:419–433

Bruijn FJ, Jing Y, Dazzo FB (1995) Potential and pitfalls of trying to extend symbiotic interactions of nitrogen-fixing organisms to presently non-nodulated plants, such as rice. Plant Soil 174:225–240

Burrill TJ, Hansen R (1917) Is symbiosis possible between legume bacteria and non-legume plants? University of Illinois. Agricultural Experiment Station. Bulletin 202

Burris RH (1980) The global nitrogen budget—science or séance? In: Newton WE, Orme-Johnson WH (eds) Nitrogen fixation. University Park Press, Baltimore, pp 7–16

Chaillou S, Rideout JW, Raper CD, Morot-Gaudry JF (1994) Responses of soybean to ammonium and nitrate supplied in combination to the whole root system or separately in a split-root system. Physiol Plant 90:259–268

Cooper JB, Long SR (1994) Morphogenetic rescue of rhizobium meliloti nodulation mutants by transzeatin secretion. Plant Cell 6:215–225

Correia CM, Pereira JMM, Coutinho JF, Björn LO, Torres-Pereira JMG (2005) Ultraviolet-B radiation and nitrogen affect the photosynthesis of maize: a Mediterranean field study. Eur J Agron 22:337–347

Coskun D, Britto DT, Shi W, Kronzucker HJ (2017) How plant root exudates shape the nitrogen cycle. Trends Plant Sci 22:661–673

Cozzolino V, Di Meo V, Piccolo A (2013) Impact of arbuscular mycorrhizal fungi applications on maize production and soil phosphorus availability. J Geochem Explor 129:40–44

Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, Marin-Spiotta E, Wang L (2015) Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. Plant Soil 396:1–26

Datta R, Meena RS, Pathan SI, Ceccherini MT (2019) Carbon and nitrogen cycling in soil. Springer Nature, Singapore

Dénarié J, Debellé F, Truchet G, Promé JC (1993) Rhizobium and legume nodulation: a molecular dialogue. In: Palacios R, Mora J, Newton WE (eds) New horizons in nitrogen fixation. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp 19–30

Diagne N, Thioulouse J, Sanguin H, Prin Y, Krasova-Wade T, Sylla S, Galiana A, Baudoin E, Neyra M, Svistoonoff S, Lebrun M, Duponnois R (2013) Ectomycorrhizal diversity enhances growth and nitrogen fixation of Acacia mangium seedlings. Soil Biol Biochem 57:468–476

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631

Ejaz S, Batool S, Anjum MA, Naz S, Qayyum MF, Naqqash T, Shah KH, Ali S (2020) Effects of inoculation of root-associative Azospirillum and agrobacterium strains on growth, yield and quality of pea (Pisum sativum L.) grown under different nitrogen and phosphorus regimes. Sci Hortic 270:109401

El-Serafy RS, El-Sheshtawy AA (2020) Effect of nitrogen fixing bacteria and moringa leaf extract on fruit yield, estragole content and total phenols of organic fennel. Sci Hortic 265:109209

Epstein E, Bloom A (2006) Mineral nutrition of plants. Sinauer Associates, Sunderland

Franklin O, Näsholm T, Högberg P, Högberg MN (2014) Forests trapped in nitrogen limitation-an ecological market perspective on ectomycorrhizal symbiosis. New Phytol 203:657–666

Frossard E, Bünemann E, Jansa J, Oberson A, Feller C (2009) Concepts and practices of nutrient management in agro-ecosystems: can we draw lessons from history to design future sustainable agricultural production systems? Die Bodenkultur 60:43–60

Gadkar V, David-Schwartz R, Kunik T, Kapulnik Y (2001) Arbuscular mycorrhizal fungal colonization: factors involved in host recognition. Plant Physiol 127:1493–1499

Gamas P, Brault M, Jardinaud MF, Frugier F (2017) Cytokinins in symbiotic nodulation: when, where, what for? Trends Plant Sci 22:792–802

Gopalakrishnan S, Sathya A, Vijayabharathi R, Varshney RK, Gowda CLL, Krishnamurthy L (2015) Plant growth promoting rhizobia: challenges and opportunities. Biotech 5:355–377

Gopalakrishnan S, Srinivas V, Samineni S (2017) Nitrogen fixation, plant growth and yield enhancements by diazotrophic growth-promoting bacteria in two cultivars of chickpea (Cicer arietinum L.). Biocatal Agric Biotechnol 11:116–123

Govindarajan M, Balandreau J, Muthukumarasamy R, Kwon S-W, Weon H-Y, Lakshminarasimhan C (2008) Effects of the inoculation of Burkholderia vietnamiensis and related endophytic diazotrophic bacteria on grain yield of rice. Microb Ecol 55:21–37

Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Gu J, Li Z, Mao Y, Struik PC, Zhang H, Liu L, Wang Z, Yang J (2018) Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. Plant Sci 274:320–331

Gueddes BA, Kearsley J, Morton R, diCenzo GC, Finan TM (2020) The genomes of rhizobia. Adv Bot Res 94:213–249

Guignard MS, Leitch AR, Acquisti C, Eizaguirre C Elser JJ, Hessen DO, Jeyasingh PD, Neiman M, Richardson AE, Soltis PS, Soltis DE, Stevens CJ, Trimmer M, Weider LJ, Woodward G, Leitch IJ (2017) Impacts of nitrogen and phosphorus: from genomes to natural ecosystems and agriculture. Front Ecol Evol 5:70

Guo J, Jia Y, Chen H, Zhang L, Yang J, Zhang J, Hu X, Ye X, Li Y, Zhou Y (2019) Growth, photosynthesis, and nutrient uptake in wheat are affected by differences in nitrogen levels and forms and potassium supply. Sci Rep 9(1):1248

Hallik L, Kull O, Niinemets Ü, Aan A (2009) Contrasting correlation networks between leaf structure, nitrogen and chlorophyll in herbaceous and woody canopies. Basic Appl Ecol 10:309–318

Han Q, Kabeya D, Inagaki Y (2017) Influence of reproduction on nitrogen uptake and allocation to new organs in Fagus crenata. Tree Physiol 37:1436–1443

Hansen AP, Choudhary DK, Agrawal PK, Varma A (2017) Rhizobium biology and biotechnology. Cham, Switzerland

Harindintwali JD, Zhou J, Yu X (2020) Lignocellulosic crop residue composting by cellulolytic nitrogen-fixing bacteria: a novel tool for environmental sustainability. Sci Total Environ 715:136912

Heidstra R, Bisseling T (1996) Nod factor-induced host responses and mechanisms of nod factor perception. New Phytol 133:25–43

Hellriegel H, Wilfarth H (1888) Untersuchungen uber die Stickstoffnahrung der Gramineen und Leguminosen. Beilageheft zu der Zeitschrift des Vereins fur Rubenzucker-Industrie Deutschen Reichs, p 234

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Hirsch AM, Bhuvaneswari TV, Torrey JG, Bisseling T (1989) Early nodulin genes are induced in alfalfa root outgrowths elicited by auxin transport inhibitors. Proc Natl Acad Sci U S A 86:1244–1248

Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci 107:13754–13759

Hodge A, Fitter AH (2012) Microbial mediation of plant competition and community structure. Funct Ecol 27:865–875

Huisman R, Geurts R (2020) A roadmap toward engineered nitrogen-fixing nodule symbiosis. Plant Comm 1(1):100019

Jack CN, Wozniak KJ, Porter SS, Friesen ML (2019) Rhizobia protect their legume hosts against soil-borne microbial antagonists in a host-genotype-dependent manner. Rhizosphere 9:47–55

Javaid A (2010) Role of arbuscular mycorrhizal fungi in nitrogen fixation in legumes. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer Wien New York, New York, pp 409–426

Jiang X, Hou X, Zhou X, Xin X, Wright A, Jia Z (2015) pH regulates key players of nitrification in paddy soils. Soil Biol Biochem 81:9–6

Kakraliya SK, Singh U, Bohra A, Choudhary KK, Kumar S, Meena RS, Jat ML (2018) Nitrogen and legumes: a meta-analysis. In: Meena RS, ADGS Y, Lal R (eds) Legumes for soil health and sustainable management. Springer, Singapore

Kaufman PB, Duke JA, Brielmann H, Boik J, Hoyt JE (1997) A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: implications for human nutrition and health. J Altern Complement Med 3:7–12

Kazmierczak T, Yang L, Boncompagni E, Meilhoc E, Frugier F, Frendo P, Bruand C, Gruber V, Brouquisse R (2020) Legume nodule senescence: a coordinated death mechanism between bacteria and plant cells. Adv Bot Res 94:181–122

Kazydub N, Kuzmina S, Ufimtseva S, Kotsyubinskaya O (2020) Leguminous crops as a valuable product in functional nutrition. Adv Soc Sci Edu Human Res 393:194–198

Lazarowitz SG, Bisseling T (1997) Plant development from the cellular perspective: integrating the signals. Plant Cell 9:1884–1900

Lea PJ, Morot-Gaudry JF (eds) (2001) Plant nitrogen. Springer Science & Business Media, New York

Liu H, Brettell LE, Qiu Z, Singh BK (2020a) Microbiome-mediated stress resistance in plants. Trends Plant Sci 25:733–743

Liu M, Adl S, Cui X, Tian Y, Xu X, Kuzyakov Y (2020b) In situ methods of plant-microbial interactions for nitrogen in rhizosphere. Rhizosphere 13:100186

Ludewig U, Neuhauser B, Dynowski M (2007) Molecular mechanisms of ammonium transport and accumulation in plants. FEBS Lett 581:2301–2308

Mahmud K, Makaju S, Ibrahim R, Missaoui A (2020) Current progress in nitrogen fixing plants and microbiome research. Plan Theory 9:97

Maillard A, Diquélou S, Billard V, Laîné P, Garnica M, Prudent M, Garcia-Mina JM, Yvin JC, Ourry A (2015) Leaf mineral nutrient remobilization during leaf senescence and modulation by nutrient deficiency. Front Plant Sci 13:317

Marin VA, Baldani VLD, Teixeira KRS, Baldani JI (1999) Fixação biológica de nitrogênio: Bactérias fixadoras de nitrogênio de importância para a agricultura tropical. Seropédica, Rio de Janeiro

Marschner H, Marschner P (2012) Marschner's mineral nutrition of higher plants, 3rd edn. John Wiley & Sons, New York

Martin F, Kohler A, Murat C, Veneault-Fourrey C, Hibbett DS (2016) Unearthing the roots of ectomycorrhizal symbiosis. Nat Rev Microbiol 14:760–773
Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann Bot 105:1141–1157
Mbengue MD, Hervé C, Debellé F (2020) Nod factor signaling in symbiotic nodulation. Adv Bot Res 94:1–39
Mergaert P (2020) Differentiation of symbiotic nodule cells and their rhizobium endosymbionts. Adv Bot Res 94:149–180
Miri M, Janakirama P, Held M, Ross L, Szcyglowski K (2016) Into the root: how cytokinin controls rhizobial infection. Trends Plant Sci 21:178–186
Misra HS (1999) Oxygen implication in the diazotrophic growth of Plectonema boryanum in dark-light cycles. Plant Sci 143:135–142
Morel MA, Cagide C, Castro-Sowinski S (2013) In: Arora NK (ed) Plants-microbe symbiosis: fundamentals and advances. Springer, Uttar Pradesh, India
Mouradi M, Bouizgaren A, Farissi M, Latrach L, Qaddoury A, Ghoulam C (2016) Seed osmopriming improves plant growth, nodulation, chlorophyll fluorescence and nutrient uptake in alfalfa (Medicago sativa L.)—rhizobia symbiosis under drought stress. Sci Hortic 213:232–242
Mus F, Crook MB, Garcia K, Costas AG, Geddes BA, Kouri ED, Paramasivan P, Ryu MH, Oldroyd GED, Poole PS et al (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Appl Environ Microbiol 82:3698–3710
Nunes-Nesi A, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol Plant Pathol 3:973–996
Okon Y, Kapulnik Y (1986) Development and function of Azospirillum-inoculated roots. Plant Soil 90:3–16
Oldroyd GED, Downie JA (2008) Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol 59:519–546
Oldroyd GED, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144
Parniske M (2018) Uptake of bacteria into living plant cells, the unifying and distinct feature of the nitrogen-fixing root nodule symbiosis. Curr Opin Plant Biol 44:164–174
Paul LR, Chapman BK, Chanway CP (2007) Nitrogen fixation associated with Suillus tomentosus Tuberculate ectomycorrhizae on Pinus contorta var. latifolia. Ann Bot 99:1101–1109
Pawlowski K, Newton WE (2008) Nitrogen-fixing Actinorhizal symbioses. Springer, Dordrecht, The Netherlands
Pellitier PT, Zak DR (2018) Ectomycorrhizal fungi and the enzymatic liberation of nitrogen from soil organic matter: why evolutionary history matters. New Phytol 217:68–73
Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas: anatomy and cell biology. National Research Council of Canada, Ottawa
Plet J, Wasson A, Ariel F, Le Signor C, Baker D et al (2011) MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordinate symbiotic nodule organogenesis in Medicago truncatula. Plant J 65:622–633
Ponte PIP, Prates JAM, Crespo JP, Crespo DG, Mourão JL, Alves JP, Bessa RJB, Chaveiro-Soares MA, Ferreira LMA, Fontes CMGA (2008) Improving the lipid nutritive value of poultry meat through the incorporation of a dehydrated leguminous-based forage in the diet for broiler chicks. Poult Sci J 87:1587–1594
Poole P, Allaway D (2000) Carbon and nitrogen metabolism in rhizobium. Adv Microb Physiol 43:117–163
Porter SS, Sachs JL (2020) Agriculture and the disruption of plant–microbial Symbi-osis. Trends Ecol Evol 35:425–439

Püschel D, Janoušková M, Voříšková A, Gryndlerová H, Vosátka M, Jansa J (2017) Arbuscular mycorrhiza stimulates biological nitrogen fixation in two Medicago spp. through improved phosphorus acquisition. Front Plant Sci 8:390

Regus JU, Wendlandt CE, Bantay RM, Gano-Cohen KA, Gleason NJ, Hollowell AC, O'Neill MR, Shahim KK, Sachs JL (2017) Nitrogen deposition decreases the benefits of symbiosis in a native legume. Plant Soil 414:159–170

Ridge RW, Rolfe BG (1985) Rhizobium sp. degradation of legume root hair cell-wall at the site of infection thread origin. Appl Environ Microbiol 50:717–720

Ríos-Ruiz WF, Torres-Chavés EE, Torres-Delgado J, Rojaz-García JC, Bedmar J, Valdez-Nuñez RA (2020) Inoculation of bacterial consortium increases rice yield (Oryza sativa L.) reducing applications of nitrogen fertilizer in san Martin region, Peru. Rhizosphere 14:100200

Romero-Gutiérrez KJ, Dourado MN, Garrido LM, Olchanheski LR, Mano ET, Dini-Andreote F, Valvano MA, Araújo WL (2020) Phenotypic traits of Burkholderia spp. associated with ecological adaptation and plant-host interaction. Microbiol Res 236:126451

Sá AGA, Moreno YMF, Carciofi BAM (2020) Plant proteins as high-quality nutritional source for human diet. Trends Food Sci Technol 97:170–184

Santos SG, Chaves VA, Ribeiro FS, Alves GC, Reis VM (2019) Rooting and growth of pre-germinated sugarcane seedlings inoculated with diazotrophic bacteria. Appl Soil Ecol 133:12–23

Sellstedt A, Richau KH (2013) Aspects of nitrogen-fixing Actinobacteria, in particular free-living and symbiotic Frankia. FEMS Microbiol Lett 342:179–186

Sharma R, Bisaria VS, Sharma S (2017) Impact of rhizobial inoculants on rhizospheric microbial communities. In: Hansen AP, Choudhary DK, Agrawal PK, Varma A (eds) Rhizobium biology and biotechnology. Cham, Switzerland

Silveira APD, Sala MRR, Cardoso EJBN, Labanca EG, Cipriano MAP (2016) Nitrogen metabolism and growth of wheat plant under diazotrophic endophytic bacteria inoculation. Appl Soil Ecol 107:313–319

Smith SE, Read D (2008) Mycorrhizal symbiosis, 3th edn. Academic Press, New York

Spohn M, Kuzyakov Y (2013) Phosphorus mineralization can be driven by microbial need for carbon. Soil Biol Biochem 61:69–75

Sprent JI, Raven JA (1985) Evolution of nitrogen-fixing symbioses. Proc R Soc Edinburgh 85B:215–237

Staal M, Rabouille S, Stal LJ (2007) On the role of oxygen for nitrogen fixation in the marine cyanobacterium Trichodesmium sp. Environ Microbiol 9:727–736

Staudinger C, Tershani VM, Quintana EG, Gonzalez EM, Hofhansl F, Bachmann G, Wienkoop S (2016) Evidence for a rhizobia-induced drought stress response strategy in Medicago truncatula. J Proteome 136:202–213

Sylvia DM, Hartel PG, Furhmann J, Zuberer D (2005) Principles and applications of soil microbiology, 2nd edn. Prentice Hall, Upper Saddle River, NJ

Taiz L, Zeiger E, Møller IM, Murphy A (2017) Plant physiology and development, 6th edn. Porto Alegre, Brazil

Tao K, Kelly S, Radutoiu S (2019) Microbial associations enabling nitrogen acquisition in plants. Curr Opin Microbiol 49:83–89

Tegeder M, Masclaux-Daubresse C (2018) Source and sink mechanisms of nitrogen transport and use. New Phytol 217:35–53

Tetteh RN (2015) Chemical soil degradation as a result of contamination: a review. J Soil Sci Environ Manag 6:301–308

Tewari S, Arora NK (2013) Plant microbe symbiosis: fundamentals and advances. Springer, Uttar Pradesh, India

Turpin DH, Elrifi IR, Birch DG, Weger HG, Holmes JJ (1988) Interactions between photosynthesis, respiration, and nitrogen assimilation in microalgae. Can J Bot 66:2083–2097

Valarini MJ, Godoy R (1994) Contribution of symbiotic nitrogen fixation on pigeon-pea yield Cajanus cajan (L.) Millsp. Sci Agrár 51:500–504

van Velzen R, Doyle JJ, Geurts R (2019) A resurrected scenario: single gain and massive loss of nitrogen-fixing nodulation. Trends Plant Sci 24:49–57
van Velzen R, Holmer R, Bu F, Rutten L, van Zeijl A, Liu W, Santuari L, Cao Q, Sharma T, Shen D et al (2018) Comparative genomics of the nonlegume Parasponia reveals insights into evolution of nitrogen-fixing rhizobium symbioses. Proc Natl Acad Sci 115:E4700–E4709
Venado RE, Liang J, Marín M (2020) Rhizobia infection, a journey to the inside of plant cells. Adv Bot Res 94:97–118
Veresoglou SD, Chen B, Rillig MC (2012) Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol Biochem 46:53–62
Vergara C, KEC A, Souza SR, Schultz N, Jaggin Júnior OJ, Sperandio MVL, Zilli JÉ (2018) Plant-mycorrhizal fungi interaction and response to inoculation with different growth-promoting fungi. Pesq Agrop Brasileira 54:e25140
Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586
Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol Appl 20:5–15
Wang ET, Chen WF, Tian CF, Young JPW, Chen WX (2019) Ecology and evolution of rhizobia: principles and applications. Gateway East, Singapore
Wang T, Tian Z, Tunlid A, Persson P (2020) Nitrogen acquisition from mineral-associated proteins by an ectomycorrhizal fungus. New Phytol 228(2):697–711
Weiss MC, Sousa FL, Mrnjavac N, Neukirchen S, Roettger M, Nelson-Sathi S, Martin WF (2016) The physiology and habitat of the last universal common ancestor. Nat Microbiol 1:16116
Wickert E, Marcondes J, Lemos MV, Lemos EG (2007) Nitrogen assimilation in citrus based on CitEST data mining. Genet Mol Biol 30:810–818
Williams LE, Miller AJ (2001) Transporters responsible for the uptake and partitioning of nitrogenous solutes. Ann Rev Plant Mol Biol 52:659–688
Yang J, Feng L, Pi S, Cui D, Ma F, Zhao H, Li A (2020) A critical review of aerobic denitrification: insights into the intracellular electron transfer. Sci Total Environ 731:139080
Zhang Y, Hou L, Li Z, Zhao D, Song L, Shao G, Ali J, Sun Q (2020) Leguminous supplementation increases the resilience of soil microbial community and nutrients in Chinese fir plantations. Sci Total Environ 703:134917
Zhu Y, Lian J, Zeng H, Gan L, Di T, Shen Q, Xu G (2011) Involvement of plasma membrane H+-ATPase in adaption of rice to ammonium nutrient. Rice Sci 18:335–342

Chapter 3
Ecological Perspectives on Soil Microbial Community Involved in Nitrogen Cycling

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Abstract Soil is known as one of the most capable microorganism survival habitats. Diverse heterotrophic microbial species in the earth and their complex network of connexions allow the cycling of micro and macronutrients in their soil environment. Demands are addressed by the maintenance of soil fertility for sustainable plant productivity. The diverse interrelationship of various agroecosystem elements, living or not, influence the resources of crops and plants. Soil organic matter is, however, affected by the inputs of plants and also its chemistry uniqueness in each ecosystem's microbium. Although it is generally recognised that the Soil Microbiome is essential, its complexity remains small. This would improve our ability to increase agricultural productivity by recognising the microbial diversity. Each environment becomes something by the inputs from plants and their chemistry special with the culture of microbials. The function of the microbiome of the soil is very important. We realise that we have still a small grasp of its complexity. Intelligence thus the microbial diversity would increase our agricultural potential performance. The soil is generally recognised as one of the world's most hostile biological ecosystems. The Antartica's ice-free regions covering about 0.44% of the overall continental land area, shelter significant and complex macro-organism populations and in particular the microorganisms of the more "hospitable" maritime regions. Nutrient cycling and habitat maintenance in soils is primarily guided by the

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microbial populations, as is the case with the McMurdo Dry Valleys of South Victoria Land, in the most extreme non-maritime areas. Nitrogen transactions are an important part of the environment maintenance. Bacteria diazotrophic and archaeal taxa add up to the genetic capacity of the elements from the whole n cycle, and nitrification processes like the anammox reaction are included. In the ensuing growth season, N cycling may have a major effect on the soil microbial population in bioavailable nitrogen cycling as well as microbial dynamics during plant dormitory season. The biogeochemical effects on bioavailable N cycles were not well defined, despite frequent observations of seasonal changes in microbial community composition in forestry. Here we investigate the relationship between microbial dynamics in communities and bioavailable N dynamics in a cool temperate Low Forest one-year environment, with an eye to sleeping season. Subsequent peaks in winter and early spring were also correlated with NH_4^+, NO^{-3}, and dissolved bio-N concentrations. These results suggest that successive growth of litter degraders, ammonifier, nitrifiers, and denitrifies in the dormant season drives the subsequent bioavailable N transformation. After summarizing the recent findings, the novel process N-cycle microbes were characterized. Also, we explored the environmental importance of population dynamics in N cycling microbes, which is critical to our understanding of ecosystem feature stabilisation.

Keywords Bioavailable nitrogen · Biogeochemical · N cycling · Microbes' communities

3.1 Introduction

The soil is known to be one of the most proficient microorganism subsistence habitats. The soil microbial community structure and activity are dependent on the structure and conditions of the soil habitat are primarily discussed. In addition to their complex web of interaction, various heterotrophic microbial communities in the soil enable micro and macronutrients to be cycled through the soil ecosystem. By managing soil fertility, the demand for sustained plant productivity is achieved. The dynamic interactions between various components of the agro ecosystem, living or non-living, control the richness of plants or crops. Soil organic matter, in particular, is affected by plant inputs, and its chemistry also makes each environment and its microbial culture very peculiar. While the function of the soil microbiome is well recognised, there is still a restricted understanding of its complexity. Knowing microbial diversity will therefore enhance our ability to increase agricultural production. In the past years, much emphasis has been placed on exploring and studying the active microbial population inhabiting the soil through increasing understanding of the potential of microorganisms. As a driver of biochemical processes that are beneficial to the natural world, soil microorganisms have taken on prominence since the beginning of the nineteenth century. These microorganisms carry out various processes in which organic compounds are depleted, components are recycled and

nutrient recycling is important for animal development, plant growth, and cultivation. However, certain microorganisms in the soil are dangerous to plant and animal life and function as pathogens that damage the host directly or introduce toxic compounds into the soil. A better understanding of soil microbes is thus important to understand their impact on agriculture and the environment. Therefore, the attention of soil microbiologists is not only on the diversity of soil microbes, but also on their contact with the atmosphere and other organisms.

The study of soil biota, its relationships, and the subject matter of biochemistry in both its research and its effects, this must strive for excellence as it Science, education, and applications are becoming increasingly important. In synthesising knowledge in a readable way and making it available to a global audience, textbooks have a fundamental role to play. At a moment when global society faces multiple barriers to preserving the environment, awareness in this area grows at an unprecedented pace. Many excellent possibilities exist. Advances in molecular techniques and analytical instrumentation are revolutionising our understanding of the structure of the microbial community and promoting the convergence of this information with principles relating to soil organic matter composition and development (SOM), its interactions with the soil matrix and its importance in the functioning of the environment. Questions regarding the role of soil biota and its processes would also need to be addressed with sound science in relation to food security for a growing global population that needs to change its diet at a time of economic globalisation. Invasive insects, contamination of water and air and plant diseases are expected to be intensified by climate change and increased control of food production and biofuels, though we are trying to protect our natural environment. Increasingly, soil microbiology, ecology, and biochemistry will be called upon to help provide the basic information required at a reasonable cost for biologically sustainable ecosystem services (Chan et al. 2013).

3.2 Soil Microbiology (Historical Perspectives)

The cornerstone of modern soil microbiology studies in the middle of the nineteenth century was set by Louis Pasteur, Selman Waksman, and Sergei Winogradsky. One remarkable research, among other studies Winogradsky was discovered as the father of soil microbiology, is the sulphur cycle, role of CO_2 and inorganic ion for the microbial (chemoautotrophic) and nitrification. He was also privileged to be named one of nitrifying bacteria by Nitrobacter winogradskii. Bacteria that were not symbiotic, Berthelot first suggested in the late nineteenth century, can achieve nitrogen fixation. Soil microbes result in litter mineralisation and facilitate the availability of essential nutrients for plant and animal growth and development. It was also emphasised that it is more effective to add stable manure to the soil than to add inorganic nutrients directly. The fastening of nitrogen by the leguminous plants is one of the main microbial processes well studied. The soil fungal and soil bacteria studies have laid the foundation for the modern era of microbiology and all these

processes have a direct effect on crops growth and productive activities in soil, compost nitrification and denitrification and chemical transformations. The general connexion between the soil microbial population and soil fertility has also given rise to the notion of inoculating desired soil microorganisms (Kizewski and Kaye 2019).

In 1939, apart from the agronomical significance of soil microbes, S. With Waksman and Rene Dubos find Streptomyces sp., a soil actinobacteria (formerly actinomycete) with antibiotic properties. Waksman received the Nobel Prize for antimicrobial soil microbes in 1952. Studies by Melin and Hayner on mycorrhizal fungi and Cutler on the actinobacterias of the soil extend the scope of the soil microbiology to include Krinsky, Conn, Waksman, and Curtis and on the ground protozoa. Soil microbes have been extensively researched and introduced since their discovery in diverse areas of human life. (Balser et al. 2010).

3.2.1 Soil Microorganism Habitat

Since it is home to several thousand different species of organisms/microorganisms, soil is an ecosystem that is not inert but dynamic. Soil microbes' population, structure, and behaviour are determined by their soil habitat. Grown land is rich in organic matter and has a much greater microbial population than sandy or eroded soils. The soil microbes breathe within this habitat, compete for food, cooperate with each other, and respond to changes in their living environment.

The soil is usually composed of varying sizes of soil aggregates and soil pores are Habitat of microorganism (Strous 2011). The forms of texture and soil impact the microbial community composition and population, and vice versa. Any dirt Polysaccharides, gums, and glycoproteins are secreted by bacteria, which sticks together minerals of the soil, to form a soil structure foundation. In addition, fungal hyphae and plant roots bind together soil aggregates that provide a positive atmosphere for plant growth. Rudakov et al. also suggested that active humus has an important function in cementing soil particles into aggregates. For the most part, soil cementing substances consist of (1) uronic acid derivatives, (2) bacterial proteins or products of their lytic action, and (3) fungal culture lysates and/or colloidal protein derivatives synthesised by soil bacteria (Makhalanyane et al. 2015).

Similarly, parameters such as temperature, moisture, and seasonal variations affect the physical differences in the soil microbial community (Yergeau et al. 2007), acidity or alkalinity (pH) of the soil, levels of oxygen and nutrient availability. The fertility of soil can be indirectly associated with the overall microbial biomass which depends on availability and nature of biomass, known as soil organic carbon, depends on the (SOC). There is a greater amount of SOC available within the initial 1 m depth of soil. The amount of carbon present aboveground is two or three times as much as (Brady and Weil 2002). Thus, the upper 20–30 cm of soil where abundance of soil microbial populations is present due to rich SOC is believed to be the most biologically active region of the soil (Ferrari et al. 2016; van der Heijden et al. 2008; Van Goethem and Cowan 2019). The availability of carbon also

decreases, as does total microbial biomass, with depth. In topsoil there are also more microorganisms with a rich carbon supply than in the subsoil. In the vicinity of plant roots (called the rhisosphere), slugged-off cells and root exudates provide carbon sources, they are abundant especially. It is understood that fungal-to-bacterial ratios decrease with increased depth. It is known that about 3×10^4 bacteria, 1.5×10^5 fungi, 6×10^4 algae, 1×10^4 protozoa, 5×10^5 nematodes, and 3×10^4.earthworms are found in the soil (Zablocki et al. 2014).

Soil fertility is one of the essential criteria for sustainable production of a natural or managed agroecosystem. Access to soil consistency, chemical, physical, and biological characteristics changes that constitute an indirect development and diversity measure are monitored. Biological metrics that have access to soil quality are called biological biomass, soil respiration, soil enzyme activity, earthworm numbers, etc. The metabolic quotient (qCO_2) is the calculation of the amount of CO_2-C emitted over-time per unit of microbial biomass that reflects the flourishing state of the microbes in the soil. In addition, in understanding soil fertility, biochemical markers such as microbial enzymes are also helpful. The most commonly studied biological reactions were nitrogen compound metabolism, where Ammonification, nitrification, denitrification, and nitrogen fixation were included. In early forecasting of any improvements in soil quality, microbial markers have the advantage of being susceptible to subtle changes in the environment. In addition to systemic diversification of soil microbes, soil species are naturally involved over sometimes of the year. Some microbes, such as late spring and early summer, when the soil is hot and damp, are active. In addition, salts, soil particle aggregation, and soil porosity have an effect on bacterial population diversification in soil microhabitats (Carson et al. 2007; Komárek et al. 2015). Typical microbial communities conducting different behaviours and multifaceted relationships with their habitat are responsible for the heterogeneity of each soil ecosystem (Wei et al. 2016).

The microbes of the soil provide essential macro-elements for plant growth and production of food, such as biomass, nitrogen, and phosphorous. A very unique community of microbes retains the symbiotic relationship with plants or plant parts. Not all microbes of the soil have healthy interactions; others damage higher seedlings. The microbes either fight for or invade the soil's vital nutrients by generating toxic chemicals from the higher plants as parasite.

Plant roots are either penetrated by various group of bacteria, fungi, and actinobacteria and remain in near proximity to the root system. To allocate the near relationship between soil microorganisms and the root systems of higher plants, the definition of the "rhizosphere" is created. The "root area," which is a high microbiological area, includes the root and rhizosphere. Typically, such a relation between true symbiosis, between bacteria root-nodule and legume plants, and parasitic phenomena, may be considered as midway. The key causes of the rhizosphere effect are the deposition of manure, decrease of the concentration of certain mineral nutrients, partial desiccation of the soil, and rise of soil carbonates after root excretion. In some seeds in plants, tuberization and protein production are caused by some fungi. According to Garrett et al., "stressed the need to differentiate between

the rhizosphere effects and the traditional features of living roots and the diseased root microenvironment" (Galloway et al. 2008).

In a variety of terrestrial ecosystem functions such as nutrient recycling, maintaining plant growth, water purification, carbon preservation, soil structure maintenance, xenobiotic depletion, nitrogen fixation and the diversity of the soil microbes plays a crucial role in or as a competitor to pathogens to create desired microbial communities (Vero et al. 2019). Similarly, in a cascading network of chemical processes, decomposition and nutrient recovery are carried out by complex classes of microbes. Soil microbes release simplified carbon compounds, nitrogen, CO_2, and minerals from dead organic material that are eventually ingested by higher plants by decomposition procedures. Decomposition by soil microbes is either aerobic (aerobic Bacteria and fungi) or anaerobic bacteria. Aerobic degradation of the plant microbial residues contains humic acid in the soil. The abundance of humic acid and fulvic acid promotes the bonding properties of humus. In addition, soil microbes such as sporogenous Bacilli, Bacillus polymyxa, B have produced numerous protopectinase enzymes. Radiobacteria, B. Yeah, mycoides, B. Laterosporus, Clostridium macerans. Few fungi often break down the residues of plants by means of humic acid synthesis. Just few examples of fungi are trichoderma lignorum, mucor intermedius, and Mortierella isabellina, mainly active in the processes of soil structure development (Falkowski and Godfrey 2008).

Solubilisation of soil nutrients, Microorganisms with the aid of CO_2; nitrous acids, nitric and sulphuric acids; and organic acids in particular are derived from carbonates, phosphates, and zeolite. Soil toxins, as a result of their activities, are toxins derived by soil microbials. The soil solution is thus a very complicated part of the soil where the bulk of biochemical reactions take place. The soil may be treated by sun, volatile antiseptics, or transparent lime, in order to maximise productivity. Soil microorganisms are documented to help plant seed germination and seed growth. For starters, the fast evolution of CO_2 in the case of microbial breathing produces unfavourable anaerobic conditions for the oxidation and germination. Recent experiments have shown that microorganisms can generate plant growth hormones and/or related substances.

3.2.2 Influence on Soil Microorganisms of Plants

While there are few laboratory studies indicating the effect of plant diversity on soil microorganisms (Bargett and Shine 1999; Voytek et al. 1999; Smith and Smith 1990), there are grounds to believe that plant diversity can influence the microbial community of the soil biome. This is due to the lack of an experimental method to provide access to the direct effect of growing plant or plant products on soil microbes. Mostly, the impact of plants on soil microbes is studied indirectly by (a) calculating the amount of microorganisms, (b) nitrifying and denitrifying soil energy, and (c) oxidising soil power in terms of aeration or output of CO_2. Effects of growing plants on the structure and role of soil microorganisms can be described as:

The growth of soil bacteria and fungi is favoured by soluble organic and inorganic compounds. Dead roots and root hairs, epidermal cells and other waste products help the growth of microorganisms. Plants excrete considerable CO_2 into the soil which increases the solubility of certain inorganic soil constituents. Soil porosity and structure are modified and affected by the plant diversity. Sometimes, plants can hinder the growing of soil microbes by removing a considerable amount of moisture from soil.

The pathways for feedback between plant and microbial communities monitor the diversity and productivity of plants. For example, soil bacteria are stimulated by cowpeas, field peas, vetch, and soya. Among the bacteria, in particular, Radiobacter sp. increased in number when legumes are grown. In the other hand, the constant development of a single plant type in the soil leaves residues which may contribute to a shift in the chemical makeup of the soil contributing to microbial imbalance. This is clear from the fact that the pathogenic fungi population improves the ecosystem of the soil through constant growing of maize, flax, or clover. While the roots of alfalfa have only a marginal stimulating effect on filamentous fungi, the effect of the aubergines was important. A range of stimulatory effects on soil microbes by cultivating alfalfa, rye, and vetch plants are observed, ranging from the least to actinobacteria, slightly to fungi, and the highest to soil bacteria.

3.2.3 Processes of Soil Biological and Microbial Diversity

The soil is considered to be the microbial storage area because the area inhabited by active microorganism's accounts for about 5% of the overall occupied area. Due to its limits of bulk, soil microorganisms are main participants in the global movement of organic matter to CO_2, H_2O, N_2, P, S, and to other nutrients, reprocessing or mineralising organic residues. As a result, soil microbes play the most important role in the control of plant growth and thereby contribute to the interdependence of diversity—fertility (Vitousek 1997; McGuire and Treseder 2010). Primary microbiological activity is limited to clusters of concentrated organic matter and rhizosphere. There is therefore a growing demand to study the composition of microbial communities in various microhabitats in nature.

As an ecosystem, soil has the most variety, contributing to neighbouring, physical and chemical properties of microhabitats. Diversified heterotrophic microbial communities living in the ecosystem of soil control of Carbon (C) and N (N) cycling in different processes which control the environment and represent a possible relationship between plant diversity and the functioning of the ecosystem. The limited supply of capital plays a key role in deciding the biotic community's (Thomas and Nielsen 2005; Tilman 1982). Chemical compounds in litters that can be used to generate cellular electricity are incarcerated for the provision of energy for soil microbial species (Silver et al. 2001). Changes in plant diversity might change the production and range of organic compounds that restrict the composition and thus regulate the microbial community function.

3.3 Nitrogen Transformations

Manure provides many of the nutrients required for crop production. Nitrogen is one of the most important nutrients and most widely applied to soil for high yields. Nitrogen undergoes several changes in the soil as it is used, reused and made available to soil microbes. It is important to consider what happens to manure nitrogen after soil application in order to use it effectively for crop production. The fate of soil nitrogen discusses the various sources of nitrogen that exist, how plants are used, and how they travel with water. Figure 3.1 displays only a part of the nitrogen cycle and involves multiple contacts and forms different nitrogen sources that exits, how plants are used and how water moves of nitrogen in the atmosphere, water, soils, and living species (Figs. 3.2 and 3.3).

No other life-critical factor takes as various forms as nitrogen (N) in soil, and transitions between these types are often regulated by microbes. Thus, soil microbiology plays yet another key position in the functioning of the ecosystem: N limits plant growth in most terrestrial ecosystems, and hence net primary production lowers

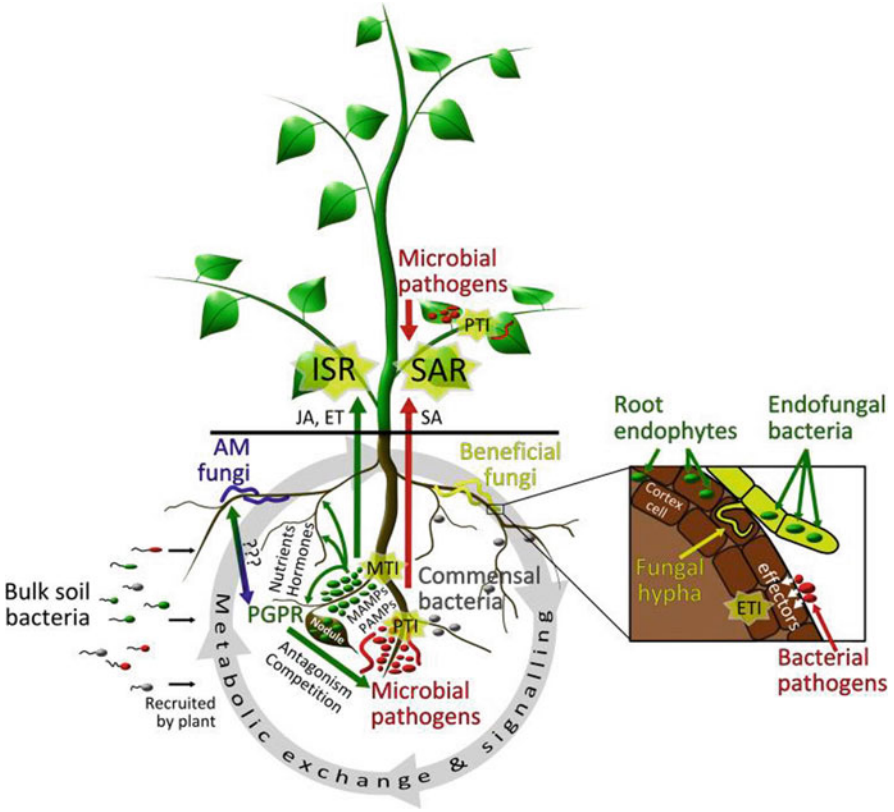

Fig. 3.1 Microbes and plant interaction. (Stephan et al. 2000) (Sect. 3.2.1)

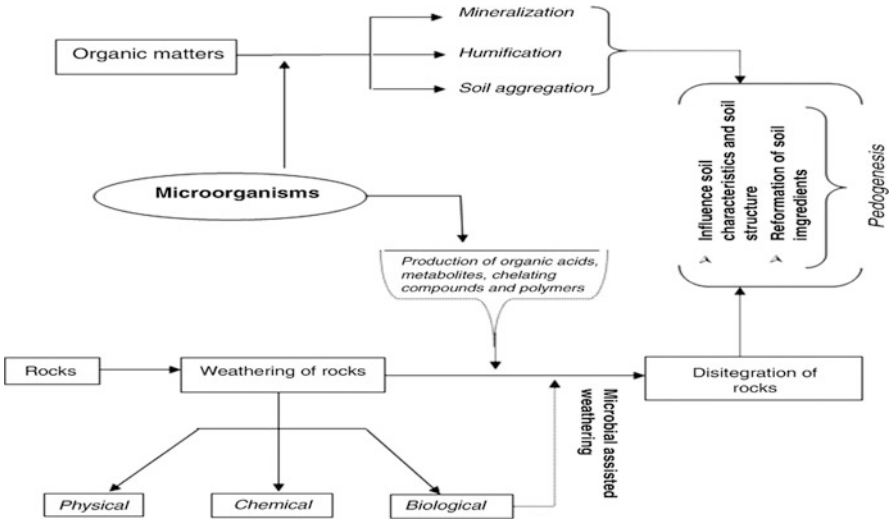

Fig. 3.2 The role of microorganisms in soil. (Kartal et al. 2011) (Sect. 3.2.2)

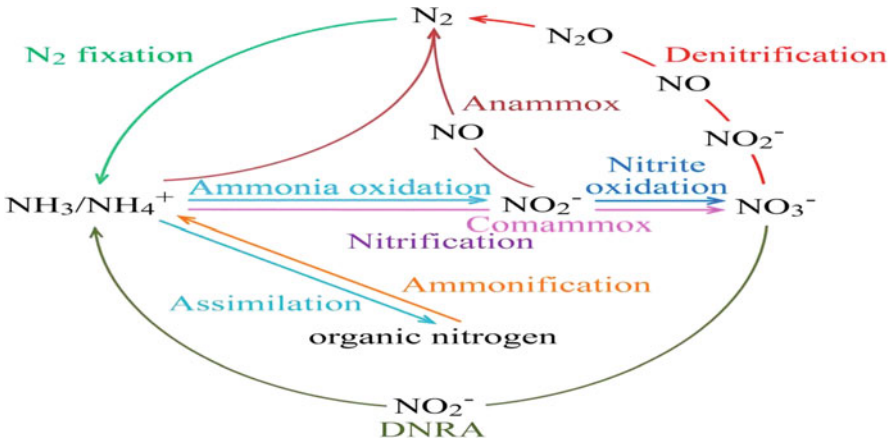

Fig. 3.3 Mechanistic link between plant diversity and ecosystem function. (Crane et al. 2018) (Sect. 3.2.2)

ecosystem production capabilities which can be controlled by the pace at which soil microbes turn N into plant-useable types. Various N forms are often pollutants, with N microbial changes to the soil often impacting human and environmental health, sometimes far from the intermediate microbes. Therefore, it is important that the transformations of N and its soil microbes are understandable for ecosystem health and productivity management and understanding. (Fig. 3.4).

Nitrogen in soil has nine distinct chemical types leading to distinct the oxidative condition. Dinitrogen gas (N_2) is made up of 79% of our gas atmospheric and is by

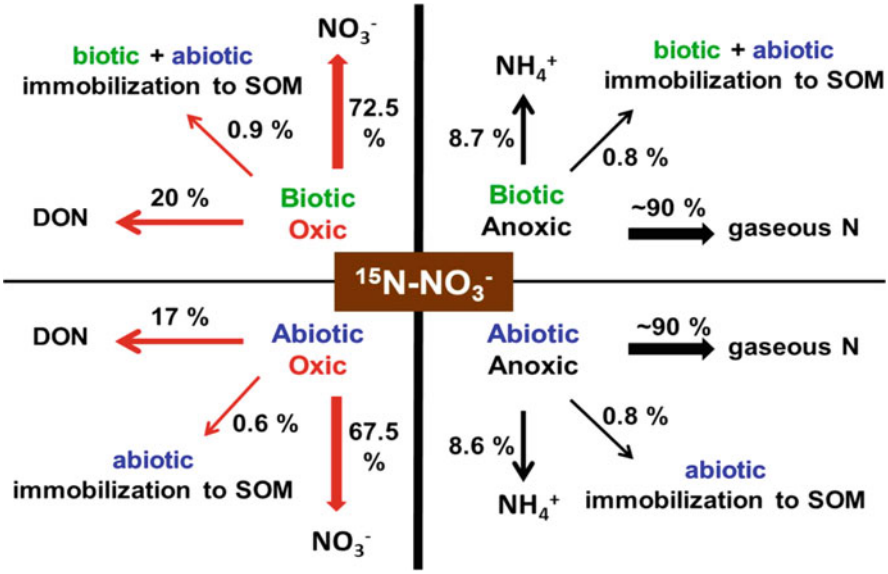

Fig. 3.4 Pathways of nitrogen transformation (15 N-NO^{3-}) as impacted by conditions of incubation. (Kingsland 1991) (Sect. 3.3)

Table 3.1 Forms of nitrogen in soil with their oxidation states (Sect. 3.3)

Oxidation states of soil N				
N form	Name	Oxidation state		
Organic-N		−3		
NH_4^+	Ammonium	−3	□	□
N_2	Dinitrogen gas	0	(Oxidation)	(Reduction)
NO_2^-	Nitrite	+3	□	□
NO_3^+	Nitrate	+5	□	□

far the most abundant source of N in the biosphere, but it is unusable for most species, including plants. Fixation of biological N_2, where N_2 is converted into organic N, it is the dominant natural mechanism by which N joins soil biological reservoirs. All corresponding soil N transformations are protected by this chapter: (1) N mineralisation and immobilisation, which is the conversion of organic N to inorganic forms; and the absorption or assimilation of inorganic N forms by microbes and other soil organisms; (2) N Cycling Taxa in Soils; (3) N Cycling Genes in Soils; (4) Nitrification, which is the conversion of ammonia (NH + 4) to nitrite (NO^{-2}) and then nitrate (NO^{-3}); and (4) Denitrification, which is the conversion of nitrate to nitrous oxide (N_2O) and to Dinitrogen gas (N_2). Other sources of N are mainly involved in these transformations as intermediaries, and may escape to the atmosphere after conversion, where they may take part in chemical reactions or are transferred elsewhere for further reactions (Table 3.1).

The idea of the N cycle first proposed by L€ohnis, which formalises the N cycle. The notion that N is transformed from one form to another in an ordered manner and predictable fashion and the same amount of dinitrogen on a global scale (Table 3.1). The gas which is set annually by the fixation of the N_2 must either be permanently fixed stored in deep ocean sediments or transformed back to N_2 by denitrification to preserve the air balance. The fact that N_2 fixation—both biological and industrial—now much exceeds historic denitrification rates is the key reason N has become a significant pollutant (Freney et al. 2000). Making controlled habitats more N conservative and eliminating N from drainage sources, such as municipal and industrial effluents, are major environmental problems involving basic awareness of microbial N soil transformation (Stephen et al. 1998). The N cycle processes have been studied to our understanding throughout microbiology, physiology, and biochemistry for more than a century. The N cycle was derived from observations on the molecular and organism scale. It had been in the lab. The structure and control of the mechanism in this chapter have been defined by lab studies and experiments, but the reductionist structure has also led us to neglect the unanticipated capacity of natural microbial activity that undermine our ability to understand the ecological significance of those processes. A single example is the denitrified occurrence of dry and even desert soils: theory and years of the laboratory research show that denitrifying can only occur on wetland and silver soils; but, as modern farming techniques became available in the 1970s, it became clear that virtually every soil supports successfully denitrifying agents (Fig. 3.5).

The study of the microbial N cycle processes has resulted in significant problems that are different from other biogeochemical mechanisms (e.g. carbon (C) and plant nutrient absorption). The physiological heterogeneity of bacteria and archaea in the wild (for example, aerobic denitrifiers, anaerobic ammonium oxidants anammox) were underestimated. This resulted in an aerobic denitrifiers. The disconnect between laboratorial and field knowledge is troublesome in soil microbial ecology but is possibly the most extreme in the field of N cycling, with a major position in the field of landscape, regional, and global scales. This issue has its own functionality. If we strive to improve our microbial awareness to address major issues of plant growth, pollution of water and atmospheric chemistry, this topic is becoming particularly relevant at ecology, countryside, and regional level (Fig. 3.6).

3.3.1 Nitrogen Mineralisation and Immobilisation

In each nutrient cycle, the conversion of organic nutrient supplies is a vital process. Nutrients that can be reused by plants and microbes in dead biomass (detritus) into simpler, more soluble forms. Microbes and other soil species releasing or mineralising nutrients as a by-product of their ingesting waste are responsible for this conversion. While microbes mainly consume waste as an energy source and C, they also have to assemble proteins, nucleic acids, and other cellular components in nutrients, especially N. When plant detritus is rich in N, microbial needs are met

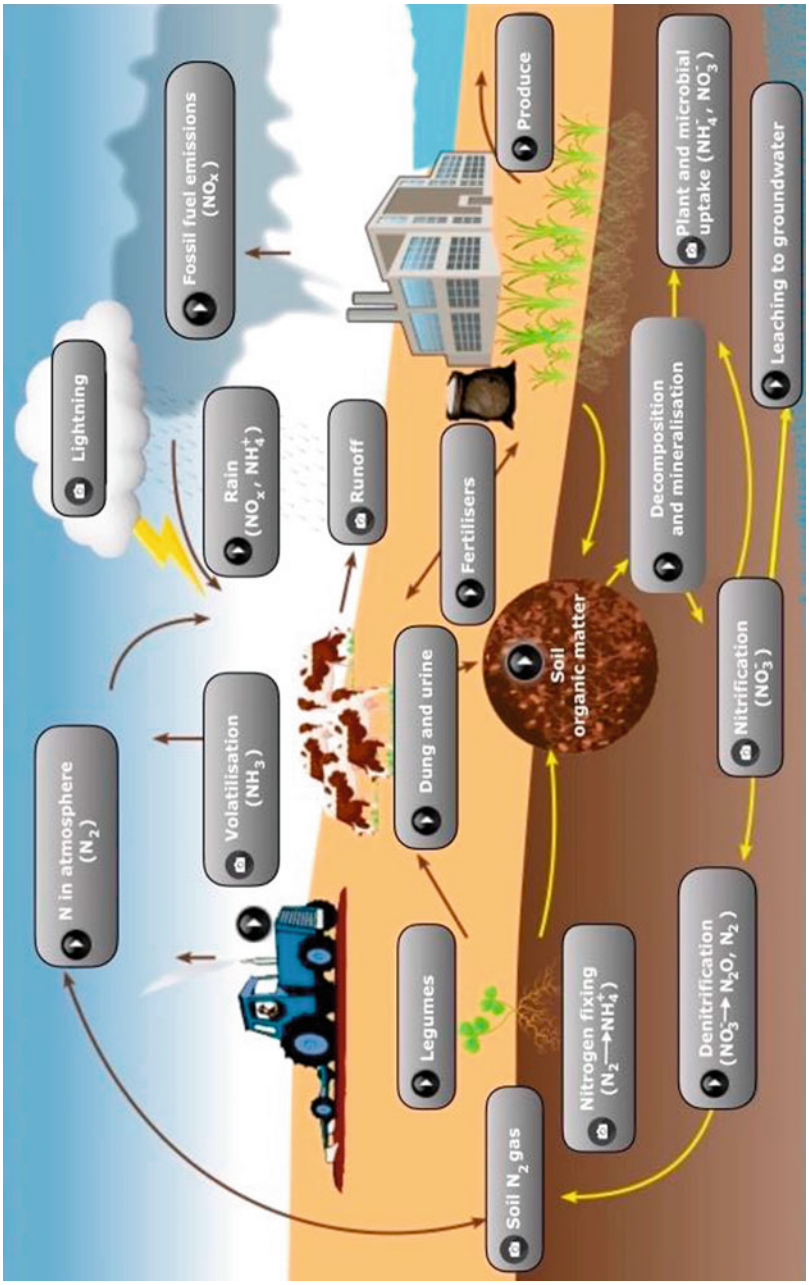

Fig. 3.5 The terrestrial nitrogen cycle with major element involves. (https://www.sciencelearn.org.nz/resources/960-the-nitrogen-cycle n.d.) (Sect. 3.3)

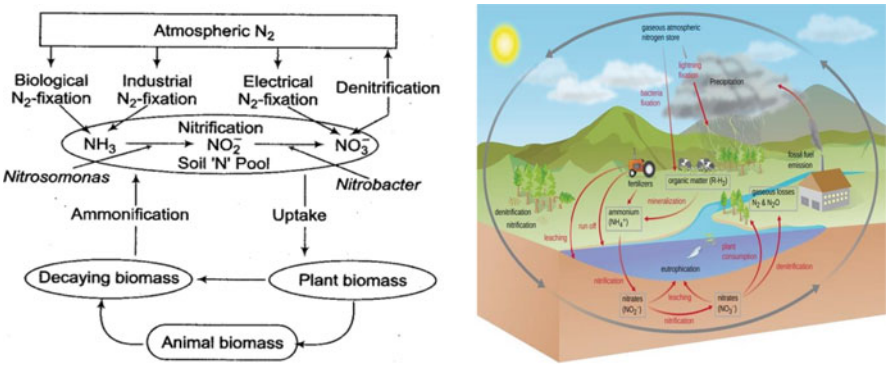

Fig. 3.6 Representing soil microbes that engage in N cycle. (Zehr et al. 2003). (Sect. 3.4.1)

rapidly, and N is continued to release or mineralise. If N is low in the plant detritus, microbes need to scavenge N from their surroundings, which will help N stable in their biomass.

In order to understand mineralisation-immobilisation the secret is to "think as a microbe", i.e. to try and live by obtaining energy and C from waste. The detritus often has all the N the microbe requires, so that excess N (mineralised) in the soil solution is released when C is ingested. Detritus also loses enough N to satisfy microbial requirements to immobilise extra N from the soil solution, because of the feeding of C. Microbes have been seen to expend more resources in the synthesis of enzymes (e.g. amidases to acquire N and phosphatases to acquire P) to receive the nutrients they require while substrates of poor quality are decomposed. Microbial N absorption is also influenced by the growth potential of the organism. Fungi have a larger C:N ratio in their tissues than bacteria and archaea and can expand more efficiently on low N substrates. Mineralisation leads to an improvement, while immobilisation leads to diminished N types in the soil present in plants. Ammonium is historically was known as the immediate mineralization product. Mineralisation is sometimes called ammonification, literature. In recent years, reconnaissance of clear, soluble, organic types of plants nutrients lead us to include all basic soluble nutrient sources that can be taken up by plants in our descriptions of mineralized products (see Schimel and Bennett 2004). The use of Amino acids and other organic sources in different environments has been found to exist in mycorrhizal waters, which can then be consumed by their hosts as amino acids, amino sugars, peptides, proteins, and chitin as an N-source.

Mineralisation is the mechanism by which microbes decompose organic N from waste, organic matter, and crop residues into ammonia. Well, because it is a biological mechanism, mineralisation rates differ with soil temperature, moisture, and soil oxygen content (aeration) ($R\text{-}NH_2 \rightarrow NH_3 \rightarrow NH_4^+$ or organic N → ammonia → ammonium). In warm (68–95°F) soils, well-aerated and damp, mineralisation occurs readily. Approximately 60–80 lbs. of N per acre of soil organic matter is mineralised annually in New York State on average.

Immobilisation is the opposite of mineralisation. All living things need N; thus, soil microorganisms compete with N crops. Immobilisation relation is made to the phase in which nitrate and ammonia are consumed by soil species and are thus not accessible to crops (NH_4^+ and/or $NO_3^- \rightarrow$ R-NH_2 and ammonium or nitrate $\rightarrow$ organic N).

The introduction of products with a high carbon to nitrogen ratio (e.g. sawdust, straw, etc.) can increase biological activity and induce increased demand for N, resulting in N immobilisation, like Immobilisation will only momentarily tie up N. As microorganisms die, the organic N found in their cells is transferred by mineralisation and nitrification to the available nitrate plant.

Mineralisation and immobilisation occur relatively simultaneously in small quantity of soil. Whereas one community of microbes might be using a Protein-rich and therefore N-rich piece of organic matter (think seed or leguminous leaf tissue), another category, perhaps $<$100 μm apart, can ingest detritus rich in C, but low in N (think leaf stalk or wood). The first party is mineralising N, while the second is immobilising it, probably even immobilising the same N that is being mineralised by the first community. Due to the simultaneity and small size of these processes, it is important to differentiate between gross and net mineralisation and immobilisation. Gross N mineralisation is the total amount of soluble N formed by microorganisms, whereas gross N immobilisation is the total amount of soluble N ingested. The equilibrium between the two is the net N mineralisation. Inorganic N rises in the soil as gross mineralisation reaches gross immobilisation (i.e. net mineralisation). Inorganic N in the soil reduces as the total immobilisations surpass total mineralisation (i.e. net immobilisation).

Soil fauna also leads role to the production mineralisation and immobilisation processes. These are responsible for most of the preliminary breakdown detritus, feed, and control bacterial and fungal populations, for a broad variety of species they may build or change environments, for instance, Earthworms build burrows, litter isopods, and macerate termites wood. Chemical energy is used by all heterotrophic soil species and C, immobilise and mineralise N simultaneously. Often influenced is the equilibrium between mineralisation and immobilisation performance of organism growth. For starters, mushrooms have broader C:N ratios. Tissues have a lower demand for N than bacteria and therefore can more quickly mineralise N. In general, C:N ratio $>$ 25:1 material stimulate immobilisation, while C:N $<$ 25:1 stimulates mineralisation (Table 3.2). Exceptions to this law include heavily decomposed compounds such as soil plants, humus, and manure that are used to deplete labile C and N. Although the C:N ratio may be poor, the undecomposed C is intrinsically resistant to decomposition in complex ways, and mineralisation is often sluggish.

There are a wide range of methods for mineralisation calculation and immobilisation. Net mineralisation and immobilisation rate measurements are much simpler and more general than gross rate measurements (Dávila-Ramos et al. 2019). Measuring net rates usually require measuring changes in inorganic N levels in a form of incubation of the entire soil. In most cases, these incubations are in tanks with no lack of plant absorption or leaching and changes in the amounts of inorganic N are measured by periodic soil extraction. Methods of incubation differ

Table 3.2 C:N ratio of different organic material (Sect. 3.3.1)

Organig\ material	C:N ratio
Soil organic matter	10
Soil microbes	5–10
Alfalfa	13
Manure	20–30:1
Wheat straw	80
Sawdust	400–600:1
Legumes	13–25:1
Microorganism	5–10:1

greatly, from short (10-day) incubations of intact soil cores buried in the field to lengthy (> 52-week) laboratory incubations of sieved soil. The gross rates are calculated using isotope dilution methods under which small quantities of 15 N-labeled ammonium are applied to the soil and the resulting dilution of 15 N with 14 N-labeled natural ammonium from mineralised organic matter is used as the basis for the measurement of the gross ammonium production and use.

3.4 N Cycling Taxa in Soils

Most of the soil nutrient cycling is thought to be driven by microbial communities with the complete absence of higher plants from most of the ice-free continental Antarctica (Certini et al. 2004; Papale et al. 2018). This prediction is corroborated by evidence that continental soils and soil-associated niche ecosystems have substantial genetic potential for nitrogen cycling (Certini et al. 2004; Cowan 2014). These adaptive cycling pathways of nitrogen are close to those found previously in bacterial and archaeal phyla (Certini et al. 2004; Wei et al. 2015). As for other terrestrial ecosystems, Antarctic terrestrial niches (Mulder et al. 1995; Cowan 2014) are occupied by bacteria. Evidence from phylogenetic gene surveys shows that the majority of primary production is regulated by bacterial taxa (Mulder et al. 1995). The key regulators of nitrogen cycling in soils are generally known to be cyanobacteria (Amarelle et al. 2019). Cyanobacteria play essential functional roles as "ecosystem engineers" as direct and indirect mediators of nutrient recycling in Antarctic soils (Cary et al. 2010). Heterocystous cyanobacteria tend to drive the fixation of nitrogen in Antarctic soils, mainly Nostoc. Most Cyanobacteria that are heterocystous Like Calothrix, Dichothrix, Nodularia, and Hydrocoryne, nitrogen in soils and rock-associated niches such as hypoliths and endoliths can play a role in sequestering nitrogen (Cary et al. 2010; Brady and Weil 2002; Yergeau et al. 2010). Several studies have documented that nitrogen sequestration genes are homologous to those previously observed in cyanobacteria, including Nitrosospira and Nitrosomonas, related to rate-limiting cycle steps including ammonia oxidation (Garrett 1951; Cowan et al. 2011).

Hypoliths and endoliths are important nitrogen sources in hyperoligotrophic soils in the McMurdo Dry Valleys (Cowan 2014; Cheeke et al. 2013). In these systems, several cyanobacteria, including Nostoc and Anabaena (Burgin and Hamilton 2007; Falkowski and Godfrey 2008), are driven by nitrification. Deltaproteobacteria, Bacteroidetes (Certini et al. 2004; Cheeke et al. 2013; Dávila-Ramos et al. 2019), and Actinobacteria (Jetten 2001; Wei et al. 2015) are mediated by denitrification, a reduction of nitrate to N_2 steam. Betaproteobacteria and Planctomycetes are also main taxa involved in the removal of soil nitrate through pathways of denitrification and anaerobic ammonium oxidation (annamox) (Certini et al. 2004). It has been suggested (Garrett 1951) that in newly colonised soils, Burkholderiales, diazotropic betaproteobacteria, can also play an important role in N input. While their quantitative contributions are uncertain, other taxa, including Actinobacteria (genus Streptomyces and family Frankeniaceae) and Chloroflexi, are involved in nitrogen fixation in Antarctic soils (Dávila-Ramos et al. 2019; Lacap-Bugler et al. 2017).

Many studies support the conclusion that diazotrophy potential is widespread in nutrient-poor Antarctic soils rather than edaphic areas of some high altitudes (Makhalanyane et al. 2013). The extent and significance of interactions between Antarctic taxa and their significance in diazotrophy, however, is not well known, although there is some evidence that co-operation between Cyanobacteria and other taxa (e.g. Actinobacteria, Bacteroidetes, and Proteobacteria) is essential to complete the nitrogen cycle (Certini et al. 2004; Brady and Weil 2002; Wixon and Balser 2009).

3.4.1 Bacterial Nitrogen Cycling in Soils

Bacterial diversity is the product of the survival of soil work. The inherent spatial component of endemism and clonality may have important consequences for soil function (Godfrey and Falkowski 2009). Even if the change is slight and with the unchanged mineral composition of the soil, soil texture affecting bacterial population structure presumably results in the dispersed organisation of bacteria (Hart et al. 1994). A variety of physicochemical properties of the soil matrix, including pore size, particle size, and water and carbon abundance, are affected by bacterial diversity and population composition (Carson et al. 2009). The overall bacterial diversity at low pore connectivity is possibly mediated by multiple interacting factors that favour coexistence and minimise competitive interactions (Carson et al. 2010).

3.4.2 The Role of Fungi in N Cycling

Eukaryotes are mainly fungal in Antarctic soils and are dominated by very few ascomycete taxa (Papale et al. 2018; Hoshino et al. 2009). Generally, free-living

fungi and yeasts are of limited abundance (Hoshino et al. 2009; Veldkamp et al. 2003) and are mainly limited to niches of lithobionts (Cheeke et al. 2013). However, low apparent abundance, as for Archaea, does not necessarily. This indicates that these species are not essential for nitrogen cycling functional processes. For starters, fungi have been shown to possess nitrification pathway genes in Miers Valley soils (Wei et al. 2015). Many fungi, including yeasts usually find on Antarctic environments, produce enzymes like urease (Kuramae et al. 2012), which may play an important role in the mineralisation or ammunition of nitrogen, such like Rhodotorula muscorum, the Rhodotorula mucilaginosa, the Cryptococcus aerius, and the Cryptococcus albidus (Buzzini et al. 2012). Many compounds of nitrogen from inorganic and organic sources may also be assimilated and play an important role in nitrogen rotation in soils, including in the retention of nitrogen. (Buzzini et al. 2012; Buzzini and Margesin 2014; Kuramae et al. 2012). Moreover, while denitrification is commonly regarded as a prokaryotic process, fungal denitrifiers have been found in Antarctic soils, known as Candida sp. and Trichosporon cutaneum (Hutchinson 1957). The role of fungi and yeast in nitrogen cycling in Antarctic soils, however, is still poorly understood to date.

3.4.3 Viruses as Drivers of N Cycling

The roles and possible impacts of the related phages and viruses to microbial dynamics and nitrogen cycling by host cell lysis are still misunderstood, while the structure of some Antarctic soil microbial populations is well known. It has been hypothesised that viruses, especially in the biogeochemical cycling of Antarctic soils, may play a very important role by causing diversification of organisms and consequent functionality (Anesio and Bellas 2011). Recent literature suggests that viruses are extremely diverse in Antarctic soils and hypoliths, dominated predominantly by phages of Mycobacterium (Wardle et al. 1999; Yergeau et al. 2007). The role of viruses in metabolic regulation has been indicated by research in other systems (Adriaenssens et al. 2017; Dang and Chen 2017). It is tempting to speculate that a lysogenic rather than lytic phage lifestyle can be promoted by extreme environmental conditions, and the Antarctic "metaviromic" experiments are circumstantial evidence of this (Yergeau et al. 2007). Although there is no corroboration of viral–Host interaction studies for processes other as the phage niche differentiation driven by infection that may play an important role in the regulation of Nitrogen Cycling in Antarctic soils.

3.5 N Cycling Genes in Soils

NarG (NarG to reduce nitrate nitrite, nirK, and nirS to reduce the nitrite, norB to reduce oxides and nosZ to reduce nitrous oxide) were the most commonly used soil surveys for n cycling functional markers involved with the processes for nitrite fixation (nifH), nitrification (amoA), and denitrification processes. Metagenomic and amplicon sequencing methods for shotguns the presence/absence and variety of main nitrogen cycling genes (Howarth 2008; Cowan et al. 2011) have been investigated, while gene abundances have been tracked using qPCR and its variations (Jetten 2001; Cowan et al. 2011) and Geochip microarray technologies (Ferrari et al. 2016; Wardle et al. 1999; Asuming-Brempong 2012). Nif genes, especially nifH, are strongly conserved and present in a large number of bacteria and archaea that are phylogenetically divergent (Elliott et al. 1980; Garrido-Benavent et al. 2020). Most studies use the robustness of the nifH gene to classify the variety of diazotrophs in natural environments as a functional marker (Zablocki et al. 2014). NifH gene analysis has been used throughout the Antarctic area to determine the abundance of autotrophic cyanobacteria, particularly in cryptic soil environments such as hypoliths and endoliths (Ferrari et al. 2016; Cowan et al. 2014; Brady and Weil 2002; Wardle et al. 1999). The appearance of Cyanobacteria, however, is not an absolute predictor of N-fixation, as reports of nifH gene loss from their genomes have been reported (Lacap-Bugler et al. 2017). Solid NifH signatures attributed to heterotrophic N-fixers, however, are now seen as evidence of significant non-phototrophic nitrogen dependent inputs into oligotrophic Antarctic soils (Ferrari et al. 2016; Wardle et al. 1999; Mulder et al. 1995; L€ohnis 1913). Examination of the diversity of nifH markers in hypolithic populations of the McMurdo Dry Valley showed that all possible diazotrophs were correlated with Proteobacteria taxa (L €ohnis 1913). The involvement of heterotrophic diazotrophs in combination with Cyanobacteria showed similar findings at the functional level, where over 50% of the overall nitrogen fixation was attributed to non-autotrophic taxa (Mulder et al. 1995).

3.5.1 Nitrification Inhibition

The oxidative component of the nitrogen (N) cycle is expressed by nitrification, the two-step phase by which ammonia is oxidised to nitrite and then to nitrate (Grundmann and Normand 2000). While most of the microbial N process surveys in Antarctic soils have concentrated on N-fixation, various studies have been undertaken in different parts of Antarctica including lakes of the Ross Sea Region (Grundmann and Normand 2000; Vitousek 1997; Ayton et al. 2010), on the Antarctic Peninsula (Rabalais et al. 2002; Tilman 1987) and on soils in the McMurdo Dry Valley (Ferrari et al. 2016; Kuramae et al. 2012; Mitsch et al. 2001) on the diversity and abundance of nitrifiers. The processes of nitrification carried out by AOA and AOB are limited to a limited number of taxa (Grundmann and Normand

2000; Kuramae et al. 2012). Just four AOA and three AOB amoA OTUs in four separate and extremely heterogeneous Dry Valley soils were found (Grundmann and Normand 2000). They were extracted from Nitrosospira-like taxa, usually related to pristine atmosphere and low levels of NH_4-N in the soil (Grundmann and Normand 2000). Although several studies have shown that AOA predominates over AOB (Kingsland 1991), for various Antarctic soils, the ratio of the two clades varies. In the Antarctic Peninsula, relative to their bacterial counterparts, archaeal amoA genes were dominant (Jetten 2001), but significant differences in the abundance of AOA and AOB amoA genes were found in four soils from the Dry Valley (Grundmann and Normand 2000). It was concluded that the geochemical properties of soils (i.e. pH, C/N, Mg, Cr, Mn, Co, Ni, and Cu) and other environmental influences such as the supply of water have a major effect on the relative abundances of AOA and AOB amoA genes (Grundmann and Normand 2000; Kuramae et al. 2012; Mitsch et al. 2001).

The relevance of nitrifiers to the work of the ecosystem is considerable: while in acid rain or as fertiliser, some nitrate enters ecosystems; nitrate is formed in situ through nitrification in most ecosystems. Since nitrate is an anion, it is more mobile in soil water than ammonium, an ionised source of NH_3:

NH_4^+ (aq) $\Leftrightarrow NH_3$(aq) + H^+ (aq)

3.5.1.1 Inhibition of Nitrification

In certain soils, nitrification is unaccountably sluggish, and in certain cases, its natural or processed substances can be inhibited. A broad array of in vitro studies reveal that plant extracts can inhibit culturable nitrifiers, even though their in-situ significance is uncertain. Likewise, consumer products such as nitrapyrin and dicyandiamide can be used with differing degrees of effectiveness to prevent nitrification in soil. Pyridines, pyrimidines, amino triazoles, and sulphur compounds, such as ammonium thiosulfate, are mainly industrial compounds. Another breakthrough is calcium carbide paraffin-coated CaC_2; (Fraser et al. 2009). Calcium carbide, which resists nitrifiers at very low partial pressures, reacts with water to form acetylene (C_2H_2), approx. Oh. 10 Pa. CaC_2 is exposed to soil moisture as the paraffin wears off, and nitrification is inhibited by the C_2H_2 produced. Similarly, neem oil derived from the Indian neem tree (Azadirachta indica) was commercially used to coat pellets of urea fertiliser to slow its nitrification to NO_3^-.

The potential importance of environmental control of nitrifiers can be easily seen from the nitrification location in the N loop overall. Ecosystem degradation, primarily after conversion of N to NO^{-3} and before planting, uptake, retains N in the form of NH^{+4} prevents it from being lost via nitrate leaching and denitrification, the two primary pathways of unintended degradation of N in certain species, and resulting atmospheric and water pollution. Since, N is preferred to be taken up as NO^{-3} by many plants, it is not desirable to block Nitrification completely. Even in intensively regulated environments, such as fertilised row crops, slowing down or limiting their operation to nitrifiers active plant growth cycles are an enticing.

3.5.2 *Denitrification*

Nitrate reductase, to end the cycle and to return the N_2 to the atmosphere, it is responsible for the reduction of nitrate to nitrite encoded by the narGHJI operon (Schimel and Bennett 2004). In the second step, the reduction of nitrite to nitric oxide is catalysed by two forms of nitrite reductase: the nirS-encoded cytochrome cd1 or the nirK-encoded Cu-containing enzyme (Silver et al. 2001; Smith and Smith 1990). Nitric oxide is subsequently made up to reduce by the norB-encoded nitrite oxide reductase that generates nitrous oxide, a potent greenhouse gas with important global warming consequences. Finally, by nitrous oxide reductase encoded by nosZ (Stephan et al. 2000), nitrous oxide is reduced to N_2.

In addition, soils have the genetic potential to complete the mechanism of denitrification (Asuming-Brempong 2012). In several different Antarctic soil environments, denitrification genes were observed, such as sub-Antarctics, desert soils, and lithic niches in the coastal Antarctic and the McMurdo Dry Valley (Cowan et al. 2011; Ferrari et al. 2016; Wardle et al. 1999; Yergeau et al. 2010). However, the abundance and diversity of functional markers for denitrification can vary significantly between sampling locations and may be influenced by temperature, form of vegetation and macrofauna (Cowan et al. 2011; Yergeau et al. 2010; Stephen et al. 1998).

Before its passage to rivers and banks, denitrification will also extract nitrate from groundwater. Nitrate-rich soil waters must cross an intersection between anaerobic and C-rich groundwater in most wetlands and ribs. Nitrate can be denitrified to N_2O and N_2 as it travels through this interface, avoiding the contamination of downstream surface waters. In the regulated habitats, it is generally optimal for N to be conserved for plant uptake to be reduced. N losses due to denitrification can interact with or surpass losses due to nitrate laughing in regions with heavy precipitation. In general, denitrificators are better managing indirectly by controlling water levels (e.g., in rice cultivation) or sources of nitrates (e.g., nitrification inhibitors). There are no technologies designed to reduce deritrification in itself.

3.6 Soil Nitrogen Transitions

N is converted into soil by many additional microbial processes, but none are its thought to be as quantitatively essential as mineralisation, nitrification, and denitrification of immobilisation. Dissimilative conversion of nitrate to ammonium (DNRA) applies to nitrate anaerobic transformation to nitrite and then to nitrate anaerobic transformation with ammonium. Like the denitrification, this form requires respiration to proceed in conditions with high C-nitrate levels, which are assumed to be favourable because the mechanism absorbs more electron than denitrification. In optional and necessarily fermenting bacteria, a potential for DNRA has been discovered and has long been considered to be confined to

high C, extremely anaerobic conditions, such as bioreactors of anaerobic waste sludge, anoxic sediments, and bovine rumen. However, in some tropical forest soils and in a variety of freshwater sediments (Burgin and Hamilton 2007), DNRA has been found to be prevalent and important in these soils flows too far as or higher than the denitrification or nitrification flux and can help protect N by transferring nitrate into ammonium instead of N_2O or N_2 in these environments. No respiratory denitrification often results in the production of N gas (mainly N_2O), such as respiratory denitrification, but the reduction does not promote development and can occur in aerobic conditions. Non-respiratory denitrification can be carried out by a number of nitrate-assimilating bacteria, fungi, and yeast, which may be responsible for some of the N_2O that is now due to nitrifiers in welded soils.

Anammox is believed to occur in water treatment plants and oceanic processes in which ammonium and nitrite are converted to N_2 (Moore and de Ruiter 2012; Insam 2001; Kurtzman et al. 2011), where they may be the primary cause of N_2 flux. In the enrichment culture, Anammox bacteria are considered to be part of a substantial anaerobic ecosystem only in regularly or permanently submerged soils, and thus only under purely anaerobic conditions. (Stephen et al. 1996).

Bacteria capable of conducting anammox in the phylum Planctomycetes exist within the single order Brocadiales. Anammox catabolism in these bacteria occurs in a specialised organelle called the anammoxosome, wherein MUCH remains to be known, particularly intermediate products, about the process of biochemistry and bioenergetics.

$NH^{+}_4 + NO^{-}_2 \rightarrow N_2H_2 \rightarrow N_2$

Chemo denitrification happens as NO^{-2} reacts to form N_2 or NO_x in the soil. This can occur through a variety of aerobic pathways. Amino groups in the alpha position of carboxyl's yield N_2 in the Van Slyke reaction:

$RNH_2 + HNO_2 \rightarrow ROH + H_2O + N_2$

Chemo denitrification is generally thought to be a minor pathway to N loss in the plurality of habitats. It is not readily measured in situ, however, except in the laboratory. A sterilisation process is necessary that does not greatly disturb the soil itself chemistry of N.

3.7 Landscape Nitrogen Movement

Reactive N microbial transformations (Fig. 3.7) are of considerable significance for at biodiversity, landscape, and provincial levels, soil fertility, water quality, and atmospheric chemistry. Differences between what we have observed in the laboratory and what we experience in the field are more noticeable on these measures.

One of the big scale approaches to thinking about microbial N cycle processes, it is to ask a set of questions that aim to decide "Is a given ecosystem is a source or sink of environmental significance for specific N organisms". Sites that are N-rich either spontaneously or subsequent to disruption have any of the reactive N forms listed in Table 3.1. Mineralisation and nitrification have a high potential to serve as sources

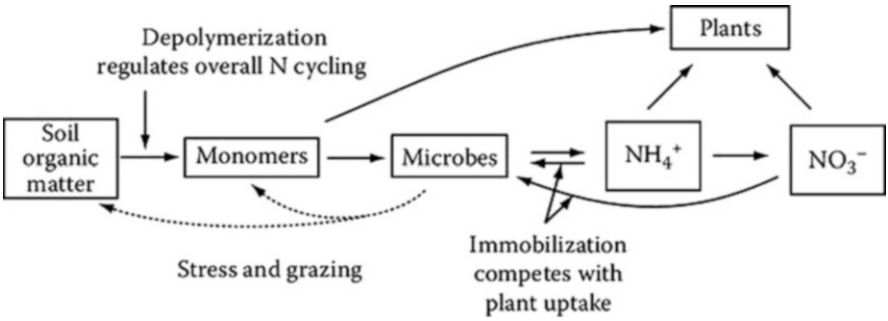

Fig. 3.7 Four forms of N of concern. (Rudakov 1951) (Sect. 3.7)

because, the processes creating most of these reactive forms, occur at high rates (Fig. 3.8).

3.8 Soil Biota Ecology and Its Function

Ecology is the study of connexions between animals and the ambience of their relationships. The name of the Greek term oikos, the family household, represents the opinion of Haeckel in 1866 as a household, the climate, and species. (Kaviya et al. 2019). Initially, ecology was developed to provide the study of natural history with a mechanistic backbone. The ecology field is associated with evolution because of this origin, and it is believed that one of the long evolutionary histories is the cornerstone of ecological relationships. Human ecosystem disruptions may lead to encounters between species, which are not dependent on evolutionary history and provide possibilities for testing this presupposition.

Since the field of ecology is concerned with the concept of habits of species, the interest of eco-populations in the function and function of both terrestrial and aquatic ecological environments is rare for ecologist to recently start to be articulated. Relationships and distributions has resulted in the development of a broad variety of studies exploring soil microbes within the meaning of ecological science. This interest has helped to develop microbial ecology and soil ecology areas that provide an ecological concept of soil biota. This emphasis maintains ecologists' essential aims of understanding the processes that decide the spread of organisms and the consequential effects of biotic and abiotic ecosystems.

In some aspects, ecological research on soil biota varies from researches that focus on the systems of plants and animals. The "species principle", for instance, accepted by many biologists of plant and animal, a species is characterised as an interbreeding community of organisms that is isolated from other organisms reproductively (and genetically). Bacteria, archaea, and even other microscopic eucaryotes are reproduced asexually. This part of the concept of biodiversity also does not apply for those microorganisms. The degree of genetic recombination within and

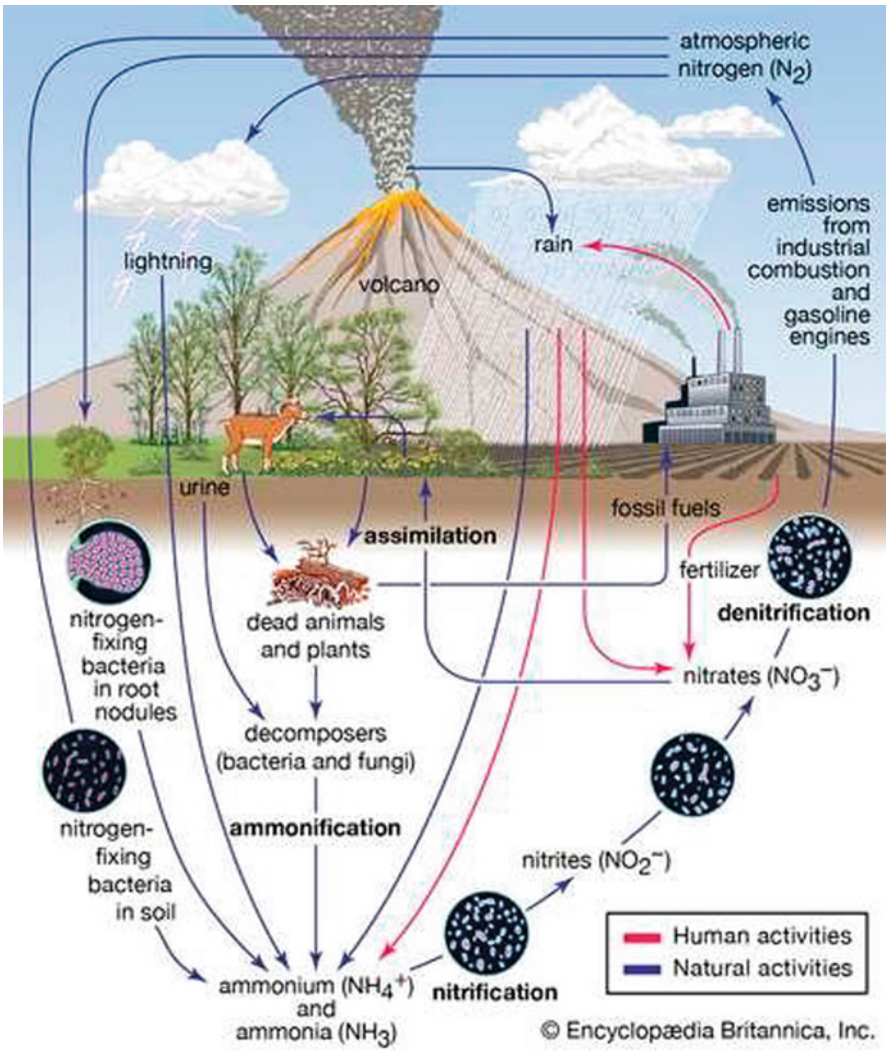

Fig. 3.8 Ecological consequences of human and natural activities modifications to the cycle of nitrogen. (https://www.cbsetuts.com/neet-biology-notes-mineral-nutrition-nitrogen-cycle n.d.) (Sect. 3.11)

among these classes has, however, been discovered by analysis of whole genome sequences. Genetic recombination in some lines appears to be widespread among closely related strains. Gene acquisition and degradation can be part of the technology of certain bacterial organisms, facilitating adjustment to a wide range of climate. For, e.g., the bacterial Pseudomonas fluorescens (Gammaproteobacteria) is widespread in soil, including varieties of plant, microorganic, archaea, fungi, oomycetes, nematode, and insect species that interact with a wide variety of other species. This

phenotypic variability can be correlated with a highly variable genome, with all other strains of P. fluorescens only 45–52% of genes within a particular genome (Lipson 2007). Genetically similar lines are less frequent due to their lack of homologous gene and promoters, as compared to the normal recombination of close-related bacterial strains between plasmids and viruses that mediate gene transferring (Sylvia et al. 2005). Therefore, the closely related strains classes with high gene exchange rates may be similar to the classes defined for plants and animals as defined for plants and animals. Microbial taxonomy and ecology technology are now being developed in the natural history of microorganisms. Methods based on microbial phylogeny genetic markers, along with study of key genes and physiological characteristics, enable us to make dramatic strides in understanding soil organisms' ecology amid an evolutionary system that is still evolving.

3.8.1 Mechanisms that Drive Community Structure

The study of how species spread is an important aspect of ecology and the climate, and it is linked to questions like: Why are those organisms found in one field but not in others? What is the stability and repeatability of the species groups discovered together? "Lourens G. M. Baas Becking, and Bejerinck's study, formulated the principle that influenced scientific perception of the propagation of microorganisms during the twentieth century:" Everything is available, but the atmosphere selects (Niederberger et al. 2012). The implication of this argument is that environmental factors exclusively decide the spread of microorganisms. However, this is vigorously questioned because molecular approaches have united the study of biogeographical microbial patterns with general ecological hypotheses. Each organism has a physiological capacity to survive and replicate, and its biotic and abiotic climate affects the environment in which an organism resides. Functional characteristics are an organism's properties that influence how well the organism performs under a certain set of conditions (McCaig et al. 1999). The population is made up of species that exist in one ecosystem and the numbers and kinds of species present are referred to as the composition of the population. Populations or subpopulations of distinct species are comprised of groups. A population is a group of all organisms with association capacity that belong to a single genus. This renders a population's spatial scale dependent on the species' mobility. Only part of a true population (e.g. migrating species) or multiple isolated populations (e.g. soil bacteria) may be covered by a study. It is common for both conditions to occur in the same sample, considering the degree to which organisms are differentially mobile.

3.8.1.1 Physiological Limitations to Survival

To forecast population dynamics under evolving environmental factors in new areas or with changes in group composition, ecologists also require detailed knowledge on

the suitability of ecosystems for a specific species. The functional characteristics of a species provide limits on the circumstances in which organisms, and therefore populations, can expand and reproduce. The Tolerance Rule of Shelford states that with each environmental cause, there is a maximum and minimum value above which a given species cannot live. With regard to environmental properties known as modulators, such as temperature, pH, or salinity, this law is generally debated. By modifying the conformation of proteins and cell membranes and the thermodynamic and kinetic favourability of biochemical reactions, modulators affect the physiology of organisms. For each environmental modulator where maximum population growth occurs, species also have an optimal selection. Modulator resistance may be interactive; in certain fungi, for example, cold temperature resistance depends on water potential (Hayashi et al. 2020). The geographical range of a species usually corresponds with regions where the environmental conditions are beyond the species' optimum range, with the most optimum conditions being at the middle of the geographical range.

Tools are physical elements of the world that are collected organisms, such as N, energy or territory, for their use. The Rule of Shelford may be applied to most resources, but the response to various resources is strongly interactive. This is partially defined in the Law of the Minimum by Liebig, which it notes that in relation to organismal needs, the resource in the lowest supply would restrict progress. The organism is unable to accumulate at very low resource levels, a fuel for metabolism in sufficient amounts. At very high quantities, sometimes resources can be toxic or hinder development. The amount of resources that the requirements of the organism (e.g., biomass nutrient stoichiometry) are essential. Functional feature to decide how the success of organisms varies with resources availability, as well as food excretion that is absorbed as waste in excess of use. Studies have found that the availability of nutrients, particularly C:N:P stoichiometry could have an effect on microbial biomass ratios of C:N:P, with limits of N and P influencing the overall amount of microbial biomass in the soil. However, these ratios are actually very conserved across soils in general, by Cleveland and Liptzin. The effect of these resource requirements feeds into the back to the microbial group's effect feature. The C:N fluctuations fungal, bacterial biomass, and other characteristic differences between bacteria and fungi, such as the efficacy of growth, have significant effects on the cycles of soil C and N.

3.8.1.2 Intraspecific Competition

When certain species expand and reproduce, resources are consumed and access to those with the same needs reduced. This reduction of one organism's efficiency by another that requires the same capital is known as rivalry. The logistical growth equation is a statistical model that explains the intraspecific competition's over-time impact on demographic change. The likelihood of a reproduction less the chance of death per unit time is proportional to the general population rise or decline over that time. Classification of organisms as r- or K-selected is common in soil microbiology.

R-selected species can be positively identified (with respect to the isolation conditions), but K-selected species cannot. Other classifications were based instead on intraspecific competition on chosen species capital. In the year 1925 the term autochthonous and zymogenous was used by Winogradsky to describe species that grow continuously in the atmosphere on resistant organic matter.

3.8.1.3 Dispersal in Space and Time

In order to escape the harmful consequences of competition, species have to transfer or scatter with energy. Passive dispersal occurs thanks to the movement of fabric the organism is attached to or caught in. Active dispersal involves the expenditure of energy by the organism. Stages for dispersal are typically more resistant, dormant, or mobile than growth stages. Plant roots, seeds, fungal spores, and chemical substrates found within several centimetres of soil bacteria have been shown to induce chemotactic responses (active dispersal) that may be important for responses, like rhizosphere colonisation.

Fungi also have various hyphal growth forms for acquisition and dispersal of nutrients. Spores, such as arbuscular mycorrhizal mushrooms or sporocarps, can also be formed in the soil. Fungal hyphae vegetative development should be viewed as a kind of aggressive dispersal, since new areas are being investigated. The ability of an organism to enter a dormant phase can be seen as a dispersal mechanism, but through time instead of space, for several organisms, life stages that facilitate passive dispersal in space also are optimal for dispersing in time. These are often as true of plant seeds and fungal spores, such as plant seeds and above- and below-ground animal behaviour. Species are often scattered, and dormancy related to several populations. Soil microorganisms tend to be usually inactive when water or nutrients are changed by increase in numbers and metabolic activity. The bulk of the soil bacterial cells contain “Dwarf” cells. The population base for colonisation of new areas of resources-rich ecosystems is the inactive, passively scattered cells.

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3.8.1.4 Interspecific Competition

Each factor contributing to each axis can be traced to the influence of abiotic factors on the population’s survival or growth rate. The area of the area ideal for species development was envisaged by Hutchinson (Hutchins and Miller 2017) as being the fundamental niche of the species. It is the accomplished niche that refers to the diminished hypervolume that a species will currently fill. At every given time, there is a small pool of resources available, but when used faster than used up, the rate of

development decreases. Despite low levels of capital, the best rivals will sustain their fastest growth rates. Tilman (Thomas and Nielsen 2005) recommended to have equal to the potentially restricting tools used by that ecosystem the number of like species that may occur in one ecosystem. The extremely heterogeneous soil conditions are also believed to support the enormous microbial range.

In addition, fluctuating death rates can help the coexistence of organisms. R-selected organisms can be production but prosper when the death rate for the dominant organisms' spikes. Soil labour is an example of a disruption that damages fungi and spreads bacteria. Fleeting species can escape rivalry by moving to habitat areas where local dominant species are extinct. Mortality rates may also be modified through competitive intervention, where one competing species has a specifically hostile effect on another.

The use of various subtypes of the same resource can also grow related organisms. It is known as the partitioning of wealth and was considered some of the first proof of competitiveness and natural choice. The pure colonies of Escherichia coli, owing to the disparity in physiology, have been shown to be different and co-existing subtypes that was seen to be pure, for instance E's cultures. Because of its physiological function, coli (Gamma proteobacterium class) grow into different subtypes concurrence was exploited in soil as a biocontrol mechanism. The reality that many soil inoculation programmes had failed was also blamed. A number of plant pathogens were found to be suppressed by fluorescent pseudomonads. Sterilised soil species often thrive while non-sterile soil communities easily decrease. In rare cases, inoculated species have survived, if the ecosystem is changed to suit their niche requirements (in lower numbers than inoculum size).

3.8.1.5 Direct and Indirect Effects of Exploitation

Biological interactions, including exploitation and mutuality, impact the assembly of microbial communities. The microbial ecosystem is full of exploitation, including predation, herbivorous infections, parasitism, and pathogenesis. Predators and parasites are aggregated in environments with high host and host populations. Predatory pressure also contributes to the quality of the prey's habitat. Predator-free patches can be used as shelter for the predators and can have major metapopulation effects. Nematode and protozoa concentrations are increased by high bacteria near their roots and N mineralisation rises in turn from the microbial biomass. A viral genome based on both its bacterial and virus hosts is an extreme example of a secondary, smaller viral genome within larger virus genomes. It is necessary to know when a consumer's predator or a parasite kills automatically and only receives a part of the prey's capital without destroying them so that in the future the same organism can be used. As in all neat environmental types, there is an extreme gradient in lifestyles.

Elliott (de Scally et al. 2016) found that a finer-textured soil contained more bacteria protected from predation by nematodes. Predation has an increase in death rates on the dynamics of the population of prey. Parasitism is a far more complicated model than predation phenomenon as prey are weakened by pests that affect

reproduction and death rates. Parasites may decrease the biomass accumulation or growth rate. They will also raise the mortality rate, either by continuing with the parasite or sensitising the prey to other causes of death.

High-quality habitats allow pathogens to further settle the new roots through the soil (to a minimum of 15 cm). Planting crops at wider ranges is known to reduce the spread of root diseases (e.g. reducing host density). Some parasites are transferred through other species or other environmental components.

The result of competitive interactions between prey species can be influenced greatly by exploitation. It can coexist with competing prey species by decreasing the size of the population of the top rivals. Defenses from exploitation can take a variety of forms, including behavioural, morphological, or biochemical. Evolution can also result in the development of new attack strategies in consumers. This results in a continual coevolutionary arms race between consumers and their prey. A dynamic web of interactions (a food web) results from the different species-specific trophy relationships between individuals in ecosystems. The increased complexity of food web complexity in the environment will contribute to the complexity of the ecosystem, according to a meta-analysis by Sackett et al. Exploitation is often the basis of biocontrol strategies of plant pests, such as the control of Rhizoctonia solani, a mycoparasitic fungus that attacks the root pathogen Rhizoctonia solani. The study of microbial dynamics in situ is difficult, and laboratory experiments do not provide the correct details about how the natural systems function. It is hard to evaluate.

In food chains of microorganisms there are no "top predators", so both organisms are being exploited by parasites. Decomposer species control primary producers' population dynamics by providing nutrients. The presence and resource of the decomposing organism must also be taken into account for food webs including microorganisms. This decomposition is critical to the recycling of nutrients that can be used in primary production. The framework for this web-based foodstuff has not been precisely studied for microbial systems, but according to Moore and De Ruiter, these loops often appear due to random encounters (Monteiro et al. 2020). In macroscopic organisms the existence of "three species circle", which is problematic, can only be accomplished when species are influenced by variations due to the stage of evolution.

3.8.1.6 Mutualistic Interactions

Soil mutualists influence community dynamics across a variety of ecosystems. Soil species function on nutrient acquisition for a wide range of plants. Mycorrhizae are one of the most ubiquitous mutualisms of the soil, a relationship between the plant root and the fungus. It is concerned that essential relationships between species could be at risk as a result of human disturbances, such as N extensions, invasive species, and global climate change, which have formed symbioses with bacterial N fixators, to acquire the needed nutrient. These are also important for stabilising the soil in easily erodible soils as macrobiotic crusts.

3.8.1.7 Community Impacts on Abiotic Factors

In terms of use of resources, interactions between organisms were discussed. But animals may also influence environmental modulators. Both nitrifying bacteria and plant roots reduce their pH and the plant and litter cover affect soil temperatures. Depending on the species niche requirements, this can affect positively or negatively in the development of another species. Any species change the spatial structure of environmental components or serve themselves as a new ecosystem. This species is known as ecosystem engineers and usually influence an ecosystem.

3.8.1.8 Community Variation among Soil Habitats

The precise spatial arrangement of environmental components is a landscape that is somehow essential for the dynamics of a species population. Patches with different ecosystems as well as variations in factors that impact ecosystem quality usually involve landscapes. Minerals and non-particulate, humidized organic matter dominate the matrix of habitat of most soils; we call it a "mineral bulky soil" with a diverse variety of microbial species. A widespread supply of nutrients or labile organic matter creates many soil habitats parches and are thus areas of increased biological activity. The rhizosphere, faecal matter, and rhizopaedia represent the most essential parts of a landscape and are vital for plant interactions and ecosystem processes. We assume that for various species, landscapes are different based on the space level at which the species communicate with the environment.

These examples of this ecosystem are essential organic matter and plant tissue decomposting. The microbial biomass with a distinct taxonomic structure has risen in these ecosystems. Some hyphal-growing microorganisms (e.g. many fungi) are more spatially interactive with the environment than individual rhizospheres or organic particles. Many forms of environmental variations are often considered to influence the composition, biomass and behaviour of the soil, and other embedded ecosystems of microbes. In the structure of soil microbial populations, growth of multiple plant species and, in some cases, of plant genotypes and developmental stages creates variation. Plant organisms impact microbial populations through the release of multiple compounds and through tissue decomposition into the rhizosphere. Plants also communicate with microbial symbionts, which may be helpful or detrimental to surface compounds. Soil pH also has the strongest correlation with the structure of the microbial population. However, the soil characteristics, habitat types, and land use vary greatly.

3.8.1.9 Community Structural Changes through Time

Succession is the change by biological interactions of populations in the ecosystem over time. The constant creation of different habitats and the gradual return to the

matrix community creates a changing mosaic of different habitats at various succession stages. In several different systems, incidents such as habitat destruction are an integral part of the nature of the group. Most of these events are stochastic over large time and space scales at an average rate. The proportion of the landscape in the Community Matrix should be equal to the stable value defined by the rate and spatial size of the events of destruction and the rate of return of the community matrix by succession.

3.9 Ecosystem Role Effects of the Microbial Population Organisation

Soil microbes include species ranging from strict aerobics and anaerobes, high water demand and low water need, basic inorganic, and complex organic substrates and autotrophe to heterotrophic lifestyles. Soil microbes include species. Many aspects of the microbial function of the soil are related to the role of microbes in ecosystem outcomes. Despite the very short time human beings have been on the earth, ecological equilibrium has been altered by cropping and burning and, most recently, by the rise in soil chemical loads and the introduction in pesticides and other human pesticides. Ecosystems are spatially characterised as interconnected structures by the association of organisms and their relationship to the physical space. For example, after the last glacial retreats, trees are still migrating, forcing new interactions between the components of ecosystems. These impacts are not yet modelled by ecologists which biotic processes are increasing or declining. Climate is one of the most critical variables in deciding processing speeds by checking moisture and temperature availability. The parent material type defines the ability of the medium in which species mature as a food and water retention. The access to water, the material movement, soil depth, the degrees weathering of the parent material are determined by the topography, slip, and aspect characteristics The time needed for soil development is interactive with the climate, because environmentally harsh conditions require a lot more time for soil development. Warmer environments with ample humidity and moderate temperatures require significantly lower soil development. The relationship between the two subsequent effects on microbial performance and the extent to which microbes are able to grow under the new constraints. Potential biota includes all organisms that can exist or have existed in an area. Topographic composition and microbial behaviour may be altered above and below the ground depending on the slope. The work of the microbial community modifies soil chemistry through processes that improve nutrient supply or decomposition rates. An understanding of soil biology and biochemistry is essential to understand the impacts of land use and climate change, says Singh and Treseder. A wide-ranging number of species have resulted, they claim, from the vast variety of organic compounds that can be found on the earth's surface. The results are also backed by metagenomic methods to determine the variability of the metabolic genes.

The authors conclude that an understanding of the impact of soil on climate change and land use on ecosystems is essential for a balanced approach to land use change and sustainability.

3.9.1 Energy Flow

The energy supply is the light for most structures and green plants are the autotrophs. The available energy is equal to the energy from solar power captured by photosynthesis in a given environment. Complete energy use by plants in an environment usable for other trophic stages is the net primary productivity (NPP). The leaf area and the contents N, season duration, temperature, lights, and carbon dioxide are regulated by NPP. Most of the true decomposers are heterotrophic osmotrophic. They release enzymes to breakdown materials and absorb pieces that increase in NPP. For mandated symbionts, the number of trees that require mycorrhizal fungus for production and survival will be decreased and the turnover of the nutrient and ecosystem characteristic will continue to be affected.

3.9.2 Nutrient Cycles

Biotic and abiotic components provide molecules for the growth and reproduction of living organisms. The massive CO_2 flux to the atmosphere has significantly affected the global C cycle. Potential human-induced shifts in the C cycle of global fluxes are the most critical ecological experiment of all time. Human response would be dictated by biological reactions to high CO_2 levels as well as indirect responses, such as changes in temperature and humidity and climatic instability. Predicting the consequences on soil microorganisms of climate change is a unique task, we say. We conclude that predicting the impact of human-induced CO_2 alterations on the C cycles on the soil will be a challenge, and that we need to act now to protect the Earth's biodiversity. We thank the authors for their interest in our understanding of the C and N cycles and the potential impacts of humans on the global environment on the biotic, abiotic, and soil cycles on CO_2 and climate change. Not all soil species depend on plant and animal energy products. Soil microorganisms contain lithotrophic substances and can be used as energy sources by materials, such as ammonium and certain sulphur compounds. In O_2 confined environments, many soil species often use nitrate and sulphate to be the greatest electron accepter, helping them to survive in anaerobic conditions. As such they play a singular part in cycles C, N, and S and have a major impact on global climate change with CH_4 and N_2O impacts. This singular energy transitions have important implications for the global climate awareness, as they change the flow rates of ecosystem C by photosynthesis and decomposition. Almost any main step in this cycle is guided by the microbial population.

The rate of return of N to the system after the plant intake is determined in large measure by plant structure, efficiency of nutrient usage and finally, mineralisation rate. In the past, the volume of N naturally found in environments has been either N set by microbes or recycled by microbes to organic materials. A doubling of N currently available to plants in many ecosystems has resulted in the generation and application of fertiliser N and pollutant dispersal. It increased soil breath, decreased microbial biomass and activity of the enzyme throughout many different soils have been found to increase N. Disturbances, such as fires, that release nutrients to soil can decrease N availability. Mutualism and the growth of decomposer bacteria and fungi have tremendous potential to shift the nature of plant community. The abundance of P and other nutrients will also determine how easily plant nutrients and the nutrient succession of an ecosystem are available. Plants need so much P that plants with mutual interplay can grow larger than plants without fungi, while plants continue to produce C for fungal growth.

Low levels of any critical nutrient will contribute to stress and lower productivity "under Liebig's Law of the Minimum". The nutrients by which plants are affected in most environments are the ones retrieved by microbial activity through recycling. Microbes alone can return up to 100% of the nutrients needed for the growth of plants in the decomposition of vegetable litter. Many systems may rely entirely on local nutrient cycle components, and many systems depend on internal cycles.

3.9.3 Emergent Properties

Elemental tasks are driven by soil dynamics and ecosystem functions are regulated. They are essentially determinants of evolving properties, such as decay rates, biodiversity, and stability of the environment. A dynamic result of the first two layers of sophistication is the soil structure. This interacts with biodiversity or in this case directly with the population system of decomposition in order to determine decomposition rates. Intellectually, the conservation of the biodiversity is necessary for the protection of the system integrity, but the entire biodiversity of a system, according to the authors, is difficult to quantify. These concerns need urgent attention in an age of diminishing global biodiversity and genetic diversity, they add. The notion that forests are more than trees translates the value of the term, but they write that they can be widely generalised to any ecosystem. Each feature of a soil ecosystem that is critical for biology involves the movement of gas, water, macro and microporous spaces, water retention capacities, etc.

A rise in diversity by expanded unwanted species such as non-native invasive species would not protect the system's integrity. In the past, several researches centred on biodiversity and the implications on the role of the ecosystems. More evidence is available that the process rate is essentially not regulated by the climate, but by biodiversity. An important link between diversity and cycling was formed with low diversity. Diversity became more important than the number of organisms

as basic characteristics became high. Return to the page which shows the quality, the amount.

Anthropogenic effects have modified how the habitats work, leading to the stress on habitats world-wide. Novel, common, or serious disruptions can threaten the stability of the ecosystem. How resistant or robust habitats are may be completely determined by biodiversity and how much tension the environment actually has. Shade et al. (2012) studied the concepts that can forecast short-term and long-term population resilience and microbial resilience. They find that the structure and role of the soil population was disturbingly sensitive and pulse- and push-resistant. There can, however, be distortions when documenting disruption responses, as studies which find no improvements in the group structure or function may be underreported. Further experiments are required to determine the effects of ecosystem stability vulnerability in microbial communities. Ecosystem stability is an emerging feature which cannot be quantitatively calculated quickly.

3.10 Evaluation of Soil Fertility by Assessing Microbiological Activity

Various approaches have been developed by researchers around the world to assess soil fertility based on its microbiological behaviour.

1. General soil fertility test mechanisms require the addition of damp soil samples with water holding potential of 60–70% and a particular nutrient solution, followed by incubation at 20–30 C for 7–30 days, and biologically-modified adjustments.
2. In order to determine soil fertility, the biochemical reactions requiring a nitrogen compound metabolism are analysed.
3. In the study of soil fertility, the normal biological reactions, such as ammonification, nitrification, denitrification, and nitrification, are used.

Dependence of these techniques is the main challenge involved with their application. Soil microbes are exposed to a variety of factors in normal scenario. It comprises of different surface conditions, temperature impacts and soil conservation.

3.11 Ecological Consequences of Human Modifications to the Cycle of Nitrogen

Most human actions have a significant effect on the nitrogen cycle. Burning fossil fuels, the use of nitrogen-based fertilisers and other practises will significantly raise the amount of naturally accessible nitrogen in the environment. And, since the supply of nitrogen also restricts the primary productivity in certain species,

important changes in nitrogen supply in both marine and terrestrial ecosystems can contribute to dramatic changes in the nitrogen cycle. Industrial nitrogen fixation has grown exponentially since the 1940s, twice the extent of global nitrogen fixation by human operation.

The inclusion of nitrogen in terrestrial ecosystems would help to mitigate forest nutrient imbalances, enhance forest quality and biodiversity. In an improved nitrogen supply, carbon storage often takes place and requires more processes than the nitrogen cycle. In agriculture, fertilisers are commonly used to increase the quality of plants, but unused nitrogen can usually leak out of the soil, infiltrate streams and rivers and ultimately make its way into our drinking water. In recent decades N_2 has significantly improved its production method for synthetic fertilisers used in agriculture by allowing N_2 to react to H_2, called the Haber Bosch mechanism. Probably, almost 80% of the nitrogen in human tissues today comes from the Haber–Bosch process (Howard-Williams and Hawes 2007).

In rural and urban areas, most of the nitrogen used gradually drains into rivers and marine waters. Nitrogen development also contributes to anoxia (no oxygen) or hypoxia (low oxygen), altered biodiversity, changes in food web structure and a general lack of habitat in nearshore marine ecosystems. A rise in dangerous algal blooms is a typical effect of increased nitrogen (Howard-Williams and Hawes 2007). In certain countries, high deaths of fish and shellfish have been linked by toxic blooms of certain forms of dinoflagellates. Even without those economically disastrous consequences, the addition of nitrogen will contribute to improvements in ecology and species distribution that may contribute to changes in the overall functioning of the ecosystem. Some have also indicated that changes to the nitrogen cycle can lead to an increase in risk of human and wildlife parasites and infectious diseases (Jobba'gy and Jackson 2000). In addition, increases in nitrogen in aquatic systems may lead to increased acidification.

3.12 Nitrogen Derivatives and Future Environmental Effects

About half of Earth's nitrogen fixation by fertilisers and the development of nitrogen fixation crops can be induced by soil. More Nitrogen (soil) inputs have helped to generate much more food, which is considered the "green revolution" and feeds more people. Nitrogen, however, will spill from the soil into the waterway, exceeding demand from plants. Nitrogen enrichment enables eutrophic therapy. During nitrification and denitrification, another problem may arise. Nitrous oxide (N_2O) may be generated if the chemical process is not completed. The balance of nitrogen compounds in the environment supports plant life and is not a danger to wildlife. N_2O is a significant greenhouse gas—contributing to global warming. The only explanation why problems arise being that the loop is not controlled.

3.13 Conclusion and Gaps in Current Knowledge

Soil microbiology is a multidisciplinary area of study that examines soil microbes and their interactions. The soil has always focused on studying its physicochemical and biological properties, as a natural habitat for survival and growth of microorganisms. The relationship between microorganisms and higher plants and the use of the ecto- and endo-microbial connexion for a soil microbial environment should be considered very significant. Soil microbiology describes, depending on spatial constraints and climate change, a short idea of nature-based bacteria and the chemical properties of soil.

Speciation and quantification of nitrogen compound data are ancient, most of which were collected decades ago. There are concerns about the relevance of these information, as the disponibility of nitrogen in the intermediate years may have shifted. Transcriptomic soil details, with some notable exceptions, are generally quite lacking. There is no question that further research ought to use metatranscriptomics to figure out which genes (and processes) and what amounts are expressed. Earlier research has shown that microbial species are susceptible to climate change. How soil microbials respond to changing temperatures and how nitrogen cycle in soils affects would be important to consider. A wider pathway of nitrogen, rates and stoichiometry are obviously essential. The benefits of future studies would be to resolve the shortcomings already found. These studies are also excellent opportunities for metabolomic analysis to get a better understanding of the effect of nitrogen on the functionality of the microbe culture.

References

Adhya TK (2017) Advances in soil microbiology: recent trends and future prospects. Springer, Singapore

Adriaenssens EM, Kramer R, Van Goethem MW, Makhalanyane TP, Hogg I, Cowan DA (2017) Environmental drivers of viral community composition in Antarctic soils identified by viromics. Microbiome 5:83

Amarelle V, Carrasco V, Fabiano E (2019) The hidden life of Antarctic rocks. In: The ecological role of micro-organisms in the Antarctic environment. In: Castro-Sowinski S (ed) Springer, Cham, Switzerland, pp 221–237

Anesio AM, Bellas CM (2011) Are low temperature habitats hot spots of microbial evolution driven by viruses? Trends Microbiol 19:52–57

Asuming-Brempong S (2012) Microarray technology and its applicability in soil science—a short review. Open J Soil Sci 2:333–340

Ayton J, Aislabie J, Barker GM, Saul D, Turner S (2010) Crenarchaeota affiliated with group 1.1 b are prevalent in coastal mineral soils of the Ross Sea region of Antarctica. Environ Microbiol 12:689–703

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681

Balser TC, Wixon D, Moritz LK, Lipps L (2010) The microbiology of natural soils. In: Dixon GR, Tilston EL (eds) Soil microbiology and sustainable crop production. Springer, Berlin, pp 27–57

Bargett RD, Shine A (1999) Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol Biochem 31:317–321. https://doi.org/10.1016/S0038-0717(98)00121-7

Brady NC, Weil RR (2002) The nature and properties of soils, 13th edn. Prentice Hall, Upper Saddle River, NJ

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? a review of nitrate removal pathways. Front Ecol Environ 5:89–96

Buzzini P, Branda E, Goretti M, Turchetti B (2012) Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. FEMS Microbiol Ecol 82:217–241

Buzzini P, Margesin R (2014) Cold-adapted yeasts: a lesson from the cold and a challenge for the XXI century. In: Buzzini P, Margesin R (eds) Cold-adapted yeasts. Springer, Berlin; Heidelberg, Germany, p 3

Carson JK, Campbell L, Rooney D, Clipson N, Gleeson DB (2009) Minerals in soil select distinct bacterial communities in their microhabitats. FEMS Microbiol Ecol 67:381–388. https://doi.org/10.1111/j.1574-6941.2008.00645

Carson JK, Gonzalez-Quinones V, Murphy DV, Hinz C, Shaw JA, Gleeson DB (2010) Low pore connectivity increases bacterial diversity in soil. Appl Environ Microbiol 76:3936–3942. https://doi.org/10.1128/AEM.03085-09

Carson JK, Rooney D, Gleeson DB, Clipson N (2007) Altering the mineral composition of soil causes a shift in microbial community structure. FEMS Microbiol Ecol 61:414–423. https://doi.org/10.1111/j.1574-6941.2007.00361.x

Cary SC, McDonald IR, Barrett JE, Cowan DA (2010) On the rocks: the microbiology of Antarctic dry valley soils. Nat Rev Microbiol 8:129–138

Certini G, Campbell CD, Edwards AC (2004) Rock fragments in soil support a different microbial community from the fine earth. Soil Biol Biochem 36:1119–1128. https://doi.org/10.1016/j.soilbio.2004.02.022

Chan Y, Van Nostrand JD, Zhou J, pointing sb, farrell rl (2013) functional ecology of an Antarctic dry valley. Proc Natl Acad Sci U S A 110:8990–8995

Cheeke TC, Coleman DC, Wall DH (2013) Microbial ecology in sustainable agroecosystems. CRC Press, Boca Raton, FL

Cowan DA (2014) Microbiology of Antarctic soils. Springer, Berlin; Heidelberg, Germany, p 328

Cowan DA, Makhalanyane TP, Dennis PG, Hopkins DW (2014) Microbial ecology and biogeochemistry of continental Antarctic soils. Front Microbiol 5:154

Cowan DA, Sohm JA, Makhalanyane TP, Capone DG, Green TGA, Cary SC, Tuffin IM (2011) Hypolithic communities: important nitrogen sources in Antarctic desert soils. Environ Microbiol Rep 3:581–586

Coyne KJ, Parker AE, Lee CK, Sohm JA, Kalmbach A, Gunderson T, León-Zayas R, Capone DG, Carpenter EJ, Cary SC (2020) The distribution and relative ecological roles of autotrophic and heterotrophic diazotrophs in the McMurdo dry valleys, Antarctica. FEMS Microbiol Ecol 96:fiaa010

Crane SL, van Dorst J, Hose GC, King CK, Ferrari BC (2018) Microfluidic qPCR enables high throughput quantification of microbial functional genes but requires strict curation of primers. Front Environ Sci 6:145

Dang H, Chen CA (2017) Ecological energetic perspectives on responses of nitrogen-transforming chemolithoautotrophic microbiota to changes in the marine environment. Front Microbiol. https://doi.org/10.3389/fmicb.2017.01246

Dávila-Ramos S, Castelán-Sánchez HG, Martínez-ávila L, Sánchez-Carbente MDR, Peralta R, Hernández-Mendoza A, Dobson ADW, Gonzalez RA, Pastor N, Batista-García RA (2019) A review on viral metagenomics in extreme environments. Front Microbiol 10:2403

de Scally SZ, Makhalanyane TP, Frossard A, Hogg ID, Cowan DA (2016) Antarctic microbial communities are functionally redundant, adapted and resistant to short term temperature perturbations. Soil Biol Biochem 103:160–170

Elliott ET, Anderson RV, Coleman DC, Cole CV (1980) Habitable pore space and microbial trophic interactions. Oikos 35:327–335
Falkowski PG, Godfrey LV (2008) Electrons, life and the evolution of Earth's oxygen cycle. Philos Trans R Soc Lond Ser B Biol Sci 363:2705–2716
Fernández-Valiente E, Quesada A, Howard-Williams C, Hawes I (2001) N2-fixation in cyanobacterial mats from ponds on the McMurdo ice shelf, Antarctica. Microb Ecol 42:338–349
Ferrari BC, Bissett A, Snape I, van Dorst J, Palmer AS, Ji M, Siciliano SD, Stark JS, Winsley T, Brown MV (2016) Geological connectivity drives microbial community structure and connectivity in polar, terrestrial ecosystems. Environ Microbiol 18:1834–1849
Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364. https://doi.org/10.1890/05-1839
Fraser C, Alm EJ, Polz MF, Spratt BG, Hanage WP (2009) The bacterial species challenge: making sense of genetic and ecological diversity. Science 323:741–746
Freney JR, Randall PJ, Smith JWB, Hodgkin J, Harrington KJ, Morton TC (2000) Slow release sources of acetylene to inhibit nitrification in soil. Nutr Cycl Agroecosyst 56:241–251
Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai ZC, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–892
Garrett SD (1951) Ecological groups of soil fungi: a survey of substrate relationships. New Phytol 50:149–166
Garrido-Benavent I, Pérez-Ortega S, Durán J, Ascaso C, Pointing SB, Rodríguez-Cielos R, Navarro F, de los Ríos A (2020) Differential colonization and succession of microbial communities in rock and soil substrates on a maritime Antarctic Glacier Forefield. Front Microbiol 11:126
Godfrey LV, Falkowski PG (2009) The cycling and redox state of nitrogen in the Archaean Ocean. Nat Geosci 2:725–729
Grundmann GL, Normand P (2000) Microscale diversity of the genus Nitrobacter in soil on the basis of analysis of genes encoding rRNA. Appl Environ Microbiol 66:4543–4546
Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JB (2012) Beyond biogeographic patterns: processes shaping the microbial landscape. Nat Rev Microbiol 10:497–506
Harris RF, Chesters G, Allen ON (1966) Dynamics of soil aggregation. Adv Agron 18:107–169
Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. In: Weaver RW, Angle JS, Bottomley PJ, Bezdicek DF, Smith MS, Tabatabai MA, Wollum AG (eds) Methods of soil analysis, part 2—microbiological and biochemical properties. Soil Science Society of America, Madison, WI, pp 985–1018
Hattori T, Hattori R (1976) The physical environment in soil microbiology: an attempt to extend principles of microbiology to soil microorganisms. Crit Rev Microbiol 4:423–460. https://doi.org/10.3109/10408417609102305
Hayashi K, Tanabe Y, Fujitake N, Kida M, Wang Y, Hayatsu M, Kudoh S (2020) Ammonia oxidation potentials and ammonia oxidizers of lichen–moss vegetated soils at two ice-free areas in East Antarctica. Microbes Environ 35:2–6
Hoshino T, Xiao N, Tkachenko OB (2009) Cold adaptation in the phytopathogenic fungi causing snow molds. Mycoscience 50:26–38
Howard-Williams C, Hawes I (2007) Ecological processes in Antarctic inland waters: interactions between physical processes and the nitrogen cycle. Antarct Sci 19:205–217
Howarth RW (2008) Coastal nitrogen pollution: a review of sources and trends globally and regionally. Harmful Algae 8:14–20
https://www.britannica.com/science/nitrogen-cycle n.d.
https://www.cbsetuts.com/neet-biology-notes-mineral-nutrition-nitrogen-cycle n.d.
https://www.sciencelearn.org.nz/resources/960-the-nitrogen-cycle n.d.
Hutchins PR, Miller SR (2017) Genomics of variation in nitrogen fixation activity in a population of the thermophilic cyanobacterium Mastigocladus laminosus. ISME J 11:78–86
Hutchinson GE (1957) Concluding remarks. Cold Spring Harb Symp 22:415–427

Insam H (2001) Developments in soil microbiology since the mid-1960s. Geoderma 100:389–402. https://doi.org/10.1007/s00374-010-0442-3
Jetten MSM (2001) New pathways for ammonia conversion in soil and aquatic systems. Plant Soil 230:9–19
Ji M, Greening C, Vanwonterghem I, Carere CR, Bay SK, Steen JA, Montgomery K, Lines T, Beardall J, Van Dorst J (2017) Atmospheric trace gases support primary production in Antarctic desert surface soil. Nature 552:400–403
Jobba'gy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–436. https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2
Johnson PTJ (2010) Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecol Appl 20:16–29
Jung J, Yeom J, Kim J, Han J, Lim HS, Park H, Hyun S, Park W (2011) Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils. Res Microbiol 162:1018–1026
Kartal BK, Keltjens JT, Jetten MSM (2011) Metabolism and genomics of anammox bacteria. In: Ward BB, Arp DJ, Klotz MG (eds) Nitrification. American Society for Microbiology Press, Washington, DC, pp 181–200
Kaviya N, Upadhayay VK, Singh J, Khan A, Panwar M, Singh AV (2019) Role of microorganisms in soil genesis and functions. In: Mycorrhizosphere and Pedogenesis, pp 25–52
Kingsland SE (1991) Defining ecology as a science. In: Real LA, Brown JH (eds) Foundations of ecology: classic papers with commentaries. University of Chicago Press, Chicago
Kizewski FR, Kaye JP, Martínez CE (2019) Nitrate transformation and immobilization in particulate organic matter incubations: influence of redox, iron and (a)biotic conditions. PLoS One 14(7):e0218752. https://doi.org/10.1371/journal.pone.0218752
Komárek J, Genuário DB, Fiore MF, Elster J (2015) Heterocytous cyanobacteria of the ulu peninsula, James Ross island, Antarctica. Polar Biol 38:475–492
Kuramae EE, Yergeau E, Wong LC, Pijl AS, van Veen JA, Kowalchuk GA (2012) Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiol Ecol 79:12–24
Kurtzman C, Fell JW, Boekhout T (2011) The yeasts: a taxonomic study. Elsevier, Amsterdam, The Netherlands
Kuypers MMM, Lavik G, Woebken D, Schmid M, Fuchs BM, Amann R, Jorgensen BB, Jetten SM (2005) Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc Natl Acad Sci U S A 102:6478–6483
L€ohnis F (1913) Vorlesungen uber Landwortschaftliche Bacterologia. Borntraeger, Berlin
L€onn A, Ga'rdonyi M, van Zyl W, Hahn-Ha¨gerdal B, Otero RC (2002) Cold adaptation of xylose isomerase from Thermus thermophilus through random PCR mutagenesis gene cloning and protein characterization. Eur J Biochem 269:157–163
Lacap-Bugler DC, Lee KK, Archer S, Gillman LN, Lau M, Leuzinger S, Lee CK, Maki T, McKay CP, Perrott JK (2017) Global diversity of desert Hypolithic cyanobacteria. Front Microbiol 8:867
Latysheva N, Junker VL, Palmer WJ, Codd GA (2012) Barker, D. the evolution of nitrogen fixation in cyanobacteria. Bioinformatics 28:603–606
Lipson DA (2007) Relationship between temperature responses and bacterial community structure along seasonal and altitudinal gradients. FEMS Microb Ecol 59:418–427. https://doi.org/10.1111/j.1574-6941.2006.00240.x
Loper JE, Hassan KA (2012) Comparative genomics of plant-associated Pseudomonas spp.: insights into diversity and inheritance of traits involved in multitrophic interaction. PLoS Genet e1002784:8
Lowrance RR, Todd RL, Fail J, Hendrickson O, Leonard R, Asmussen L (1984) Riparian forests as nutrient filters in agricultural watersheds. Bioscience 34:374–377

Magalhães C, Machado A, Bordalo AA (2009) Temporal variability of relative abundance of ammonia oxidizing bacteria vs. archaea in the sandy at of the Douro River estuary, Portugal. Aquat Microb Ecol 56:13–23

Magalhães C, Machado A, Frank-Fahle B, Lee CK, Cary CS (2014) The ecological dichotomy of ammonia-oxidizing archaea and bacteria in the hyper-arid soils of the Antarctic dry valleys. Front Microbiol 5:515

Makhalanyane TP, Valverde A, Lacap DC, Pointing SB, Tuffin MI, Cowan DA (2013) Evidence of species recruitment and development of hot desert hypolithic communities. Environ Microbiol Rep 5:219–224

Makhalanyane TP, Valverde A, Velázquez D, Gunnigle E, Van Goethem MW, Quesada A, Cowan DA (2015) Ecology and biogeochemistry of cyanobacteria in soils, permafrost, aquatic and cryptic polar habitats. Biodivers Conserv 24:819–840

McCaig AE, Phillips CJ, Stephen JR, Kowalchuk GA, Harvey SM, Herbert RA, Embley TM, Prosser JI (1999) Nitrogen cycling and community structure of proteobacterial beta-subgroup ammonia-oxidizing bacteria within polluted marine fish farm sediments. Appl Environ Microbiol 65:213–220

McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol 21:178–184

McGuire KL, Treseder KK (2010) Microbial communities and their relevance for ecosystem models: decomposition as a case study. Soil Biol Biochem 42:529–535

Mitchell CE (2003) Trophic control of grassland production and biomass by pathogens. Ecol Lett 6:147–155. https://doi.org/10.1046/j.1461-0248.2003.00408.x

Mitsch WJ, Day JW, Gilliam JW, Groffman PM, Hey DL, Randall GW, Wang N (2001) Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River basin: strategies to counter a persistent ecological problem. Bioscience 51:373–388

Monteiro M, Baptista MS, Séneca J, Torgo L, Lee CK, Cary SC, Magalhães C (2020) Understanding the response of nitrifying communities to disturbance in the McMurdo dry valleys, Antarctica. Microorganisms 8:404

Moore J, de Ruiter PC (2012) Energetic food webs. Oxford University Press, Oxford, UK

Mulder A, van de Graaf AA, Robertson LA, Kuenen JG (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiol Ecol 16:177–184

Niederberger T, Sohm J, Tirindelli J, Gunderson T, Capone D, Carpenter E, Cary S (2012) Diverse and highly active diazotrophic assemblages inhabit ephermally wetted soils of the Antarctic dry valleys. FEMS Microbiol Ecol 82:376–390

O'Malley MA (2007) The nineteenth century roots of 'everything is everywhere'. Nat Rev Microbiol 5:647–651

Pankhurst CE (1997) Biodiversity of soil organisms as an indicator of soil health. In: Pankhurst C, Doube BM, Gupta VVSR (eds) Biological indicators of soil health. CAB International, Oxfordshire, pp 297–324

Papale M, Conte A, Mikkonen A, Michaud L, La Ferla R, Azzaro M, Caruso G, Paranhos R, Cabral Anderson S, Maimone G (2018) Prokaryotic assemblages within permafrost active layer at Edmonson point (northern Victoria land, Antarctica). Soil Biol Biochem 123:165–179

Pointing SB, Chan Y, Lacap DC, Lau MCY, Jurgens JA, Farrell RL (2009) Highly specialized microbial diversity in hyper-arid polar desert. Proc Natl Acad Sci U S A 106:19964–19969

Pradhan A, Baisakh B, Mishra BB (2014) Plant growth characteristics of bacteria isolated from rhizosphere region of Santalum album. J Pure Appl Microbiol 8:4775–4781

Rabalais NN, Turner ER, Scavia D (2002) Beyond science into policy: Gulf of Mexico hypoxia and the Mississippi River. Bioscience 52:129–152

Rao S, Chan Y, Lacap DC, Hyde KD, Pointing SB, Farrell RL (2012) Low-diversity fungal assemblage in an Antarctic dry valleys soil. Polar Biol 35:567–574

Richter I, Herbold CW, Lee CK, McDonald IR, Barrett JE, Cary SC (2014) Influence of soil properties on archaeal diversity and distribution in the McMurdo dry valleys, Antarctica. FEMS Microbiol Ecol 89:347–359

Robertson GP, Tiedje JM (1987) Nitrous oxide sources in aerobic soils: nitrification, denitrification, and other biological processes. Soil Biol Biochem 19:187–193
Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: balancing the cost of an essential resource. Annu Rev Environ Resour 34:97–125
Rodriguez PA, Rothballer M, Chowdhury SP, Nussbaumer T, Gutjahr C, Falter-Braun P (2019) Systems biology of plant-microbiome interactions. Mol Plant 12:804–821
Rudakov KI (1951) Mikroorganizmy i struktura pochvy (microorganisms and soil structure). SeFkhozgiz, Moscow
Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602
Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, Huber DH, Langenheder S, Lennon JT, Martiny JBH, Matulich KL, Schmidt TM, Handelsman J (2012) Fundamentals of microbial community resistance and resilience. Front Microbiol. https://doi.org/10.3389/fmicb.2012.00417
Silver WL, Herman DJ, Firestone MK (2001) Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. Ecology 82:2410–2416
Smith SE, Smith FA (1990) Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytol 114:1–38
Stephan A, Meyer AH, Schmid B (2000) Plant diversity affects culturable soil bacteria in experimental grassland communities. J Ecol 88:988–999
Stephen JR, Kowalchuk GA, Bruns MAV, McCaig AE, Phillips CJ, Embley TM, Prosser JI (1998) Analysis of β-subgroup proteobacterial ammonia oxidizer populations in soil by denaturating gradient gel electrophoresis analysis and hierarchical phylogenetic probing. Appl Environ Microbiol 64:2958–2965
Stephen JR, McCaig AE, Smith Z, Prosser JI, Embley TM (1996) Molecular diversity of soil and marine 16S rRNA gene sequences related to b-subgroup ammonia-oxidizing bacteria. Appl Environ Microbiol 62:4147–4154
Strous M (2011) Beyond denitrification: alternative routes to dinitrogen. Nitrogen cycling in bacteria: molecular analysis. Caister Academic Press, Norfolk, UK
Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (2005) Principles and applications of soil microbiology, 2nd edn. Prentice Hall, Upper Saddle River
Thomas CM, Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol 3:711–721
Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton, NJ
Tilman D (1987) Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecol Monogr 57:189–214
Tolar BB, Ross MJ, Wallsgrove NJ, Liu Q, Aluwihare LI, Popp BN (2016) Contribution of ammonia oxidation to chemoautotrophy in Antarctic coastal waters. ISME J 10:2605–2619
van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–301. https://doi.org/10.1111/j.1461-0248.2007.01139.x
Van Dorst J, Benaud N, Ferrari B (2017) New insights into the microbial diversity of polar desert soils: a biotechnological perspective. In: Microbial ecology of extreme environments. Springer, Cham, Switzerland, pp 169–183
Van Goethem MW, Cowan DA (2019) Role of cyanobacteria in the ecology of polar environments. In: Castro-Sowinski S (ed) The ecological role of micro-organisms in the Antarctic environment. Springer Polar Sciences, Cham, Switzerland, pp 3–23
Veldkamp E, Becker A, Schwendenmann L, Clark DA, Schulte-Bisping H (2003) Substantial labile carbon stocks and microbial activity in deeply weathered soils below a tropical wet forest. Glob Chang Biol 9:1171–1184. https://doi.org/10.1046/j.1365-2486.2003.00656.x

Vero S, Garmendia G, Martínez-Silveira A, Cavello I, Wisniewski M (2019) Yeast activities involved in carbon and nitrogen cycles in Antarctica. In: Castro-Sowinski S (ed) The ecological role of micro-organisms in the Antarctic environment. Springer, Cham, Switzerland, pp 45–64

Vishniac HS (2006) Yeast biodiversity in the Antarctic. In: Rosa CA, Péter G (eds) Biodiversity and ecophysiology of yeasts. Springer, Berlin; Heidelberg, Germany, pp 419–440

Vitousek PM (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Voytek MA, Priscu JC, Ward BB (1999) The distribution and relative abundance of ammonia-oxidizing bacteria in lakes of the McMurdo Dry Valley, Antarctica. Hydrobiologia 401:113–130

Wardle DA, Bonner KI, Barker GM, Yeates GW, Nicholson KS, Bardgett RD, Watson RN, Ghani A (1999) Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecol Monogr 69:535–568. https://doi.org/10.1890/0012-9615(1999)069[0535:PRIPGV]2.0.CO;2

Wei STS, Higgins CM, Adriaenssens EM, Cowan DA (2015) Pointing, S.B. genetic signatures indicate widespread antibiotic resistance and phage infection in microbial communities of the McMurdo dry valleys, East Antarctica. Polar Biol 38:919–925

Wei STS, Lacap-Bugler DC, Lau MCY, Caruso T, Rao S, de los Rios A, Archer SK, Chiu JMY, Higgins C, Van Nostrand JD (2016) Taxonomic and functional diversity of soil and hypolithic microbial communities in Miers Valley, McMurdo dry valleys, Antarctica. Front Microbiol 7:1642

Wixon DL, Balser TC (2009) Investigating biological control over soil carbon temperature sensitivity. Glob Chang Biol 15(12):2803–3065. https://doi.org/10.1111/j.1365-2486.2009.01946.x

Yergeau E, Hogues H, Whyte LG, Greer CW (2010) The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. ISME J 4:1206–1214

Yergeau E, Kang S, He Z, Zhou J, Kowalchuk GA (2007) Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. ISME J 1:163–179

Zablocki O, van Zyl L, Adriaenssens EM, Rubagotti E, Tuffin M, Cary SC, Cowan D (2014) High-level diversity of tailed phages, eukaryote-associated viruses, and virophage-like elements in the metaviromes of Antarctic soils. Appl Environ Microbiol 80:6879–6887

Zehr JP, Jenkins BD, Short SM, Steward GF (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. Environ Microbiol 5:539–554

Chapter 4
Pedological Assessment of Soil Organic Carbon and Total Nitrogen Contents in Wetland Rice Ecosystems of Majuli River Island, Assam, India

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Abstract The study of soil organic carbon and total nitrogen in relation to texture, landforms, and soil pH in riverine rice growing wetlands is very important for both agrarian economy and land use related issues in Majuli island of Upper Brahmaputra valley, Assam. Thus, thirteen soil series classified under the subgroups of Inceptisols and Entisols under different alluvial landforms in Majuli river island were selected to investigate the distribution of organic carbon (OC) and Total nitrogen (Total N) in pedological point of view. Our results showed an enrichment in OC and Total N on superficial Ap horizons (silt loam to silty clay loam) to the detriment of the deep sandy C horizons. Nevertheless, the irregular distribution of OC and total N contents with depth in stratified soils of the Island showed differential rates of leaching and its subsequent accumulations due to depositional episodes during seasonal floods in the region. These soils are slightly acid to neutral with mean densities of 30.49 Mg/ha of OC in Majuli series (P5) to 196.78 Mg/ha in Dakshinpat series (P7) with significant variations between the horizons ($F = 5.904$). The data further shows that silty clay texture have mean SOC of 25.24 $\pm$ 10.48 Mg/ha but low with value of 6.26 $\pm$ 2.81 Mg/ha for sand texture whereas total N stocks were high for sand (mean 4.71 $\pm$ 3.45 Mg/ha). The stratification of Total N and its stocks are highly variable having positive relation with cation exchange capacity ($R^2 = 0.57^{**}$) and Clay ($R^2 = 0.35^{*}$). The mean C/N ratio of soils was 8.3 $\pm$ 10.33 but highly variable (Cv of 124%). The regional study shows that geographically explicit information on soil carbon and total N pools must be combined with seasonal flooding history and depositional episodes for better rice management factors in the island.

Keywords Assam · Brahmaputra valley · Bulk density · Flood deposits · Organic carbon · Rice ecosystems · Soil series · Total Nitrogen

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4.1 Introduction

The agricultural ecosystems are designed mostly to produce food and other valuable products such as fiber and fuel to the environment (Costanza et al. 1997; Millennium Ecosystem Assessment (MEA) 2005; de Groot et al. 2012). The ecosystem services (ES) from arable lands are largely depended on the inputs used for cultivation and the crop management practices (Wossink and Swinton 2007; Ma et al. 2012). In India, rice is cultivated in 43.4 million hectares (GOI 2016) and is widely grown in eastern parts of India with consumption of 32% fertilizer (FAO 2005), 22% of pesticide (Krishna et al. 2003), and about 60% of water (Raju et al. 2005). Rice-based systems lead to soil loss, nutrient depletion, the hosting of pests and diseases, and greenhouse gas (GHG) emissions (Barrios 2007; Stallman 2011).

In this chapter, we present current knowledge on the soil nitrogen, in rice ecosystems due to low productivity per unit of land and often pay compensation in the form of subsidies to the farmers and policy agendas of local government (Dahal and Bajracharya 2013). Soil nitrogen (N) stocks, soil pH, and carbon-nitrogen ratio (C/N ratio) are important indicators of carbon sequestration potentials and also soil microbial structure and activities (Rousk et al. 2010). The soil carbon and nitrogen data sets are important in attaining target yields and to conserve SOC in rice ecosystems. Generally, the vertical patterns of SOC (soil organic carbon) in relation to total nitrogen, other physico chemical properties were examined to assess the response of the ecosystems to global change (Mi et al. 2008). Most of the soil nitrogen studies in rice ecosystems (Nayak et al. 2019) primarily focused on the top soil but poorly understood nitrogen dynamics in deeper soil layers (Liu and Greaver 2010). It is therefore needed to assess the carbon and nitrogen status in the unique rice ecosystem of riverine floodplain wetlands of Majuli island. The vertical distribution of soil organic carbon and nitrogen information in relation to soil pedological properties of rice are scanty and are useful for understanding the pedogenic behavior of the paddy soils for increasing crop productivity (Singaravel et al. 1996; Vijayakumar et al. 2013). The major challenge to enhance the productivity of rice and rice-based systems requires better crop management and crop care techniques (Barah and Pandey 2005). The soil resource data base generated in riverine wetlands of Majuli island was chosen for pedological assessment of the vertical distribution of SOC and nitrogen across different soil depths in alluvial landforms and worked out its relation with texture and chemical properties.

4.2 Materials and Methods

4.2.1 Study Area

The Majuli island (93°30′–94°35′E and 26°50′–27°10′N) is located in the north of Jorhat district in Assam state of India. The elevation varies from 60 to 85 meters

above mean sea level. The island is bounded by Kherkutia Suti, Subansiri, and Brahmaputra rivers. The island is marked by 70 *bils* (local name for small ponds and *oxbow* lakes). The climate is subtropical with warm humid summer and cool dry winter with mean annual rainfall of 1900 mm. Barthakur (2004) defined the climate of island as humid mesothermal gangetic type (CWg) in accordance with Kopper. It is further reported that south west monsoon contributes 62–65% of annual rainfall during April through October (Bhaskar et al. 2010) The island has maximum temperature of 23.6–31.7 °C and minimum temperature of 10 °C but drops to as low as 6.0 °C in some years. On an average, the relative humidity is more than 80% throughout the year.

4.2.2 Rice System in Majuli

The rice growing seasons (Ahmed et al. 2011) along with varieties and general package of practices were presented (Table 4.1). The rice is the principle crop grown to an extent in three seasons of 787 ha in Ahu, 15,857 ha in Sali, and 7857 ha in Boro but area under Bao rice approximately accounts to 1197 ha. The island has 142 villages with total geographical area of 924.6 km^2. This island has 42 chapori's covering an area of 26315.97 ha supporting population of 21,650.The chronically flood prone areas in three circles (Garmura, Kamalabari, and Jenjari) of Majuli are recorded as 39,298 ha with total cropped area of 47,348 ha. The major rice-based cropping systems are mustard, wheat, with black gram. The general fertilizer dose for rice is 40 N:20P_2O_5:K_2Okg/hectare. The commonly grown rice varieties include: semi dwarf:-Govind, IR-50, IR-36, Luit, Kopilee, Disang and Jaya, where tall varieties include Rangdoria, Banglami, Dubaichang, Fapori, Guni, and Ihajit.

4.2.3 Soil Data Base

A reconnaissance soil survey was carried out using 1:50,000 scale topsheets in combination with geocoded Indian Remote Sensing Satellite (IRS)-1D images (taken on 18th January, 2003). Thirteen soil series were identified and described as per Schoeneberger et al. (2012). The soil database from soil survey report of Majuli was used in the present study (Bhaskar et al. 2008).

4.2.4 Laboratory Analysis

The horizon-wise soil samples were collected for laboratory analysis. The samples were air dried, ground, and passed through a 2 mm sieve for laboratory analysis. The international pipette method was used to estimate particle size as per procedure

Table 4.1 Distribution and estimation of total N, organic carbon stocks, and C/N ratio in paddy soils of Majuli island

Soil series	Depth (cm)	Horizon	Total		Stock estimation (Mg/ha)		C/N	Clay (%)	pH	CEC (cmol/kg)
			N	OC	OC	Total N (TN)				
			g/kg							
P1.Boritika series—Fluvaquentic Endoaquepts	0–26	Ap	0.81	12.3	39.45	2.60	15.19	24.0	7.0	17.9
	26–52	C1	1.46	1.6	5.67	5.17	1.10	6.0	7.5	11.9
	52–64	2Bw1	0.81	11.0	15.70	1.16	13.58	34.5	7.2	19.6
	64–79	2Bw2	1.46	4.3	8.01	2.72	2.95	29.5	7.3	20.0
	79–113	C2	0.49	0.6	2.78	2.27	1.22	6.5	7.6	8.9
P2.Puranibari series—Fluvaquentic Endoaquepts	0–14	Ap	1.14	6.1	11.14	2.08	5.35	14.5	6.1	14
	14–27	A/B	0.81	4.9	8.28	1.37	6.05	16.5	6.6	15.9
	27–46	C1	0.65	1.2	3.11	1.68	1.85	6.5	6.4	9.57
	46–71	C2	0.65	2.9	9.60	2.15	4.46	13	6.5	13.2
	71–86	2Bw1	2.44	5.3	9.98	4.60	2.17	25.5	6.4	18.9
	86–105	2Bw2	1.3	6.3	14.48	2.99	4.85	34.5	6.5	20.6
	105–175	2Bw3	0.97	1.8	15.77	8.50	1.86	29.5	6.5	20.1
P3.Adi elengi series—Typic Endoaquepts	0–13	Ap	0.16	9.7	16.46	0.27	60.63	11	7.1	11.1
	13–34	Bw1	1.79	16.7	41.13	4.41	9.33	33	6.8	14.1
	34–60	Bw2	0.97	5.8	19.23	3.22	5.98	21	7	12.5
	60–98	C1	1.14	1.4	7.18	5.85	1.23	9	7.2	7.89
	98–225	C2	0.81	1.4	24.33	14.08	1.73	5	7.2	3.48
P4.Chilakala series—Typic Endoaquepts	0–16	Ap	1.62	13.8	25.59	3.00	8.52	38.5	5.5	11.6
	16–35	Bw1	1.46	10.5	22.60	3.14	7.19	47	6.4	11
	35–59	Bw2	1.14	8.1	23.14	3.26	7.11	37	6.8	11.5
	59–170	C1	0.65	0.6	9.18	9.95	0.92	3.5	7.2	4.78
P5.Majuli series—Typic Psammaquents	0–33	Ap	0.65	1.5	6.78	2.94	2.31	4.5	7.5	5.54
	33–57	AC1	1.3	3.8	12.24	4.19	2.92	8.5	7.2	9.02
	57–82	AC2	0.81	3.4	11.46	2.73	4.20	7.5	7.2	7.28

P6.Bangaon series—Typic Fluvaquents	0–13	Ap	1.3	8.3	14.33	2.24	6.38	7.5	7.7	9.78
	13–38	Ac	0.81	5.0	16.86	2.73	6.17	6	7.8	8.8
	38–68	C1	0.65	3.5	14.11	2.62	5.38	8.5	7.8	7.17
	68–80	C2	0.49	3.3	5.36	0.80	6.73	6.5	7.9	6.41
	80–105	C3	0.49	2.3	7.79	1.66	4.69	7	7.7	5.54
	105–170	C4	0.49	1.2	10.76	4.39	2.45	3	7.7	1.41
P7.Dakhinpath series—humic Endoaquepts	0–13	Ap	4.87	57	73.49	6.28	11.70	35.5	5.5	25.6
	13–34	Bw1	1.79	19.3	45.09	4.18	10.78	43.5	6.3	19.7
	34–55	Bw2	0.97	7.3	19.20	2.55	7.53	24.5	6.9	14.5
	55–105	Bw3	0.49	5	32.37	3.17	10.20	17.5	7	15.7
	105–200	BC	0.49	2.1	26.64	6.22	4.29	11.5	7.2	10
P8.Kamalabari series—Humaqueptic Fluvaquents	0–19	Ap	1.62	12.2	29.15	3.87	7.53	19	5.9	12.6
	19–39	AC	0.97	4.8	12.48	2.52	4.95	16.5	6.8	12.3
	39–61	C1	0.81	1.9	5.67	2.42	2.35	7	7	10.2
	61–89	C2	0.49	2.5	9.43	1.85	5.10	8.5	7.1	11.2
	89–130	C3	0.32	0.8	4.51	1.80	2.50	4.5	7.2	7.61
P9.Garumara series—Fluvaquentic Endoaquepts	0–14	Ap	0.97	9.6	17.17	1.74	9.90	17	4.8	10.6
	14–43	Bw1	0.65	7.7	28.40	2.40	11.85	20	5.8	13.5
	43–64	Bw2	0.65	3.9	10.78	1.80	6.00	14.0	6.3	12.2
	64–75	BC	0.32	1.4	2.10	0.48	4.38	6.0	6.3	8.7
	75–160	C	0.65	0.6	7.10	7.70	0.92	0.5	6.7	4.35
P10.Gayangaon series—Typic Endoaquepts	0–13	Ap	1.3	10	16.10	2.09	7.69	25.0	6.0	17.1
	13–39	Bw1	0.39	5.1	17.26	1.32	13.08	16.0	7.2	14.4
	39–54	Bw2	0.97	6.1	11.61	1.85	6.29	22	7.3	16.5
	54–72	Bw3	1.14	10.9	24.09	2.52	9.56	26.5	6.9	14.7
	72–94	Bw4	0.81	7.4	20.61	2.26	9.14	21.5	6.9	10.7
	94–169	C	0.32	0.8	10.66	4.26	2.50	4.5	7.1	4.78

(continued)

Table 4.1 (continued)

Soil series	Depth (cm)	Horizon	Total		Stock estimation (Mg/ha)		C/N	Clay (%)	pH	CEC (cmol/kg)
			N	OC	OC	Total N (TN)				
			g/kg							
P11.Sonaribari series—Typic Endoaquepts	0–13	Ap	0.33	19.5	29.25	0.49	60.00	34.5	6.7	17.5
	13–31	B/A	0.97	10.2	23.11	2.20	10.52	20.5	7.7	14.2
	31–38	Bw1	0.16	5.0	4.60	0.15	31.25	13.5	7.7	11.7
	38–47	Bw2	0.49	3.9	4.69	0.58	8.02	9.5	7.9	10.9
	47–56	Bw3	0.49	4.2	5.02	0.58	8.64	11	7.7	11.5
	56–66	Bw4	0.65	6.2	8.03	0.84	9.57	16.5	7.8	12.6
	66–90	C	0.65	6.4	20.19	2.04	9.88	12	7.6	10.2
P12.Bharaki series—Fluvaquventic Endoaquepts	0–20	Ap	1.46	12	30.20	3.67	8.23	19.0	6.6	14.8
	20–30	C1	0.65	3.9	5.17	0.86	6.02	12.0	7.3	11.2
	30–48	2Bw1	0.65	5.8	13.14	1.47	8.95	24.5	7.2	12.6
	48–68	2Bw2	0.65	2.5	6.62	1.72	3.86	13.5	7.4	13.5
	68–99	2Bw3	0.16	3.1	12.63	0.65	19.38	15	7.5	14.4
	99–140	2Bw4	1.46	3.9	20.03	7.50	2.67	27.5	7.4	12.4
P13.Bhakat series—Typic Fluvaquents	0–27	Ap	0.33	4.9	17.45	1.16	15.08	12.5	7.7	11
	27–46	AC	0.49	1.5	3.83	1.25	3.06	10.0	7.6	9.54
	46–85	C1	0.49	1.3	6.87	2.59	2.65	8.0	7.9	9.57
	85–98	C2	0.16	1.9	3.31	0.28	11.88	10.5	8.1	9.7
	98–180	C3	0.97	0.4	4.53	10.98	0.41	3.5	8.1	3.96

described by Gee and Bauder (1986). The pH of the soil samples was determined in 1:2.5 soil:water ratio. The quantity of organic carbon in the soil was estimated by using Walkey Black method (Walkley and Black 1934; Jackson 1973). The exchangeable K and Na were determined by flame photometer while Ca and Mg were determined using atomic absorption spectrometer. The cation exchange capacity (CEC) was determined by distillation method as described by Jackson (1979). Soil bulk density was determined using the soil core sampler having a diameter of 5.7 cm (Blake and Harte 1986). Total nitrogen (TN) was determined by the Kjeldahl digestion-distillation method (Bremner and Mulvaney 1982).

4.2.5 Calculation of Stocks of Total Nitrogen and Organic Carbon in Soil

1. Soil organic carbon in ton per hectare (SOC, Mg/ha) = organic carbon content (%) × soil bulk density × depth of soil layer,
2. Total nitrogen in ton per hectare (TN, Mg/ha) = nitrogen content (%) × soil bulk density (Mgm^{-3}) × depth of soil layer (cm).

4.2.6 Statistical Analysis

The correlation test was applied to find out relationship between the variables of soils. The ANOVA, multiple and bivariate correlation were out using SPSS software. The depth distribution functions were constructed for soil parameters under study using Microsoft excel and picture manager.

4.3 Soil Organic and Total N in Relation to Soil Types and Soil Properties

4.3.1 Depth Distribution Function of Organic Carbon (OC) and Total Nitrogen (TN)

The depth wise distribution of the TC and TN contents in rice growing soils of majuli island, respectively, is presented in Table 4.1. The Fluvaquentic Endoaquepts (P1, P2, P9, and P12) have recorded irregular distribution with the contents in Ap horizons of 6.1 g/kg (P2) to 12.3 g/kg in Boritika profile (P1) but decreases to 0.6 g/kg in C horizons of Boritika (P1). In Typic Endoaquepts (P3-Adi Elengi series; P4-Chilkala series, P11-Sonaribari series), the Ap horizons have 9.7 g/kg in P3 to 19.5 g/kg in P11. In P3, the OC shows increase in cambic B horizon (16.7 g/kg) but

Table 4.2 Relation of geomorphic units on stocks of SOC, TN, and C/N

Source	Degrees of freedom (df)	Sum of squares	Mean sum of squares	*F* statistics	*P* value	Critical value of Turkey test (HSD at 0.05 level)
OC stock (mg/ha)						
Soils	3	567.02	189.21	1.72	0.1793	9.97
Geomorphic units	2	1245.26	622.63	5.66	0.006	7.77
Soils X geomorphic units	6	1769.02	294.84	2.68	0.0242	22.66
Error	52	5725.24	110.1			
Total	63					
TN (mg/ha)						
Soils	3	35.27	11.76	2.13	0.107	2.23
Geomorphic units	2	14.83	7.42	1.35	0.268	1.74
Soils X geomorphic units	6	43.52	7.25	1.21	0.265	5.07
Error	52	286.67	5.51			
Total	63	380.29				
C/N ratio						
Soils	3	497.38	165.79	1.57	0.2077	9.77
Geomorphic units	2	24.17	12.09	0.11	0.896	7.61
Soils X geomorphic units	6	1064.73	177.46	1.68	0.145	22.2
Error	52	5494.07	105.66			
Total	63	7080.35				

gradually decreases to 1.4 g/kg in C horizons. In P4 and P11, the gradational decrease of OC is visible but C horizons have 0.6 g/kg in P4 and of 6.2 g/kg in C horizons of P11. The OC in Typic Fluvaquents (P6-Bangaon series and P13-Bhakat series) shows a slight infliction with depth and have values of 8.3–4.3 g/kg in A horizons but decreased to less than 1.5 g/kg in C horizons. The profile distribution of TN in Fluvaquentic Endoaquepts (Table 4.1) have shown irregular with its contents more than 2 g/kg in Bw horizon s (P2, P12) and of 0.49 g/kg in C horizons (P1). In case of Typic Endoaquepts show variable patterns with depth except Chilkala series (P4) where TN shows gradational decrease. In case of Sonaribari series (P11) the TN is 0.97 g/kg in transitional B/A horizon (0.97 g/kg) but decreased to less than 0.5 g/kg in other B horizons. In AdiElengi series (P3), the TN values are low but varied from 1.79 g/kg in Bw horizon to 0.89 in C horizons but Ap horizons have 0.16 g/kg due to recent flood deposit on the top layers.

4.3.2 Vertical Distribution of Organic Carbon and Total N Stocks

The depth functions of total organic carbon stock of paddy growing soils in Majuli island show irregular trends with value of 30.49 Mg/ha in Majuli series (P5, Typic Psammaquents) to 196.78 Mg/ha in Dakshinpat series (P7, Humic Endoaqupets, Table 4.1). The reported values are in agreement with the values reported in depositional riverine soils of Wisconsin (Adhikari et al. 2019). The results showed that the mean carbon stock is only 15.58 ± 12.01 Mg/ha and its distribution is highly variable (Cv of 77.14%). The distribution pattern of SOC stock in wetlands of majuli was highly variable due to seasonal erosion/depositional processes operating in the island. Out of thirteen, nine soil series were classified in the subgroups of Inceptisols (Typic Endoaqupets-P3,P4, P10, and P11), Fluvaquentic Endoaquepts (P1, P2, P9, and P12), and Humic Endoaquepts (P7) whereas other soils were placed in the subgroups of Entisols, viz., Typic Psammaquents (P5), Typic Fluvaquents (P6, P13), and Humaquentic Fluvaquents (P8). The mean carbon stock is 21.25 ± 6.61 Mg/ha for Typic Endoaquepts (P3, P4, P10, P11) with moderate variability (Cv of 30.22%) whereas Fluvaquentic Endoaquepts have mean of 21.25 ± 3.2 Mg/ha with high Cv of 62.18%. The anova analysis shows that Fluvaquentic Endoaquepts have a significant variation in carbon stocks between the horizons with calculated F value of 5.904 (p value of 0.0069 at 2, 30 degrees of freedom) but not significant in case of Typic Endoaqupets ($F = 0.722$ at 2, 19 degrees of freedom).

With respect to the depth distribution of total N stock in these soils, the mean for Total N stock in C horizons is 14.31 ± 7.51 Mg/ha and Cv of 52.47% in Typic Endoaquepts whereas B horizons have a mean of 3.23 ± 2.59 TN Mg/ha but highly variable (Cv of 84%). The depth distribution pattern of total N stock in studied soils is similar to that of SOC but with low contents (range from 0.27 in Ap horizon to 14.8 Mg/ha in C horizons of P3, Table 4.1). The Ap horizons have low TN stock as compared to cambic B and C horizons. The distribution of TN stock between the horizons is not significant with F value less than unit value in both soils types under study. The C/N ratio follows similar pattern with depth to that of SOC and TN stocks with a mean of 8.3 ± 0.33 and Cv of 124.48%. The values C/N ratio in submerged rice soils of Majuli is in agreement with values reported for paddy soils of India (Sahrawat et al. 2005). The wide C/N ratio's within these soils is due to recurring seasonal floods and also due to triple rice crop systems in the region (Olk et al. 1996).

4.3.3 Relation of Geomorphic Units on Stocks of SOC, TN, and C/N

The results of anova analysis shows that to findout variation in stocks of OC, Total N and C/N ratio between horizons and within geomorphic units and interaction between horizons and the estimation of stocks. The results showed significant

relation of OC stocks with respect to geomorphic units (F value of 5.66, p value of 0.006) but nonsignificant for Total N and C/N ratio in these soils (Table 4.2).

4.3.4 *Stratification of Clay, Organic Carbon, Total Nitrogen and Carbon to Nitrogen Ratio in Paddy Growing Soils*

The distribution clay in soil profiles from rice ecosystems is presented in Fig. 4.1. The distribution of clay is duplex positive in Boritika (P1), bulged in Adielengi (P2), Chilkala (P4) and Dakshinpat (P6), variable in Puranibari (P2) and Bangaon (P5). Similar kind of observations were reported in paddy soils of Thailand (Kyuma and Kawaguchi 1977) and in poorly drained soils of Ohio (Smeck et al. 1981). The organic carbon at Ap horizons is more than 10 g/kg in P1, P4, and P6 but low organic carbon in other soils. All soils show decreasing trend of organic carbon with depth. It is further reported here that there is irregular distribution of organic carbon in Puranibari soils (P3) but a perceptible increase of carbon below 50 cm in Boritika (P1) and Adielengi (P3). The high content of organic carbon in A horizons of rice soils in Majuli is ascribed to application of manure in top layer, its slow translocation process and lack of incorporation into the deeper layer (Anshori et al. 2020). The variable distribution of total nitrogen in the soil profiles of paddy soils is in the similar pattern of organic carbon. The stratification of Total N shows an increase of its content below 1 m depth indicating leaching of residual nitrogen and its subsequent accumulation in deeper layers (Zhao et al. 2015). The regression equations

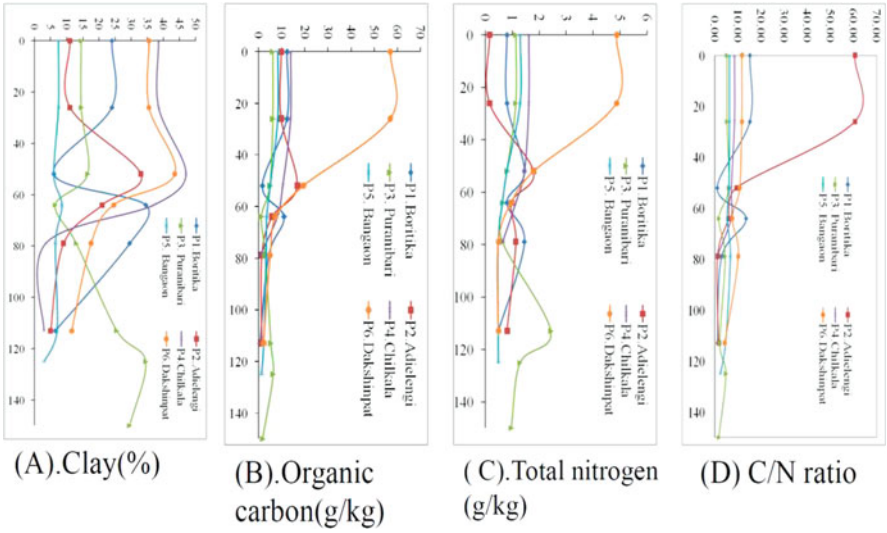

Fig. 4.1 Depth distribution of clay, organic carbon, total nitrogen, and Carbon to Nitrogen in paddy soils

were constructed between total N versus organic carbon ($R^2 = 0.62^{**}$) and cation exchange capacity ($R^2 = 0.571^{**}$) as given under:

$$\text{Total N}(\text{g/kg}) = 0.604 + 0.036(\text{organic carbon, g/kg}) \tag{4.1}$$

$$\text{Total N}(\text{g/kg}) = 0.166 + 0.001(\text{CEC, cmol/kg}) + 0.306(\text{CEC})) - 0.031(\text{CEC})^2 \tag{4.2}$$

Over all, the mean C/N ratio is 8.33 ± 10.33 with variation of 124 per cent. The Ap horizons have mean C/N ratio of 9.41 ± 12.11 but varies to mean of 7.025 ± 7.66 in B horizons and of 3.00 ± 5.02 in C horizons. In all soils, the C/N ratio in surface layer ranges from 5.63(Puranibari P3) to 60.63(Adielengi P2) and the reported values are in agreement with rice soils of riverine floodplains with less than 25% of clay and of variable proportions of sand and silt (Yang et al. 2010; Zhou et al. 2019).The C/N ratio has yielded significant positive relation with clay and silt and its relation is expressed in regression equation as under:

$$\text{C/N} = -0.195 + 0.001(\text{clay}, \%) + .198(\text{silt}, \%) \text{ with } F \text{ value of } 8.079 \text{ at } 2 \text{ and } 66 \text{ degrees of freedom.} \tag{4.3}$$

4.4 Pedogenic Assessment of SOC, TN, and C/N in Paddy Soils

The dominant pedogenic process was "gleyzation" with mottle formation (P1, P2, P4, P8, P9, P11, P12, & P13). These soils have horizon sequence of gleyed plowed layer (Apg) and gley cambic horizon (Bwg*, in P1, P4, P5, P6, P7, P8, P9, P12, & P13). These soils are "saturated and flooded" resulted in characteristic morphologies with stratified layers starting with in 15 cm of soil surface (Bharaki series, P4 with value 3 and chroma of 1 and ochric horizon was depleted in all soil profiles with matrix value of 5 or more and chroma of 1 (Bhaskar and Sarkar 2013). Occurrence of distinct brown mottles (10YR4/4 to 4/3) below 30–150 cm depth are the indicators of translocation of Fe, Mn oxides (Mokma and Sprecher 1994) and also due to diffusion of oxygen into the soil aggregates (Ponnamperuma 1972). The soil profile has silty loam /clay loam to silty clay textures with underlying sandy horizons. The distribution of silt and clay is irregular with depth in all the pedons. The silt content varies from 26.1% (P12) to 74.3% in Ap horizons of P1 and 42.8% in P4 to 75.4% in Bwg horizons of P13. The particle size classes of P1, P7, P9, and P13 are fine silty as the silt content exceeds 50 per cent in soil control section. The infliction in sand to silt ratio is useful to identify lithological discontinuities. The illuvial process is evident in the cambic B horizons with an increase in clay content (33% clay in

Bwg1 horizon in P1, 47% in P6, 43.5% in P7, 34.5% in 2Bwg2 horizon of P12). The distribution of clay is duplex positive in P5 and P12, bulged in P1 and P6, variable in P2, P3, P4, gradationally negative in P7 and P10 and duplex negative in P9 and P13. The downward increase of clay was recorded in cambic Bw horizons of Adielengi (P1), Chilkala (P6), and Dakshinapat series (P7). Similar kind of observations were reported in paddy soils of Thailand (Kyuma and Kawaguchi 1977) and in poorly drained soils of Ohio (Smeck et al. 1981). The Chilkala (P6) and Dakshinpat (P7) soils have strongly acid Ap horizons but moderately acid to neutral subsoils in Garumara (P9), Gayangaon (P10), and Kamalabari (P8). The mean organic carbon in Ap horizons is 12.66 g/kg with coefficient of variation of 113.5 per cent but organic carbon decreases to 6.57 g/kg in Bw horizon and then to 2.07 g/kg in C horizons (Table 4.1). These soils have 14.9 cmol/kg of CEC for cambic B horizons but have 11 cmol/kg for Ap horizon and 7.8 cmol/kg in C horizons. Similar pattern of CEC, organic carbon, total Fe and Mn in soils were reported from Brahmaputra valley (Karmakar 1985; Chakravarthy et al. 1984; Bhaskar et al. 2009).

Generally, these soils undergo "ferrolysis" (Brinkman 1970; Bhaskar et al. 2005) that has resulted in low cation exchange capacity (1.41 $cmol^{(+)}$/kg in C4 horizon of Bangaon series (P2) to 25. $cmol^{(+)}$/kg in Apg horizon of Dakshinpat series (P7). Earlier, soils under paddy cultivation can be separated at lower subgroup level as "Aquorizem" (Kawaguchi and Kyuma 1969) and the movement of iron in hydra-agric horizon, is reported in paddy soils of China (Zhang 1985). The Bw horizon is an illuvial horizon, where ferrous iron is formed and absorbed on the exchange sites. The absorbed iron is oxidized and form iron illuvial horizon by which a typical paddy soil is characterized and eluviation of organic matter had resulted in grayization (Mitsuchi 1974).

Most of the rice soils classified under subgroups of inceptisols and Entisols are rich in silt and sand with clay content less than 25% and moderate CEC less than 25 cmol/kg. The Majuli island has a complex mosaic of soils belonging to subgroups of "Inceptisols and Entisols" with young and intermediate pedogenetic development levels (Bhaskar et al. 2008). Most of these soils are less weathered and were influenced by the uprising of Brahmaputra valley (Sarma and Phukan 2004), thus having much younger geological ages. The very low nutrient content of these soils, often associated with high groundwater levels, results in the formation of thick root mats in the soil surface (Herrera et al. 1978) which then strongly influences the amount and vertical distribution of their SOC stocks. The soil mats close to surface layers in bil environs may reasonably be expected to exert a strong influence on soil SOC concentrations, in seasonally waterlogged soils (Dakshin part series, P7, AdiElengi-P3). It is therefore not a surprise that we observed some of the highest carbon in this soil.

All soils show irregular distribution of organic carbon in Puranibari soils (P2) but a perceptible increase of carbon below 50 cm in Boritika (P1) and Adielengi (P3). The high content of organic carbon in A horizons of paddy soils in Majuli is ascribed to application of manure in top layer, its slow translocation process and lack of incorporation into the deeper layer lowered its concentration in the deeper layer (Anshori et al. 2020). The variable depth distribution of total nitrogen in profiles of

paddy soils is similar pattern to that of organic carbon distribution. The stratification of Total N shows an increase of its content below 1 m indicating leaching of residual nitrogen and its subsequent accumulation in deeper layers (Zhao et al. 2015). The polynominal equations derived from the regression analysis clearly shows that TN has a significant positive relation with CEC ($R^2 = 0.571^{**}$ significant at 1% level) and also with clay ($R^2 = 0.352^{*}$ significant at 5% level). The results are in agreement with the findings of Hassink (1994) who reported that TOC contents were positively correlated with clay and silt contents and also help to stabilize soil organic matter (Baldock and Skjemstad 2000; Quesada et al. 2020).

Mean soil organic carbon content in each soil subgroup of inceptisols and Entisols of paddy growing soils (Table 4.1) shows that the high values recorded for Humic Endoaquepts in bil environs is due to periodical addition of aquatic weeds (Water hycianth, P7, Dakshinpat series, 196.78 Mg/ha) and also reflects a lower degree of decomposition of the organic materials present in poorly drained environments. This region experiences air temperature below 4 °C and foggy days during winter. The mean carbon density is 15.58 ± 12.01 with high coefficient of variation (74.14%). These soils have nitrogen density of 27.82 Mg/ha for Typic Endoaquepts (P3, Table 4.3) to 9.8 Mg/ha in Typic Psammaquents (P5) Typically the mean C/N ratios is varied from 8.3 ± 10.33 with high coefficient of variation (120%). The C/N ratio above 12–14 often is considered indicative for a shortage of nitrogen in the soil (Batjes and Dijkshoonn 1999). The C/N ratio of these soils is below to that of reported value and reflected the continuous rice–rice–rice cultivation in the region.

The results from two-way ANOVA analysis show a significant influence of geomorphic units on SOC stocks of each soil types (F value of 5.66) but non-significant for Total N and C/N ratio. These soils are slightly acid to slightly alkaline but have insignificant influence on SOC and Total N. This finding is in agreement with findings of Zhou et al. (2019). These paddy soils of Majuli were grouped into 8 textural classes and worked out the influence of texture on stocks of SOC and TN. The results showed that the stocks of SOC and Total N have significant relation with texture and yielded an F value of 2.89 for SOC and 2.237 for total N but significant at 0.05% probability level. The data further shows that silty clay texture has mean SOC of 25.24 ± 10.48 Mg/ha but low with value of 6.26 ± 2.81 Mg/ha for sand texture. The moderate coefficient of variation for loamy sand is recorded with value of 25.37 but rated as high in other textures. Similarily, the total N stocks are high for sand (mean 4.71 ± 3.45 Mg/ha) and low in silt loam texture (1.4 ± 1.02 Mg/ha). This region receives mean annual rainfall of 1900 mm and subjected to serious losses of calcium and magnesium ions (Bhaskar 2019) but also have sandy flood deposits in C horizons. In general, these soils are neutral with rich in organic matter in bil environs but low in sand deposit zones of active and old floodplains. The soils in bil environs have shown lowering of top soil pH (Hong et al. 2019). These soils have mottles and also iron manganese nodules (Bhaskar et al. 2008) with increase of pH values in the middle section of soil profiles. In interpreting the use of soil C / N ratios as an indicator for litter quality, the whole part correlation such as log (C: N) = log[C] − log[N], (Chayes 1971). The logarithamic expression of C:N was used to estimate the extent to which slopes

and correlation coefficients are biased by the presence of the same terms on both sides (Lloyd et al. 2013). In the present study, the whole part correlation yielded significant correlation ($R^2 = 0.652$ $n = 67$, Fig. 4.1) but, cautious in using this equation in any sort of predictive framework specially in riverine floodplains.

4.5 Conclusion

The wet land rice growing soil data base for Majuli island on was made with thirteen soil series and twenty five soil mapping units designated as soil series association. This soil data base was used to workout the stocks of organic cabon (OC) and total N (TN) for each soil subgroup. These soils are endosaturated throughout the year with continuous rice cultivation and eight textural groups were identified. The study showed the strong influence of textural class on stocks of OC and TN but not soil pH. The vertical variation in stock of C and N in each soil subgroups and its horizons suggests that rice management factors practices would be a major factor influencing the degree of soil organic matter and total N storage. The profile distribution of C and N stocks showed irregular depth trends indicating periodic depositional episodes occurred in the Island. Our study using remote sensing and GIS techniques allows to put reasonable hurdles on soil organic and total N stocks for Majuli region (1.2 lakh hectares) with a suitable baseline data base for further studies of pedogenic changes in rice ecosystems. Understanding regional characteristics of SOC and Total N are of great importance for designing best management practices for rice. The study suggested that modeling approaches to estimate SOC changes with respect to land use, flooding events, depositional layering and spatial heterogeneity of soil properties are to taken up on priority in future for upgrading and monitoring pedogenic changes using soil base line data as reference and to improve management strategies of rice ecosystems with long-term field measurements in the region.

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References

Adhikari K, Phillip R, Owens, Libohova Z et al (2019) Assessing soil organic carbon stock of Wisconsin, USA and its fate underfuture land use and climate change. Sci Total Environ 667:833–845

Ahmed T, Chetia SK, Chowdhury R, Ali S (2011) Status paper on Rice in Assam. RKMP:1–49

Anshori A, Pramono A, Mujiyo (2020) The stratification of organic carbon and nitrogen in top soils as affected by the Management of Organic and Conventional Rice Cultivation. Caraka Tani J Sustain Agric 35:126–134

Baldock JA, Skjemstad JO (2000) Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Org Geochem 31:697–710

Barah BC, Pandey S (2005) Rainfed Rice production Systems in Eastern India: an on-farm diagnosis and policy alternatives—National Symposium—a report. Ind J Agric Econ 60:110–136

Barrios E (2007) Soil biota, ecosystem services and land productivity. Ecol. Econ. Biodiversity synthesis. In: World resources institute. D.C, Washington

Barthakur M (2004) Weather and climate. The Brahmaputra basin water resources. In: Singh VP et al (eds) The Brahmaputra Basin water resource, vol 47. Kluwer Academic Publishers, Dordrecht, pp 17–23

Batjes NH, Dijkshoonn JA (1999) Carbon and nitrogen stocks in the soils of the Amazon region. Geoderma 89:273–286

Bhaskar BP (2019) Evaluating potentials of riverine floodplain soils for management of Rice(Oryza sativa L) in Majuli river island, Assam. Ind Int J Ecol Environ Sci 45:145–156

Bhaskar BP, Sarkar D (2013) Capability and quality assessment of rice growing hydric soils in Majuli river island, Assam, India. J Agric Environ Int Development 107:13–32

Bhaskar BP, Baruah U, Vadivelu S, Butte PS (2005) Characterization in the bile environs of Brahmaputra valley in Jorhat district, Assam for land use interpretation. J Indian Soc Soil Sci 53:3–10

Bhaskar BP, Baruah U, Vadivelu S, Sarkar D (2008) Characterization of depositional soils in dynamic fluvial landforms of Majuli island for land use related issues. Agropedology 18:33–43

Bhaskar BP, Baruah U, Vadivelu S et al (2009) Pedogenesis in some subaqueous soils of Brahmaputra Valley, Assam, India. J Indian Soc Soil Sci 57:237–244

Bhaskar BP, Baruah U, etal VS (2010) Remote sensing and GIS in the management of wetland resources of Majuli Island, Assam, India. Trop Ecol 51:31–40

Blake GR, Harte KH (1986) Bulk density, in methods of SoiAnalysis part 1. Physical and mineralogical methods-AgronomyMonograph. In: Klute A (ed) , 2nd edn. American Society of Agronomy-Soil Science Society of America, Madison, WI, pp 363–375

Bremner JM, Mulvaney CS (1982) "Nitrogen—total—1," in methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Soil Science Society of America, Madison, WI, pp 595–624

Brinkman R (1970) Ferrolysis. A hydromorphic soil forming process. Geoderma 3:199–206

Chakravarthy SK, Sinha H, Mathur BS (1984) Morphological and Physico-chemical characteristics of some alluvial soils of Assam. J Ind Soc Soil Sci 32:128–136

Chayes F (1971) Ratio correlation: a manual for students of petrology and geochemistry, vol 99. University of Chicago Press

Costanza R, d'Arge R, de Groot R et al (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260

Dahal H, Bajracharya RM (2013) Prospects of soil organic carbon sequestration: implications for Nepal's mountain agriculture. J Forest Livelihood 9:45–56

FAO (2005) Fertilizer use by crop in India. First version, published by FAO. Rome

Gee G, Bauder JW (1986) Particle size analysis in methods of soil analysis, Part-1. In: Klute A (ed) Agronomy 9. American Society of Agronomy, Madison, WI, pp 383–411

Government of India (GOI) (2016) Ministry of agriculture agricultural statistics at a glance 2016: 83–84. http://eands.dacnet.nic.in/PDF/Glance-2016.pdf

de Groot R, Brander L, van der Ploeg S et al (2012) Global estimates of the value of ecosystems and their services in monetary units. Ecosyst Serv 1:50–61

Hassink J (1994) Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol Biochem 26:1221–1231. https://doi.org/10.1016/0038-0717(94)90147

Herrera R, Jordan CF, Klinge H, Medina E (1978) Amazon ecosystems: their structure and functioning with particular emphasis on nutrients. Interciencia 3:223–232
Hong S, Gan P, Chen A (2019) Environmental controls on soil pH in planted forest and its response to nitrogen deposition. Environ Res 172:159–165. https://doi.org/10.1016/j.envres.2019.02.020
Jackson ML (1973) Soil chemical analysis. Prentice Hall of India, New Delhi
Jackson ML (1979) Soil chemical analysis. Advanced course. 2nd edn. Published by the author, Madison, WI
Karmakar KM (1985) Genesis and classification of soils in the northern Brahmaputra valley of Assam. PhD. Thesis. IARI. New Delhi
Kawaguchi K, Kyuma K (1969) Lowland rice soils in Thailand centre for southeast Asian studies, Kyoto University, Kyoto
Krishna VV, Byju NG, Tamizheniyan S (2003) Integrated pest management in Indian agriculture: a developing economic perspective. In: Radcliff EB, Hutchson WD (eds) IPM World Text book, St Paul, MN. https://ipmworld.umn.edu/krishna-indian-ag
Kyuma K, Kawaguchi K (1977) Paddy soils in tropical Asia, their maerial, nature and fertility. University Press of Hawaii. Honolulu 1977:258
Liu L, Greaver TL (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecol Lett 13:819–828
Lloyd J, Bloomfield K, Domingues TF, Farquhar GD (2013) Photosynthetically relevant foliar traits correlating better on a mass vs. an area basis of ecophysiological relevance or just a case of mathematical imperatives and statistical quicksand? New Phytol 199:311–321
Ma S, Swinton SM, Lupi F (2012) Farmer's willingness to participate in payment for-environmental-services programmes. J Agric Econ 63:604–626
MEA (Millennium Ecosystem Assessment) (2005) Ecosystems and human wellbeing:biodiversity synthesis. World Resources Institute, Washington, DC
Mi N, Wang SQ, Liu JY, Yu G, Zhang W, Jobbagy E (2008) Soil inorganic carbon storage in China. Global Change Biology 14(10):2380–2387
Mitsuchi M (1974) Pedogenic characteristics of paddy soils and their significance in soil classification. Bull Nat Inst Agric Sci B 1974:29–115. (in Japanese)
Mokma DI, Sprecher SW (1994) Water table depths and colour patterns in Spodosols of two hydrosequences in northern Michigan, USA. Catena 22:275–286
Nayak AK, Md Shahid AD, Nayak AD (2019) Assessment of the C/N ratio as an indicator of the decomposability of organic matter in forest soils. Ecol Indic 49:104–109
Olk DC, Cassman KG, Randall EW (1996) Changes in chemical properties of organic matter with intensified rice cropping in tropical lowland soil. Eur J Soil Sci 47:293–303
Ponnamperuma FN (1972) The chemistry of submerged soils. Adv Agron 24:29–90
Quesada CA, Paz C, Oblitas Mendoza E, Phillips OL, Saiz G, Lloyd J (2020) Variations in soil chemical and physical properties explain basin-wide Amazon forest soil carbon concentrations. Soil 6:53–88. https://doi.org/10.5194/soil-6-53-2020
Raju DSR, Reddy VR, Reddy PP, Reddy MS (2005) Water use efficiency: a study ofsystem of rice intensification (SRI) adoption in Andhra Pradesh. Ind J Agric Econ 60:458–472
Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across pH gradients in arable soils. The ISME J 4:1340–1351
Sahrawat KL, Bhattacharyya T, Wani SP (2005) Long-term lowland rice and arable cropping effects on carbon and nitrogen status of some semi-arid tropical soils. Curr Sci 89:2159–2162
Sarma JN, Phukan MK (2004) Origin and some geomorphological changes in Majuli island of the Brahmaputra river in Assam, India. Geomorphology 60:1–19
Schoeneberger PJ, Wysocki DA, Benham EC, Soil Survey Staff (2012) Field book for describing and sampling soils. Version 3.0., Natural Resources Conservation Service, National Soil Survey Center, Lincoln, NE
Singaravel R, Balasundaram CS, Johnson K (1996) Physico-chemical characteristics and nutrient status of coastal saline soils of Tamil Nadu. J Ind Soc Coastal Agric Res 14:59–61

Smeck NE, Ritchie A, Wilding LP, Drees LR (1981) Clay accumulation in sola of poorly drained soils of Western Ohio. Soil Sci Soc Am J 45:95–102

Stallman HR (2011) Ecosystem services in agriculture: determining suitability for provision by collective management. Ecol Econ 71:131–139

Vijayakumar M, Lakshmi GV, Madhuvani P (2013) Appraisal of soil fertility status in salt-affected soils of Ongole division, Prakasam district, Andhra Pradesh. J Indian Soc Soil Sci 61:333–340

Walkley AJ, Black IA (1934) An examination of theDegtjareff method for determining soil organic matter, and a proposed modification of the chromic acidtitration method. Soil Sci 37:29–38

Wossink A, Swinton SM (2007) Jointness in production and farmers' willingness to supply non-marketed ecosystem services. Ecol Econ 64:297–304

Yang YH, Fang JY, Guo DL, Ji C (2010) Vertical patterns of soil carbon, nitrogen and carbon: nitrogen stoichiometry in Tibetan grasslands. Biogeosci Discuss 7:1–24

Zhang X (1985, 1974) Ion adsorption, physical chemistry of paddy soils. Science Press, Springer Verlag, Beijing/Berlin

Zhao X, Xue JF, Zhang XQ (2015) Stratification and storage of soil organic carbon and nitrogen as affected by tillage practices in the North China plain. PLoS One 10(6):e0128873. https://doi.org/10.1371/journal.pone.0128873

Zhou W, Han G, Liu M, Li X (2019) Effects of soil pH and texture on soil carbon and nitrogen in soil profiles under different land uses in Mun River basin. Northeast Thailand Peer J 7:e7880. https://doi.org/10.7717/peerj.7880

Part II
Nitrogen Cycle and Pathway

Chapter 5
Functional Nitrogen in Rhizosphere

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Abstract Rhizosphere, a zone of intense microbial activity is a hub of soil–plant–microbial interactions playing a vital role in the growth and yield of plants. Physical, chemical, and biological properties of rhizosphere are greatly influenced by root exudates, energy source for proliferation, and activity of microbes. Various microorganisms in rhizosphere greatly influence nutrient availability and play an important role in all aspects of nitrogen cycling. Nitrogen is one of the essential macronutrient elements considered critical in limiting plant growth and development. Majority of plant nitrogen uptake is accounted by organic nitrogen pool of soil; highlighting the role of plant roots and associated nitrogen transformation in the rhizosphere. In-depth understanding of various soil–plant–microbial interactions in rhizosphere governing nitrogen availability and uptake is essential for increasing plant nitrogen acquisition, thereby enhancing use efficiency.

Keywords Rhizosphere · Root exudates · Microorganisms · N cycle · Biological nitrogen fixation

5.1 Introduction

Striking increase in the use of nitrogen (N) fertilizers has been witnessed round the globe since 1960 by following intensive agricultural practices for meeting the food requirements of ever increasing population. For achieving sustainability in food

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production prime focus must be towards greater resource efficiency and conservation. Maintenance of soil fertility and plant nutrition is highly dependent on soil microbiological processes and soil biodiversity. N being a critical macronutrient element for crop growth cycle is a highly demanded fertilizer input and production of N fertilizers has increased by a factor of 20 since green revolution (Glass 2003). Excess N from fertilizer poses a serious threat to environment by air and water pollution by processes like denitrification and nitrate leaching. It is high time to increase the efficiency of plant N acquisition and reduce dependency on N fertilizers. Rhizosphere environment supports a diverse array of microorganisms that play crucial role in soil nitrogen ecology. As majority of N transformations are mediated by rhizosphere inhabiting microbes, this millimeter zone surrounding the plant roots has got special significance in governing N availability and uptake. This chapter discusses about N cycle and nitrogen dynamics in rhizosphere and microbial interactions and their role in plant N nutrition.

5.2 Rhizosphere

It was a German agronomist and plant physiologist named Lorentz Hiltner in 1904 to first use the term rhizosphere to describe the zone of intense bacterial activity in the soil surrounding legume roots (from Greek "*rhiza"* means root and "*sphere*" means field of influence). Rhizosphere is a soil microzone under the influence of plant roots and is different from the remaining soil volume consequent to the exudation of organic and inorganic substances, nutrient and water uptake, and proliferation of microorganisms on the root surface. This soil–plant interface is known to be the most dynamic environment in soil as it is a hub of soil–plant–microbe interactions as well as an exchange site for water and nutrients, thereby playing a vital role in growth and yield of plants. There are distinct regions recognized within the rhizosphere. The *endorhizosphere* includes the area within the tissues of the plant root, i.e. root epidermis cortex zone colonized by microbes, the *ectorhizosphere*; the soil colonized by microbes adjacent to the root and the *rhizoplane* is the root surface where endorhizosphere and ectorhizosphere merge (Lynch 1987). The *mycorrhizosphere* is an additional component of ectorhizosphere that exists in plants colonized by mycorrhizal fungi and known to extend for a considerable distance from the root. Research on rhizosphere shows that no clearly defined zone can be demarcated for rhizosphere as its extent varies with the plant type, growth stage, root development, soil properties, and nutrient development, it is widely accepted that it covers at least 2 mm from the rhizoplane (Liu 1997). With the advancement of experimental techniques studies on rhizosphere soil are carried out by using several microcosm called rhizo-boxes or rhizobox set-up known to be very sophisticated and use proper microsensors for measurement of pH, redox potential, and soil moisture (Wenzel et al. 2001).

5.2.1 Root Exudates and Composition

Root activity primarily governs the properties of the rhizosphere environment by release of organic and inorganic substances, nutrient and water uptake, and proliferation of microorganisms, thereby making it different from rest of soil in all aspects. Plant roots allocate nearly 20% to 40% of photosynthetically fixed carbon into the rhizosphere (Badri and Vivanco 2009; Jones et al. 2009). Distribution of net carbon fixed by plant to belowground region was also studied by Uren (2007) and it is observed that 50% is devoted to roots, 15% is respired by roots as CO_2, and 10% is released as root debris including border cells, whereas diffusates and secretions accounted for less than 1%. The release of organic compounds by living plant roots is often referred to as rhizodeposition and the term root exudates is generally used to describe low molecular weight compounds released by roots into the rhizosphere (Table 5.1). Root exudates are the energy source for the microbial

Table 5.1 Compounds released by plant roots into rhizosphere

Class of compounds	Identified compounds	Function
Sugars	Arabinose, deoxyribose, fructose, fucose, galactose, glucose, maltose, mannitol, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose, complex polysaccharides	Energy source for microbial growth, chemoattractants, lubrication
Amino acids and amide	All 20 proteinogenic amino acids, γ-aminobutyric acid, cystathionine, cysteine, homoserine, mugineic acid, ornithine, phytosiderophores, betaine, stachydrine	Plant and microbe N source, iron scavengers, inhibit nematode and root growth
Organic acids	Acetic, butyric, citric, glutaric, lactic, maleic, malic, malonic, oxalic, propionic, pyruvic, succinic, tartaric, valeric, gallic, benozoic	Role in plant and microbial growth, chemoattractants
Phenolics	Flavonols, flavones, flavanones, anthocyanins, isoflavonoids, acetosyringone	Initiate legume-rhizobia, arbuscular mycorrhizal and actinorhizal interactions, plant defense
Phenolic acid	Caffeic acid, cinnamic acid, coumarin, ferulic acid, salicylic acid, syringic acid, vanillic acid	Plant defense, plant growth regulation
Enzyme and proteins	Amylase, invertase, phosphatase, protease, polygalacturonase, hydrolase, lectin	Plant defense, Nod factor degradation
Growth factors, viz. vitamins	*p*-Amino benzoic acid, auxins, biotin, choline, inositol, *n*-methyl nicotinic acid, niacin, pantothenate, pyridoxine, thiamine	Plant growth regulation microbial growth stimulation
Others	Nucleotide, fatty acids, sterols, lipids, reactive oxygen species, strigolactones	Quorum quenching, plant defense, microbial growth stimulation

Source: Modified from Bertin et al. (2003)

growth and activity. The physical and chemical composition of rhizodeposition varies with plant species and their developmental stages.

It is a well-known fact that microbes play a key role in nutrient recycling. Plants, by means of exudation of several biomolecules by their actively growing roots have the ability to modify the pool of microbial diversity in the rhizosphere. Physical, chemical, and biological properties of rhizosphere are determined by various root exudates, their nature and amount. A wide range of such organic substances released into the rhizosphere potentially determines the diversity of microbial communities inhabiting the root surface.

5.2.2 *Rhizosphere Versus Bulk Soil*

Rhizosphere is a dynamic environment in soil as well as is heterogenous in resource availability. The process of root exudation makes rhizosphere "a microbial hotspot" by carrying 1011 microbial cells per gram of root and more than 30,000 prokaryotic species (Pinton et al. 2001; Egamberdieva et al. 2008; Mendes et al. 2013). A study by Nannipieri et al. (2003) reported that life of microbes in soil is restricted to a minor volume being localized in hot spots such as the rhizosphere soil with a continuous access to low and high molecular weight organic substrates derived from roots. Gray and Williams (1971) studied influence of rhizosphere of wheat (*Triticum aestivum* L.) or in short, rhizosphere effect on microbes by comparing the population density (colony forming units, CFU) of both rhizosphere (R) and non-rhizosphere or bulk soil (S), for which scientists later employed R/S ratio. It is reported that rhizosphere effect was higher for bacteria followed by fungi and least for algae (Table 5.2). Among some of functional groups of bacteria involved in N cycle, i.e. ammonifiers and denitrifiers, higher density was found in rhizosphere as compared to that bulk soil as evidenced by high R/S ratio.

The organic substances as part of root exudation released to the rhizosphere soil support higher microbial biomass and microbial activity in the rhizosphere than in

Table 5.2 Microbial population in rhizosphere (R) soil and bulk soil (S) of wheat (*Triticum aestivum* L.) and R/S ratio

Microorganisms	Rhizosphere soil (R) (CFU g^{-1} soil)	Bulk soil (S)	R/S ratio
Bacteria	1.2×10^9	5.3×10^7	23
Actinomycetes	4.6×10^7	7.0×10^6	7
Fungi	1.2×10^6	1.0×10^5	12
Protozoa	2.4×10^3	1.0×10^3	2
Algae	5.0×10^3	2.7×10^4	0.2
Ammonifiers	5.0×10^8	4.0×10^6	125
Denitrifiers	1.26×10^8	1.0×10^5	1260

Source: Gray and Williams (1971)

the bulk soil. In addition to the differential microbial load of both environment, rhizosphere soil differs from bulk soil in other properties too. Marschner (1995) reported that rhizosphere pH is observed lower that of bulk soil by 1–2 units, attributed to several mechanisms like production of CO_2 by respiration processes, extrusion of H^+ during nutrient uptake by plant and microbes, exudation of organic acids by roots and microbes, organic matter decomposition, and N_2 fixation by *Rhizobium*-legume symbiosis.

Rhizosphere environment is characterized by low oxygen levels due to high demand of oxygen required for respiration of carbonaceous compounds getting released from time to time. Redox potential is also found much more negative in rhizosphere in comparison to bulk soil owing to higher oxygen consumption by respiration of plant roots and microbes. Consequent to this, CO_2 levels are also high and a significant contribution towards the higher levels can be attributed to root and microbial respiration. An anaerobic condition exists favoring reduction reactions.

High population of denitrifiers (anaerobic bacteria) (Table 5.1) in the soil–plant interface facilitates reduction of nitrogen. Nitrogenase enzyme for N fixation is also known to be sensitive to aerobic conditions; rhizosphere with limited oxygen supply provides the best platform N_2 fixation processes. In contrast to this, rice rhizosphere is known to be more aerobic than bulk soil (Vega 2007). Evidences from several studies showed that higher diversity of functional genes such as *amo*A and *nif*H genes was present in rhizosphere soil than bulk soil (Briones et al. 2003; Cocking 2003).

5.3 Nitrogen

Nitrogen exists in nature dominantly as N_2 gas contributing up to 78% of the earth's atmosphere. Nitrogen is a structural component in plant cell components and is directly involved in processes like plant photosynthesis, protein synthesis signifying its importance as primary nutrient in plant growth. It is a key constituent of amino acids, nucleic acids, nucleotides, proteins, chlorophyll, ribosomes, chromosomes, genes, and a component of all enzymes. Since it is a constituent of chlorophyll, nitrogen is responsible for dark-green color to plants and it helps in the vegetative growth of crop plants (Karthika et al. 2018).

5.3.1 Plant Available N Sources in Soil–Root Interface

Soil gains nitrogen from various sources as given below.

1. Soil reservoirs, i.e. soil organic matter
2. Biological N fixation: by symbiotic and non-symbiotic N fixing organisms
3. Industrial fixation: commercial fertilizers

4. Crop residues, green manure, farmyard manure, compost
5. Proteins, amino acids, and amino sugars
6. Non-biological N fixation or abiotic nitrogen fixation has been estimated to account for only <1/10th of biological nitrogen fixation

Plant roots possess a mucilage layer at the soil–root interface. This consists of several organic and inorganic nutrients, including proteins, polypeptides, and amino acids that contain N. The mucilaginous layer also acts as a home for many soil microbes. Root surface too is inhabited by many microorganisms. Many genera of bacteria aid in non-symbiotic N fixation at the root surface which results in an increased number of microorganisms in the rhizosphere soil than the bulk soil (Liu 1997).

5.3.2 *N cycle and Microbial Nitrogen Transformations in Rhizosphere*

The most critical macronutrient element, N is distributed on earth in two large pools: as inert form of atmospheric molecular nitrogen and biologically reactive form of nitrogen, viz. NO_3, NH_4, and organic nitrogen. These two large pools are interconnected and controlled by microbially mediated processes, nitrogen fixation, and denitrification (Gruber and Galloway 2008). Losses of mineral N from terrestrial ecosystem are mainly through microbially mediated processes nitrification and denitrification. Dissimilatory nitrate reduction (DNRA) is a process mediated by bacteria and fungi wherein respiratory reduction of nitrate to more stable ammonium form takes place, thereby limiting losses of N from soil system. DNRA is more favored in the rhizosphere due to higher C near the roots and it is also reported that bacteria possessing key gene involved in DNRA is overrepresented in rhizosphere than bulk soil (Li et al. 2014). N cycle begins with this inert dinitrogen (N_2) form and follows it through the process of fixation, mineralization, immobilization, nitrification, ammonia volatilization, leaching, runoff, plant assimilation and completes with denitrification. Microorganisms are involved in all major aspects of the cycle. Plant available N in soil depends on the balance between rates of mineralization, nitrification, and denitrification.

Rhizosphere plays a very important role in soil N cycle and the processes related to it (Wen et al. 2019). Nitrogen in rhizosphere gains huge importance due to the major form of N available in rhizosphere soil, i.e. organic forms of N that actually contribute to plant uptake (Liu 1997). Plant N uptake takes place as a result of several transformations to convert the organic forms of N to inorganic forms. As plants cannot take up N in its atmospheric form, it needs to be transformed to either as nitrate (NO_3^-) or ammoniacal (NH_4^+) to facilitate its uptake by plants (Karthika et al. 2018). Atmospheric N is converted to these forms by N fixation by soil microorganisms. This could be by either symbiotic or non-symbiotic N fixations which are microbial mediated processes. Once the N is incorporated as amino acids

or proteins into the plant body, it undergoes recycling processes several times through the activities of decomposers (Jhonson 2009). The entire process of fixing of atmospheric N_2 into rhizosphere and/or plant compartment, transformation from one form of nitrogen into the other, scavenging of organic N by microbial action and reabasorption by plants altogether comprises the "nitrogen cycle".

5.3.2.1 Nitrogen Fixation in Rhizosphere

Nitrogen plays a major role in enhancing the agricultural production and since it is a constituent of proteins and amino acids, it adds to the food value. In soil, nitrogen is present in both organic and inorganic forms. However, the bulk of the soil nitrogen is in organic form ~90 to 95 percent. Nitrogen fixation can be considered as one of the prime mechanisms contributing to large additions of mineral N into the soil ecosystem. Rhizosphere dwelling microorganisms especially those belonging to prokaryotes are called diazotrophs, has got a unique ability to convert very inert atmospheric N_2 to reactive nitrogen (NH_3). In 1888 Dutch microbiologist Martinus Beijerinck was the first to identify *Rhizobium*, a class of bacteria for its ability to fix elemental N from atmosphere into the root of leguminous plants, by forming nodules. Importance of legumes in N nutrition is widely discussed in several literatures. Besides legumes, certain non-legume crops also tend to fix atmospheric nitrogen, though their contribution is lesser than legume N fixation. Scientific literatures disclose that the rhizosphere dwellers of non-legume crops belong to the nitrogen fixing families of Azotobacteriaceae, Spirillaceae, Enterobacteriaceae, Bacillaceae, Pseudomonadaceae, and Achromobacteriaceae.

Biological N Fixation

Effective nutrient management requires effective management of biological N fixation. Biological N fixation includes the processes through which atmospheric N is converted to ammonia with the involvement of nitrogenase enzyme (Smercina et al. 2019). It depends on biological activity as it is carried out by microorganisms present in soil. The availability of N in terrestrial ecosystems is mainly governed by the processes of biological N fixation (Vitousek et al. 2002). Biological N fixation can be symbiotic N fixation, non-symbiotic or associative, and free living N fixation. These are carried out by symbiotic N fixers, associative N fixers as well as free living N fixers. Free living N fixation is mainly a microbially driven process when compared to the plant driven symbiotic N fixation. In free living N fixation, a diverse array of N fixers are involved and live in a community than that of symbiotic N fixation which is carried out by a few bacteria like *Rhizobia* and *Frankia* living in a population (Smercina et al. 2019).

$$N_2 + 8H^+ + 8e^- + 16ATP \xrightarrow[\text{N fixing microbes}]{\text{Nitrogenase}} 2NH_3 + H_2 + 16ADP + 16Pi$$

Symbiotic N Fixation

Symbiotic N fixation is the well-known example for symbiotic association that exists between roots of leguminous crops and N fixing bacteria. This results from the complex interaction between the host plant and rhizobia (a term used collectively to *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium*) (Sulieman and Tran 2014). *Rhizobium* and *Bradyrhizobium*, mainly belonging to α proteobacteria, are the main N fixers that inhabit the root nodules of leguminous crops. These usually occur as free living bacteria and are found in plenty in the rhizosphere of legumes. However, a very small number of β proteobacteria are also found as tropical legume symbionts (Fenchel et al. 2012). In case of rhizobium–legume symbiosis the interaction between the partners, i.e. bacteria and host legume is so intricate that a particular species of *Rhizobium* or other related genera will nodulate only a specific plant genera (Table 5.3). This host specificity of bacteria is called as cross inoculation group cell signaling for between the bacteria and host legume plant.

There are several other symbiotic systems but less important than legume–*Rhizobium* symbiosis. These include *Anabaena azollae* (Cyanobacterium) which has a symbiotic association with *Azolla*. This symbiosis enhances N fixation in rice fields. Another important symbiosis is the one that exists between actinorhizal trees and shrubs, such as Alder (*Alnus* sp.), with the actinomycete *Frankia*.

Nitrogenase Enzyme and Process of Nodulation

Nitrogenase, a protein complex composed of enzymes with metal co-factors makes N fixation possible in plants. It consists of dinitrogenase and dinitrogen reductase; three types of dinitrogenase exist in nature with differences in their active site co-factor binding metal. Most abundantly seen type of enzyme in the N fixing

Table 5.3 Examples of *Rhizobium* species and compatible host legume plants

Nodulating bacteria	Associated plant species	References
Bradyrhizobium japonicum	Soybean (*Glycine max*)	Jordan (1982)
Rhizobium meliloti	Lucerne (*Medicago sativa*)	Rome et al. (1996)
Rhizobium lupine	Lupine (Lupinus sp.)	Eckhardt et al. (1931)
Rhizobium leguminosarum biovar phaseoli	Beans *(Phaseolus vulgaris*)	Segovia et al. (1991)
Rhizobium leguminosarum biovar trifoli	Clover (Trifolium spp)	Frank (1889)
Mesorhizobium loti	Lotus (*Lotus corniculatus*)	Jarvis et al. (1997)
Rhizobium mediterraneum	Chickpea	Nour et al. (1995)

bacteria is Molybdenum (Mo) nitrogenase and other types are Vanadium (V) and iron-only (Fe) nitrogenase. The components of nitrogenase are encoded by bacterial *nif* genes. *nif* H and *nif* D genes are known to encode structural subunit of dinitrogenase reductase and *nif* K encodes 2 subunits of dinitrogenase (Buren and Rubio 2017). Legume–rhizobium symbiosis starts with molecular signaling between the partners. Legumes release flavonoids like flavone 7, 4 dihydroxyflavone and isoflavone genistein as signaling molecules that are capable of inducing expression of Nod factors (NF) for initiating nodulation. These signals are picked by the compatible bacteria in the rhizosphere environment and infection starts after a chain of events. These lipo-oligosaccharide nod factors determine host range and specificity of Rhizobium spp. Infection of legume with *Rhizobium* begins with adhesion to root hairs, following which infection thread, a tubular structure develops through which bacteria enter the roots. Upon entry, bacteria invade root cells to transform to bacteroids which swell and deform losing its ability to divide. Thus the roots develop nodules that host the infected cells. *Rhizobium* once they enter the nodules of plants, an encapsulated chamber and a strict microaerophilic (lower oxygen concentration) environment differentiates into bacteroids, fixing diffused nitrogen gas using their nitrogenase enzyme. The oxygen level inside the nodules is regulated by leghemoglobin which protects the enzyme nitrogenase involved in N fixation. The pink color of mature nodules is attributed to leghemoglobin. *Rhizobium* bacteria residing inside the nodules fix atmospheric N_2 as NH_3 and in return bacteria derive energy from plant synthesized carbohydrate mainly malate (Biswas and Gressho 2014).

Alternative N Fixation Systems

(1) *Non-symbiotic N fixation or Associative N fixation*

The method of N fixation, also termed as rhizosphere-associated N fixation refers to the process in which bacteria fix nitrogen by using carbon rich compounds viz exudates or material sloughed off or root tissue secreted into the rhizosphere as energy source and bacteria releases the fixed N at the root surface and in the cellular interstices of root tissue only after lysis of bacterial cell (James 2000; White et al. 2012). *Azotobacter*, *Azospirillum*, and *Enterobacter* are the major bacteria involved in non-symbiotic N fixation in the rhizosphere. Associative N fixation in the rhizosphere or plant root interiors has gained high significance and agronomic importance in sugarcane (*Saccharum* spp.), sweet potato (*Ipomea batatus* L.), and rice (*Oryza sativa* L.). Available N and partial pressure of oxygen are the main factors affecting non-symbiotic N fixation. Nitrogenase is highly sensitive to oxygen supply. Moderate level of N and low partial pressure will be conducive for N fixation. Among crop plants with non-symbiotic N fixation, C4 plants like sugarcane and maize have a higher rate than C3 crops like barley and wheat (Table 5.4).

Table 5.4 Examples for associative symbiotic N fixation in crops

Bacteria	Plant	References
Azotobacter beijerinckia	Sugarcane	Dobereiner (1961)
Azotobacter paspali	Buckwheat, wheat, and maize	Mengel and Viro (1978)
Azospirillum brasiliense	Wheat	Mengel and Viro (1978)
Azospirillum spp.	Sweet potato	Yoneyama et al. (1997)
Anabaena (Cyanobacteria)	Rice	Yoneyama et al. (2017)
Burkholderia spp.	Switchgrass	Compant et al. (2008)
Ralstonia taiwanensis	Switchgrass	Compant et al. (2008)

Quantification of N Fixed through Non-symbiotic N Fixation

Non-symbiotic N fixation was estimated to be 4–8 μ N g soil^{-1} day^{-1} and 2–5 μ N g soil^{-1} day^{-1} due to algae and bacteria, respectively, in paddy soils (Rinaudo et al. 1971) and in temperate forest ecosystems it ranged from<0.01 to 5 kg N ha^{-1} year^{-1}, which decreased with stand age (Son 2001).

(2) *Free living Nitrogen Fixation (FLNF)*

Free living N fixers contribute only a small fraction of plant rhizosphere ecosystem. In free living N fixation, the fixation of N occurs without a formal microbe plant symbiotic relationship. Several studies find FLNF as a subcategory of symbiotic N fixation. However, FLNF was described as an entirely different process carried out by wholly different bacterial species by Smercina et al. 2019. The process is ubiquitous in terrestrial ecosystems and the major free living N fixers include many bacterial phyla such as alphaproteobacteria (*Rhizobia, Bradyrhizobia, Rhodobacter*), betaproteobacteria (*Burkholderia, Nitrosospira*), gammaproteobacteria (*Pseudomonas, Xanthomonas*), deltaproteobacteria, firmicutes, cyanobacteria, etc. A simple briefing of such free living N fixers is given hereunder.

(1) Obligate anaerobes, e.g. *Clostridium pasteurianum, Desulfovibrio, Desulfotomaculum*
(2) Facultative anaerobes, e.g. *Klebsiella, Bacillus, Citrobacter*
(3) Photosynthetic bacteria, e.g. *Rhodobacter*
(4) Many cyanobacteria, e.g. *Plectonema*
(5) Obligate aerobes such as *Azotobacter, Beijerinckia, Derxia*
(6) Green Sulfur bacteria (Gaby and Buckley 2015)
(7) Some methanogens (Gaby and Buckley 2015)

The perennial bioenergy cropping systems were found to be very important in FLNF and these included Miscanthus (*Miscanthus giganteus*) (Davis et al. 2010) and switchgrass (*Panicum virgatum*) (Ruan et al. 2016). Switchgrass rhizosphere was found to have many free living N fixers and *Azotobacter vinelandii* was the major bacterium among those (Smercina et al. 2019).

5.3.2.2 N Mineralization

As discussed in previous sections of this chapter, majority share of N in the rhizosphere and soil in general is present as complex organic molecules which need to be converted to plant usable forms by microorganisms present at the soil–root interface. Mineralization of organic nitrogen is a three step process, namely aminization, ammonification, and nitrification. The pace of these processes is affected by carbon:nitrogen (C:N) ratio of the organic substrate present. In case of fresh and easily decomposable organic material with a narrower C:N ratio, mineralization takes place faster whereas for high molecular weight compounds like lignin and humus with a wider C:N ratio mineralization is very slow. Release of photosynthates by plant roots is thought to trigger rhizosphere priming, that is, the acceleration of microbial soil organic matter (SOM) decomposition by increased availability of saprophytic microbes (Kuzyakov 2002). Extracellular enzymes produced by these microbes aids in degradation of high molecular weight organic compounds, resulting in increased availabilityof inorganic nitrogen (N) to plants as well as soil microbes.

Aminization

Heterotrophic bacteria, actinomycetes, and fungi are capable of doing by enzymatic digestion of proteins and other proteinous compounds released from roots or from organic residues in the rhizosphere to amino acids and amines and the process is known as aminization.

$$\text{Protein} \xrightarrow[\text{Heterotrophic microbes}]{\text{Enzymes}} \text{Polypeptides} \xrightarrow[\text{hydrolysis}]{\text{Enzymatic}} \text{Amino acids/amines} + CO_2 + \text{ATP}$$

These amino acids so produced are either utilized by microbes, i.e. immobilization or further gets mineralized to NH_3 by ammonification. Amino acids act as an important source of N uptake for plants under some circumstances and the existence of amino acid uptake systems in plant roots is also reported.

Ammonification

In this process amino acids or amines present in rhizosphere or released by root exudation undergo transformation to ammonia by ammonifiers (bacteria, fungi, and actinomycetes) by using enzymes.

$$\text{R-NH}_2 + \text{HOH} \xrightarrow[\text{Ammonifiers}]{\text{Enzymes}} \text{NH}_3 + \text{R-OH} + \text{Energy}$$
$$\text{NH}_3 + \text{HOH} \rightarrow \text{NH}_4^+ + \text{OH}^-$$

Many microorganisms are involved in this and it is carried out in an efficient way in aerobic conditions. Studies reported that a higher population of ammonifiers and high diversity *amo*A in the rhizosphere favor the process of ammonification in this narrow zone.

Nitrification

Nitrification is a two step oxidation process wherein oxidation of ammonium ion (NH_4^+) is carried out to nitrate ion (NO_3^-) by nitrifying organisms. In the first step, NH_4^+ is converted to nitrite (NO_2^-) by *Nitrosomonas, Micrococcus, Nitrospira,* and *Nitrogela* and then conversion of NO_2^- to NO_3^- is mediated by the *Nitrobacter* and *Nitrocystis*. Nitrification is negatively influenced by low soil pH, anaerobic conditions, lack of soil water, and temperatures below 5 °C and above 40 °C.

$$\text{NH}_4^+ \xrightarrow[\text{Nitrosomonas}]{[\text{O}]} \text{NO}_2^- \xrightarrow[\text{Nitrobacter}]{[\text{O}]} \text{NO}_3^-$$

5.3.2.3 Immobilization

Soil microorganisms utilize inorganic N forms for building up their body tissues, thereby leading to temporary unavailability of nitrogen for plant uptake. This process of conversion of inorganic nitrogen into organic forms is termed as immobilization. It is a reversal of mineralization process. Main factor governing immobilization process is the C:N ratio of the substrate available for rhizosphere dwellers. More complex the organic substrate, wider will be the C:N ratio leading to a higher N demand by saprophytes feeding on this. To meet their elevated N demand, decomposers utilize either added or native N source immediately available to them. The process of immobilization leads only to short term unavailability of N for plants.

5.3.2.4 Denitrification

The process of denitrification mainly happens under anoxic conditions in which bacteria of the genera *Thiobacillus denitrificans, Thiobacillus thioparus, Pseudomonas, Micrococcus, Achromobacter,* and *Bacillus* reduce NO_2^- and NO_3^- leading to the release of gases NO, N_2O, and N_2 back to atmosphere.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \uparrow \rightarrow N_2 \uparrow$$

These microorganisms use organic compounds as their energy source which is available in plenty in the rhizosphere and higher population of denitrifying bacteria are present at soil–root interface than in bulk soil. Favorable conditions for denitrification process include limited O_2 supply, high concentration of NO_3^-, soil moisture presence, carbohydrates source, and warm temperatures (Luo et al. 2000; Strong and Fillery 2002). Nitrate and nitrite act as electron acceptors instead of O_2 for respiration by microorganisms in denitrification.

5.3.3 *Nitrogen Cycling in Rice*

Rice is an important food crop of the majority of the population around the world and deserves special attention because of its cultivation practices. Though upland rice also shares considerable area, wetland rice occupies the major share. The chemistry of wetland rhizosphere is entirely different from those of upland which plays a major role in the crop growth by influencing physicochemical properties of the soil and nutrient availability. The rhizosphere of flooded rice exhibits an environment more aerobic than the bulk soil. Rice plants have got aerenquima tissue which permits the transport of O_2 to the roots and its release into the rhizosphere (Marschner 1995). Nitrogen being an important essential element for crops, its transformation and chemistry in anaerobic condition is necessary to understand as it is highly varied from that of aerobic soil.

Rhizosphere of wetland paddy system was found different from that of paddy soil without rice plants and in upland rice soil system and the former was found having higher nitrogenase activities than the latter. Long term experiments conducted by International Rice Research Institute (IRRI) studied the nitrogen fixation by heterotrophic bacteria utilizing the carbon source by root secretions from rhizosphere in addition to photosynthetic cyanobacteria contributing significantly to atmospheric N input in paddy fields (Yoneyama et al. 2017; Ladha et al. 2016).

The transformations of nitrogen in the soil are wholly mediated by microorganisms. The reactions of N cycle in rice soils include ammonification, N immobilization, nitrification, denitrification, dissimilatory nitrate reduction to ammonium, anaerobic ammonium oxidation, and nitrogen fixation. Apart from these microbial processes, newly recognized processes such as fungal denitrification, anaerobic methane oxidation coupled with denitrification, archaeal ammonia oxidation, and anaerobic ammonium oxidation also contribute to N cycling in rice fields (Ishii et al. 2011).

Biological nitrogen fixation can occur in the surface of rice rhizosphere by free-living or plant-associated bacteria. As depicted in Fig. 5.1 the process of nitrification occurs in the oxidized layer, viz. thin surface layer and in the rhizosphere. When nitrate and nitrite diffuses to the reduced layer, there stepwise reduction occurs to gaseous products (NO, N_2O, and N_2) leading to denitrification process by microorganisms. Anaerobic ammonium oxidation (anammox) may potentially occur in rice

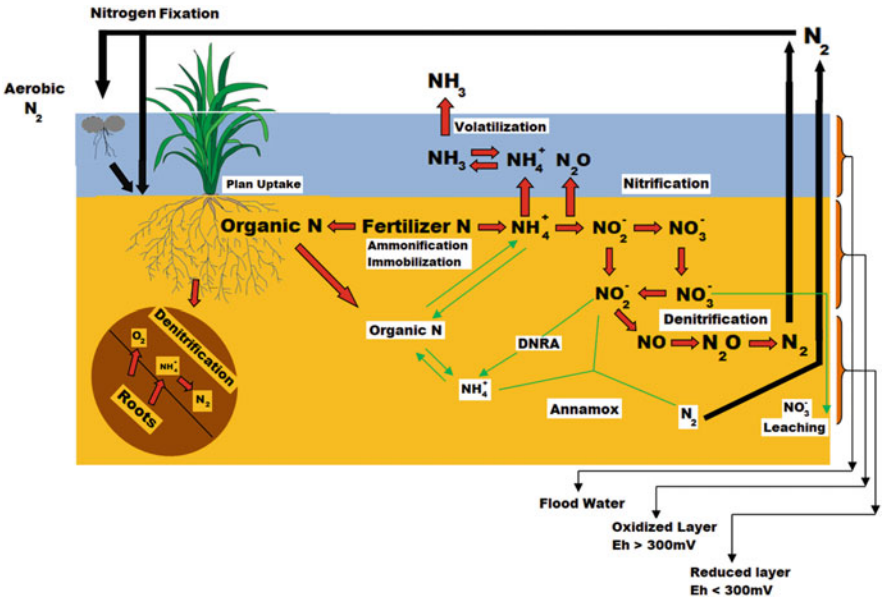

Fig. 5.1 Nitrogen cycle in rice rhizosphere

soils when conditions allow. Leaching of nitrate/nitrite rarely arose in rice ecosystem, which might be due to strong denitrification activity in rice soils. In addition, ammonia volatilization is also mostly negligible in most rice environment until the pH of the soil is more than 8.5.

5.3.3.1 Nitrogen Mineralization–Immobilization Reactions and its Relevance with Respect to Plant N Uptake in Rice Rhizosphere

Under anaerobic condition also mineralization–immobilization reactions play significant role in the availability and consequently the plant N uptake. The conversion of organic form of N to inorganic form (mineralization) and the reverse process (immobilization) are widely governed by plant characteristics, C:N ratio, and soil properties. The first step of mineralization is aminization where complex organic moieties are converted to amines. Later these amines are biochemically transferred to ammonium by heterotrophic microorganisms. Ammonium is transferred to nitrate by the process of nitrification which is recognized as two step oxidation process, wherein ammonium is first oxidized into nitrite (NO_2), being known as ammonia oxidation and the second step, nitrite oxidation involves the oxidation of nitrite to nitrate (NO_3). Ammonia oxidation is carried out by ammonia oxidizing bacteria (AOB), viz. *Nitrosomonas* spp. and *Nitrospira* spp. of Betaproteobacteria and *Nitrosococcus* spp. of Gammaproteobacteria. Besides bacteria, certain archaea can also perform ammonia oxidation and are documented as ammonia oxidizing archaea

(AOA). Contribution of AOA and AOB towards nitrification may vary depending upon the soil type or land characteristics. Francis et al. (2007) depicted abundance of AOA over AOB in ocean, whereas the case was reverse with respect to the upland agricultural soils. The predominance of microbial community also varies in the paddy soil environment. Bowatte et al. (2006) discussed about the difference in microbial community in connection with the difference in fertilization.

In the next step of nitrification i.e. nitrite oxidation, the conversion of nitrite to nitrate is performed by nitrite oxidizing bacteria, viz. *Nitrobacter, Nitrospira, Nitrospina, Nitrococcus* and *Nitrospira.* Similar to ammonia oxidizers, community of nitrite oxidizers may also vary depending on the nitrogen fertilization practices. Among the various species of nitrite oxidizers, *Nitrospira* spp. were predominant in rice rhizosphere (Iguchi et al. 2009).

Environmental Perspectives of Nitrification and Denitrification in Rice Rhizosphere

In rice rhizosphere under reduced condition, nitrate (NO_3^-) is reduced to gaseous end products such as NO, N_2O, and N_2 by microbes through a process termed as denitrification which causes major loss of N from the paddy fields. The major end product is reported to be N_2 because of strong denitrification activity in the rice rhizosphere (Nakaya et al. 2009). Like nitrification, denitrification is not exclusively carried out by microbes and can be either bio-denitrification or chemo-denitrification and the latter occurs only when highly acidic soils are submerged. The seat of nitrification is at the surface thin oxidized layer, whereas denitrification occurs just below the oxidized layer. Deep placement of ammoniacal fertilizers reduces the extent of denitrification. Denitrification is performed by a variety of microbial communities such as bacteria, fungi, and archaea (Mayumi et al. 2010). Recently, fungal denitrification gains special focus (Hayatsu et al. 2008) and a typical example of which is *Fusarium oxysporum* carrying *nir* K genes responsible for nitrate reduction.

Dissimilatory nitrate reduction (DNRA) and ammonia fermentation are the two terminologies relating to denitrification discussed recently. DNRA implies the dissimilatory reduction of nitrate to ammonium via nitrite which depends on carbon content, type of carbon, and the electron acceptors (Hayatsu et al. 2008). In addition to DNRA, nitrate can be reduced to ammonia by ammonia fermentation as ethanol is oxidized to acetate along with nitrate reduction and is widely being carried out by fungi (Zhou et al. 2002). Anaerobic ammonium oxidation (anammox) is another process wherein N_2 is produced when both ammonium and nitrite are present in rice soils. The process is of less significance in rice rhizosphere (Suwa et al. 2008).

Though occurrence of nitrification-denitrification reactions in rice rhizosphere may cause loss of applied fertilizer N, it can reduce the risk of environmental pollution by eliminating or by decreasing leaching of nitrate nitrogen and emission of N_2O, a potential greenhouse gas. Besides less emission of N_2O, rice rhizosphere has been occupied with a greater number of denitrifiers which helps in significant removal of N_2O (Ishii et al. 2011).

5.3.3.2 Nitrogen Fixation in Rice Soils

Biological nitrogen fixation (BNF) helps to incorporate atmospheric N to soil N, particularly in rhizosphere soil which helps in compensating the denitrification losses of N. Besides cyanobacteria and phototrophic bacteria found in the surface of rice soils can also fix atmospheric N_2. Nitrogen fixing microbes in the water-logged rice ecosystem contribute around 40–50 kg N ha^{-1}, which is cumulative effect of photoautotrophs such as *Rhodopseudomonas* and blue green algae and heterotrophs like *Azotobacter, Beijerinckia, Clostridium, Desulfovibrio, Klebsiella, Enterobacter, Flavobacterium, Pseudomonas, Rhizobium,* and *Azospirillum* (Subbarao 2005). BNF is influenced by factors such as soil types, application of chemical fertilizers and pesticides, organic matter, field management practices, and genotype (Ishii et al. 2011). Application of fertilizer N, particularly ammonium sulfate has adverse impact on BNF (Yasuda et al. 2010). Andisols have weak BNF capacity because of high phosphate adsorption which induces P deficiency to microbes. Application of herbicides may tend to decline the population of N fixers in rice field. Application of rice straw is found to enhance the BNF potential of rice soils. Rice genotypes also differ in their potential to BNF. Population of N fixers varies with the crop growth stage, higher number at the tillering stage than at flowering stage (Ishii et al. 2011).

5.3.4 Nitrogen Uptake in Rhizosphere

Application of fertilizers alone without knowing the chemistry of both soil and the fertilizer nutrient may not ensure the food security which has a prime role in quenching the global hunger. Plant nutrient availability is a major constraint limiting the crop growth and development particularly in soils low in available nutrients. Plants take up the mineral nutrients through a microzone of soil (~ 2 mm) present in the immediate vicinity of the roots called "rhizosphere" where soil microorganisms, plant roots, and the nutrients interact with each other and vary with plant species and soil types. The importance of this zone is often neglected due to lack of adequate knowledge on its role in plant nutrition and mechanisms leading for nutrient uptake. Manipulation of rhizosphere either through agronomic or breeding practices can enhance the nutrient use efficiency as the rhizosphere microbes are actively involved in the geochemical cycling of nutrients, viz. nitrogen, phosphorus, sulfur, and some micronutrients.

Nitrogen (N) one of the most important nutrients needed for the vital growth of the plants is described as "universally deficient nutrient" since its shortage is widely spread and limits crop production across the world. Besides an essential element, its role as an environmental pollutant is also widely recognized. Hence, it is important to understand the chemistry of N transformations and uptake taking place in the rhizosphere. Among the three mechanisms of nutrient uptake, viz. mass flow,

diffusion, and root interception, N is mostly carried from the soil to roots via combination of mass flow and diffusion since it is a mobile nutrient. Root interception is thought to account for 1% of total N uptake. McMurtrie and Nasholm (2017) pointed out that root-N uptake is sensitive to the rate of mass flow for widely spaced roots with high N uptake capacity, but not for closely spaced roots or roots with low uptake capacity. They also highlighted that nitrogen traverses the rhizosphere faster in the presence of mass flow, dropping the possibility of its immobilization before reaching the root surface.

In general, plants take up nitrogen in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions. Among the essential elements for plant growth, mineral N is the only plant nutrient that is available in both as a cationic and an anionic form and plant roots have transport mechanisms for absorbing both NO_3^- and NH_4^+ from the soil. The uptake of NH_4^+ has been studied less intensively than that of NO_3^-. Most of the crops take up N in NO_3^- form except rice, oat, potato, tea, etc. which preferably take NH_4^+ form. Maathuis (2009) reported that plants grown in low pH and reducing environment as in mature forests or arctic tundra preferably take up ammonium or amino acids, whereas plants acclimatized to higher pH and aerobic condition preferentially take up N in nitrate form. It is also clear that many species that normally use NO_3^- also have an efficient system(s) for absorbing NH_4^+ which is constitutively expressed at high levels. Indeed, where such species are presented with a mixed NO_3^-/NH_4^+ source, NH_4^+ is absorbed more rapidly, e.g. in perennial ryegrass and barley. The concentration of these two ions in the rhizosphere soil and bulk soil may vary and may also induce certain chemical changes and nutritional transformations in the former which will be discussed hereunder.

5.3.4.1 Forms of N Uptake in the Rhizosphere

Plant roots are exposed to diverse forms of N as latter exists in various organic and inorganic forms in rhizosphere which has influence on both availability and uptake into the plant system. Nitrogen is present as three major forms in soil: organic nitrogen, ammonium nitrogen (NH_4^+–N), and nitrate N (NO_3^-–N). Organic forms contribute more than 90% of the total N in the soil, whereas only a small portion is in the plant preferred inorganic or mineral forms. Organic forms of N in soils are amino acids, amino sugars, nucleic acids, glycerol-phosphatides, amines, vitamins, and DNA as well as more complex organic molecules like proteins.

Plants absorb nitrogen in both organic and inorganic forms (Jones et al. 2005; Glass 2009). The most preferentially absorbed inorganic forms are nitrate and ammonium (Courty et al. 2015). Certain literature depicted that N can be taken up by the plant roots as organic forms which may vary from simpler low organic compounds such as amino acids, oligopeptides, nucleotides, and urea to complex polymeric moieties such as proteins (Hill et al. 2011). Amino acids are one of the main components of root exudates, are omnipresent in the soil environment at low concentrations, and might therefore represent important triggers of plant responses to changing N availability in soil. It was demonstrated by certain studies that amino

acids can be taken as the main indicator of the N status of plants, which is important for the regulation of plant N uptake (Forde 2014; Gent and Forde 2017).

It has been proven physiologically that different transporters are responsible for the uptake of inorganic (NO_3^- and NH_4^+) and organic N from the soil into roots. Nitrate is absorbed by plants against an electrochemical gradient and can be considered as an energy demanding (active) process and hence uptake of NO_3^-–N is less energetically efficient than ammonium uptake (Wang et al. 1993), which may be either active or passive, reliant upon the ammonium concentration in soils. If N is taken up by the plants as NO_3^-, it has to be assimilated in order to participate in the plant biochemical reactions. Nitrate taken up by roots is either reduced in situ to NH_4^+ in the root or stored in vacuoles or transported to the shoot. The reduction of NO_3^-–N to NH_4^+–N is carried out by nitrite and nitrate reductases. Later NH_4^+–N is assimilated into glutamate and glutamine by glutamine synthetase and glutamate synthase. Hence, uptake of NO_3^-–N can be viewed as an energy expensive process as preliminary reduction of nitrate to ammonium is essential, whereas absorbed ammonium can be directly assimilated in order to synthesize amino acids and other organic compounds (Moreau et al. 2019). Being a cation, NH_4^+ will bind to the cation exchange sites of the soil and is less mobile than NO_3^- and hence more available for plant N uptake (Courty et al. 2015). Chapin et al. (2002) reviewed that uptake of N in organic form is the least energy expensive since the assimilation process is bypassed.

5.3.4.2 Factors Influencing Forms of Nitrogen Uptake in the Rhizosphere

The plants will take up nitrogen in different forms and proportions which may be influenced by many factors. Though the exact mechanisms are not well documented, the various factors affecting nitrogen uptake in the rhizosphere can be grouped broadly in to plant characteristics, soil factors, and biotic factors (Fig. 5.2).

1. **Plant characteristics**: Plant factors which majorly influence the forms of N uptake in the rhizosphere are genotype, plant N content, and growth stage. Genotypic variations significantly influence the form of N taken up by the plants in rhizosphere. A wide spread notion with regard to N form uptake is that plants prefer to take up NO_3^-–N than any other forms. Marschner et al. (1991) reported that coniferous trees prefer to take up NH_4^+–N than NO_3^-–N unlike many other plant species which may vary depending upon the concentration and proportions from of N supplied. Tea plants are adapted to take up N in NH_4^+ form (Ruan et al. 2000). Generally, plants grown in low pH will be NH_4^+ tolerant. von Wiren et al. (1997) opined that the genotypic variations with regard to N form uptake may be linked to different transporters present in the plant species. Root architecture is another major factor influencing the plant N uptake. Arai-Sanoh et al. (2014) reported that deeper rooting in rice facilitates N uptake particularly from N deficient soils. Britto and Kronzucker (2002) pointed out that susceptibility to

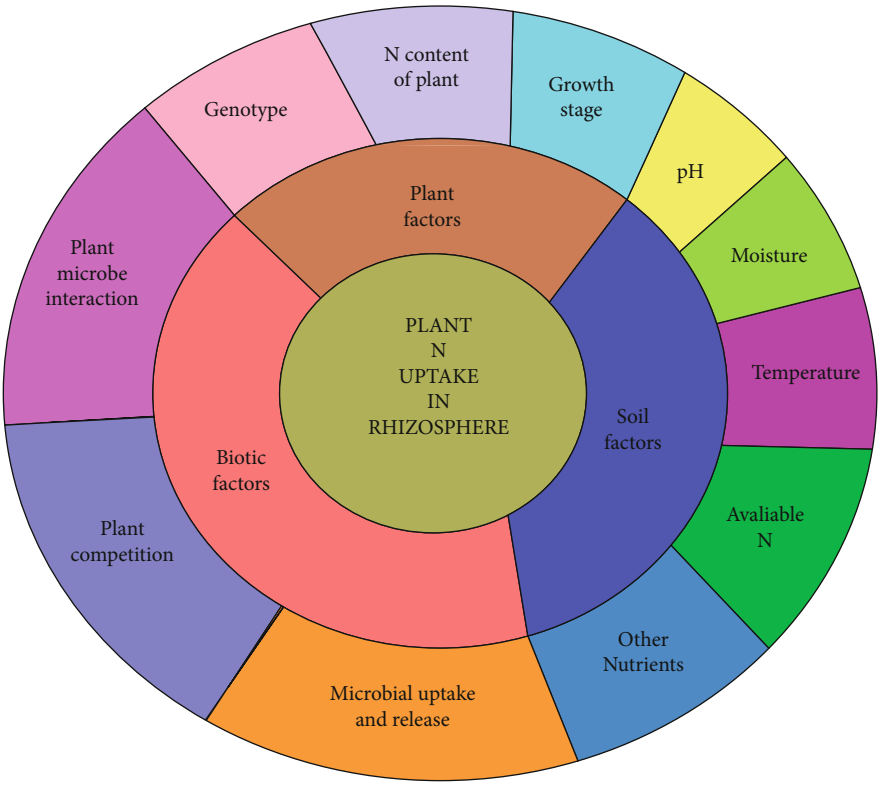

Fig. 5.2 Factors influencing N uptake in the rhizosphere

ammonium toxicity may trigger the plants to take up nitrate form. The growth and developmental stage of the crop as well as the plant N content can also influence the forms of N taken up the plant (Cui et al. 2017; Britto and Kronzucker 2002; Houlton et al. 2007). Cui et al. (2017) observed that legumes preferred NO_3^- throughout their growth, while the grasses preferred NH_4^+ at the early stages but switched the preference for NO_3^- later. A family of transporters (mainly NRT2 and NRT1) mediate root responses to NO_3^- by modulating auxin transport (and other plant hormones and signal molecules) so as to regulate the root system architecture (Sun et al. 2017).

2. ***Soil factors***: Soil properties such as pH, temperature, moisture, available N, and other nutrients greatly affect the forms of N taken up by plants. Among these, amount of available N present in the soil is the major one determining the form of N uptake by plants which indeed is influenced by soil pH, moisture, and temperature and hence these also affect the forms of N uptake by plants (Britto and Kronzucker 2013). Concentration of NH_4^+ and NO_3^- in soil solution also affects the form of N taken up by plants in rhizosphere.

When NH_4^+ was present in the external solution at concentrations of about 100 gM and higher, net uptake of NO_3^- was very low, even at NO_3^- concentrations up to 1000 gM (Marschner et al. 1991). The inhibition of NO_3^- by NH_4^+ is caused by various mechanism like a decrease in nitrate reductase activity (NRA) (Peuke 1987), NH_4^+ induced NO_3^- efflux (Deane-Drummond and Glass 1983) or by direct inhibition of NO_3 influx through inducible carrier system for NO_3 uptake (Siddiqi et al. 1989). Presence of other nutrients particularly potassium has a role in prompting the N form uptake due to its ability to alleviate ammonium toxicity in nitrate specialist plants (Li et al. 2012).

3. ***Biotic factors***: Microbial uptake and release of N, plant–microbe interaction, and plant competition are the biotic factors influencing N uptake in the rhizosphere. Plants take up N through microbial symbiosis and other multitrophic interactions. Transformations of N in the soil, viz. mineralization, immobilization, and denitrification are microbial mediated and are driven by the carbon availability in the rhizosphere. Rhizodeposition is a major source of C and N for the soil and its inhabitants (Jensen 1996). Composition of root exudates may vary depending upon the plant species and the form of N applied. Plants supplied with NH_4^+ tend to exude higher concentrations of carbohydrates and amino acids than that from roots supplied with NO_3^- (Cramer and Titus 2001; Mahmood et al. 2002).

Changes in Soil Properties as Affected by N Form Uptake

The form of N taken up by the plants significantly causes certain changes in the properties of soil particularly in the rhizospheric soil and hence there is difference in the former from the bulk soil.

***Soil pH*:** Uptake of nitrogen as NH_4^+ decreases and uptake of NO_3^- increases the pH of rhizosphere soil not only in annual species but also in coniferous trees such as Norway spruce (Marschner et al. 1991). The rhizosphere and rhizoplane pH may vary from the bulk soil pH by up to 2 units (Marschner and Romheld 1983). The increase in pH of the rhizosphere soils of NO_3^- fed plants may be caused by H^+/NO_3 cotransport (1/1 or $>$ 1/1) at the plasma membrane (McClure et al. 1990) or may also due to preferential reduction of NO_3 in the plant roots.

Relative concentration of NO_3^- or NH_4^+: Uptake of N either as NO_3^- or NH_4^+ causes a decline or increase in the concentration of NO_3^- or NH_4^+ in the rhizosphere soil from that of the bulk soil and it depends upon the plant species and relative proportion of NO_3^- or NH_4^+ in the rhizosphere soil (Marschner et al. 1991).

Availability of other nutrients: Uptake of N either as NO_3^- or NH_4^+ affects the availability of other nutrients primarily through modifying the soil pH. Ruan et al. (2000) reported that concentration of Al and Mn was greater in the rhizosphere soil with NH_4^+N nutrition. Available P content in the soil may vary depending upon the native P content irrespective of the forms of N taken up by the plants (Ruan et al. 2000). Solubility and toxicity of mineral elements like aluminum and availability and uptake of other mineral nutrients are influenced by the shift in pH in the

rhizosphere soil and at the rhizoplane caused by the uptake of N forms (Marschner et al. 1991).

5.3.5 *Other Microbial Interactions and their Role in N Dynamics*

Plants absorb mineral nutrients from the soil solution predominantly in ionic forms and microorganisms in the rhizosphere mediate nutrient transformation reactions to make these into plant usable forms. There are so many interactions belowground between plant and microflora and fauna that plays a role in nutrient dynamics. It is evidenced that arbuscular mycorrhizal (AM) fungi take up nitrate, ammonium, and organic forms of N from the mycorrhizal interface and transfer to the plant partner in return to carbon supply from host plant (Govindarajulu et al. 2005; Fellbaum et al. 2012). Behie et al. (2012) reported that plants can acquire N from soil insects through their endophytic associations with Metarhizium spp. (a ubiquitous soil-dwelling insect–pathogenic fungi). This insect-derived N represented up to 48% of the plant N content and was driven by allocation of C from the plant roots to the fungal mycelium (Behie et al. 2017). In addition, there is increasing evidence that dark septate endophytic (DSE) fungi have the potential to facilitate the transfer N to plants (Vergara et al. 2017).

5.3.6 *Conclusion*

This chapter gives evidences for the fact that plant–microbe interactions in rhizosphere environment significantly influence N cycle and transformation processes in many ways. Any alterations in the properties of this microzone are governed by nature and amount of root exudates released over a period of time. Consequently, the fate of N in the soil has got a strong relation with the highly specific plant root activity, as most of processes affecting nutrient management are governed by roots and soil adhering to root surface in the rhizosphere. Comprehensive knowledge on complex interactions taking place in rhizosphere in relation to nutrient availability would help us to understand and work on regulating the fertilizer application and enhancing nutrient use efficiency. Future research studies focussing more on efficient and strategic use of nitrogen fixing plants are highly required as biological nitrogen fixation in plants acts as a sustainable source for nitrogen would help in reducing over dependency on industrial nitrogen production. In this context, more in situ rhizosphere studies are urgently demanded to address our lack of knowledge in relation to N dynamics in rhizosphere as it is the plant roots which determine the nature of rhizosphere environment and associated microbiome.

References

Arai-Sanoh Y, Takai T, Yoshinaga S, Nakano H, Kojima M, Sakakibara H, Kondo M, Uga Y (2014) Deep rooting conferred by DEEPER ROOTING 1 enhances rice yield in paddy fields. Sci Rep 3(4):55–63

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681. https://doi.org/10.1111/j.1365-3040.2009.01926.x

Behie S, Zelisko P, Bidochka M (2012) Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577. https://doi.org/10.1126/science.1222289

Behie SW, Moreira CC, Sementchoukova I, Barelli L, Zelisko PM, Bidochka MJ (2017) Carbon translocation from a plant to an insect-pathogenic endophytic fungus. Nat Commun 8:14245. https://doi.org/10.1038/ncomms14245

Bertin C, Yang XH, Weston LA (2003) The role of root exudates and allelochemicals in rhizosphere. Plant Soil 256:67–83

Biswas B, Gressho PM (2014) The role of symbiotic nitrogen fixation in sustainable production of biofuels. Int J Mol Sci 15:7380–7397

Bowatte S, Jia Z, Ishihara R, Nakajima Y, Asakawa S, Kimura M (2006) Molecular analysis of the ammonia-oxidizing bacterial community in the surface soil layer of Japanese paddy field. Soil Sci Plant Nutr 52:427–431

Briones AM, Satoshi O, Yoshiaki U, Niels-Birger R, Wolfgang R, Hidetoshi O (2003) Ammonia-oxidizing bacteria on root biofilms and their possible contribution to N use efficiency of different rice cultivars. Plant Soil 250:335–348

Britto DT, Kronzucker HJ (2002) NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Britto DT, Kronzucker HJ (2013) Ecological significance and complexity of N-source preference in plants. Ann Bot 112:957–963

Buren S, Rubio LM (2017) State of the art in eukaryotic nitrogenase engineering. FEMS Microbiol Lett 365

Chapin FS, Matson PA, Mooney HA (2002) Terrestrial plant nutrient use. Prin Terres Ecosyst Ecol:176–196

Cocking EC (2003) Endophytic colonization of plant roots by nitrogen-fixing bacteria. Plant Soil 252:169–175

Compant S, Nowak J, Coenye T, Clément C, Ait Barka E (2008) Diversity and occurrence of Burkholderia spp. in the natural environment. FEMS Microbiol Rev 32:607–626

Courty PE, Smith P, Koegel S, Redecker D, Wipf D (2015) Inorganic nitrogen uptake and transport in beneficial plant root -microbe interactions. Critical Reviews in Plant Sci 34:4–16

Cramer MD, Titus CHA (2001) Elevated root zone dissolved inorganic carbon can ameliorate Al toxicity in tomato seedlings. New Phytol 152:29–39

Cui J, Yu C, Qiao N, Xu X, Tian Y, Ouyang H (2017) Plant preference for NH4+ versus NO_3^- at different growth stages in an alpine agroecosystem. Field Crops Res 201:192–199

Davis SC, Parton WJ, Dohleman FG, Smith CM, Del Grosso S, Kent AD, DeLucia EH (2010) Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a Miscanthus X giganteus agro-ecosystem. Ecosystems 13:144–156. https://doi.org/10.1007/s10021-009-9306-9

Deane-Drummond CE, Glass ADM (1983) Short term studies of nitrate uptake into barley plants using ion-specific electrodes and 36C1O3. II. Regulation of NO3 efflux by NH4. Plant Physio 1 (73):105–110

Dobereiner J (1961) Nitrogen-fixing bacteria of the genus *Beijerinckia* Derx in the rhizosphere of sugar cane. Plant Soil 15:211–216

Eckhardt MM, Baldwin IL, Fred EB (1931) Studies of the root-nodule organism of Lupinus. J Bacteriol 21:273–285

Egamberdieva D, Kamilova F, Vadilov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S et al (2012) Carbon availability triggers fungal nitrogen uptake and transport in arbuscular Mycorrhizal symbiosis. Proc Natl Acad Sci U S A 109:2666–2671. https://doi.org/10.1073/pnas.1118650109

Fenchel T, King GM, Blackburn TH (2012) Symbiotic systems. In: Bacterial biogeochemistry, 3rd edn, pp 163–181. https://doi.org/10.1016/B978-0-12-415836-8.0009-8

Forde BG (2014) Nitrogen signalling pathways shaping root system architecture: an update. Curr Opin Plant Biol 21:30–36

Francis CA, Bemanand JM, Kuypers MMM (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J 1:19–27

Frank B (1889) Ueber die Pilzsymbiose der Leguminosen. Berichte der Deutschen Botanishen Gesellschaft 7:332–346

Gaby JC, Buckley DH (2015) Assessment of nitrogenase diversity in the environment. In: de Bruijn FJ (ed) Biological nitrogen fixation. Wiley, Hoboken, NJ, pp 209–216

Gent L, Forde BG (2017) How do plants sense their nitrogen status? J Exp Bot 68:2531–2539. https://doi.org/10.1093/jxb/erx013

Glass AD (2003) Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. Critical Rev in Plant Sci 22:453–470. https://doi.org/10.1080/07352680390243512

Glass ADM (2009) Nitrate uptake by plant roots. Botany 87:659–667

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J et al (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823. https://doi.org/10.1038/nature03610

Gray TRG, Williams ST (1971) Soil microorganisms. Oliver and Boyd, Edinburgh

Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. Nature 451:293–296

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45

Hill PW, Quilliam RS, DeLuca TH, Farrar J, Farrell M, Roberts P, Jones DL (2011) Acquisition and assimilation of nitrogen as peptide bound and D-enantiomers of amino acids by wheat. PLoS One 6:e19220

Houlton BZ, Sigman DM, Schuur EA, Hedin LO (2007) A climate-driven switch in plant nitrogen acquisition within tropical forest communities. Proc Natl Acad Sci U S A 104:8902–8906

Iguchi A, Terada T, Narihiro T, Yamaguchi T, Kamagata Y, Sekiguchi Y (2009) In situ detection and quantification of uncultured members of the phylum Nitrospirae abundant in methanogenic wastewater treatment systems. Microbes Environ 24:97–104

Ishii S, Ikeda S, Minamisawa K, Senoo K (2011) Nitrogen cycling in rice paddy environments: past achievements and future challenges. Microbes Environ 26:282–292

James EJ (2000) Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res 65:197–209

Jarvis BDW, Van Berkum P, Chen WX, Nour SM, Fernandez MP, Cleyet Marel JC, Gillis M (1997) Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum,* and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int J Syst Bacteriol 47:895–898

Jensen ES (1996) Barley uptake of N deposited in the rhizosphere of associated field pea. Soil Biol Biochem 28:159–168

Jhonson C (2009) Microbiological studies of soil. In: Jhonson C (ed) Biology of soil science. Oxford Book Company, p 301

Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? Soil Biol Biochem 37:413–423

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant Soil 321:5–33. https://doi.org/10.1007/s11104-009-9925-0

Jordan DC (1982) Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing, root nodule bacteria from leguminous plants. Int J Syst Bacteriol 32:136–139

Karthika KS, Rashmi I, Parvathi MS (2018) Biological functions, uptake and transport of essential nutrients in relation to plant growth. In: Hazanuzzaman et al (eds) Plant Nutrients and Abiotic stress tolerance. Springer. https://doi.org/10.1007/978-981-10-9044-8_1

Kuzyakov Y (2002) Factors affecting rhizosphere priming effects. J Plant Nutr Soil Sci 165:382–396. https://doi.org/10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-%23

Ladha JK, Tirol-Padre A, Reddy CK, Cassman KG, Verma S, Powlson DS, Van Kessel C, Richter DDB, Chakraborty D, Pathak H (2016) Global nitrogen budgets in cereals: a 50-year assessment for maize, rice and wheat production systems. Sci Rep 6:19355

Li G, Dong G, Li B, Li Q, Kronzucker HJ, Shi W (2012) Isolation and characterization of a novel ammonium overly sensitive mutant, amos2, in *Arabidopsis thaliana*. Planta 235:239–252. https://doi.org/10.1007/s00425-011-1504-y

Li X, Rui J, Xiong J, Li J, et al (2014) Functional potential of soil microbial communities in the maize rhizosphere. PLoS One 9 e112609 https://doi.org/10.1371/journal.pone.0112609

Liu ZY (1997) Nitrogen in the rhizosphere. In: Zhu Z, Wen Q, Freney JR (eds) Nitrogen in soils of China. Developments in plant and soil sciences, vol 74. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-5636-3-10

Luo J, Tillman RW, Ball PR (2000) Nitrogen loss through denitrification in a soil under pasture in New Zealand. Soil Biol Biochem 32:497–509

Lynch JM (1987) The rhizosphere. Wiley Interscience, Chichester

Maathuis F (2009) Physiological functions of mineral nutrients. Curr Opin Plant Biol 12:250–258

Mahmood T, Woitke M, Gimmler H, Kaiser WM (2002) Sugar exudation by roots of kallar grass [Leptochloa fusca (L.) Kunth] is strongly affected by the nitrogen source. Planta 214:887–894

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press Ltd, London

Marschner H, Romheld V (1983) In-vivo measurement of root-induced pH changes of the soil-root interface: effect of plant species and nitrogen source. Z Pflanzenphysiol 111:241–251

Marschner H, Haussling M, George E (1991) Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce [Picea abies (L.) Karst.]. Trees 5:14–21

Mayumi D, Yoshimoto T, Uchiyama H, Nomura N, Nakajima-Kambe T (2010) Seasonal change in methanotrophic diversity and populations in a rice field soil assessed by DNA-stable isotope probing and quantitative real-time PCR. Microbes Environ 25:156–163

McClure PR, Kochian LV, Spanswick RM, Shaft JE (1990) Evidence for cotransport of nitrate and protons in maize roots. II. Measurement of NO_3^- and H^+ fluxes with ion-selective microelectrodes. Plant Physiol 93:290–294

McMurtrie RE, Nasholm T (2017) Quantifying the contribution of mass flow to nitrogen acquisition by an individual plant root. New Phytol 218:119–130

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663

Mengel K, Viro M (1978) The significant of plant energy status for the uptake and incorporation of ammonium nitrogen by young rice plants. Soil Sci Plant Nutr 24:407

Moreau D, Bardgett RD, Finlay RD, Jones DL, Philippot L (2019) A plant perspective on nitrogen cycling in the rhizosphere. Funct Ecol 33:540–552

Nakaya A, Onodera Y, Nakagawa T, Satoh K, Takahashi R, Sasaki S, Tokuyama T (2009) Analysis of ammonia monooxygenase and archaeal 16S rRNA gene fragments in nitrifying acid-sulfate soil microcosms. Microbes Environ 24:168–174

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670

Nour SM, Cleyet-Marel JC, Normand P, Fernandez MP (1995) Genomic heterogeneity of strains nodulating chickpeas (*Cicer arietinum* L.) and description of rhizobium mediterraneum sp. nov. Int J Syst Evol Microbiol 45:640–648

Peuke AD (1987) Der Effect yon Schwefeldioxid-, Ozon- und Stickstoffdioxid-Begasung auf den Stickstoff-Metabolismus steril kultivierter Fichtenkeimlinge [Picea abies (L.) Karst.]. Ph.D. Thesis, University of Gottingen

Pinton R, Varanini Z, Nannipieri P (2001) The Rhizosphere. Biochemistry and organic substances at the soil-plant Interface. Marcel Dekker, New York

Rinaudo G, Balandreau J, Dommergues Y (1971) Algal and bacterial non symbiotic nitrogen fixation in paddy soils. Plant Soil 35:471–479

Rome S, Fernandez MP, Brunel B, Normannd P, Cleyet-Marel JC (1996) *Sinorhizobium medicae* sp. nov., isolated from annual *Medicago* spp. Int J Syst Evol Microbiol 46:972–980

Ruan J, Gerendas J, Sattelmacher B (2000) Effect of nitrogen form and root zone pH on growth and nitrogen uptake of tea (Camellia sinensis) plants. Ann Bot 99:301–310

Ruan L, Bhardwaj AK, Hamilton SK, Robertson GP (2016) Nitrogen fertilization challenges the climate benefit of cellulosic biofuels. Environ Res Lett 11:064007. https://doi.org/10.1088/1748-9326/11/6/064007

Segovia L, Pifiero D, Palacios R, Martinez-Romero E (1991) Genetic structure of a soil population of non-symbiotic *Rhizobium leguminosarum*. Appl Environ Microbiol 57:426–433

Siddiqi MY, Glass ADM, Ruth TJ, Fernando M (1989) Studies of the regulation of nitrate influx by barley seedlings using $^{13}NO_3$. Plant Physiol 90:806–813

Smercina DN, Evans SE, Friesen ML, Tiemann LK (2019) To fix or not to fix: controls on free-living nitrogen fixation in the rhizosphere. Appl Environ Microbiol 85:e02546–18. https://doi.org/10.1128/AEM.02546-18

Son Y (2001) Non symbiotic nitrogen fixation in forest ecosystems. Ecol Res 16:183–196. https://doi.org/10.1046/1703.2001.00385x

Strong DT, Fillery IRP (2002) Denitrification response to nitrate concentrations in sandy soils. Soil Biol Biochem 34:945–954

Subbarao NS (2005) Soil Microbiology, 4th edn. Oxford and IBH Publishing Company Pvt Ltd, New Delhi

Sulieman S, Tran LS (2014) Symbiotic nitrogen fixation in legume nodules: metabolism and regulatory mechanisms. Int J Mol Sci 15:19389–19393. https://doi.org/10.3390/ijms151119389

Sun CH, Yu JQ, Hu DG (2017) Nitrate: a crucial signal during lateral roots development. Front Plant Sci 8:485. https://doi.org/10.3389/fpls.2017.00485

Suwa Y, Ostrom NE, Ostrom PH, Breznak JA, Gandhi H, Pitt AJ, Li F (2008) Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. Appl Environ Microbiol 72:638–644

Uren NC (2007) Types, amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R, Varanini Z, Nannipieri P (eds) The Rhizosphere. Biochemistry and organic substances at the soil- plant Interface. Taylor and Francis Group, Boca Raton, FL, pp 1–21

Vega NWO (2007) A review on beneficial effects of rhizosphere bacteria on soil nutrient availability and plant nutrient uptake. Rev Fac Nal Agr Medellın 60:3621–3643

Vergara C, Araujo KEC, Urquiaga S, Schultz N et al (2017) Dark septate endophytic fungi help tomato to acquire nutrients from ground plant material. Front Microbiol 8:2437. https://doi.org/10.3389/fmicb.2017.02437

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, Sprent JI (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57:1–45. https://doi.org/10.1023/A:1015798428743

Von Wiren N, Gazzarrini S, Frommer WB (1997) Regulation of mineral nitrogen uptake in plants. Plant Soil 196:191–199

Wang MY, Siddiqi MY, Ruth TJ, Glass AD (1993) Ammonium uptake by rice roots (II. Kinetics of 13NH4+ influx across the plasma- lemma). Plant Physiol 103:1259–1267

Wen X, Hu C, Sun X, Zhao X, Tan Q (2019) Research on the nitrogen transformation in the rhizosphere of the winter wheat (*Triticum aestivum*) under Mo addition. Enviro Sci Pollution Res 26:2363–2374

Wenzel WW, Wieshammer G, Fitz WJ, Puschenreiter M (2001) Novel rhizobox design to assess rhizosphere characteristics at high spatial resolution. Plant Soil 237:37–45

White JF, Crawford H, Torres MS, Mattera R, Irizarry I, Bergen M (2012) A proposed mechanism for nitrogen acquisition by grass seedlings through oxidation of symbiotic bacteria. Symbiosis 57:161–171

Yasuda T, Kuroda K, Hanajima D, Fukumoto Y, Waki M, Suzuki K (2010) Characteristics of the microbial community associated with ammonia oxidation in a full-scale rockwool biofilter treating malodors from livestock manure composting. Microbes Environ 25:111–119

Yoneyama T, Terakado J, Masuda T (1997) Natural abundance of 15 N in sweet potato, pumpkin, sorghum and castor bean: possible input of N_2 -derived nitrogen in sweet potato. Boil Fertil Soils 26:152–154

Yoneyama T, Terakado-Tonooka J, Minamisawa K (2017) Exploration of bacterial N2-fixation systems in association with soil-grown sugarcane, sweet potato, and paddy rice: a review and synthesis. Soil Sci Plant Nutr 63:578–590

Zhou Z, Takaya N, Nakamura A, Yamaguchi M, Takeo K, Shoun H (2002) Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. J Biol Chem 277:1892–1896

Chapter 6
Potential Contribution of Soil Microflora and Fauna in Nitrogen Cycle: A Comprehensive Study

Mitali Mishra, Kanchan B. M. Singh, Snehlata Rao, Veerendra Kumar, and Shweta Rai

Abstract Soil biota plays a critical role in the functioning of ecosystem. In terrestrial regions nitrogen is limiting constituent that determines the growth of plants, primary productivity therefore net productivity of an ecosystem can be controlled by combined activities of microflora and fauna of soil which facilitate the conversion of nitrogen to usable forms. Varieties of microorganisms that are highly diverse in terms of taxa and activities are engaged in N cycle including nitrogen fixers, ammonia oxidizers, autotrophic nitrifiers, heterotrophic nitrifiers, anammox bacteria, denitrifiers, archaea, and fungi. In addition, soil fauna perform the significant role in maintaining the structure and function of soil ecosystems including organic matter mineralization, productivity regulation, and nutrient cycling (N, S, C). Soil fauna generally includes nematodes, micro-arthopods, and macrofauna. These soil fauna play pivotal role in nitrogen cycling via performing mineralization process. In order to improve the effectiveness of agricultural systems and practices that ultimately lead to better productivity of food, understanding of different phases of N cycle along with activities of microflora and fauna is necessary. In this regard, present chapter centers on exploring variety of soil microflora and fauna and their potential roles in nitrogen cycle. Here, we also review critical insights related to various processes of nitrogen cycling in soil ecosystem and their ecological importance.

Keywords Productivity · Microflora · Nitrogen cycle · Soil fauna · Nutrient cycling · Mineralization · Soil ecosystem

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6.1 Introduction

Nitrogen is one of the important constituents of proteins, chlorophyll, DNA, and other biomolecules, therefore it is essential for survival of all living organisms present in the nature but is not directly accessible for organisms. The inaccessibility of nitrogen in dinitrogen form makes its flow restrict throughout the ecosystem. By the means of nitrogen cycle, nitrogen is converted into usable forms so that it becomes available to living organisms this makes the understanding of this cycle of great concern. Dinitrogen is converted to different states in the ecosystem in cyclic manner, these transformed states of nitrogen are used by living organisms of ecosystem for their growth and development (Pajares and Bohannan 2016). Also these transformations (conversion of nitrogen to different oxidation states) play important role in determining overall productivity of an ecosystem. Various processes in nitrogen cycle are responsible for generating nitrogen in various oxidation states. These processes are: N fixation, nitrification, mineralization, denitrification, anammox reactions, and ammonification. The reaction mechanisms of aforementioned processes are mainly dependent on activities of diverse soil microflora and fauna in orchestral manner. Soil microflora form a large fraction of the soil biodiversity and facilitate various processes in soil habitat (Fierer and Jackson 2006; Schimel and Schaeffer 2012). Wide variety of microbes like bacteria, archaea, and fungi possess astonishing diversity in their functioning and phylogenies play important roles in N cycling in soil. Many studies revealed that diverse varieties of bacteria facilitate N fixation, nitrification, anammox reactions therefore are the key players of nitrogen cycling soil (Hayatsu et al. 2008). Moreover, there are archaea that also mediate nitrification and anammox reactions. Recent studies have been carried out to explore the role of fungi in nitrogen cycling and it has been speculated that many types of fungi are potent contributors in producing nitrous oxide and dinitrogen in different ecosystems such as grasslands, forest, and semiarid areas. Fungi perform the respiration of oxygen, nitrite as well as fermentation of ammonia as substrate to generate energy for their survival in varying oxygen conditions.

There is plethora of literatures available explaining mechanisms of processes involved in soil N cycle (Wallenstein et al. 2006; Philippot et al. 2007; Hayatsu et al. 2008; Braker and Conrad 2011; Levy-Booth et al. 2014). Chief processes of N cycle in soil are depicted in Fig. 7.1**.**

Nitrogen cycle starts with nitrogen fixation in which nitrogen present in the air is transformed into ammonia by nitrogen fixers including *Azotobacter* and archaea. Some nitrogen fixers exhibit symbiotic relations with higher plants while some are free-living diazotrophs. Nitrification is the next process of N cycling in which ammonia in firstly converted into nitrite (NO_2^-) and ultimately into nitrate (NO_3^-). This oxidation is performed by taxonomical variants of microbes involving bacteria and archaea having oxidizing properties to oxidize ammonia and nitrite. These microorganisms are aerobes and mainly autotrophs while some are heterotrophic in nature. Autotrophic microbes mediated nitrification is observed to be predominant nitrification, however, heterotrophs mediated nitrification is found in

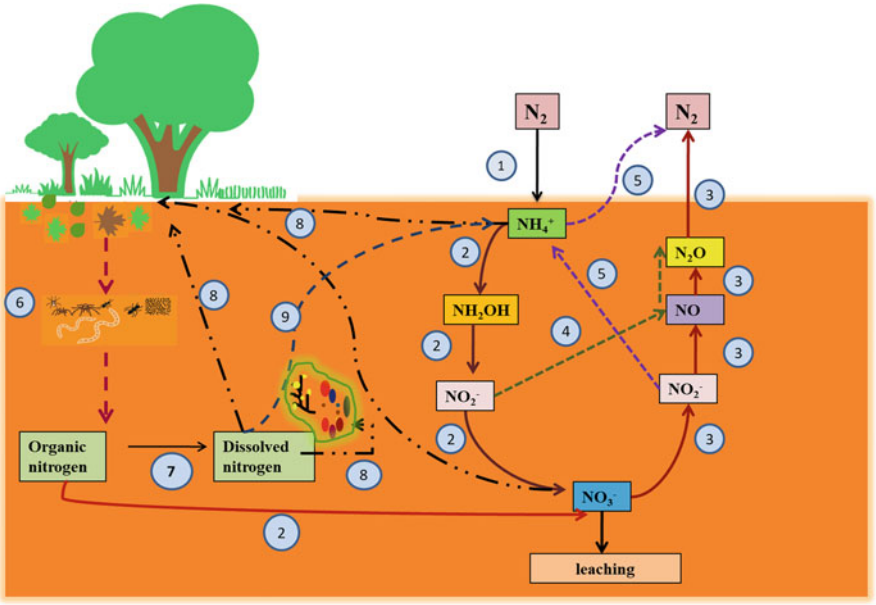


Fig. 6.1 Chief operations of N cycle in soil ecosystem (1) N fixation (2) Nitrification (3) Denitrification (4) Nitrifier denitrification (5) Anammox reaction (6) Decomposition (7) Mineralization (8) Assimilation (9) Ammonification

acidic soils of tropical and subtropical regions. In addition, direct oxidation of ammonia is carried out by some specific group of microbes called anammox organisms under anaerobic conditions. Nitrification process is one of the important processes in N cycling as it determines the level of nitrogen in soil. Denitrification is the anaerobic process of converting nitrite/nitrates into dinitrogen via forming nitrous oxide (N_2O) and nitric oxide (NO) in sequential manner. It is the ultimate pathway of nitrogen cycle for releasing fixed nitrogen to environment. It is also the only process of releasing N_2O and NO from the soil ecosytem (Houlton and Bai 2009). Taxonomically diverse assemblages of microbes are involved in this process. In soil, main players of this process are heterotrophic bacteria belonging to facultative aerobic groups (Philippot et al. 2007; Demanèche et al. 2009). Other denitrifiers are archaea and fungi (Cabello et al. 2004; Bartossek et al. 2010; Shoun et al. 1992; Hayatsu et al. 2008).

In addition soil fauna also plays pivotal role in N cycling. The wide distribution of fauna in soil and their activities are determining factors in regulating allocating organic components, storage of nitrogen and its discharge. In soil, wide variety of animals are present such as microfuana (nematodes, rotifera, protozoa), micro-arthopods/mesofauna (mites, collembola, chilopoda), and macrofauna (beetles, spiders, snails, earthworms, etc.) (Van Groenigen et al. 2015). Among these, earthworms are considered as protagonist as they affect structure and distribution of litter therefore they are linked with many aspects of nitrogen cycle (Shipitalo and

Bayon 2004; Blouin et al. 2013). These animals are found to increase the nitrogen mineralization by affecting the microbial communities of soil directly or indirectly. Microflora and fauna interact with each other either in facilitative manner or competitive manner (Heemsbergen et al. 2004; De Ruiter et al. 1995), in order to perform soil processes including N cycle.

Nitrogen cycle is a significant ecological process that determines the overall dynamics of soil in terms of net productivity, mineral composition, and biodiversity. In this context, this chapter provides detailed knowledge in order to reconcile mechanisms of processes involved in nitrogen cycling in soil and their ecological importance. In addition, this chapter presents the comprehensive portrayal of role of different microflora and macrofauna in nitrogen cycle as well as many factors, enzymes responsible for facilitating the processes.

6.2 Soil Microflora: Potential Player in Nitrogen Cycling

Microorganisms of soil perform a very significant function in nitrogen cycle. Diverse variety of soil microflora are involved in the N cycle like biological N fixing bacteria, NH_4^+ oxidizing bacteria, archea, heterotrophic nitrifiers and anammox bacteria, along with denitrifiers (archea, bacteria, and fungi) (Pajares and Bohannan 2016). Nitrogen is present in our environment in different oxidation states and inter-conversion between these different forms are mediated by microbes.

6.2.1 Bacteria

Bacteria are the key players of nitrogen cycle in soil. A large variety of bacteria are involved in this cycle like nitrogen fixers (e.g. *Cyanobacteria*, *Anabaena,* and *Nostoc*) that converts atmospheric nitrogen into ammonia; nitrifiers (e.g. *Nitrosomonas, Nitrobacter Nitrosococcus, Nitrococcus*) converts soil ammonia into nitrate; anammox bacteria (e.g. Ca. *Kuenenia,* Ca. *Anammoxoglobus,* Ca. *Jettenia,* Ca. *Brocadia*) converts ammonia into gaseous nitrogen with nitrate as electron acceptor, denitrifying bacteria (e.g. *Pseudomonas, Alcaligenes, Bacillus*) converts nitrates into nitrogen gas and archae bacteria (e.g. *Crenarchaeota, Pyrobaculum aerophilum, Halofera denitrificans*) can perform nitrification as well as denitrification process (Pajares and Bohannan 2016).

6.2.1.1 Nitrogen Fixers

Nitrogen fixers are the group bacteria which convert atmospheric nitrogen into ammonia. It is performed by free-living bacteria as well as by bacteria which form mutualistic association with plants. The process of biological nitrogen fixation is

very important for plants and animals as it provide ammonia. The nitrogen fixers also show symbiotic relationship with leguminous or non-leguminous plants. Non-symbiotic fixation can contribute nitrogen to the soil that is deficient in nitrogen. At least 26 genera of bacteria were recorded as nitrogen fixers in soil (Dilfuza 2012).

The atmospheric nitrogen is reduced by an enzyme known as Nitrogenase which is a protein complex and consists of two metal proteins. These are- *nitrogenase-molybdenum-iron protein* (*Mo-Fe protein*) and *nitrogenase iron protein* (*Fe protein*) or *the nitrogenase reductase*. The definite attachment and substrate (N) reduction location lies in the Mo-Fe-S-homocitrate clusters of the nitrogenase. Nitrogenase reductase (Fe protein) transfers e^- to dinitrogenase (Mo-Fe protein) by utilizing ATP (Dilfuza 2012). Mo-Fe protein is reducing site of the substrates, mainly N_2. Genes that code for various subunits of nitrogenase are NifH, NifD, NifK. Total 16 Nif genes are required for synthesizing active nitrogenase complex in *K. pneumoniae.* In terms of energy requirements of N fixation, it is energetically very expensive process as 2 moles of NH_3 are generated from 1 mole of N_2 with total expense of 16 ATP. Overall reaction of this mechanism is

$$N_2 + 16\,Mg - ATP + 8e^- + 8H+ \rightarrow 2NH_3 + H_2 + 16MgADP + 7.16Pi$$

Diazotrophs are the main performers of nitrogen fixation. Diazotrophs are those prokaryotes that can grow in devoid of fixed nitrogen and have features that can convert nitrogen into ammonia. Diazotrophs are either free living or symbiotic. Various types of diazotrophs have been discussed in Fig. 7.2**.**

The root exudates are the source of energy and carry out important ecological functions, principally N fixation by heterotrophs of soil. The favorable conditions for fixation in soil are moisture condition, oxygen concentration, presence of organic carbon substrates, and plants that are capable of releasing exudates (Shin et al. 2016).

6.2.1.2 Nitrifying Bacteria

Nitrifying bacteria converts ammonia into nitrates by stepwise oxidation. Nitrification is a main process and mediate an important function in dinitrogen availability. The process of nitrification can cause leaching of nitrite to ground water and production of nitrous oxide. Nitrous oxide can be produced either directly by the means of chemical degradation of hydroxylamine or indirectly by denitrification and nitrifier denitrification. Two types of nitrification pathways are take place in soil: Autotrophic nitrification and Heterotrophic nitrification (Hayatsu et al. 2008).

Autotrophic nitrification is performed by *AOB* or ammonium oxidizing bacteria involving *Nitrosomonas, Nitrosococcus, Nitrosospira*, species and *AOA* or ammonium oxidizing archaea including lineages of *Thaumarchaeota,* i.e. *Nitrososphaera* and *Nitrosotalea*. Other Group of bacteria involved in this process are *NOB* or nitrite-oxidizing bacteria, e.g. *Nitrobacter, Nitrospina, Nitrococcus,* and *Nitrospira* species.

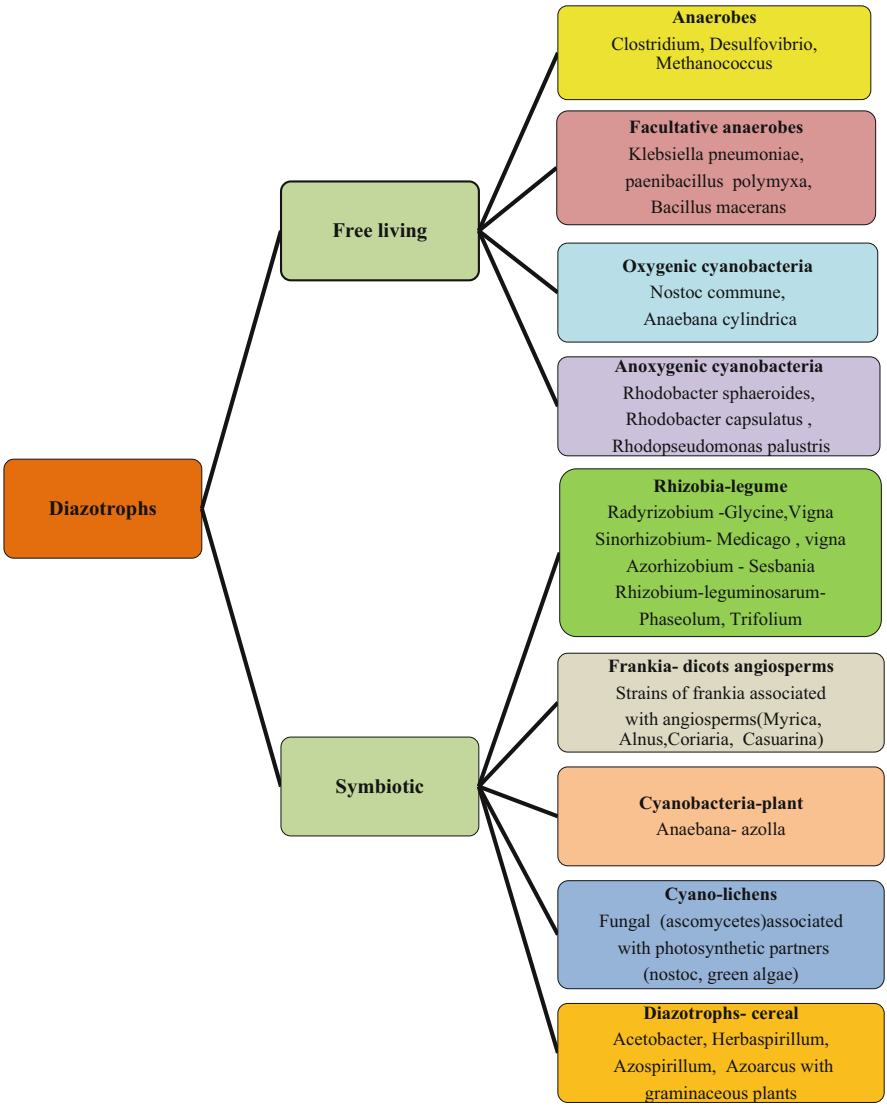
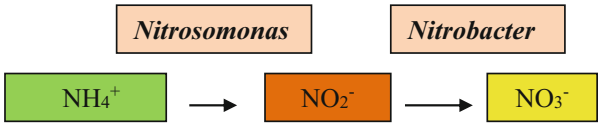


Fig. 6.2 Typical diazotrophs involved in nitrogen fixation

The heterotrophic nitrification can be done by the means of heterotrophs and fungal cells which can oxidize both organic and inorganic nitrogen compounds. Heterotrophic nitrification observed to play significant function in soil having acidic nature. Although the other physiological aspects of this process are not so clear, recent studies speculated that *P. dinitrificans, A. faecalis, P. putida,* and some other species of bacteria are involved in heterotrophic nitrification (Moir et al. 1996; Joo et al. 2005; Daum et al. 1998).

In nitrification, soil bacteria converts ammonium which accumulates in soil by many sources like waste of animals, composites, degrading crop residues, decomposing cover crop or fertilizers made up of urea or ammonium into nitrite/nitrates (Pajares and Bohannan 2016).

Nitrification is a two-step mechanism:



In first step, chemoautotrophic bacteria oxidize the ammonium to nitrite by using CO_2 as Carbon source. These microbes derive energy from this conversion. There are several microorganisms that are known to carry out this conversion, but the focus is mainly acquired by bacteria from *Nitrosomonas* genus.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{Energy}$$

Later the nitrite converts into nitrate by bacteria belonging to *Nitrobacter* and obtain energy. The nitrite should be completely converted into nitrate because it is toxic to plants.

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{Energy}$$

The energy obtained from both the processes is utilized by suite of microbes belonging to AOB and NOB categories for their biomass production.

Nitrification is one of the processes of ecological importance as it determines the availability of nitrogen in soil. Nitrate can be lost due to leaching, run off or denitrification from soil. In order to maintain its concentration in soil the nitrogen should present in ammonium form to minimize the loss. There are different factors of soil that affect nitrification process such as pH, oxygen content, moisture, temperature, organic constituents, agricultural practices, and occurrence of nitrifiers (Jalota et al. 2018).

6.2.1.3 Anammox Bacteria

Anaerobic ammonium oxidation (anammox) is a type of microbial process in which oxidation of NH_4^+ occurs into N_2 gas and nitrate acts as e^- acceptor. A chemist Engelbert Broda in 1997 envisaged the presence of bacteria which are previously unknown lithographic microbes that have capabilities to oxidize NH_4^+ to N_2 gas using NO_2^- or NO_x^- as an electron acceptor (Broda 1977). The occurrence of these bacteria was found in sludge in early 1990s. The anammox process was demonstrated in a bioreactor which was conducted in the Netherland (Mulder et al. 1995).

The anammox bacteria plays a specific role in N cycle, they were instigated to be a main source of N_2 gas globally.

Anammox process is mediated by bacterial members affiliated to the phylum *Planctomycetes* of the bacterial domain. These are ultra-structurally distinct from other bacteria in having intracytoplasmic membranes that compartmentalize the cell. The phylum Plantomycetes have been classified into five genera based on the 16S rRNA and 23S rRNA gene sequence identities: *Candidatus Kuenenia, Candidatus Brocadia, Candidatus Jettenia,* and *Candidatus Scalindua* (Jetten et al. 1997). The stoichiometry of the anammox process was first described in 1998 (Strous et al. 1998) and was re-proposed utilizing an enriched annamox planktonic culture (Lotti et al. 2014). The reaction proposed was

$$\mathrm{NH4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+} \rightarrow \mathrm{0.986\ N_2 + 0.161NO_3^- + 2.002H_2O + 0.071CH_{1.74}O_{0.31}N_{0.20}}$$

In the anammox process following three enzymatic reactions are involved (Kartal et al. 2013).

1. *Reduction of NO_2^- to NO*: $NO_3^- \rightleftarrows NO_2^- \rightarrow NO$, in this reaction the conversion of NO_3^- into NO_2^- is catalyzed by nitrate/nitrite oxido-reductase (nxrAB); conversion of NO_2^- into NO is catalyzed by *nir*S (cyt cd_1-type nitrite reductase and *nir*K (Cu containing nitrite reductase).
2. *Biosynthesis of hydrazine from NO and NH_4^+*: $NO \rightarrow N_2H_4$ and $NH_4^+ \rightarrow N_2H_4$, these reactions are catalyzed by *hzs* (hydrazine synthase).
3. *Dehydration of hydrazine to N_2 gas*: $N_2H_4 \rightarrow N_2$, this reaction is catalyzed by *hdh/hao* (hydrazine dehydrogenase/hydroxylamine dehydrogenase).

Varieties of nitrite reductase are used among the anammox bacterial group. As their physiological pathways are species specific, some physiological aspects are deduced from the genomic information and biochemical experiments. The biosynthesis and dehydration of hydrazine takes place in a membrane linked intracytoplasmic cellular compartment of anammox bacteria, known as the anammoxosome (Van der Star et al. 2010; de Almeida et al. 2015). The anammoxosome contains remarkable lipids, termed as ladderane lipids. These lipids are made up of cyclobutene moieties which are linear and present in the form of chain (Rattray et al. 2008; Damsté et al. 2002). From the microbiological and physiological perspectives, the ecological and physiological properties of anammox bacteria are of great interest.

In the various types of anoxic ecosystem including marine, brackish, freshwater, and terrestrial environments the activities and population of anammox bacteria have been found. Some populations of these bacteria were also found in harsh and adverse environment like hydrothermal vents, hypersaline basins, sea ice, permafrost soil and oil contaminated fields. Many researchers speculated that anammox bacteria are mainly found in ecosystems devoid of oxygen. These habitats of these microbes are determined by their genus and species. Parameters that affect the activity of

anammox bacteria like NO_x^- concentration, salinity, sediment reactivity (Nicholls and Trimmer 2009), MnO_2 concentration (Engström et al. 2005), pH (Li and Gu 2013), temperature (Brin et al. 2017), the molar ratio of NH_4^+ to NO_x^- (Li and Gu 2013).

6.2.1.4 Denitrifying Bacteria

Denitrification is a process in which microbes use NO_3^- or NO_2^- as a substitute of oxygen to acquire energy. The nitrates are reduced to nitrogen gas in a sequential manner via intermediary compounds NO and N_2O. Denitrification comprises of four reactions catalyzed by different types of metallic enzymes (nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase). Among these, Nitrite (NO_2^-) reductase is the crucial enzyme as it catalyzes the rate limiting step in which nitrite oxide is reduced to gaseous products (Henry et al. 2004; Zumft 1997).

Several facultative aerobic heterotrophic bacteria that reside in soil mainly carry out the denitrification. In the absence of oxygen as the terminal electron acceptor they switch from the aerobic respiration to denitrification. For example, some of the bacteria involved in denitrification are *Pseudomonas, Bacillus, Alcaligenes,* and *Paraccocus* (Philippot et al. 2007). Some autotrophic bacteria like *Thiobacillus denitrificans* also performs the denitrification process (Demanèche et al. 2009). The polyphyletic distribution of denitrifying genes in several microbial species can lead to the co-occurrence of these genes with the ammonia oxidation and N fixation genes (Bedmar et al. 2005; Hayatsu et al. 2008). The genus *Nitrospora* or *Nitrosomonas* and many species of diazotrophic bacteria, like *Azospirillum* and *Bradyrhizobium* are capable of denitrification (Rösch et al. 2002; Shaw et al. 2006).

The most common denitrification process, which is the reduction of nitrogen oxides into gaseous N_2 is outlined in Eq. (7.1).

$$2\,NO3- + 10\,e^- + 12\,H+ \rightarrow N2 + 6\,H2O \tag{6.1}$$

The reaction in Eq. (7.1) can be divided into different half reactions;

1. Reduction of nitrate to nitrite catalyzed by nitrate reductase (Nar)

$$NO_3^- + 2\,H^+ + 2\,e^- \rightarrow NO_2^- + H_2O$$

2. Reduction of nitrite into the nitric oxide catalyzed by nitrite reductase (Nir).

$$2\,NO2^- + 4\,H + + 2\,e^- \rightarrow 2\,NO + 2\,H2O$$

3. Reduction of nitric oxide into nitrous oxide catalyzed by nitric oxide reductase (Nor).

Table 6.1 Gene and enzymes involved in denitrification process

Reduction process	Gene	Enzymes
$NO_3^- \rightarrow NO_2^-$	*nar*G	Nitrate reductase (Nar)
$NO_2^- \rightarrow NO$	*nir*K/S	Cu/Cd—Nitrite reductase (Nir)
$NO \rightarrow N_2O$	*nor*B	Nitric oxide reductase (Nor)
$N_2O \rightarrow N_2$	*nos*Z	Nitrous oxide reductase (Nos)

$$2\,NO + 2\,H^+ + 2\,e^- \rightarrow N_2O + H_2O$$

Reduction of nitrous oxide to dinitrogen catalyzed by nitrous oxide reductase (Nos).

$$N_2O + 2\,H^+ + 2\,e^- \rightarrow N_2 + H_2O$$

Products generated at any stage in the denitrification process can be interchanged with the soil environment. Denitrifiers are very diverse on the basis of functional genes involved (Table 7.1) in the process which are used as the genetic markers, such as *nar*G and *nap*A (nitrate reductase), *nir*K and *nir*S (nitrite reductase), *nor*B (nitric oxide), and *nos*Z (nitrous oxide) (Henry et al. 2006; Kandeler et al. 2006; Smith et al. 2007; Yu et al. 2014). Denitrifying microorganisms follow different denitrification pathway due to difference in the combination of enzymes involved in their specific process.

Denitrification process plays a significant role in the N_2–cycle, as it affects the soil nitrogen concentration. The denitrifying soil microbes are capable of converting nitrate into various gases under certain conditions. It occurs mostly in warm, wet, and waterlogged soil with an abundance of nitrate. Although, denitrification plays a key role in the aquatic ecosystem and waste water treatment in lowering the nitrate concentration, this process is not very beneficial for the soil crop as the loss of NO_3^- by denitrification has negative economic impact since crucial N-fertilizer get lost from the soil.

Denitrification is very sensitive process to the various environmental factors, so the ecology of these microorganisms mediates a pathway which can be used in the research for the alleviation of the climate changes (Richardson et al. 2009). Most of the studies are going on in the temperate soil as it more sensitive to the environmental factors and its role in nitrogen loss. Denitrification mechanism and denitrifiers in tropical forest varies to a great extent from the temperate soil areas as tropical forest is the major repositories of the biodiversity, defined by high precipitation, small annual temperature variability, high heterogeneity in plant diversity and distinctive soil profile (Zhang et al. 2009; Xu et al. 2013). They contribute through the exchange of gases, water, energy, and nutrients in the biogeochemical cycles. Tropical forest soil, nevertheless, has a relatively lower denitrification propensity than temperate forest soil owing to heterogeneity in edaphic conditions.

Denitrification is an important microbial process by which nitrogen oxides are reduced to N_2 gas to maintain the continuity of the nitrogen cycle and governing the

climate change by analyzing the effect of environmental factors. The loss of nitrogen by denitrification process leads to the nitrogen fixation in the atmosphere (Philippot et al. 2007), river, lake, and coastal marine ecosystems. In the waste water treatment denitrifying bacteria plays a key role in reduction of nitrate into the gaseous nitrogen. In fresh water sediments denitrification appears to remove a greater amount of the mineralized nitrogen.

There are several physiological traits that are important to the denitrification process, such as soil pH, humidity, temperature, and redox potential (Zhang et al. 2014) of the reduction process. Soil microbial process are affected by the temperature, denitrification occurs more rapidly at temperature between 80 and 100 °C. Soil pH positively affects both the denitrifying and the total bacterial population (Yu et al. 2014), but the denitrifiers are more sensitive to acidic environment. O_2 partial pressure is also an important physiological aspect in the denitrification process as the enzymes involved in the process are more sensitive towards the oxygen. Soil moisture is also an essential aspect in N_2O emission as it influences the oxygen availability in soil microbes. In the oxygen deficiency condition, certain bacteria develop new strains using nitrate as the terminal electron acceptor, whereas some others require ammonium or growth factors for denitrification to occur in growth media. Denitrification occurs rapidly when the pore space filled with water exceeds 60% (Davidson et al. 2000) and the final product depends on the soil condition and microbial community.

6.2.1.5 Archaea Bacteria

Archaea is a domain which acts as a connecting link between the prokaryotes and the eukaryotes. These are metabolically very diverse in nature. They have unique ability to exist in extremely adverse habitats like hypersaline environment, high temperature, and low pH and acute anoxia. The archaeal adaptations in harsh environments can be represented by three ecological categories: thermophilic, methanogenic, and halophilic. Archaea bacteria are ubiquitous in nature as they also thrive in freshwater sediments, temperate soil, and other less adverse environment conditions. Crenarchaeota are archaea that are present in moderate habitats conditions (Bintrim et al. 1997; Buckley et al. 1998; Nicol et al. 2005; Oline 2006). Archaea is considered as the reservoir of novel genes.

Archaea bacteria are different from other bacteria in structure as they lack peptidoglycan in the cell wall and contain cell membrane that enclose lipids with the hydrocarbons not fatty acids. The archaea bacteria are evolutionary different from the other bacteria in having distinct features. Archaea resembles eukaryotes more than the eubacteria and their replication mechanism are also more like eukaryotes.

These bacteria are capable of performing different types of reductive pathways of the N cycle which includes both assimilatory process like N_2 fixation and NO_3^- reduction, and dissimilatory process like denitrification. Archae bacteria also work as an ammonium oxidizer in the N cycle.

Both archae bacteria and bacteria require a functional enzyme for the ammonium oxidation that is ammonium monooxygenase (AMO) for the oxidation of ammonium into hydroxylamine. *Nitrosopumilus maritimus* (Könneke et al. 2005) a Crenarchaeota is an ammonium oxidizing bacteria is phylogenetically included in the marine group. The gene which encodes the ammonium monooxygenase is known as *amo* gene. The *amo* gene differs greatly in the Crenarchaeota and Proteobacteria (Treusch et al. 2005).

Nitrification reaction: oxidation of ammonium into hydroxylamine catalyzed by *amo*A (Eq. 7.2).

$$NH_4^+ - amoA \rightarrow NH_2OH \tag{6.2}$$

And further oxidation of NH_2OH into NO_2^- takes place for the continuation of N cycle.

Several archaea bacteria are capable of the denitrification process, such as *Pyrobaculum aerophilum* (Hyperthermophile) and *Haloferax denitrificans* (Halophile) (Cabello et al. 2004). They follow a dissimilatory pathway by reduction of various nitrogen oxides like nitric oxide and nitrous oxide into nitrogen gas.

Ammonium oxidizing archaea bacteria (AOA) has been found in higher temperature in both soil and in freshwater (predominately *Nitrosotenuis*) (Santoro et al. 2015). These archaea bacteria are classified in five genera: *Nitrososphaera, Nitrosocosmicus, Nitrosocaldus, Nitrosotalea,* and *Nitrosopumilus* (Pester et al. 2012). Environmental pH and temperature appear to be a major driver of the diversification of ammonium oxidizing archae bacteria. These bacteria are also found in the extremely harsh condition where ammonium oxidizing bacteria (AOB) are unable to do the denitrification process. In contrast with the ammonium oxidizing bacteria (AOB), ammonium oxidizing archaebacteria (AOA) can also grow in cyanate medium (Palatinszky et al. 2015) other than urea. Ammonium concentration is critical to the distribution of AOB and AOA (Bates et al. 2011; Verhamme et al. 2011). The ammonium oxidizing bacteria prefer higher ammonium concentration for the nitrification process but ammonium oxidizing archaebacteria prefer lower ammonium concentration. Salinity also affects the ecological distribution of archaebacteria.

There is a difference between the nitrification process of the archaea bacteria and the bacteria as they have difference in their rRNA structure and gene involved in the process. Substrate affinity is lower in the ammonium oxidizing bacteria (AOB) than the ammonium oxidizing archaebacteria (AOA). The AOB and AOA show different affinity towards the substrates. Kinetics of the process is also different for the ammonium oxidizing bacteria and ammonium oxidizing archaebacteria. AOB have higher K_m constant (half-saturation value) than the AOA (Kits et al. 2017; Martens-Habbena et al. 2009; Prosser and Nicol 2012). Archaea bacterial nitrification dominates in the low nitrogenous soil and in the hypersaline oceans.

Archaea bacteria and eubacteria follow different carbon assimilation mechanisms. Bacteria follow Calvin cycle for carbon assimilation and archaebacteria fix

HCO_3^- via hydroxy propionate-hydroxybutyrate cycle (Berg et al. 2007). Soil pH also affects the kinetics of the nitrification process by archaea bacteria. On decreasing the pH, the availability of ammonium ion decreases in the soil which is favorable for the archaea bacterial nitrification, due to this reason ammonium oxidizing archae bacteria have lower half-saturation value (K_m constant).

So, the physiological traits are also responsible for the distribution of the archaea bacteria. Archaea bacterial denitrification process is not favorable in waste water treatment. The archaeal denitrification occurs in the open oceans, hypersaline environments and in hyperthermal conditions where bacterial denitrification is not favorable.

Archaea bacteria solves the mystery that how nitrification process is possible in the acidophilic soil and in hot-spring. They contribute to the Nitrogen cycle at a global scale. Previously it was assumed that ammonium oxidizing bacteria contributes majorly in the nitrification process, but with the discovery of the ammonium oxidizing bacteria (AOA), the statistics need to be reevaluated. Although archae bacteria are not majorly involved in the nitrification and denitrification process in the nitrogen cycle but these primarily contribute to the process in many extremophilic environments.

6.2.2 Role of Fungi in Nitrogen Cycling

Fungi are adapted to the diverse range of habitats. In biogeochemical cycles their role is mainly focused in the decomposition of plant litters. They participate in the conversion of dead remains of plant or animals or their excretory product to ammonia (NH_3) with bacteria. The involvement of fungi in the nitrogen cycle can be observed in different types of processes like denitrification, codenitrification, and ammonium fermentation.

Denitrification is a process of formation of nitrous oxide or nitrogen via reduction of NO_3^- /NO_2^- respectively, by microbes. It is the process of dissimilation. In many studies, it has been believed that this process is solely performed by prokaryotic organisms and process of bacteria (*Paracoccus, Pseudomonas*) mediated denitrification has been discussed (Zumft 1997). Further the role of fungi in reduction of nitrite to nitrous oxide (N_2O) has been depicted in a study, in which it has been observed that *F. oxysporum* and *F. solani* have nitrite reducing capabilities in low O_2 state (Bollag and Tung 1972). Shoun et al. (1992) found that several phyla of fungi are involved in the denitrification activity including Ascomycota (*C. tonkinense, Gibberella fujikuroii)* as well as Basidiomycota (*Trichosporon cutaneum*). The biochemical and physiological role of soil fungi in denitrification was investigated by isolating pure cultures fungi (Shoun 2006). Recent studies have shown that fungi can perform reduction process in the presence of oxygen as well as in the absence of oxygen. According to the ability of reducing NO_3^- or NO_2^- in dissimilatory manner two categories of denitrifying fungi have been defined. One category of fungi reduces both NO_3^-or NO_2^- into N_2O, such as *Fusarium oxysporum* and *Gibberella*

fujikuroii (Shoun and Tanimoto 1991) and the other category that can reduce only NO_2^- into N_2O (Shoun et al. 1992).

Fungi mediated denitrification specifically take place in its mitochondrial chamber, later is also involved in performing respiration in anaerobic state as observed in many bacterial cells (Kobayashi et al. 1996). Nitrate reductase (*Nar*) involved in dissimilation process when extracted from denitrifier *F. oxysporum,* it was observed that this enzyme was not identical to soluble nitrate reductase enzyme involved in assimilation process (Uchimura et al. 2002). This enzyme (Nar) found to be similar to nitrate reducing enzyme involved in dissimilation process performed by other denitrifier bacteria.

Nitrite reductase (Nir) also plays the key role in the dissimilation process. This enzyme is divided into two types on the basis of their structure:

1. copper containing Nitrite reductase (Cu-Nir),
2. heme c and heme d1 containing Nitrite reductase (cd1-Nir).

Genes involved in expression of these enzymes are *nirK* and *nirS,* respectively (Zumft 1997). Although both of nitrite reductases is similar in function. Fungal nitrite reductase (Nir) contains copper and orthologous to the *nir*K of bacterial cells (Kobayashi et al. 1996). Fungal nitric oxide reductase differs from bacterial ones due to having different types cytochromes. Based on 1° and 3° structures of cytochromes, the fungal nitric oxide reductase has been placed in the cytochrome oxidases superfamily of P450. Several gene analysis predicted the extensive occurrence of P450nor in many fungal cells. Nitric oxide reductase (P450nor) of fungal origin reduces NO to N_2O using direct electron donor like NADPH or NADH (Kudo et al. 2001; Nakahara et al. 1993). This reaction can be represented as equation given below is catalyzed by P450nor (Shiro et al. 1995) and occurs mainly in limited supply of oxygen

$$2NO + NAD(P)H + H^+ \text{———P450nor} \rightarrow N_2O + NAD(P)^+ + H_2O$$

6.2.2.1 Role of Arbuscular Mycorrhizal Fungi in Nitrogen Cycle

Arbuscular mycorrhizas (AM) are the symbiotic association of the plant roots and specific soil fungi that can boost the plant nutrient accession. In this type of symbiosis, fungi enter into root cortical cells of tracheophytes by the means of arbuscules (Smith and Read 2010). AMF are obligate symbiotic microbes due to having restricted saprophytic activities and therefore predominantly dependent on the host plant for their nutrition (Harley and Smith 1983).

Arbuscular mycorrhizal fungi (AMF) play significant function nitrogen nutrition for plants. It has been observed that the positive effect of AMF symbiosis on productivity of crops rely solely on the type of AMF associated (Johnson et al. 1992; Sanders 2003), nutrient available (Liu et al. 2000a, b), ecological conditions

generated by the practices performed in crop field (Johnson et al. 1992; Johnson 1993; Corkidi et al. 2002), as well as genetic constitution of the generated crops (Liu et al. 2000b, 2003). AMF have potential to enhance the plant nitrogen uptake by proliferating their hyphae, and acquire nitrogen (N) from the organic sources. These can transfer inorganic nitrogen in the form of NO_3^- or NH_4^+ to their host plants.

Nitrogren transfer mechanism mediated by fungi to their respective host was instigated in many studies (Bago et al. 2001). This mechanism includes a pathway through which nitrogen is transferred from ERM to IRM (extra-radical mycelia to intra radical mycelia) in the form of an amino acid, i.e. Arg (Arginine). This pathway is commonly known as GOGAT pathway or glutamine synthetase (GS)/glutamate synthase pathway. Nitrogen moves from the soil into the fungal ERM through a stepwise metabolic conversion into Arg, which is transported into the IRM within the plant root and converted into ammonia. This ammonia is transported to the host root and get assimilated. In order to carryout of this process, array of genes are constitutively expressed. About 11 genes were cloned from *G. intaradices* which are found to be expressed during the assimilation as well as in metabolism.

The nitrogen cycling is an extremely dynamic process in the soil. In addition, to improve the plant nitrogen uptake AM plays a pivotal part in soil nitrogen cycling. For instance, nitrogen uptake from decaying organic matter is mediated by huge amount of AMF in the soil, indicates their potential involvement in soil N cycling (Hodge and Fitter 2010). As AMF increase the immobilizing potential of nitrogen therefore it can be concluded that root-mycorrhizal symbiosis may have higher capability of decreasing gaseous loss of nitrogen in comparison to non-symbiotic roots (Asghari and Cavagnaro 2011).

AMF are commonly associated with plants developing in the nutrient rich soils as well as are of greater significance for plants developing in soil belongs to volcanic regions and sand dune as these soil systems are nutrient deficient. AM fungi are mostly found in regions like tropical forests and temperate grasslands as these regions provide broad range of highly diverse hosts to colonize (Smith and Read 2010). They are also of greater importance as biofertilizers and in enhancing crop productivity by improving the soil fertility.

6.2.2.2 Other Fungi

Many studies have instigated the role of fungi in generation of N_2O and dinitrogen in different soil ecosystems. Some fungi mediate codinitrification and ammonia fermentation in order to survive in different oxygenic conditions.

Codenitrification

Under denitrifying condition, denitrifying fungi converts azides, ammonium ions and other nitrogen compounds into hybrid Dinitrogen or Nitrous oxide molecules. This phenomenon was named as “codenitrification” as azide and ammonium

ions which themselves are not capable of inducing denitrification mechanism, can be denitrified by a system induced by nitrites/nitrates (Shoun et al. 1992; Tanimoto et al. 1992). *Fusarium solani* and *Cylindrocarpon tonkinense* produce N_2 by codenitrification in the presence of amino acids. In the presence of co-substrates like ammonia *F. oxysporum* produce N_2O from NO_2^- through both codenitrification and denitrification. Codenitrification reaction catalyzed by the fungal P450 nor to form a hybrid N_2O from NO and other co-substrates (N_3^- or NH_4^+) in absence of an e^- donor, like NADH. Therefore, in *F. oxysporum* P450nor is considered as a multi-functional enzyme as it catalyzes denitrification and codenitrification both in the presence of NADH or co-substrates, correspondingly (Su et al. 2004). There is a difference between the denitrification and codenitrification as the denitrification pathway combines two molecules of NO_3^- or NO_2^- in stepwise manner into N_2 or N_2O, whereas codenitrification pathway joins nitrogen atoms from nitrite and other nitrogeneous compounds.

Fermentation of Ammonia

In nitrate metabolism, ammonia fermentation is another type of dissimilatory process which has also been observed in the denitrier *F. oxysporum* (Takasaki et al. 2004; Zhou et al. 2001). In this process nitrate which gets reduced to ammonia and ethanol is simultaneously get oxidized into acetic acid to form ATP. Nitrate act as the terminal electron acceptor for fermentation in this pathway but not for anaerobic respiration. Ammonia fermentation needs more anoxic condition than denitrification process for the fungal growth. Fermentation of ammonia has been observed in many other soil fungi as well.

6.3 Role of Soil Fauna in Nitrogen Cycle

Soil fauna consist many invertebrates which participate in Nitrogen Cycle directly or indirectly (Coleman and Wall 2015). Soil fauna consist many invertebrates which participate in Nitrogen Cycle directly or indirectly (Coleman and Wall 2015). About 30% of total mineralization is contributed by soil fauna communities. These soil fauna are categorized into microfauna that feeds on bacteria (nematodes, protozoa), mesofauna include mites, collembola, Acari and macrofauna include earthworm, ants, termites, etc. Soil fauna influence the nitrogen mineralization by forming interactions with different trophic levels via predating microbes, fragmentizing organic constituents of soil, amalgamating organic constituents into soil. Activities of these fauna alter the soil porosity and aggregates formation thereby mediate changes in soil structure. Burrow formation by earthworms create favorable flow trail resulting in the loss of inorganic nitrogen as well as dissolved nitrogen via leaching (Domínguez et al. 2004). In addition, smaller fauna also alter the soil structure by producing fecal pellets and increasing porosity of soil (Topoliantz

et al. 2000; Van Vliet et al. 2004). Soil fauna also make the litter constituents available to microbes by decomposing process thereby directly affects soil microflora communities hence nitrogen dynamics of soil (Edwards 2000).

Various types of interactions are established by soil fauna with other organisms present in soil. Among them is facilitative interaction (Heemsbergen et al. 2004) in which one fauna community benefits other, e.g. isopod alters the structure of soil as well as facilitates litter decomposition thus promotes growth of microbes (Wardle 2006). Some soil fauna competitively interacts with other but affects the mineralization process positively (Loreau 1998), for example, mites predate on fungi consuming mites, springtails, potworms, nematodes (de Ruiter et al. 1995) resulting in increase in the numbers of microbes as their feeders are eaten up by microbial feeders. Although these interactions play pivot roles in nutrient cycling still there are gaps in understanding these mechanisms and their importance.

In this section we have discussed some of the important fauna related to N cycling of soil.

6.3.1 Annelids

The annelids are a phylum of invertebrates containing vast variety of species including earthworms. Earthworms, scientifically known as *Lumbricus terrestris*, belong to the class Oligochaeta and play a very important role in nitrogen cycle. These worms play a crucial role in forming soil structure (e.g. aggregate or crumb formation, pore formation) as well as breakdown the organic matter applied to the soil (fragmentation, burial, and mixing of plant residues). The importance of earthworm as a biological agent came into existence after Darwin's book "The formation of Vegetable Mould" which forms the basis of modern era research. Earthworm contributes nitrogen to the soil by mineralization of organic matters. This mineralization is increased by enhancing the process of nitrification in their casts. Earthworms also affect the microbial community directly or indirectly by increasing the process of nitrogen mineralization (Bhadauria and Saxena 2010).

In addition, these worms also play role in passing significant amount of nitrogen to terrestrial ecosystem. Upon decomposition, 70% of nitrogen present in their tissues are mineralized in 10–20 days. Nitrogen fixing bacteria present in the gut and casts of the earthworms are responsible for the nitrogen fixation and increased activity of nitrogen in cast as compare to soil (Chauhan 2014).

Earthworms provide more amount of soil nitrogen to the plant as compared to the total input provided by addition of slashed vegetation, inorganic and organic manure, recycled crop residues and weeds. The cropping system and fertilizer used (i.e., mineral or organic) are the factor which affect or increase the functioning of earthworm during Nitrogen cycle (Bhadauria and Saxena 2010).

6.3.2 *Nematoda*

Nematoda is a group of invertebrates, consisting of nematodes or commonly known as roundworms. These worms seem to be closely related to the molting animals (Ecdysozoa), such as Nematophora and present in majority on the Earth (every 4 in 5 animals are nematodes). They commonly live in water film or water filled pores spaces in soils. As below ground parasite as well as plant pathogen, nematodes plays an important role in increasing the net primary productivity and regulating decomposition, hence maintaining the functioning of ecosystem (Coleman and Wall 2015).

Nematodes present in soil help in nitrogen cycling by increasing the secretion of ammonium from microbes (bacterial communities and fungal communities). About 27% of nitrogen present in soil is contributed by free-living nematodes that are feeders of bacteria and fungi. It has been seen that the diversity of soil nematodes increases as nitrogen is added in soil, which in turn, increases the productivity of plant. On the other hand, it can also affect the nematode communities due to allocation of belowground carbon to soil biota. The response of nematodes to nitrogen addition vary with time, soil nature, sampling depth, and ecosystem variety. In addition, it is also affected by precipitation as nematodes depend upon water for their movement and migration towards prey (Song et al. 2016).

6.3.3 *Arthropoda*

Arthropoda are the invertebrates and in soil it present about 85%. Arthropoda contain five classes—Isopoda, Myriapoda, Insecta, Acari, and Collembola. Arthropoda works as plant litter transformers and ecosystem engineers in soil food web. Soil fauna consist of 23% of arthropods or about 360,000 species (Nardi et al. 2002). Arthropoda needs higher nitrogen and carbon ratios for survival and growth but they do not obtain sufficient nitrogen from their diet and they depend on other source for nitrogen requirements. Some arthropods fulfill their requirement of nitrogen by symbiotic microbes present in their hindguts by obtaining additional nitrogen as a result of nitrogen fixation (Nardi et al. 2002). Arthopodes like termites are also able to assimilate non-dietary nitrogen through fixation of atmospheric nitrogen by intestinal bacteria (Culliney 2013).

6.4 Conclusion

Nitrogen cycle is one of the significant biological cycles that determine overall productivity of an ecosystem. Previously it was thought that only bacterial communities are potent contributors of N cycle and nitrogen fixation, aerobic nitrification, anaerobic denitrification are only the processes of nitrogen transformations. As

described in the present study, however, other novel processes are also persist in soil ecosystem that transforms the nitrogen in other forms and are driven bacteria as well as archaea and fungi. Although, some forms of nitrogen act as pollutants and can adversely affect human and environmental health hence, understanding of microflora and fauna mediated different processes involved in transformations of nitrogen is essential for understanding and managing health and productivity of ecosystem. Soil fauna also play pivot role in nitrogen cycling mainly in mineralization process, however, there is a need of exploring interactive mechanisms of soil fauna with other living organisms extensively as well as their functional diversities relative to nitrogen cycling beyond mineralization. In order to investigate diversity in microflora, enzymes and processes specific isolation techniques as well as molecular tools should be developed.

References

Asghari HR, Cavagnaro TR (2011) Arbuscular mycorrhizas enhance plant interception of leached nutrients. Funct Plant Biol 38:219–226. https://doi.org/10.1071/FP10180

Bago B, Pfeffer P, Shachar-Hill Y (2001) Could the urea cycle be translocating nitrogen in the arbuscular mycorrhizal symbiosis? New Phytol 149:4–8. https://doi.org/10.1046/j.1469-8137.2001.00016.x

Bartossek R, Nicol GW, Lanzen A, Klenk H-P, Schleper C (2010) Homologues of nitrite reductases in ammonia-oxidizing archaea: diversity and genomic context. Environ Microbiol 12:1075–1088. https://doi.org/10.1111/j.1462-2920.2010.02153.x

Bates ST, Berg-Lyons D, Caporaso JG, Walters WA, Knight R, Fierer N (2011) Examining the global distribution of dominant archaeal populations in soil. ISME J 5:908–917. https://doi.org/10.1038/ismej.2010.171

Bedmar EJ, Robles EF, Delgado MJ (2005) The complete denitrification pathway of the symbiotic, nitrogen-fixing bacterium Bradyrhizobium japonicum. Biochem Soc Trans 33:141–144. https://doi.org/10.1042/BST0330141

Berg IA, Kockelkorn D, Buckel W, Fuchs G (2007) A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in archaea. Science 318(5857):1782–1786. https://doi.org/10.1126/science.1149976

Bhadauria T, Saxena KG (2010) Role of earthworms in soil fertility maintenance through the production of biogenic structures. Appl Environ Soil Sci 2010:1687

Bintrim SB, Donohue TJ, Handelsman J, Roberts GP, Goodman RM (1997) Molecular phylogeny of archaea from soil. Proc Natl Acad Sci U S A 94:277–282. https://doi.org/10.1073/pnas.94.1.277

Blouin M, Hodson ME, Delgado EA, Baker G, Brussaard L, Butt KR, Dai J, Dendooven L, Peres G, Tondoh JE, Cluzeau D, Brun J-J (2013) A review of earthworm impact on soil function and ecosystem services. Eur J Soil Sci 64:161–182. https://doi.org/10.1111/ejss.12025

Bollag J-M, Tung G (1972) Nitrous oxide release by soil fungi. Soil Biol Biochem 4:271–276. https://doi.org/10.1016/0038-0717(72)90021-1

Braker G, Conrad R (2011) In: Laskin AI, Sariaslani S, Gadd AM (eds) Diversity, structure, and size of N2O-producing microbial communities in soils—what matters for their functioning? Academic Press, Amsterdam, pp 33–70. https://doi.org/10.1016/B978-0-12-387046-9.00002-5

Brin LD, Giblin AE, Rich JJ (2017) Similar temperature responses suggest future climate warming will not alter partitioning between denitrification and anammox in temperate marine sediments. Glob Chang Biol 23:331–340. https://doi.org/10.1111/gcb.13370

Broda E (1977) Two kinds of lithotrophs missing in nature. Z Allg Mikrobiol 17:491–493. https://doi.org/10.1002/jobm.19770170611

Buckley DH, Graber JR, Schmidt TM (1998) Phylogenetic analysis of nonthermophilic members of the kingdom *Crenarchaeota* and their diversity and abundance in soils. Appl Environ Microbiol 64:4333–4339. https://doi.org/10.1128/AEM.64.11.4333-4339.1998

Cabello P, Roldán MD, Moreno-Vivián C (2004) Nitrate reduction and the nitrogen cycle in archaea. Microbiology 150(Pt 11):3527–3546. https://doi.org/10.1099/mic.0.27303-0

Chauhan RP (2014) Role of earthworms in soil fertility and factors affecting their population dynamics: a review. Int J Res 1:642–649

Coleman DC, Wall DH (2015) Soil fauna: occurrence, biodiversity, and roles in ecosystem function. Soil Microbiol Ecol Biochem 4:111–149

Corkidi L, Rowland DL, Johnson NC, Allen EB (2002) Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands. Plant and Soil 240:299–310. https://doi.org/10.1023/A:1015792204633

Culliney TW (2013) Role of arthropods in maintaining soil fertility. Agri 3:629–659

Damsté JSS, Strous M, Rijpstra WIC, Hopmans EC, Geenevasen JAJ, van Duin ACT, van Niftrik LA, Jetten MSM (2002) Linearly concatenated cyclobutane lipids form a dense bacterial membrane. Nature 419:708–712. https://doi.org/10.1038/nature01128

Daum M, Zimmer W, Papen H, Kloos K, Nawrath K, Bothe H (1998) Physiological and molecular biological characterization of ammonia oxidation of the heterotrophic nitrifier pseudomonas putida. Curr Microbiol 37:281–288

Davidson EA, Keller M, Erickson HE, Verchot LV, Veldkamp E (2000) Testing a conceptual model of soil emissions of nitrous and nitric oxides: using two functions based on soil nitrogen availability and soil water content, the hole-in-the-pipe model characterizes a large fraction of the observed variation of nitric oxide. Bioscience 50:667–680. https://doi.org/10.1641/0006-3568(2000)050[0667:TACMOS]2.0.CO;2

de Almeida NM, Neumann S, Mesman RJ, Ferousi C, Keltjens JT, Jetten MSM, Kartal B, van Niftrik L (2015) Immunogold localization of key metabolic enzymes in the Anammoxosome and on the tubule-like structures of Kuenenia stuttgartiensis. J Bacteriol 197:2432–2441. https://doi.org/10.1128/JB.00186-15

de Ruiter PC, Neutel A-M, Moore JC (1995) Energetics, patterns of interaction strengths, and stability in real ecosystems. Science 269:1257–1260. https://doi.org/10.1126/science.269.5228.1257

Demanèche S, Philippot L, David MM, Navarro E, Vogel TM, Simonet P (2009) Characterization of denitrification gene clusters of soil bacteria via a metagenomic approach. Appl Environ Microbiol 75:534–537. https://doi.org/10.1128/AEM.01706-08

Dilfuza E (2012) Role of microorganisms in nitrogen cycling in soils. Soil Nutrients. Nova Science Publishers, New York, pp 159–176

Domínguez J, Bohlen PJ, Parmelee RW (2004) Earthworms increase nitrogen leaching to greater soil depths in row crop agroecosystems. Ecosystems 7:672–685

Edwards CA (2000) Soil invertebrate controls and microbial interactions in nutrient and organic matter dynamics in natural and agroecosystems. In: Invertebrates as Webmasters in Ecosystems. CABI, Wallingford, pp 141–159

Engström P, Dalsgaard T, Hulth S, Aller RC (2005) Anaerobic ammonium oxidation by nitrite (anammox): implications for N2 production in coastal marine sediments. Geochim Cosmochim Acta 69:2057–2065. https://doi.org/10.1016/j.gca.2004.09.032

Fierer N, Jackson RB (2006) Prokaryotes: the unseen majority. PNAS 95:6578–6583. https://doi.org/10.1073/pnas.95.12.6578

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, New York

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45. https://doi.org/10.1111/j.1747-0765.2007.00195.x

Heemsbergen DA, Berg MP, Loreau M, van Hal JR, Faber JH, Verhoef HA (2004) Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science 306 (5698):1019–1020. https://doi.org/10.1126/science.1101865

Henry S, Baudoin E, López-Gutiérrez JC, Martin-Laurent F, Brauman A, Philippot L (2004) Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. J Microbiol Methods 59:327–335. https://doi.org/10.1016/j.mimet.2004.07.002

Henry S, Bru D, Stres B, Hallet S, Philippot L (2006) Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, & nirK, and nosZ genes in soils. Appl Environ Microbiol 72:5181–5189. https://doi.org/10.1128/AEM.00231-06

Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci 107:13754–13759. https://doi.org/10.1073/pnas.1005874107

Houlton BZ, Bai E (2009) Imprint of denitrifying bacteria on the global terrestrial biosphere. Proc Natl Acad Sci 106:21713–21716. https://doi.org/10.1073/pnas.0912111106

Jalota SK, Vashisht BB, Sharma S, Kaur S (2018) Understanding climate change impacts on crop productivity and water balance. Academic Press, Amsterdam

Jetten MSM, De Bruijn P, Kuenen JG (1997) Hydroxylamine metabolism in pseudomonas PB16: involvement of a novel hydroxylamine oxidoreductase. Antonie Van Leeuwenhoek 71:69–74

Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? Ecol Appl 3:749–757. https://doi.org/10.2307/1942106

Johnson NC, Copeland PJ, Crookston RK, Pfleger FL (1992) Mycorrhizae: possible explanation for yield decline with continuous corn and soybean. Agron J 84:387–390. https://doi.org/10.2134/agronj1992.00021962008400030007x

Joo H-S, Hirai M, Shoda M (2005) Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by Alcaligenes faecalis no. 4. J Biosci Bioeng 100:184–191

Kandeler E, Deiglmayr K, Tscherko D, Bru D, Philippot L (2006) Abundance of *narG, nirS, nirK*, and *nosZ* genes of denitrifying bacteria during primary successions of a Glacier foreland. Appl Environ Microbiol 72:5957–5962. https://doi.org/10.1128/AEM.00439-06

Kartal B, de Almeida NM, Maalcke WJ, Op den Camp HJM, Jetten MSM, Keltjens JT (2013) How to make a living from anaerobic ammonium oxidation. FEMS Microbiol Rev 37:428–461

Kits KD, Sedlacek CJ, Lebedeva EV, Han P, Bulaev A, Pjevac P, Daebeler A, Romano S, Albertsen M, Stein LY, Daims H, Wagner M (2017) Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. Nature 549:269–272. https://doi.org/10.1038/nature23679

Kobayashi M, Matsuo Y, Takimoto A, Suzuki S, Maruo F, Shoun H (1996) Denitrification, a novel type of respiratory metabolism in fungal mitochondrion. J Biol Chem 271:16263–16267

Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546. https://doi.org/10.1038/nature03911

Kudo T, Takaya N, Park S-Y, Shiro Y, Shoun H (2001) A positively charged cluster formed in the heme-distal pocket of cytochrome P450nor is essential for interaction with NADH. J Biol Chem 276:5020–5026

Levy-Booth DJ, Prescott CE, Grayston SJ (2014) Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol Biochem 75:11–25. https://doi.org/10.1016/j.soilbio.2014.03.021

Li M, Gu J-D (2013) Community structure and transcript responses of anammox bacteria, AOA, and AOB in mangrove sediment microcosms amended with ammonium and nitrite. Appl Microbiol Biotechnol 97:9859–9874. https://doi.org/10.1007/s00253-012-4683-y

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000a) Acquisition of cu, Zn, Mn and Fe by mycorrhizal maize (Zea mays L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9:331–336. https://doi.org/10.1007/s005720050277

Liu A, Hamel C, Hamilton RI, Smith DL (2000b) Mycorrhizae formation and nutrient uptake of new corn (Zea mays L.) hybrids with extreme canopy and leaf architecture as influenced by soil N and P levels. Plant and Soil 221:157–166. https://doi.org/10.1023/A:1004777821422

Liu A, Hamel C, Elmi AA, Zhang T, Smith DL (2003) Reduction of the available phosphorus pool in field soils growing maize genotypes with extensive mycorrhizal development. Can J Plant Sci 83:737–744. https://doi.org/10.4141/P02-199

Loreau M (1998) Ecosystem development explained by competition within and between material cycles. Proc Royal Socf London 265:33–38

Lotti T, Kleerebezem R, Lubello C, van Loosdrecht MCM (2014) Physiological and kinetic characterization of a suspended cell anammox culture. Water Res 60:1–14. https://doi.org/10.1016/j.watres.2014.04.017

Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation of nitrifying archaea and bacteria. Nature 461:976–979. https://doi.org/10.1038/nature08465

Moir JWB, Crossman LC, Spiro S, Richardson DJ (1996) The purification of ammonia monooxygenase from Paracoccus denitrficans. FEBS Lett 387:71–74

Mulder A, van de Graaf AA, Robertson LA, Kuenen JG (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiol Ecol 16:177–183. https://doi.org/10.1016/0168-6496(94)00081-7

Nakahara K, Tanimoto T, Hatano K, Usuda K, Shoun H (1993) Cytochrome P-450 55A1 (P-450dNIR) acts as nitric oxide reductase employing NADH as the direct electron donor. J Biol Chem 268:8350–8355

Nardi JB, Mackie RI, Dawson JO (2002) Could microbial symbionts of arthropod guts contribute significantly to nitrogen fixation in terrestrial ecosystems? J Insect Physiol 48:751–763

Nicholls JC, Trimmer M (2009) Widespread occurrence of the anammox reaction in estuarine sediments. Aquat Microb Ecol 55:105–113

Nicol GW, Tscherko D, Embley TM, Prosser JI (2005) Primary succession of soil Crenarchaeota across a receding glacier foreland. Environ Microbiol 7:337–347

Oline DK (2006) Phylogenetic comparisons of bacterial communities from serpentine and nonserpentine soils. Appl Environ Microbiol 72:6965–6971

Pajares S, Bohannan BJM (2016) Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. Front Microbiol 7:1045. https://doi.org/10.3389/fmicb.2016.01045

Palatinszky M, Herbold C, Jehmlich N, Pogoda M, Han P, von Bergen M, Lagkouvardos I, Karst SM, Galushko A, Koch H, Berry D, Daims H, Wagner M (2015) Cyanate as an energy source for nitrifiers. Nature 524:105–108. https://doi.org/10.1038/nature14856

Pester M, Rattei T, Flechl S, Gröngröft A, Richter A, Overmann J, Reinhold-Hurek B, Loy A, Wagner M (2012) amoA-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA genes from soils of four different geographic regions. Environ Microbiol 14:525–539. https://doi.org/10.1111/j.1462-2920.2011.02666.x

Philippot L, Hallin S, Schloter MBT-A (2007) Ecology of denitrifying prokaryotes in agricultural soil. Adv Agron 96:249–305. https://doi.org/10.1016/S0065-2113(07)96003-4

Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends Microbiol 20:523–531. https://doi.org/10.1016/j.tim.2012.08.001

Rattray JE, van de Vossenberg J, Hopmans EC, Kartal B, van Niftrik L, Rijpstra WIC, Strous M, Jetten MSM, Schouten S, Sinninghe Damsté JS (2008) Ladderane lipid distribution in four genera of anammox bacteria. Arch Microbiol 190:51–66. https://doi.org/10.1007/s00203-008-0364-8

Richardson D, Felgate H, Watmough N, Thomson A, Baggs E (2009) Mitigating release of the potent greenhouse gas N2O from the nitrogen cycle – could enzymic regulation hold the key? Trends Biotechnol 27:388–397. https://doi.org/10.1016/j.tibtech.2009.03.009

Rösch C, Mergel A, Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68:3818–3829. https://doi.org/10.1128/AEM.68.8.3818-3829.2002

Sanders IR (2003) Preference, specificity and cheating in the arbuscular mycorrhizal symbiosis. Trends Plant Sci 8:143–145. https://doi.org/10.1016/S1360-1385(03)00012-8

Santoro AE, Dupont CL, Richter RA, Craig MT, Carini P, McIlvin MR, Yang Y, Orsi WD, Moran DM, Saito MA (2015) Genomic and proteomic characterization of Candidatus Nitrosopelagicus brevis: an ammonia-oxidizing archaeon from the open ocean. Proc Natl Acad Sci 112:1173–1178. https://doi.org/10.1073/pnas.1416223112

Schimel J, Schaeffer S (2012) Microbial control over carbon cycling in soil. Front Microbiol 3:348. https://doi.org/10.3389/fmicb.2012.00348

Shaw LJ, Nicol GW, Smith Z, Fear J, Prosser JI, Baggs EM (2006) Nitrosospira spp. can produce nitrous oxide via a nitrifier denitrification pathway. Environ Microbiol 8:214–222. https://doi.org/10.1111/j.1462-2920.2005.00882.x

Shin W, Islam R, Benson A, Joe MM, Kim K, Gopal S, Samaddar S, Banerjee S, Sa T (2016) Role of diazotrophic bacteria in biological nitrogen fixation and plant growth improvement. Korean J Soil Sci Fertilizer 49:17–29

Shipitalo MJ, Le Bayon R-C (2004) 10 quantifying the effects of earthworms on soil aggregation and porosity. In: Earthworm ecology. CRC, New York, pp 183–199

Shiro Y, Fujii M, Iizuka T, Adachi S, Tsukamoto K, Nakahara K, Shoun H (1995) Spectroscopic and kinetic studies on reaction of cytochrome P450nor with nitric oxide implication for its nitric oxide reduction mechanism. J Biol Chem 270:1617–1623

Shoun H (2006) Denitrification and anaerobic energy producing mechanisms by fungi. Protein Nucleic Acid Enzyme 51:419–429

Shoun H, Tanimoto T (1991) Denitrification by the fungus fusarium oxysporum and involvement of cytochrome P-450 in the respiratory nitrite reduction. J Biol Chem 266:11078–11082

Shoun H, Kim D-H, Uchiyama H, Sugiyama J (1992) Denitrification by fungi. FEMS Microbiol Lett 94:277–281. https://doi.org/10.1111/j.1574-6968.1992.tb05331.x

Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic Press, Amsterdam

Smith CJ, Nedwell DB, Dong LF, Osborn AM (2007) Diversity and abundance of nitrate reductase genes (narG and napA), nitrite reductase genes (nirS and nrfA), and their transcripts in estuarine sediments. Appl Environ Microbiol 73:3612–3622. https://doi.org/10.1128/AEM.02894-06

Song M, Li X, Jing S, Lei L, Wang J, Wan S (2016) Responses of soil nematodes to water and nitrogen additions in an old-field grassland. Appl Soil Ecol 102:53–60

Strous M, Heijnen JJ, Kuenen JG, Jetten MSM (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl Microbiol Biotechnol 50:589–596. https://doi.org/10.1007/s002530051340

Su F, Takaya N, Shoun H (2004) Nitrous oxide-forming codenitrification catalyzed by cytochrome P450nor. Biosci Biotechnol Biochem 68:473–475

Takasaki K, Shoun H, Yamaguchi M, Takeo K, Nakamura A, Hoshino T, Takaya N (2004) Fungal ammonia fermentation, a novel metabolic mechanism that couples the dissimilatory and assimilatory pathways of both nitrate and ethanol. Role of acetyl CoA synthetase in anaerobic ATP synthesis. J Biol Chem 279:12414–12420

Tanimoto T, Hatano K, Kim D, Uchiyama H, Shoun H (1992) Co-denitrification by the denitrifying system of the fungus fusarium oxysporum. FEMS Microbiol Lett 93:177–180

Topoliantz S, Ponge J-F, Viaux P (2000) Earthworm and enchytraeid activity under different arable farming systems, as exemplified by biogenic structures. Plant and Soil 225:39–51

Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk H, Schleper C (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ Microbiol 7:1985–1995

Uchimura H, Enjoji H, Seki T, Taguchi A, Takaya N, Shoun H (2002) Nitrate reductase-formate dehydrogenase couple involved in the fungal denitrification by fusarium oxysporum. J Biochem 131:579–586. https://doi.org/10.1093/oxfordjournals.jbchem.a003137

van der Star WRL, Dijkema C, de Waard P, Picioreanu C, Strous M, van Loosdrecht MCM (2010) An intracellular pH gradient in the anammox bacterium Kuenenia stuttgartiensis as evaluated by 31P NMR. Appl Microbiol Biotechnol 86:311–317. https://doi.org/10.1007/s00253-009-2309-9

van Groenigen JW, Huygens D, Boeckx P, Kuyper ThW, Lubbers IM, Rütting T, Groffman PM (2015) The soil N cycle: new insights and key challenges. Soil 1(1):235–256

Van Vliet PCJ, Beare MH, Coleman DC, Hendrix PF (2004) Effects of enchytraeids (Annelida: Oligochaeta) on soil carbon and nitrogen dynamics in laboratory incubations. Appl Soil Ecol 25:147–160

Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. ISME J 5:1067–1071. https://doi.org/10.1038/ismej.2010.191

Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Ecol Appl 16:2143–2152. https://doi.org/10.1890/1051-0761(2006)016[2143:ECODCA]2.0.CO;2

Wardle DA (2006) The influence of biotic interactions on soil biodiversity. Ecol Lett 9:870–886

Xu Y, Xu Z, Cai Z, Reverchon F (2013) Review of denitrification in tropical and subtropical soils of terrestrial ecosystems. J Soil Sediment 13:699–710. https://doi.org/10.1007/s11368-013-0650-1

Yu Y, Zhang J, Chen W, Zhong W, Zhu T, Cai Z (2014) Effect of land use on the denitrification, abundance of denitrifiers, and total nitrogen gas production in the subtropical region of China. Biol Fertil Soils 50:105–113. https://doi.org/10.1007/s00374-013-0839-x

Zhang J, Cai Z, Cheng Y, Zhu T (2009) Denitrification and total nitrogen gas production from forest soils of eastern China. Soil Biol Biochem 41:2551–2557. https://doi.org/10.1016/j.soilbio.2009.09.016

Zhang J, Yu Y, Zhu T, Cai Z (2014) The mechanisms governing low denitrification capacity and high nitrogen oxide gas emissions in subtropical forest soils in China. J Geophys Res Biogeo 119:1670–1683. https://doi.org/10.1002/2014JG002662

Zhou Z, Takaya N, Sakairi MA, Shoun H (2001) Oxygen requirement for denitrification by the fungus Fusarium oxysporum. Arch Microbiol 175:19–25. https://doi.org/10.1007/s002030000231

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Molecular Biol Rev 61:533–616

Chapter 7
Unravelling Microbial Nitrogen Pathway in Rhizosphere

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Abstract The rhizosphere is a biologically active zone of the soil around plant roots containing soil microbes including bacteria and fungi, where plant–microbe interactions taking place are often beneficial to the plant, the microbes or to neither of them. The developments in molecular biology methods of recent times are focusing on rhizospheric microbial diversity. The rhizosphere is a microenvironment which is very much different from non-rhizosphere soil. The activity of the microbes in the rhizosphere is high and leads to better cycling and availability of nutrients improving the chemical soil quality indicators. With the advances in second generation sequencing and omic technologies, several important mechanisms underlying plant–microbe interactions have been revealed. Metagenomics has revolutionized microbial ecology by facilitating the genomic characterization of microbial communities in various segments of the environment. This has provided a deep insight to the genes that are present in varying environments and constitutes powerful reference material for studying microbial gene expression. Another approach, metatranscriptomics, enables the characterization of community transcription patterns and is a significant advancement in understanding of plant and microbe gene expression. This chapter discusses about how metatranscriptomics could rapidly improve our understanding of plant–microbe interactions in the rhizospheric region.

Keywords Rhizosphere · Plant–microbe interactions · Metagenomics · Metatranscriptomics

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7.1 Introduction

The nitrogen cycle is an important collection of biogeochemical pathways responsible for transforming atmospheric nitrogen (not usable by plants) to organic and inorganic forms which can be used by plants in different ecosystem. Microbial communities are responsible for mediating these pathways. The process of nitrogen cycle is transforming one form of nitrogen to another with the help of different pathways that include nitrogen fixation, assimilation, ammonification, nitrification and some other oxidation-reduction reactions. Different microbes are responsible for providing plants with different sources of nitrogen (Cropnutrition.com).

Nitrogen is the most essential macronutrient required by plants to survive because it is an essential component of nucleotide and protein and because it is a major component of chlorophyll. Without proteins, plants would wither out and die, and without chlorophyll, they would not be able to carry out photosynthesis. Nitrogen is also a compound of ATP (which is used for energy driven reactions), forms certain enzymes in plants and is also a component of DNA (stores genetic information). Without nitrogen there will be no plant life. Nitrogen in soil exists in two forms, i.e. organic form and inorganic form (which include ammonium and nitrate) (Jones et al. 2005). Although most of the nitrogen present in the soil is in organic form (90–95%), most of it is not available directly for plant use. Therefore NO_3^- (nitrate)- and NH_4^+ (ammonium) make up most of the plant available nitrogen. Unlike ammonium ions, nitrate ions are free flowing in soil and do not bind to negatively charged cations in soil. Thus, it is more readily available for plants (Courty et al. 2015). Despite most of the organic nitrogen sources being unavailable, some of them can be converted to available forms by microorganisms. Uptake of organic nitrogen is very energy efficient for plants because the assimilation step (conversion of inorganic nitrogen compounds into organic products) is bypassed (Chapin et al. 2002).

The relative proportion of different forms of nitrogen absorbed by plants can depend on various interacting factors (Fig. 7.3). Even though a lot is not known about these factors and how they interact, the relative amount of nitrogen present in soil is considered one of the major factors (Chapin et al. 2002; von Wirén et al. 1997). Other factors like pH of the soil, moisture, temperature, availability of a particular nitrogen form and amount of potassium available in the soil (alleviate ammonium toxicity) might be some of the soil properties determining N form uptake (Britto and Kronzucker 2013). Competition experienced by other plants and microorganisms might play their role in N-availability. Symbiotic relationships on the other hand can increase plant's ability to absorb certain N forms (Kuzyakov and Xu 2013; Li et al. 2012). The species of a plant also might determine the form of nitrogen uptake because the transporters of N vary in every plant species. The development stage of the plant can also be a factor (Britto and Kronzucker 2002). Rhizosphere is basically a micro-zone of soil biota surrounding the root and root hair of plants. This is the region which accounts for plant–microbe interactions. The size of rhizosphere however depends on the species of plant and microbes (Supriya 2018)

Rhizosphere can be classified into three zones, i.e. the inner zone (Closest to the root), Rhizoplane (Root system) and Outer zone (adjoining epidermis). Rhizosphere is favourable for microbial growth due to the presence of root exudates (the organic and in-organic compounds that ooze out of the root during unfavourable conditions). This helps in forming a link between plants and microbes (Krasilnikov 1961). Organic root exudates include compounds like proteins, vitamins, sugars, enzymes, etc. Inorganic root exudates include compounds like water, gases like oxygen, nitrogen, etc. Other than these, some miscellaneous compounds like auxins, glycosides, etc. are also released which might create a negative impact.

In the rhizosphere, deposition of root exudates serves as food and nutrition for microbes. These microbes convert the complex compounds into simpler compounds and these simple compounds can now be consumed by plants through roots (Supriya 2018). Recent studies have explored the role of rhizosphere microbes in providing plants with different forms of nitrogen. There are many omics approaches used for soil ecology (Fig. 7.1). Studies have also suggested that the nitrogen cycling abilities of these microbes change with changes in external conditions, i.e. soil structure, temperature, pH, etc. (Cobo-Díaz et al. 2015). Even after such major advances, in understanding the interactions between microbes and plants, we are still not very sure about how uptake of different nitrogen forms is modulated. In this chapter, we will be talking in detail about the microbial community present in rhizosphere, relation between plants and microorganisms to finally understand and metagenomically analyse the nitrogen cycling pathways carried out in rhizosphere microorganisms. This genomic study would help us understand the very basics of how nitrogen sources are mediated by plant–microbe interactions and why different microbes process different sources of nitrogen.

7.2 Microbial Communities in Rhizosphere

The existence of huge microbial diversity in the rhizosphere zone is due to the nutrition provided by root exudates and the favourable conditions like optimum pH, temperature, moisture, etc. These communities include bacteria, actinomycetes, fungi, algae and protozoa majorly (Fig. 7.2). These microbes can be either beneficial, deleterious or neutral in nature (Supriya 2018).

Beneficial ones include the group of microbes which are of benefit to the root system of plant, for example, mycorrhizal fungi, N_2 fixing bacteria, etc. They help in N_2 fixation, promoting plant growth, colonizing root, increasing absorption capacity of roots. Deleterious ones are those which cause harm to the root systems which includes nematodes, pathogens, etc. Neutral ones are those which show a neutral effect, i.e. neither harm nor benefit the plants (Table 7.1).

Certain organic growth factors like vitamins, auxins, some biotic elements are of great importance for microbial growth. It was observed that small doses of these substances were able to increase the growth of microbes and plants by promoting various biochemical processes. The organic compounds/soil humus is of greatest

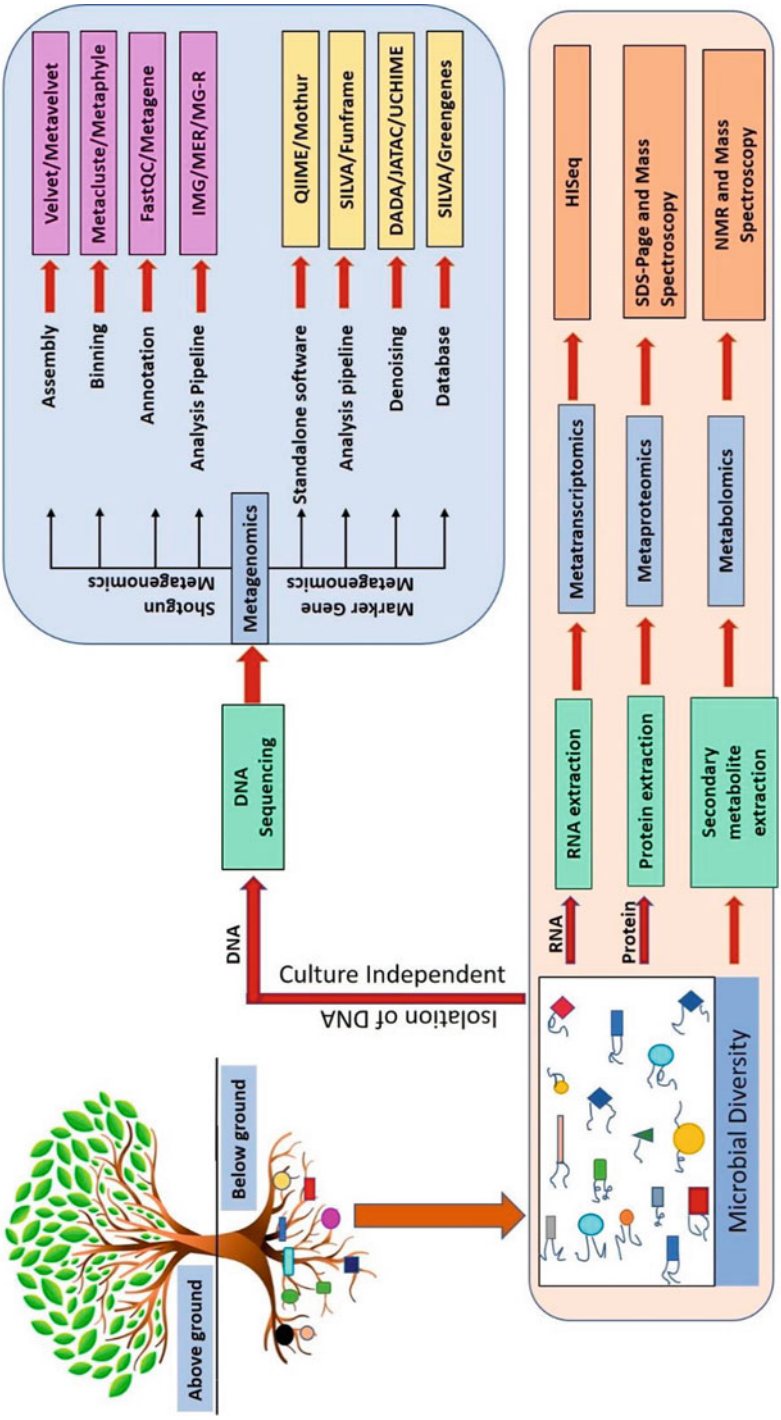

Fig. 7.1 Schematic representation of different omics approaches in soil ecology

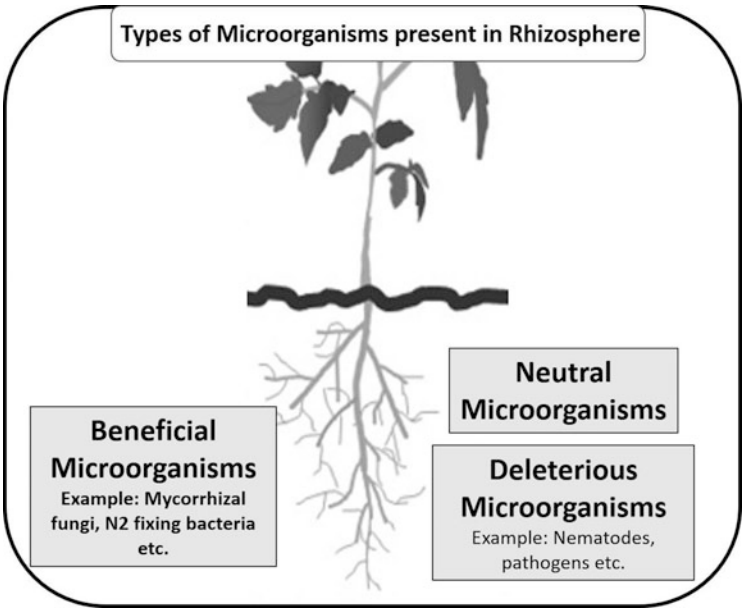

Fig. 7.2 Types of microorganisms present in rhizosphere

importance and thus its quantity and total number of microbial populations can be assumed to be directly proportional (Krasilnikov 1961).

7.3 Relation Between Plants and Microorganisms

Over the course of hundreds of years, plants have evolved various mutualistic associations with certain microbes which majorly include some fungi and bacteria. The most prominent example being that of Arbuscular mycorrhizal (AM) fungi which forms association with plant roots (Parniske 2008). 20% of the Carbon (C) is allocated to mycelia. Other than carbon it takes up nitrate, ammonium and provide Nitrogen to plants in return (Hodge and Storer 2015). This increases the nutrient absorption by plants and thus increases plant growth. Talking about bacteria, plants form mutualistic interactions with bacteria that can fix atmospheric N_2 to produce ammonia. This interaction takes place in the root nodules of plants where photosynthetically fixed carbon is provided to plant in return of fixed nitrogen (Moreau et al. 2019). Since, now we have an idea about the importance of plant and microorganisms in each other's life, we will discuss their effects in detail.

Table 7.1 Microbes found in rhizosphere with their features and functions

Microorganisms	Features	Functions	Examples	Reference
Bacteria	Small size, high in number, present in root hair, mostly gram negative	Fixing of nitrogen, solubilizing inorganic nitrogen	*Pseudomonas sp., Bacillus sp., Rhizobium sp., Mycobacterium sp.*	Bashan et al. (2005)
Fungi	Second highest in number, work in association with roots	Symbiotic association, might be pathogenic, provides nutrition to roots, colonize in roots	*Aspergillus niger, Penicillium pinophilum*	Shaikh and Mokat (2018)
Actinomycetes	Forms a link between fungi and bacteria, gram +ve in nature	Compete with bacteria, recycle nutrients	*Allokutzneria sp., Amycolatopsis sp.*	Poomthongdee et al. (2015)
Algae	Aerobic in nature, photoautotrophic	Symbiotic association with roots, maintain soil fertility	*Chlorella sp., Chlamydomonas sp.*	Mendes et al. (2013)

7.3.1 Effect of Plants on Soil Microflora

Plants influence microorganism while alive and after their death. When they are alive, the root exudates and dying parts of roots provide nutrition for the microorganism's optimal growth in rhizosphere region. After their death, the leftovers of the plants nourish the soil and indirectly create favourable conditions for microbes to proliferate (Krasilnikov 1961).

7.3.1.1 Root Mass of Plants

Surface layer of soil constitutes the major root mass, i.e. the density of roots is highest in surface layer (Kachinskiĭ et al. 1950). This density keeps on decreasing as we move deeper into the soil. Climatic conditions, type of flora and fauna also affect root mass.

The significance of root system is dependent on its activity, i.e. ability to absorb nutrition from the surroundings. In return, products of metabolism are excreted, which are used by microbes to grow. Thus, higher root mass means more microbes (Krasilnikov 1961). This clearly shows that root mass has a direct and immense influence on the growth and development of microorganisms. It may be positive or negative.

7.3.1.2 Root Excretions

Roots are not only responsible for absorbing nutrients from the soil, but also excrete certain substances which play major role in determining fertility of soil. CO_2 is one of the major excretions by roots. The intensity of CO_2 formation by roots is directly influenced by the number of microorganisms in soil. Root excretions also include elements like Phosphorus, Potassium, Calcium, Sodium, etc. (Minina 1927; Sabinin 1940). Excretion from roots is achieved by the process of exosmosis. This happens in accordance with the concentration gradients across root membrane (Minina 1927; Sabinin 1940).

Plants were also found to excrete acetic, formic and oxalic acid through roots (Stoklasa et al. 1909). It was also observed that metabolic products can be transferred from one plant to another in an experiment where two plants were grown in a vessel, one was sprayed with methoxyphenyl acetic acid while the other was not. After some time of incubation, the substance was detected in the unsprayed plant, hence showing that the non-sprayed plant absorbed the substance from the sprayed plant via soil (Preston et al. 1954).

Roots of vegetative plants also secrete certain enzymes in soil. The nature and amount of enzyme secreted vary in different plant species (Virtanen et al. 1937). All these substances are in some way or the other, nutritional sources for microorganisms in soil and are responsible for increase in growth.

7.3.1.3 Root Residue

Root residue refers to dead roost cells, hair, epidermis, etc. Different plants attract different types of microflora, which are responsible for decomposition of roots. Different products are formed as a result of this decomposition. These products are the basis of nutrition for some of the other species of microbes (Krasilnikov 1961). This shift of microbial population causes versatility in the final fauna.

7.3.2 Effect of Soil Microorganisms on Plants

The effect of soil microflora on life of plants has not been studied very vastly and only a little information is available. Microbes can either effect plant growth positively or negatively. We will talk about both the aspects.

7.3.2.1 Microbial Activators

These are the microorganisms which can produce biotic factors like vitamins, amino acids, etc. They are responsible for activating processes in soil (Zimmermann 1902).

Pure cultures of microbes including bacteria, fungi and actinomycetes affect the growth of plants positively. They increase percentage of germinating seeds and increase biochemical processes. Symbionts are responsible for nodule formation on roots of leguminous plants, they considerably improve growth and crop yield. Root nodule bacteria which act as microbial activators are thought to fix molecular nitrogen and supply it to plants (Krasilnikov 1961).

7.3.2.2 Microbial Inhibitors

These are microorganisms which during metabolism produce certain substrates that are responsible for suppression of growth and development of plant (Greig-Smith 1911). Some fungi and bacteria are known to produce toxins which act upon animals through food products, fodder and results is poisoning of those animals (Pidoplichko 1953). Talking about suppression of plant growth, these toxins are responsible for suppression of seed germination, sprout growth, sporulation process of lower plant, zygote formation in phycomycetes (Krasilnikov 1961). Some common microbes responsible for toxin production are some Protozoa, bacteria belonging to genus *Pseudomonas*. Under certain conditions, the toxin produced might endow soil with its toxicity. Level of toxicity depends upon rate of toxin production by microorganisms.

7.4 Microbial Nitrogen Pathways

In terrestrial ecosystems, the availability of Nitrogen is limited, which results in competition in soil fauna and flora. To deal with this shortage of nitrogen, plants and microbes have developed ways to utilize other organic and mineral compounds as a source of Nitrogen (Merrick and Edwards 1995). Main sources of organic Nitrogen are usually plant and animal residue (Kögel-Knabner 2002). Proteins are the most important of them all, constituting 60% of organic nitrogen in soil (Cochrane 1958; Sinha 2004). A small proportion is also fulfilled by mineral sources like ammonium and nitrate (Schimel and Bennett 2004). Glutamate and glutamine are also used as nitrogen sources by various microorganisms (Wong et al. 2008). There are two broad pathways of utilizing nitrogen from soil:

1. *MIT route*: This stands for mineralization-immobilization turnover. This is the step where nitrogen is first mineralized to NH_4^+ before use by microbes and plants. When MIT route is dominant in soil, competition is experienced between plants and microbes (Manzoni and Porporato 2007).
2. *Direct route*: This involves uptake of organic molecules directly like amino acids (Barak et al. 1990; Hadas et al. 1992). In this case, microbes fulfil their requirement from organic resources while plants use mineral sources, hence no competition is experienced (Manzoni and Porporato 2007).

7.4.1 *Extracellular Depolymerase*

Organic polymerase released as residue from plants and microbes needs to be degraded to soluble forms before being utilized by microbe. This is done with the help of extracellular depolymerases. For hydrolysing/degrading nitrogen containing molecules, depolymerases like protease, chitinase and peptidoglycan hydrolases are most common. Protease can be produced by a wide variety of bacteria and fungi (Ahearn et al. 1968; Gupta et al. 2002) and has wide substrate specifities (Kalisz 1988). Extracellular protease is responsible for hydrolysing large protein molecules into smaller peptides and amino acids.

Chitinase is a polymerase secreted by wide variety of bacteria, fungi, plants but not archaea (Gooday 1990). It is found abundantly in nature. Chitinase produced by bacteria hydrolyses chitin. When produced by fungi, they also play role in cell wall development (Adams 2004; Bhattacharya et al. 2007).

7.4.1.1 Regulation

Production of extracellular hydrolases is regulated by the presence or absence of substrate. Here, substrate refers to the residues. For example, protease is activated by presence of protein in the medium (Kalisz 1988) so is chitinase activated by the presence of chitin (Felse and Panda 1999). Carbon sources in the medium might supress production of these enzymes. For example, glucose might repress product of protease and chitinase (Duo-Chuan 2006). It was also found that inducing substrate in soil might affect production of enzymes by microbes. For example, protein induction increased the protease activity in Tundra soil (Zanuta and Bremner 1976). Similar results were obtained in case of chitinase (Rodriguez-Kabana et al. 1983).

Protease synthesis was repressed under limiting Nitrogen and Sulphur condition (Sims and Wander 2002) but in contrast it was observed that glucose addition to agar increases protease activity (Asmar et al. 1992). Due to different results in different conditions, it was believed that certain factors like soil age, fertility, type of microbes, etc. determine the result of an addition or reduction.

7.4.2 *Nitrogen Mineralization*

Now, that the organic molecules have been depolymerized by respective depolymerases, they are ready for mineralization. This is via the MIT route we talked about earlier. It includes mineralization of organic molecules to finally form ammonium (NH_4^+) (Geisseler et al. 2009). Mineralization of Nitrogen can be done with the help of enzymes like urease and amino-acid oxidase.

7.4.2.1 Urease

Urease is an enzyme which acts as a catalyst for hydrolysing urea to form NH_4^+ (Mobley et al. 1995). It has been extensively studied because of its presence in environment. It is released by mammals as an excretion product. It is also a product of degradation of uric acid extracted by birds (Cunin et al. 1986). More importantly urease can be produced by bacteria, algae, fungi and even plants (Mobley and Hausinger 1989; Follmer 2008). As in the case of protease and chitinase, urease synthesis too is repressed in presence of a more suitable nitrogen source and increased when urea is added (Mobley et al. 1995). It is estimated that most of the urease activity is extracellular, i.e. about 60% of total in soil (Pettit et al. 1976). Later it was believed that urease activity in soil was not a result of urea released by animals but because of urea produced by microbes and plants. This was believed on the basis of experiment carried out by (Zanuta and Bremner 1976). When tests were carried out in soil, it was found that addition of urea did not promote urease activity (Lloyd and Sheaffe 1973). Presence of protected extracellular urease could be one of the reasons or repression by NH_4^+. Addition of Carbon increases urease activity, this is because of the increased nitrogen demand by microorganisms when carbon is added (Lloyd and Sheaffe 1973; Zanuta and Bremner 1976).

7.4.2.2 Amino-Acid Oxidase

Deamination of amino acids and uptake of ammonium are key reactions in nitrogen fixation, and these are carried out by amino-acid oxidase. This enzyme is mainly intracellular but may be produced by some bacteria and fungi extracellularly a well (on cell surface) (Böhmer et al. 1989; Braun et al. 1992; Davis et al. 2005). Some amino acids are very substrate specific; some have a broad spectrum (Braun et al. 1992). It has been found that production of amino-acid oxidases is repressed by NH_4+ (Palenik and Morel 1990; Vallon et al. 1993), enhanced by Nitrogen starvation (Davis et al. 2005). Amino acid in the presence of Carbon induces amino-acid oxidases production (Vallon et al. 1993). Several fungal species like basidiomycetes and ascomycetes as well as some bacterial species can produce amino-acid oxidases (Braun et al. 1992; Davis et al. 2005) (Fig. 7.3).

7.5 Metagenomic Analysis

Metagenomics refers to study of genetic material recovered directly from environmental samples. This can also be referred to as environmental genomics.

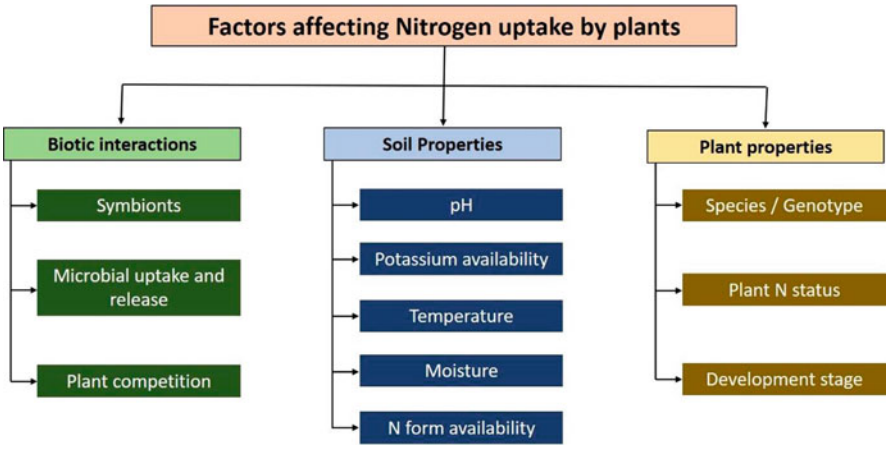

Fig. 7.3 factors affecting nitrogen uptake by plants

7.5.1 Metagenomic Analysis of Nitrogen Cycle

Nitrogen metabolism involves about 7–8 pathways including ammonia assimilation, nitrate and nitrite ammonification, nitric oxide synthase, allantoin utilization, cyanate hydrolysis, Dissimilatory nitrate reductase, denitrification, and Nitrogen fixation. To analyse nitrogen cycle pathway, metagenomics (Cobo-Díaz et al. 2015) was carried out in a study where soil sample was collected from Sierra Nevada Natural and National park and pyrosequencing was performed. About 520,430 reads were obtained which were trimmed to 412,302 sequences. These sequences obtained were studied and analysed with the help of genome library on the basis of functions a particular set of genome performs. At subsystem level (2) 52% of sequence were responsible for ammonia assimilation, 16 of sequence for Nitrate ammonification. In subsystem 3, Nitric oxide synthetase was 9.1%, nitrosative stress was 0.65%, Allantonian utilization 5.18%, Dissimilatory nitrate reductase 4.19%, Denitrification 2.33%, Nitrogen fixation 1.33%. This analysis also helped in figuring bacterial community based on similarities found in proteins which are responsible for nitrogen cycle. The percentage abundance of microbes in soil is as follows: Bacteroidetes—42%, Proteobacteria—27%. Actinobacteria—14%, Firmicutes—2%, Acidobacteria—6%, and others.

7.5.2 Response of Nitrogen Cycle to CO_2

Earlier studies were focussed on gene families like nifH (important for Nitrogen fixation) (Collavino et al. 2014), amoA (Nitrification) (Bru et al. 2011; Leininger et al. 2006).

These studies suggested the importance of uncultured organisms in carrying out these processes. When CO_2 concentration is increases, it results in affecting several processes that affect N-cycling. Increase in photosynthesis and plant growth release more carbon into soil and in return demand more nitrogen. Therefore, microbial nitrogen fixation tends to increase under high concentration of CO_2 (Luo et al. 2006; Norby et al. 2010). Decrease in N-fixation was also observed in some studies (Hungate et al. 2004). Experiments (Tu et al. 2017) showed that long CO_2 exposure, produced a significant change in the number of gene families responsible for dissimilatory nitrate reduction, N-Metabolism, N_2 fixation while no such change was observed in case of nitrification, assimilatory Nitrate reduction and denitrification.

Overall, elevated CO_2 increased plant biomass both above ground and in root, increased concentration of ammonium, no change was observed in concentration of nitrate and an increase was observed in C/N ratio both above and below ground.

7.6 Conclusion

After unravelling the nitrogen cycling pathways followed in rhizosphere region with the help of metagenomics and metatranscriptomics approach, it can be concluded that the abundance or absence of certain nutrients in soil can be responsible for the nitrogen cycling pathways followed by microorganisms. These conditions also decide activity level of microbes and their ability to produce soluble nitrogen for plants to consume. The production of enzymes like urease, amino-acid oxidase also varies depending upon soil nutrient availability. This shows that microbes can follow a wide variety of N-cycling pathways ranging from MIT route to direct route as discussed above depending upon the requirements of plants and nutrient availability in soil.

References

Adams DJ (2004) Fungal cell wall chitinases and glucanases. Microbiology 150:2029–2035

Ahearn DG, Meyers SP, Nichols RA (1968) Extracellular proteinases of yeasts and yeast like fungi. Appl Microbiol 16:1370–1374

Asmar F, Eiland F, Nielsen NE (1992) Interrelationship between extracellular enzyme activity, ATP content, total counts of bacteria and CO 2 evolution. Biol Fertil Soils 14:288–292

Barak P, Molina JAE, Hadas A, Clapp CE (1990) Mineralization of amino acids and evidence of direct assimilation of organic nitrogen. Soil Sci Soc Am J 54:769–774

Bashan Y, De-Bashan LE (2005) Plant growth-promoting. Encyclopedia Soil Environ 1:103–115

Bhattacharya D, Nagpure A, Gupta RK (2007) Bacterial chitinases: properties and potential. Crit Rev Biotechnol 27:21–28

Böhmer A, Müller A, Passarge M, Liebs P, Honeck H, Müller HG (1989) A novel l-glutamate oxidase from Streptomyces endus: purification and properties. Eur J Biochem 182:327–332

Braun M, Kim JM, Schmid RD (1992) Purification and some properties of an extracellular L-amino acid oxidase from Cellulomonas cellulans AM8 isolated from soil. Appl Microbiol Biotechnol 37:594–598
Britto DT, Kronzucker HJ (2002) NH4+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584
Britto DT, Kronzucker HJ (2013) Ecological significance and complexity of N-source preference in plants. Ann Bot 112:957–963
Bru D, Ramette A, Saby NPA, Dequiedt S, Ranjard L, Jolivet C, Arrouays D, Philippot L (2011) Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME J 5:532–542
Chapin FS, Matson PA, Mooney HA (2002) Terrestrial decomposition. In: Principles of terrestrial ecosystem ecology. Springer, New York, pp 151–175
Cobo-Díaz JF, Fernández-González AJ, Villadas PJ, Robles AB, Toro N, Fernández-López M (2015) Metagenomic assessment of the potential microbial nitrogen pathways in the rhizosphere of a Mediterranean forest after a wildfire. Microb Ecol 69:895–904
Cochrane VW (1958) Physiology of fungi. Wiley, New York
Collavino MM, Tripp HJ, Frank IE, Vidoz ML, Calderoli PA, Donato M, Zehr JP, Aguilar OM (2014) nifH pyrosequencing reveals the potential for location-specific soil chemistry to influence N 2-fixing community dynamics. Environ Microbiol 16:3211–3223
Courty PE, Smith P, Koegel S, Redecker D, Wipf D (2015) Inorganic nitrogen uptake and transport in beneficial plant root-microbe interactions. Crit Rev Plant Sci 34:4–16
Cunin R, Glansdorff N, Pierard A, Stalon V (1986) Biosynthesis and metabolism of arginine in bacteria. Microbiol Rev 50:314
Davis MA, Askin MC, Hynes MJ (2005) Amino acid catabolism by an areA-regulated gene encoding an L-amino acid oxidase with broad substrate specificity in aspergillus nidulans. Appl Environ Microbiol 71:3551–3555
Duo-Chuan L (2006) Review of fungal chitinases. Mycopathologia 161:345–360
Felse PA, Panda T (1999) Regulation and cloning of microbial chitinase genes. Appl Microbiol Biotechnol 51:141–151
Follmer C (2008) Insights into the role and structure of plant ureases. Phytochemistry 69:18–28
Geisseler D, Horwath WR, Doane TA (2009) Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon and nitrogen availability. Soil Biol Biochem 41:1281–1288
Gooday GW (1990) Physiology of microbial degradation of chitin and chitosan. Biodegradation 1:177–190
Greig-Smith R (1911) The bacteriotoxins and the agricere of soils. Centr Bakt 30:154–156
Gupta R, Beg Q, Khan S, Chauhan B (2002) An overview on fermentation, downstream processing and properties of microbial alkaline proteases. Appl Microbiol Biotechnol 60:381–395
Hadas A, Sofer M, Molina JAE, Barak P, Clapp CE (1992) Assimilation of nitrogen by soil microbial population: NH4 versus organic N. Soil Biol Biochem 24:137–143
Hodge A, Storer K (2015) Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant and Soil 386:1–19
Hungate BA, Stiling PD, Dijkstra P, Johnson DW, Ketterer ME, Hymus GJ, Hinkle CR, Drake BG (2004) CO2 elicits long-term decline in nitrogen fixation. Science 304(5675):1291
Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? Soil Biol Biochem 37:413–423
Kachinskii NA, Vadĭnina AF, Korchagina ZA (1950) Soil agrophysical analysis, experience of the Central Ural region
Kalisz HM (1988) In: Fiechter A (ed) Advances in biochemical engineering/biotechnology. Springer, Berlin, pp 1–65
Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162

Krasilnikov NA (1961) Soil microorganisms and higher plants. Moscow, Academy of Sciences of the USSR
Kuzyakov Y, Xu XL (2013) Competition and mutualism between roots and rhizosphere microorganisms by nitrogen acquisition and their ecological consequences. New Phytol 198:656–669
Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809
Li G, Dong G, Li B, Li Q, Kronzucker HJ, Shi W (2012) Isolation and characterization of a novel ammonium overly sensitive mutant, amos2, in Arabidopsis thaliana. Planta 235:239–252
Lloyd AB, Sheaffe MJ (1973) Urease activity in soils. Plant and Soil 39:71e80
Luo Y, Hui D, Zhang D (2006) Elevated CO2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87:53–63
Manzoni S, Porporato A (2007) A theoretical analysis of nonlinearities and feedbacks in soil carbon and nitrogen cycles. Soil Biol Biochem 39:1542–1556
Mendes R, Garbeva P, Raaijmakers JM (2013) FEMS Microbiol Rev 37:634–663
Merrick MJ, Edwards RA (1995) Nitrogen control in bacteria. Microbiol Rev 59:604–622
Minina EG (1927) On the acidity of root secretions. Bull Inst Recherches Biol Univ de Perm 5:238–258
Mobley HL, Hausinger RP (1989) Microbial ureases: significance, regulation, and molecular characterization. Microbiol Mol Biol Rev 53:85–108
Mobley HL, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59:451–480
Moreau D, Bardgett RD, Finlay RD, Jones DL, Philippot L (2019) A plant perspective on nitrogen cycling in the rhizosphere. Funct Ecol 33:540–552
Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) CO2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci 107:19368–19373
Palenik B, Morel FM (1990) Amino acid utilization by marine phytoplankton: a novel mechanism. Limnol Oceanogr 35:260–269
Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775
Pettit NM, Smith ARJ, Freedman RB, Burns RG (1976) Soil urease: activity, stability and kinetic properties. Soil Biol Biochem 8:479–484
Pidoplichko NM (1953) Fungus flora of coarse fodders. Izdatel'stvo Akademii Nauk Ukrainskoj SSR, Kiev
Poomthongdee N, Duangmal K, Pathom-aree W (2015) Acidophilic actinomycetes from rhizosphere soil: diversity and properties beneficial to plants. J Antibiot 68:106–114
Preston WH, Mitchell JW, Reeve W (1954) Movement of alpha-methoxyphenylacetic acid from one plant to another through their root systems. Science 119:437–438
Rodriguez-Kabana R, Godoy G, Morgan-Jones G, Shelby RA (1983) The determination of soil chitinase activity: conditions for assay and ecological studies. Plant and Soil 75:95–106
Sabinin DA (1940) Mineral nutrition of plants. Publishing House AN USSR, Moscow
Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602
Shaikh MN, Mokat DN (2018) Role of rhizosphere fungi associated with commercially explored medicinal and aromatic plants: a review. Curr Agri Res J 6:72–77
Sims GK, Wander MM (2002) Proteolytic activity under nitrogen or sulfur limitation. Appl Soil Ecol 19:217–221
Sinha RK (2004) Absorption and translocation of water. Modern plant physiology. 1st ed. Alpha Science International, Pangbourne, pp 64–82
Stoklasa J, Brdlik V, Ernest A (1909) Zur Frage des Phosphorgehaltes des Chlorophylls. Berichte der Deutschen Botanischen Gesellschaft 27:10–20
Supriya N (2018) Rhizosphere. https://biologyreader.com/rhizosphere.html

Tu Q, He ZWL, Xue K, Xie G, Chain P, Reich PB, Hobbie SE, Zhou J (2017) Metagenomic reconstruction of nitrogen cycling pathways in a CO2-enriched grassland ecosystem. Soil Biol Biochem 106:99–108

Vallon, O., Bulté, L., Kuras, R., Olive, J. And Wollman, F.A., 1993. Extensive accumulation of an extracellular l-amino-acid oxidase during gametogenesis of Chlamydomonas reinhardtii. Eur J Biochem, 215: 351–360

Virtanen AI, von Hausen S, Laine T (1937) Investigations on the root nodule bacteria of leguminous plants: XX. Excretion of nitrogen in associated cultures of legumes and non-legumes. J Agric Sci 27:584–610

von Wirén N, Gazzarrini S, Frommer WB (1997) Regulation of mineral nitrogen uptake in plants. Plant and Soil 196:191–199

Wong KH, Hynes MJ, Davis MA (2008) Recent advances in nitrogen regulation: a comparison between Saccharomyces cerevisiae and filamentous fungi. Eukaryot Cell 7:917–925

Zanuta MI, Bremner JM (1976) Production and persistence of urease activity in soils. Soil Biol Biochem 8:369–374

Zimmermann A (1902) Ueber einige an tropischen Kulturpflanzen beobachtete Pilze II. Centralblatt fur Bakteriologie, Parasitenkunde und Infektionskrankheiten II Abteilung 8:216–221

Chapter 8
Roles of Root Exudates in Different Processes in the Nitrogen Cycle in the Rhizosphere

Ees Ahmad, Pawan K. Sharma, and Mohd Saghir Khan

Abstract The nitrogen cycle is greatly influenced by soil microbes through their transformation of different nitrogen compounds. Additionally, microbial diversity is profoundly modified by plant root exudates in the rhizosphere. Hence, root exudates indirectly control different processes in the nitrogen cycle by modifying the microbial community in the rhizosphere. We are beginning to understand more about the roles of plant root exudates in nitrogen fixation, nitrification, denitrification, anaerobic ammonium oxidation (anammox), dissimilatory nitrate reduction to ammonium (DNRA), nitrate reduction, nitrogen mineralization, and, finally, nitrogen uptake in the rhizosphere. Root exudates release chemoattractant compounds (flavonoids) into the rhizosphere; as a result, rhizobia move toward legume roots for colonization through a chemotactic process. The rhizobium–legume interaction is a very complex process involving root exudates, *nod* genes, and other compounds released from rhizobia and legume plants. Moreover, after nodulation, atmospheric nitrogen can be fixed and transformed into ammonia through biological processes involving the nitrogenase enzyme. Root exudates are also used as a carbon energy source by different microbial communities involved in asymbiotic nitrogen fixation, denitrification, and the DNRA and anammox processes. Chemical fertilizers, including synthetic nitrogen fertilizers, are also used for improving crop yields of different cereals and other vegetables in modern agricultural practices. Excess ammonia is further oxidized and converted into nitrite by *Nitrosomonas*, and, finally, nitrate is formed by *Nitrobacter* in a nitrification process in freshwater and soil ecosystems. In contrast, anammox, which is a two-step process, operates mainly in marine ecosystems and sediments. Better knowledge of these processes is needed so that urgent attention can be paid to optimizing the use of nitrogen fertilizers and minimizing their contributions to climate change and nitrogen pollution.

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8.1 The Nitrogen Cycle: An Overview

In general, the nitrogen (N) cycle in different ecosystems and environments can be summarized as a process of oxidation–reduction chemical reactions catalyzed by archaea, rhizospheric microorganisms, algae, and plants (Fig. 8.1). Of the total six nitrogen compounds, nitrate is completely oxidized, while ammonium is a fully reduced form of nitrogen are regulated by these organisms. Free nitrogen gas, an inorganic form of N, is present in the atmosphere and is not accessible to most living organisms, but N can be fixed (biochemically), transported into plants and other living things, and converted into its organic forms by diazotrophic prokaryotes and also by lightning (geochemically) (Vitousek et al. 2013). These prokaryotes may be archaea or bacteria, free living or in mutualistic cooperation, and can reduce nitrogen to ammonia (Hoffman et al. 2014). Further, ammonia is biologically incorporated into amines, transported from soil into different parts of plants, and finally converted into diverse organic compounds (Krapp 2015). Additionally, through the nitrification process, ammonium can easily be oxidized by soil microbes and converted into nitrite, nitrate, and hydroxylamine (Hayatsu et al. 2008). The nitrification and two-step oxidation processes are biologically performed by bacteria or archaea, known as ammonium-oxidizing archaea (AOAs) and ammonium-oxidizing bacteria (AOBs), and nitrite-oxidizing bacteria, respectively (Prosser and Nicol 2012). Recently, the complete ammonia oxidation (comammox) process has been described; through this biological process, both oxidative steps (conversion of ammonium into nitrite and into nitrate) are performed by a single organism, *Nitrospira* (Daims et al. 2015; van Kessel 2015). In contrast, the denitrification process—including reduction of nitrate to nitrite, nitric oxide, nitrous oxide, and, ultimately, free nitrogen gas—is executed by soil microbes, including fungi, bacteria, and archaea (Hayatsu et al. 2008). In addition to nitrification and denitrification, two other processes—dissimilatory nitrate reduction to ammonia and anaerobic ammonium oxidation (anammox)—are included in the N cycle (Rütting et al. 2011). The DNRA process—in which nitrate is used as an electron acceptor under microaerophilic/anaerobic conditions, reduced to nitrite, and finally converted into ammonia—is performed by fungi and bacteria, while nitrogen (Welsh et al. 2014) and free N_2 are finally produced in the anammox process from nitrate via nitrous oxide and hydrazine as intermediate forms (Kartal et al. 2011; van Niftrik et al. 2012).

The concept of the N cycle changed at the beginning of the twenty-first century after the discovery of archaea and as a result of human interference in the form of chemical fertilizer manufacturing to enhance crop production in current agricultural practices (Offre et al. 2013). After the discovery of archaea and their nitrogen fixation capabilities in freshwater and marine sediments, the newly discovered

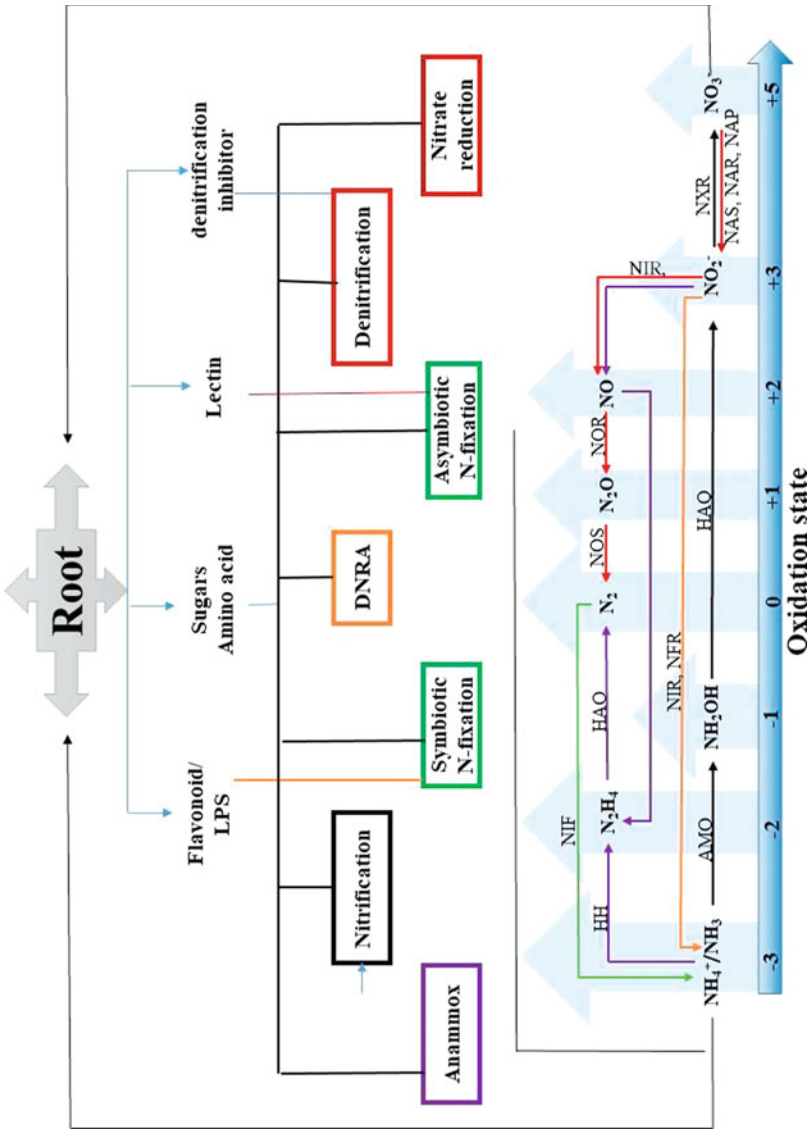

Fig. 8.1 Schematic outline of root exudates involved in different biological processes in the nitrogen cycle. *Colored arrows* show different processes in the nitrogen cycle, which are catalyzed by various enzymes, including nitrate reductases (NAS, NAR, and NAP), nitric oxide reductase (NOR), nitrite reductases (NIR and NFR), ammonia monooxygenase (AMO), nitrous oxide reductase (NOS), nitrogenase (NIF), nitrite oxidoreductase (NXR), hydroxylamine oxidoreductase (HAO), and hydrazine hydrolase (HH). *DNRA* dissimilatory nitrate reduction to ammonium, *LPS* lipopolysaccharide [Adapted from Coskun et al. (2017)]

Table 8.1 Nitrogen fertilizer use in production of three major crops worldwide

Crops	N fertilizer use [Tg]	Biomass production [Tg]	Ammonia production [Tg]	Nitrate production [Tg]	N_2O emissions [Tg]
Rice	19.2	6.10 (36%)	1.92–9.6	1.08–9.6	0.006–0.131
Wheat	21.6	10.37 (48%)	0.22–4.54	0.5–9.4	0.051–0.261
Maize	19.2	10.75 (56%)	2.11–9.22	1.06–10.27	0.202–0.257

roles of prokaryotes in the N cycle were discussed by researchers (Prosser and Nicol 2012). AOAs are broadly disseminated and capable of nitrification in acidic soil, but this process may be inhibited by high concentrations of ammonium (Verhamme et al. 2011). The impact of human activities on the N cycle has been estimated by data showing that the total amount of nitrogen fixation by the Haber–Bosch process in addition to other anthropogenic activity by human (210 Tg N/year) is greater than than the total amount of N-fixation by asymbiotic and symbitic process (203 Tg N/year). The use of chemical fertilizers in legume cultivation is essential for human nutrition (Erisman et al. 2008). A total of 120 Tg N $year^{-1}$ of N fertilizer is synthesized by chemical catalysis in the Haber–Bosch process, and 50% of the total N fertilizer that is produced is used in three major crops (Table 8.1): wheat (18%), rice (16%), and maize (16%) (Ladha et al. 2016). Plants themselves cannot fix atmospheric nitrogen and are not directly involved in the nitrification process, but they can uptake or assimilate nitrate (Vitousek et al. 1997) or ammonium from soil or water through their roots, depending on which substrate is suitable for uptake in different environments (Smith et al. 1999). Production and use of chemical fertilizers pose serious threats to the environment because they result in eutrophication of marine water and freshwater, and emission of the potent greenhouse gas N_2O (Ravishankara et al. 2009). After nitrogen fertilization of soil, nitrate and ammonium ions are generated, and some are taken up by plant roots, but most of the fertilizer is used as a substrate by nitrifiers and denitrifiers, causing substantial loss of N through production of N_2O in the denitrification process (Mosier et al. 1998; Shcherbak et al. 2014). Moreover, it is now very clear that plants are involved indirectly and regulate the N cycle by controlling the population of prokaryotes and fungi by releasing root exudates (Bardgett et al. 2014; Finzi et al. 2015). In this chapter, we discuss recent progress in research on root exudates and their involvement in pathways of N cycle nitrification, denitrification, etc.

8.2 Root Exudates: Current-Status

The rhizosphere is the active zone in soil where nutrients secreted from plant roots support microbial growth and biological activity, and exchange of nutrients is mobilized (Hamilton and Frank 2001; Landi et al. 2006; Zhu et al. 2014). This is a very densely populated area of soil, where the root system of one plant competes

with others by invasion into their root systems to acquire mineral nutrients, water, and space (Ryan and Delhaize 2001). Soil microorganisms (protozoa, bacteria, fungi, etc.) also contend with each other to utilize nutrient sources of organic materials (Bais et al. 2004). One of the most important metabolic processes in plant roots is secretion of a variety of compounds into the rhizosphere (Badri and Vivanco 2009). In this process, 5–21% of photosynthetically fixed carbon is secreted through plant roots in the form of root exudates (Marschner 1995; Derrien et al. 2004). The quality and amount of root exudates varies with the age and species of the plant and also depends on abiotic and biotic factors (Jones et al. 2004).

Root exudates are composed of low and high molecular weight compounds (Badri and Vivanco 2009) and include soluble and insoluble compounds produced by specialized cells, including border cells (Huang et al. 2014) in the roots of all plants (Table 8.2). The exudates regulate the microbial community in the rhizosphere (Hirsch et al. 2003) and act as signaling molecules that attract or repel microorganisms in the rhizosphere and provide nutrient support for microbes to establish a plant–microbe relationship (Hirsch et al. 2003; Dennis et al. 2010). Exopolysaccharides and some soluble and antagonist compounds help to regulate biotic and abiotic conditions for plants (Huang et al. 2014).

Mucilage is a type of root exudate secreted from aerial and underground roots of plants (Bennett et al. 2020). Compounds in mucilage secreted from aerial roots of Sierra Mixe corn and from underground roots of maize have been analyzed (McCully and Boyer 1997). Different polysaccharides, phospholipids, and proteins are found in mucilage (Read et al. 2003). Secretion of mucilage from roots is a common process in cereal crops, including barley, wheat, and sorghum (Kislev and Werker 1978; Sinha et al. 2002; Carter et al. 2019). In an in vitro analysis, the amount of mucilage synthesized was 11–47 mg of dry matter per gram of root (Nguyen 2003). The mucilage diffusion rate and quantity are determined by whether the root is grown in a nutrient solution or in water (Sealey et al. 1995). Mucilage secreted into soil helps to enhance the aggregation capability of soil, which promotes aeration of soil, prevents soil erosion, and supports root growth to maintain a continuous flow of water in the rhizosphere. Moreover, mucilage also protects the meristem of the root from toxic compounds (Read et al. 2003). To date, the quantity of mucilage secreted from plant roots into the rhizosphere remains unknown.

Plant roots also release different gases (CO_2, H_2, and ethylene) after different metabolic activities by microbes and plants in the rhizosphere. For example, CO_2 diffuses into the rhizosphere after carbohydrate respiration in the process of plant–microbe interaction (Phillips et al. 1999). Accumulation of CO_2 in the rhizosphere enhances Ca^{2+} production and uptake by plants through dissolution of $CaCO_3$ (Dakora and Phillips 2002).

Table 8.2 Chemical compounds identified in different root exudates, and their functions

Exudate compounds	Functions	Compounds identified in root exudates
Organic acids	Nutrient sources for microbes and plants, signaling to microbes in chemotaxis, chelating agents for low-solubility minerals, soil acidification, aluminum detoxification, *nod* gene induction	Citric acid, oxalic acid, pyruvic acid, glutaric acid, malonic acid, aldonic acid, malic acid, fumaric acid, succinic acid, erythronic acid, ferulic acid, acetic acid, butanoic acid, syringic acid, rosmarinic acid, glycolic acid, butyric acid, valeric acid, lactic acid, piscidic acid, trans-cinnamic acid, aconitic acid, formic acid, vanillic acid, tetronic acid
Amino acids	Nutrient sources for microbes and plants, chelating agents for low-solubility minerals, chemoattractant/repellent signaling to microbes	Proline, asparagine, aspartic acid, α- and β-alanine, valine, tryptophan, threonine, cysteine, cystine, ornithine, glutamate, arginine, histidine, glycine, isoleucine, lysine, homoserine, leucine, phenylalanine, serine, methionine, homoserine, γ-aminobutyric acid, α-aminoadipic acid
Sugars and vitamins	Essential nutrient sources for microbes and plants, chemoattractant/repellent signaling to microbes	Glucose, fructose, rhamnose, ribose, arabinose, desoxyribose, galactose, xylose, raffinose, maltose, oligosaccharides, biotin, riboflavin, thiamine, niacin, pantothenate, exopolysaccharide
Enzymes and proteins	P mineralization from immobilized organic molecules, conversion of complex organic forms of matter to simple forms, plant defense	Protease, acid/alkaline phosphatase, invertase, β-1,3-glucanases, amylase, lipases, pathogenesis-related proteins
Purines	Nutrient sources	Guanine, adenine, uridine, cytidine
Gases and inorganic ions	Chemoattractant/repellent signaling to microbes	CO_2, H_2, N_2O, HCO_3^{-1}, OH^{-1} and H^+
Phenolics	Nutrient sources, chemoattractant/repellent signaling to microbes, growth promotion, *nod* gene induction and inhibition in rhizobia, induction of resistance against phytoalexins, chelating agents for low-solubility minerals, aluminum detoxification, defense against soil pathogens	Liquiritigenin, daidzein, luteolin, 4′,7-dihydroxyflavanone, genistein, 4′,7-dihydroxyflavone, coumestrol, 4′,7-dihydroxy-2′-ethoxychalcone, eriodictyol, 4′,7-dihydroxyflavone, 3,5,7,3′-tetrahydroxy-4′methoxyflavone, naringenin, isoliquiritigenin, 7,3′-dihydroxy-4-′-methoxyflavone, umbelliferone, (+)- and (−)-catechin
Root border cell exudates	Production of signals that control mitosis and gene expression, stimulation of microbial growth, chemoattractant release, synthesis of defense molecules for the rhizosphere, decoys that keep the root cap infection free, release of mucilage and proteins	

Adapted from Jones et al. (2004), Badri and Vivanco (2009), Vranova et al. (2013), and Haichar et al. (2014).

8.3 Root Exudates and Different Processes in the Nitrogen Cycle

8.3.1 Root Exudates, Asymbiotic Relationships, and Nitrogen Fixation

Diazotrophic bacteria in the rhizospheres of cereals, grasses, and nonleguminous crops can fix environmental N_2 asymptotically in temperate and tropical agricultural systems. Asymbiotic N fixation is also performed in soil by different endophytic and free-living bacteria (Roper and Ladha 1995). Upto 60 kg ha^{-1} of N can be fixed asymbiotically by diazotrophic bacteria in soil around different varieties of nonleguminous crops (Cleveland et al. 1999; Gupta et al. 2006). One modern technique includes use of a radioisotope tracer in which a ^{15}N-enriched radiolabeled compound is used for quantification of asymbiotically fixed N in graminaceous plants. This has enabled estimation of a significant economic profit from asymbiotic fixed N in soil (Kennedy and Islam 2001; Hurek et al. 2002). Moreover, through molecular approaches, a diversity of culturable and nonculturable N-fixing microbes have been identified in the rhizospheres of cereals and nonleguminous plants (Hurek et al. 2002; Buckley et al. 2007).

Root exudates are continuously secreted from plant roots (Greer-Phillips et al. 2004) and influence the population and metabolic activity of diazotrophic and free-living bacteria in the rhizosphere (Fig. 8.1). Bacteria move in a favorable direction in the rhizosphere by flagellar rotation in response to release of specific chemical compounds from root exudates; this is known as chemotaxis (Eisenbach 1996). Thus, root exudates can indirectly control asymbiotic N fixation (Steenhoudt and Vanderleyden 2000). For example, *Azospirillum brasilense* is chemotactically attracted to compounds secreted from root exudates in the rhizosphere and consequently colonizes the root surface (Steenhoudt and Vanderleyden 2000). In contrast, different N sources [NH_4Cl, KNO_3, NH_4NO_3, and urea ($CO[NH_2]_2$)] can interfere with colonization by *Azospirillum* in rice and wheat plants (Naher et al. 2018). The root volume, shoot dry biomass, and N content in shoots is reduced in corn when the population of *Azospirillum* is reduced and less nitrogenase activity occurs. A total of nine amino acids (asparagine, serine, aspartic acid, glutamic acid, phenylalanine, valine, threonine, alanine, and tryptophan), six sugars (galactose, glucose, xylose, sucrose, arabinose, and fructose), and four organic acid (fumarate, malate, citrate, and succinate) have been identified in exudates from corn roots. The organic acids and five of the sugars (excluding glucose) secreted from root exudates are used by *Azospirillum* as energy sources in the rhizosphere (Pereira et al. 2020). Interestingly, indole acetic acid (IAA) has been synthesized in vitro by *Azospirillum*, using root exudates from lentil (*Lens culinaris*), bean (*Phaseolus vulgaris* L.), radish (*Raphanus sativus* L.), tomato (*Lycopersicum esculentum*), rice (*Oryza sativa* L.), canola (*Brassica napus* L.), and clover (*Trifolium alexandrinum* L.) plants grown in a medium supplemented with L-tryptophan, a precursor of IAA. A supernatant of *Azospirillum* A3 grown in a medium containing root exudates from different plants

enhanced the growth of rice roots (Moghaddam et al. 2012). In a recent study, glutamic acid (30μM L^{-1}) stimulated chemotaxis by 2.9 and 7.4 times (in comparison with control conditions) in the rhizospheres of cabbage and lettuce plants, and it acted as a signaling molecule for chemotaxis and colonization of the cabbage and lettuce roots. In a pot assay, the biomass, chlorophyll content, and available N significantly increased in lettuce bacterized with *Azospirillum* Ac63 by improving the quality of root exudates and enhancing secretion of glutamic acid in the rhizosphere (Wang et al. 2020).

8.3.2 Symbiotic Nitrogen Fixation and Root Exudates

N fixation is a metabolic activity that synthesizes ammonia from environmental N by use of nitrogenase enzymes. In this process, the system consumes 5% of the energy produced by plant photosynthates (Dong and Layzell 2001). H_2 is released as a by-product of N fixation in legumes. Some rhizobia have a hydrogenase (Hup) enzyme for uptake of H_2 gas to produce energy by oxidation, but most rhizobia lack a hydrogenase enzyme and are unable to use H_2 gas. Ultimately, therefore, the H_2 gas diffuses into the rhizosphere from root nodules (Golding et al. 2012). H_2 gas release after N fixation is also helpful for modification of the soil microbiome and contributes indirectly to plant growth (Dong and Layzell 2001). Simultaneously, H_2 gas stimulates the hydrogen-oxidizing rhizobial community, which can indirectly boost plant growth–promoting activities, such as root elongation, by syntjesizing IAA (Ahmad et al. 2020) and retarding ethylene releases from plant roots (Ahmad et al. 2013). This is beneficial to nonleguminous and leguminous plants (Maimaiti et al. 2007).

It is well established that root exudates are involved in development of symbiotic relationships between legumes and rhizobia (*Bradyrhizobium*, *Sinorhizobium*, *Rhizobium*, *Mesorhizobium*, etc.) in the rhizosphere. This is known as *Rhizobium*–legume symbiosis (Oldroyd 2013; Philippot et al. 2013). Chemical compound releases from legume roots, especially releases of flavonoids (hesperetin, genistein, and naringenin), activate and synthesize nodulation factors (Hassan and Mathesius 2012) through initiation or catalysis of expression of *nod* genes in rhizobia (Begum et al. 2001). Nodulation factors are host specific and are classified as lipochitooligosaccharides (LPOs). They are secreted by rhizobia and stimulate initiation of the nodulation process (Limpens et al. 2015). Mechanistically, these discharged LPO molecules bind to special receptors located in the plasma membrane of epidermal cells on legume root hairs and initiate the process of nodulation by stimulating a calcium-dependent cascade (Ahmad et al. 2012; Oldroyd 2013). Flavonoids are continuously secreted into the rhizosphere from legume roots, but their concentrations increase and they act as chemoattractants when compatible *Rhizobium* species are present in the rhizosphere (Zuanazii et al. 1998). Moreover, symbiotic N-fixation is influenced by intercropping of faba beans and wheat. For example, the number of nodules and total dry weight of nodule/plant increases in

faba beans after intercropping of faba beans and wheat, in comparison with monocropping. The nodulation process in faba beans is enhanced after intercropping through increases in the concentrations of chalcone, flavanol, hesperetin, and isoflavone in plant root exudates (Table 8.3). Furthermore, symbiotic N fixation is influenced by intercropping of faba beans and wheat (Liu et al. 2017). In a recent in vitro study, the effect of bis(2-ethylhexyl) phthalate (DEHP), a stress compound, on the quality and quantity of secreted root exudates was analyzed. Root exudates were collected from the roots of alfalfa (*Medicago sativa*), grown in hydroponic solution, and analyzed. This revealed that the root exudates were composed mainly of carbohydrates (28.6%), organic acids (15.58%), and lipids (13.87%), among a total of 314 identified compounds. Moreover, DEHP indirectly alters the nodulation process by retarding the rate of flavonoid diffusion from plant roots. Mechanistically, a lower concentration of DEHP suppresses the concentration of 4′,5-dihyrroxy-7- methoxyisoflavone (a flavonoid) in the root exudates and also influences carbohydrate metabolism (Wang et al. 2020).

Proteins secreted from rhizobia are also involved in determining the host specificity of rhizobium–legume interactions. There are three known mechanisms of protein secretion in rhizobia. The first study on secretion of proteins elicited that the type I secretion system was induced by nodulation factors (NodD and NodO) and flavonoids involved in symbiosis of *Rhizobium leguminosarum* bv. *viciae* (de Maagd 1989). In another mechanism, nodulation outer proteins are released by the type III secretion system in rhizobia. Activation of the type III secretion system in *Bradyrhizobium japonicum* requires NodW factors, NodD1, and flavonoids (Krause et al. 2002). Type III secretion systems have also been reported in *Sinorhizobium fredii* USDA257 (Krishnan et al. 2003), *Bradyrhizobium elkanii* (Okazaki et al. 2009), and *Mesorhizobium loti* MAFF303999 (Okazaki et al. 2010). Nodulation outer proteins (NOPs)—including nopA, nopC, nopB, nopL, nopX, and nopP—are secreted from the type III secretion system of *S. fredii* USDA257 (Deakin and Broughton 2009).

Several studies have shown that the rhizobial nodulating capacity of legumes at the genus and species levels of rhizobia is influenced by proteins secreted by the type III secretion system (Krishnan et al. 2003; Ausmees et al. 2004; Okazaki et al. 2009, 2010).

8.4 Root Exudates Control Loss of Nitrogen Through Denitrification and the Anammox Process in the Nitrogen Cycle

One of the key processes in the N cycle is nitrification, in which different nitrogen compounds are converted into nitrate through microbial processes (Fig. 8.1). The nitrification process depends on the types of nitrogen compounds and microbial metabolic activity in the rhizosphere (Subbarao et al. 2007). In this process, less

Table 8.3 Roles of root exudates in different processes in the nitrogen cycle

N cycle processes	Plants	Bacteria	Mechanisms	References
Symbiotic N fixation	*Medicago sativa*	*Sinorhizobium meliloti*/ 3654	*nod* gene and chemotaxis induction	Hartwig et al. (1990)
	Cicer arietinum	*Rhizobium ciceri*	*nod* gene induction	Srivastava et al. (1999)
	Vigna unguiculata	*Rhizobium* sp. NGR234	*nod* gene and chemotaxis induction	Dakora (2000)
	Medicago truncatula	*Sinorhizobium meliloti*	*nod* gene induction	Zhang et al. (2007)
	Trifolium spp.	*Rhizobium leguminosarum*	Exopolysaccharide secretion by *Rhizobium* to establish effective symbiosis	Janczarek and Skorupska (2011)
	Vicia sativa	*Rhizobium leguminosarum* bv. *viciae*	Flavonoid-induced calcium signaling	Moscatiello et al. (2010)
	Faba bean		Influences of intercropping on components and content change of flavonoids in root exudates and nodulation	Liu et al. (2017)
	Phaseolus vulgaris	*Rhizobium tropici* CIAT899, *Rhizobium etli* ISP42	Lipochitooligosaccharide profile changes induced by exudates collected in the presence of *Chryseobacterium balustinum*	Dardanelli et al. (2012)
	Glycine max L., *Zea mays*	Bacterial community in the rhizosphere	Changes in bacterial community structure	Guo et al. (2017)
	Soybean	*Bradyrhizobium diazoefficiens*	Upregulation of proteins involved in nodulation in response to root exudates	Liu et al. (2015)
	Lotus corniculatus	Rhizobial strains	Presence of citramalate in *Lotus* root exudates	Kidd et al. (2018)

Asymbiotic N fixation	*Zea mays*	*Azospirillum brasilense*	Stimulation of root carboxylate exudation by *Azospirillum brasilense* inoculation, influencing the rhizospheric microbial community	D'Angioli et al. (2017)
	Zea mays	*Azospirillum*	Root exudation that helps *Azospirillum* to colonize plants	Pereira et al. (2020)
	Chickpea	*Azotobacter*	Root colonization	Velmourougane et al. (2017)
	Wheat	*Azospirillum brasilense*	Chemical receptor involvement in chemotaxis	O'Neal et al. (2019)
	Amaranthus tricolor	*Azotobacter chroococcum*	Glutamic acid (in root exudates) involvement in chemotaxis	Wang et al. (2020)
	Triticum aestivum, *Medicago sativa*	*Azospirillum brasilense*	Influence of root exudates on chemotaxis	O'Neal et al. (2020)
	Oryza sativa	*Azospirillum* sp.	Inhibition of chemotactic bacterial movement toward root exudates by NH^{4+}	Naher et al. (2018)
	Hosta plantaginea, *Iris tectorum*, *Ophiopogon japonicus*		*nrfA* gene abundance; root exudates in the topsoil may provide carbon sources for local activity of dissimilatory nitrate reduction to ammonium	Chen et al. (2019)
	Corn, cotton, soy, millet	Bacterial community	Activity of dissimilatory nitrate reduction to ammonium measured in rhizospheric and nonrhizospheric soil planted with different crops	Pan et al. (2020)
	Spartina alterniflora		*nrfA* gene abundance measured in rhizospheric and nonrhizospheric soil	Li et al. (2019)
	Iris pseudacorus, *Canna glauca*, *Scirpus validus*, *Cyperus alternifolius*	Bacterial community	Activity of dissimilatory nitrate reduction to ammonium measured in in vitro analysis of a wetland microcosm fed with Hoagland solution	
	Wheat		Influence of roots on denitrifying gene (*nirK*, *nirS*, *nosZ*) abundance	Usykin-Tonne et al. (2020)
	Wheat		Denitrifying gene (*nirK*, *nirS*, *nosZ*) abundance; roots contribute to denitrification	Ai et al. (2017)

(continued)

Table 8.3 (continued)

N cycle processes	Plants	Bacteria	Mechanisms	References
Nitrification	*Picea asperata*	*Nitrobacter hamburgensis*	Stimulation of nitrifying bacteria by root exudates	Zhang et al. (2016)
Ammonification	*Picea asperata*	*Bacillus subtilis*	Stimulation of ammonia-oxidizing bacteria by root exudates	Zhang et al. (2016)
Denitrification	*Picea asperata*	*Brachymonas denitrificans*	Stimulation of denitrifying bacteria by root exudates	Zhang et al. (2016)
	Phragmites australis, *Iris pseudacorus*, *Juncus effusus*		Potential importance of root exudates as an organic C source for denitrification	Zhai et al. (2013)
	Triticum aestivum, *Brassica napus*, *Medicago truncatula*, *Arabidopsis thaliana*		Denitrifying gene (*nirK*, *nirS*, *nosZ*) abundance; host plants control the microbial community and denitrification	Achouak et al. (2019)
	Spirodela polyrrhiza, *Lemna minor*		Possible roles of root exudates as carbon sources as well as nonnutrient signals for denitrification	Lu et al. (2014)

mobile ammonium is converted into mobile nitrate through enzymatic processes (Subbarao et al. 2009). Nitrate formed in the rhizosphere is less utilized by plants than ammonium. Nitrification may not be beneficial, because it may increase loss of fertilizer N through leaching and denitrification (Subbarao et al. 2006). Recovery of N and improvement of nitrogen use efficiency through inhibition of nitrification is a key strategy to control loss of N in the rhizosphere (Subbarao et al. 2009). Some plants release certain compounds from root exudates that inhibit or suppress the nitrification process, and this is known as biological nitrification inhibition (BNI) (Subbarao et al. 2006). Recently, to evaluate the BNI process, a luminescent assay was developed for detection of the ammonium oxidation process (conversion of ammonia into nitrite) in the rhizosphere, in which recombinant *Nitrosomonas europaea* was used as a bioindicator (Subbarao et al. 2007). BNI compounds were tested in various species of plants, such as cereals, legumes, and plants from tropical and temperate regions. BNI activity ranged between 0 and 18.3 AT (inhibitor allylthiourea/gm of root dry weight day^{-1}) unit in root exudates from 18 different species of field grass, pearl millet, cereals, legumes, and vegetables. Among pasture grasses, BNI activity was greatest in *Brachiaria decumbens* and *B. humidicola* (Subbarao et al. 2007). Many low- and high-BNI genotypes have been detected in *B. humidicola* pasture grass. In a pot experiment, *B. humidicola* suppressed around 90% of the nitrification process by releasing BNI compounds into the rhizosphere, and the soil concentration of ammonium, as an inorganic form of nitrogen, remained unchanged (Subbarao et al. 2007). Plants that release only small amounts of BNI compounds have been shown not to inhibit the nitrification process; most of the ammonia is oxidized and converted into nitrate in soil (Zakir et al. 2008; Subbarao et al. 2012). Similarly, after screening, studies have revealed that other cereal crops (maize, wheat, rice, and barley) do not secrete BNI compounds in their root exudates (Lata et al. 1999). Moreover, legumes do not interfere in the nitrification process, because they lack BNI capacity. Synthesis and exudation of BNI compounds by sorghum, *B. humidicola*, and *Leymus racemosus* is influenced by the form of nitrogen applied to soil. When nitrate was applied to soil, BNIs were not released from the roots, whereas after ammonia application to soil, BNIs compounds were synthesized (Subbarao et al. 2013). Methyl 3-(3-hydroxyphenyl) propionate (MHPP) inhibits the denitrification process and has been identified in root exudates from sorghum grown in ammonia-treated soil. MHPP inhibits the nitrification process via the ammonia monooxygenase (AMO) enzymatic pathway but does not interfere in the hydroxylamine oxidoreductase (HAO) enzymatic pathway of *Nitrosomonas* (Zakir et al. 2008). The BNI process can be exploited for management of soil denitrification processes in agronomic approaches.

Root exudates are equally important in serving as a C source for growth of the bacterial population involved in the denitrification process (Zhai et al. 2013). The denitrification process is inhibited by BNI compounds in root exudates, while bacterial use of this C source to oxidize ammonia creates equilibrium in the N cycle in the rhizosphere. After the denitrification process in the rhizosphere of wheat, N_2O is released into the environment (Table 8.3). The rate of emission of N_2O is directly influenced by *nirS* (nitrite reductase) gene abundance in

Rhodobacterales and Pseudomonadales in the rhizosphere (Ai et al. 2017). Two interlinked and important key processes in the N cycle that operate in different ecosystems (estuarine water, freshwater, and the ocean) are the anammox process and denitrification (Francis et al. 2007; Zhu et al. 2010). Between 31% and 41% of N_2 is emitted from the rhizosphere of rice, while only 2–3% of N_2 is released from nonrhizospheric soil via the anammox process (Nie et al. 2018). A total of 79% of N loss in marine ecosystems occurs through the anammox process. In contrast, denitrification accounts for 87% of N loss in freshwater and soil (Schubert et al. 2006). The diversity of anammox bacteria in the rhizospheres of submerged plants and sediments is influenced by the concentrations of nitrate, ammonia, and organic matter, and by redox potential and oxygen availability (Lee and Francis 2017). The most important parameters for the anammox process are the availability of dissolved oxygen (Oshiki et al. 2016) and salinity (Sonthiphand et al. 2014) in submerged ecosystems. The ammonia-to-nitrate molar ratio also influences the anammox process. A phylogenetic analysis indicated that the anammox bacteria *Brocadia fuigida* and *Scalindua wagneri* and the *nirS* denitrifying bacteria *Herbaspirillum* and *Pseudomonas* were the dominant species in sediment around declined *Potamogeton crispus*. It was suggested that a sudden decline in submerged macrophytes would increase the abundance of anammox bacteria and denitrifying bacteria in a relatively short time (Hu et al. 2020).

8.5 Root Exudates and the DNRA Process

The DNRA process is very critical in our understanding of soil ecosystems in microaerophilic conditions and in the presence of nitrate (Stein and Klotz 2016). While free N releases in atmosphere resulted loss of N during the denitrification and anammox processes (Canfield et al. 2010). Through N gas emissions, significant loss of N occurs in the denitrification and anammox processes, although the DNRA process helps to retain N in the form of ammonia in aquatic systems (An and Gardner 2002). Ammonia is further taken up by plant roots, enhancing plant growth. In the DNRA process, highly mobile nitrate and nitrite are reduced to ammonia (An and Gardner 2002). DNRA activity is widely detected in soils or environments with microaerophilic conditions, such as wetland systems (Gao et al. 2017; Zhang et al. 2017), terrestrial (forest, grassland, agriculrural land, dessert) habitats, floodplains (Jones et al. 2017), and marine sediments (Cheng et al. 2016). The DNRA process is also influenced by the C-to-N and carbon-to-nitrate ratios in both terrestrial and aquatic systems (Robertson et al. 2016; Zhou et al. 2017).

Chemolithoautotrophic and heterotrophic bacteria and other diverse microbes are involved in the DNRA process (Pang and Ji 2019). Additionally, the *nrfA* gene, which encodes nitrite reductase, has been developed as a biomarker gene for the DNRA process (Welsh et al. 2014). This gene has been identified in diverse groups of bacteria: Planctomycetes, Chloroflexi, Bacteroides, Acidobacteria, Planctomycetes, Firmicutes, and Verrucomicrobia (Welsh et al. 2014). The

microbial diversity surrounding plant roots is greatly influenced by root exudates in terrestrial and aquatic habitats, and the DNRA process is indirectly affected by root exudates as the population of microbes is manipulated. For example, in a recent study, the DNRA rate, the abundance of *Chthiniobacter*, and the total organic matter content were correlated in rhizospheric and nonrhizospheric soil. The DNRA rates were higher in rhizospheric soils where larger populations of *Chthiniobacter* were recorded than in nonrhizospheric soil because of the greater availability of C sources in rhizospheric soil (Pan et al. 2020).

8.6 Nitrogen Mineralization and Uptake by Plant Roots

Proteins and peptides from decomposed material from living organisms in soil is an immobilized form of an organic source of nitrogen. This complex form of organic N is converted into amino acids by protease and further degraded into NH_4 by the bacterial community in the rhizosphere (Ahmad et al. 2014). Peptidases secreted in root exudates from *Medicago* enhance N mineralization in the rhizosphere (De-la-Pena and Vivanco 2010). Additionally, Godlewski and Adamczyk (2007) reported that proteases were secreted in root exudates from 15 different types of wild and agricultural plant species. Later, they concluded that secretion of proteases from wheat (Adamczyk et al. 2008) and allium (Adamczyk et al. 2009) was a strategy on the part of the plants to mineralize complex organic forms of N into simple forms for utilization of nitrogen. Root uptake of amino acids from soil was studied using a proteomic and isotopic method in which radiolabeled glycine was used as a source of nitrogen for uptake by *Lolium perenne* plants (Thornton et al. 2007). The microbial community in the rhizosphere also releases proteases and break down proteins into amino acids. Proteases in root exudates or in the rhizosphere digest protein and convert it into amino acids to facilitate N uptake by plant roots. These limited findings clearly indicate that root exudates facilitate mineralization of N and its further uptake by plants. In the N mineralization process, rhizospheric microbes secrete proteases that break down complex forms of N (in proteins and peptides) into simple organic forms (in amino acids) and further convert them into ammonium. Carbon is one of the growth-limiting factors for microbes. Thus, this limitation is partially controlled by exudates that are secreted from roots, move through soil, and transform it into rhizospheric soil (Lynch and Whipps 1990).

8.7 Conclusion

The biochemistry of root exudates is still not fully understood and varies between different species of plants. Because the exact mechanisms of plant root exudate secretion are not fully understood, many aspects of the biological processes of plant–microbe interactions are still unknown. Characterization of molecules that influence

microbial diversity in the rhizosphere and metabolic profiling of root exudates are ongoing processes aimed at increased understanding of the roles of root exudates in plant–microbe interactions. Rhizospheric microbial diversity and root exudate compounds are involved directly and indirectly in different processes in the nitrogen cycle in the rhizosphere and in other ecosystems. More physiological study of root exudation mechanisms is needed for greater understanding of the biochemistry of the nitrogen cycle in the rhizosphere.

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References

Achouak W, Abrouk D, Guyonnet J, Barakat M, Ortet P, Simon L, Lerondelle C, Heulin T, Haichar FE (2019) Plant hosts control microbial denitrification activity. FEMS Microbiol Ecol 95:fiz021

Adamczyk B, Godlewski M, Zimny J, Zimny A (2008) Wheat (*Triticum aestivum*) seedlings secrete proteases from the roots and, after protein addition, grow well on medium without inorganic nitrogen. Plant Biol 10:718–724

Adamczyk B, Godlewski M, Smolandera A, Kitunen V (2009) Degradation of proteins by enzymes exuded by *Allium porrum* roots—a potentially important strategy for acquiring organic nitrogen by plants. Plant Physiol Biochem 47:919–925

Ahmad E, Zaidi A, Khan MS, Oves M (2012) Heavy metal toxicity to symbiotic nitrogen-fixing microorganism and host legumes. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer-Verlag, Wien, pp 29–44

Ahmad E, Khan MS, Zaidi A (2013) ACC deaminase producing *Pseudomonas putida* strain 328 PSE3 and *Rhizobium leguminosarum* strain RP2 in synergism improves growth, nodulation and 329 yield of pea grown in alluvial soils. Symbiosis 61:93–104

Ahmad E, Zaidi A, Khan MS (2014) Response of PSM inoculation to certain legumes and cereal crops. In: Khan MS, Zaidi A, Musarrat J (eds) Phosphate solubilising microorganisms: principles and application of microphos technology. Springer-Verlag, Cham, pp 175–206

Ahmad E, Sharma SK, Sharma PK (2020) Deciphering operation of tryptophan-independent pathway in high IAA producing Micrococcus aloeverae DCB-20. FEMS Microbiol Lett 367: fnaa 190

Ai C, Liang G, Wang X, Sun J, He P, Zhou W (2017) A distinctive root-inhabiting denitrifying community with high $N_2O/(N_2O+N_2)$ product ratio. Soil Biol Biochem 109:118–123

An S, Gardner WS (2002) Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). Marine Ecol Prog Ser 237:41–50

Ausmees N, Kobayashi H, Deakin WJ, Marie C, Krishnan HB, Broughton WJ, Perret X (2004) Characterization of NopP, a type III secreted effector of *Rhizobium* sp. strain NGR234. J Bacteriol 186:4774–4780

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Bardgett RD, Mommer L, De Vries FT (2014) Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol 29:692–699

Begum AA, Leibovitch S, Migner P, Zhang F (2001) Specific flavonoids induced *nod* gene expression and pre-activated *nod* genes of *Rhizobium leguminosarum* increased pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) nodulation in controlled growth chamber environments. J Exp Bot 52:1537–1543

Bennett AB, Pankievicz VC, Ané JM (2020) A model for nitrogen fixation in cereal crops. Trends Plant Sci 25:226–235

Buckley DH, Huangyutithan V, Hsu SF, Nelson TA (2007) Stable isotope probing with N15 reveals novel noncultivated diazotrophs in soil. Appl Environ Microbiol 73:3196–3204

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of Earth's nitrogen cycle. Science 330:192–196

Carter AY, Ottman MJ, Curlango-Rivera G, Huskey DA, D'Agostini BA, Hawes MC (2019) Drought-tolerant barley: II. Root tip characteristics in emerging roots. Agronomy 9:220

Chen T, Liu Y, Zhang B, Sun L (2019) Plant rhizosphere, soil microenvironment, and functional genes in the nitrogen removal process of bioretention. Environ Sci Process Impacts 21:2070–2079

Cheng L, Li X, Lin X, Hou L, Liu M, Li Y, Liu S, Hu X (2016) Dissimilatory nitrate reduction processes in sediments of urban river networks: spatiotemporal variations and environmental implications. Environ Pollut 219:545–554

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. Global Biogeochem Cycles 13:623–645

Coskun D, Britto DT, Shi W, Kronzucker HJ (2017) How plant root exudates shape the nitrogen cycle. Trends Plant Sci 22:661–673

D'Angioli AM, Viani RA, Lambers H, Sawaya AC, Oliveira RS (2017) Inoculation with *Azospirillum brasilense* (Ab-V4, Ab-V5) increases *Zea mays* root carboxylate-exudation rates, dependent on soil phosphorus supply. Plant and Soil 410:499–507

Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH (2015) Complete nitrification by *Nitrospira* bacteria. Nature 528:504–509

Dakora FD (2000) Commonality of root nodulation signals and nitrogen assimilation in tropical grain legumes belonging to the tribe Phaseoleae. Aust J Plant Physiol 27:885–892

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil 245:35–47

Dardanelli MS, de Córdoba FJ, Estévez J, Contreras R, Cubo MT, Rodríguez-Carvajal MÁ, Gil-Serrano AM, López-Baena FJ, Bellogín R, Manyani H, Ollero FJ (2012) Changes in flavonoids secreted by *Phaseolus vulgaris* roots in the presence of salt and the plant growth–promoting rhizobacterium *Chryseobacterium balustinum*. Appl Soil Ecol 57:31–38

de Maagd RA, Wijfjes AH, Spaink HP, Ruiz-Sainz JE, Wijffelman CA, Okker RJ, Lugtenberg BJ (1989) NodO, a new *nod* gene of the *Rhizobium leguminosarum* biovar viciae sym plasmid pRL1JI, encodes a secreted protein. J Bacteriol 171:6764–6770

Deakin WJ, Broughton WJ (2009) Symbiotic use of pathogenic strategies: rhizobial protein secretion systems. Nat Rev Microbiol 7:312–320

De-la-Pena C, Vivanco JM (2010) Root–microbe interactions: the importance of protein secretion. Curr Proteomics 7:265–274

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327

Derrien D, Marol C, Balesdent J (2004) The dynamics of neutral sugars in the rhizosphere of wheat: an approach by ^{13}C pulse–labelling and GC/C/IRMS. Plant and Soil 267:243–253

Dong Z, Layzell DB (2001) H_2 oxidation, O_2 uptake and CO_2 fixation in hydrogen treated soils. Plant and Soil 229:1–12

Eisenbach M (1996) Control of bacterial chemotaxis. Mol Microbiol 20:903–910

Erisman JW, Sutton MA, Galloway J, Klimont Z, Winiwarter W (2008) How a century of ammonia synthesis changed the world. Nat Geosci 1:636–639

Finzi AC, Abramoff RZ, Spiller KS, Brzostek ER, Darby BA, Kramer MA, Phillips RP (2015) Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. Glob Chang Biol 21:2082–2094

Francis CA, Beman JM, Kuypers MM (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J 1:19–27

Gao D, Li X, Lin X, Wu D, Jin B, Huang Y, Liu M, Chen X (2017) Soil dissimilatory nitrate reduction processes in the Spartina alterniflora invasion chronosequences of a coastal wetland of southeastern China: dynamics and environmental implications. Plant Soil 421:383–399

Godlewski M, Adamczyk B (2007) The ability of plants to secrete proteases by roots. Plant Physiol Biochem 45:657–664

Golding A, Zou Y, Yang X, Flynn B, Dong Z (2012) Plant growth promoting H_2-oxidizing bacteria as seed inoculants for cereal crops. Agric Sci 3:510–516

Greer-Phillips SE, Stephens BB, Alexandre G (2004) An energy taxis transducer promotes root colonization by *Azospirillum brasilense*. J Bacteriol 186:6595–6604

Guo M, Gong Z, Miao R, Su D, Li X, Jia C, Zhuang J (2017) The influence of root exudates of maize and soybean on polycyclic aromatic hydrocarbons degradation and soil bacterial community structure. Ecol Eng 99:22–30

Gupta VV, Roper MM, Roget DK (2006) Potential for non-symbiotic N2-fixation in different agroecological zones of southern Australia. Soil Res 44:343–354

Haichar FZ, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated interactions belowground. Soil Biol Biochem 77:69–80

Hamilton EW III, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82:2397–2402

Hartwig UA, Maxwell CA, Joseph CM, Phillips DA (1990) Chrysoeriol and luteolin released from alfalfa seeds induce *nod* genes in *Rhizobium meliloti*. Plant Physiol 92:116–122

Hassan S, Mathesius U (2012) The role of flavonoids in root–rhizosphere signalling: opportunities and challenges for improving plant–microbe interactions. J Exp Bot 63:3429–3444

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45

Hirsch AM, Bauer WD, Bird DM, Cullimore J, Tyler B, Yoder JI (2003) Molecular signals and receptors: controlling rhizosphere interactions between plants and other organisms. Ecology 84:858–868

Hoffman BM, Lukoyanov D, Yang ZY, Dean DR, Seefeldt LC (2014) Mechanism of nitrogen fixation by nitrogenase: the next stage. Chem Rev 114:4041–4062

Hu J, Zhou Y, Lei Z, Liu G, Hua Y, Zhou W, Wan X, Zhu D, Zhao J (2020) Effects of Potamogeton crispus decline in the rhizosphere on the abundance of anammox bacteria and nirS denitrifying bacteria. Environ Pollut 260:114018

Huang XF, Chaparro JM, Reardon KF, Zhang R, Shen Q, Vivanco JM (2014) Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92:267–275

Hurek T, Handley LL, Reinhold-Hurek B, Piché Y (2002) Azoarcus grass endophytes contribute fixed nitrogen to the plant in an unculturable state. Mol Plant-Microbe Interact 15:233–242

Janczarek M, Skorupska A (2011) Modulation of *rosR* expression and exopolysaccharide production in *Rhizobium leguminosarum* bv. *trifolii* by phosphate and clover root exudates. Int J Mol Sci 12:4132–4155

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Jones ZL, Jasper JT, Sedlak DL, Sharp JO (2017) Sulfide-induced dissimilatory nitrate reduction to ammonium supports anaerobic ammonium oxidation (anammox) in an open-water unit process wetland. Appl Environ Miccrobiol 83(15)

Kartal B, Maalcke WJ, de Almeida NM, Cirpus I, Gloerich J, Geerts W, den Camp HJ, Harhangi HR, Janssen-Megens EM, Francoijs KJ, Stunnenberg HG (2011) Molecular mechanism of anaerobic ammonium oxidation. Nature 479:127–130

Kennedy IR, Islam NJ (2001) The current and potential contribution of asymbiotic nitrogen fixation to nitrogen requirements on farms: a review. Austr J Exp Agric 41:447–457

Kidd DR, Ryan MH, Hahne D, Haling RE, Lambers H, Sandral GA, Simpson RJ, Cawthray GR (2018) The carboxylate composition of rhizosheath and root exudates from twelve species of grassland and crop legumes with special reference to the occurrence of citramalate. Plant and Soil 424:389–403

Kislev M, Werker E (1978) Mucilage on the root surface and root hairs of sorghum: heterogeneity in structure, manner of production and site of accumulation. Ann Bot 42:809–816

Krapp A (2015) Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. Curr Opin Plant Biol 25:115–122

Krause A, Doerfel A, Göttfert M (2002) Mutational and transcriptional analysis of the type III secretion system of *Bradyrhizobium japonicum*. Mol Plant Microbe Interact 15:1228–1235

Krishnan HB, Lorio J, Kim WS, Jiang G, Kim KY, DeBoer M, Pueppke SG (2003) Extracellular proteins involved in soybean cultivar–specific nodulation are associated with pilus-like surface appendages and exported by a type III protein secretion system in *Sinorhizobium fredii* USDA257. Mol Plant Microbe Interact 16:617–625

Ladha J, Tirol-Padre A, Reddy C et al (2016) Global nitrogen budgets in cereals: a 50-year assessment for maize, rice, and wheat production systems. Sci Rep 6:19355

Landi L, Valori F, Ascher J, Renella G, Falchini L, Nannipieri P (2006) Root exudate effects on the bacterial communities, CO_2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils. Soil Biol Biochem 38:509–516

Lata JC, Durand J, Lensi R, Abbadie L (1999) Stable coexistence of contrasted nitrification statuses in a wet tropical savanna system. Funct Ecol 13:762–763

Lee JA, Francis CA (2017) Spatiotemporal characterization of San Francisco Bay denitrifying communities: a comparison of *nirK* and *nirS* diversity and abundance. Microb Ecol 73:271–284

Li X, Gao D, Hou L, Liu M (2019) Soil substrates rather than gene abundance dominate DNRA capacity in the *Spartina alterniflora* ecotones of estuarine and intertidal wetlands. Plant Soil 436:123–140

Limpens E, van Zeijl A, Geurts R (2015) Lipochitooligosaccharides modulate plant host immunity to enable endosymbioses. Annu Rev Phytopathol 53:311–334

Liu Y, Guan D, Jiang X, Ma M, Li L, Cao F, Chen H, Shen D, Li J (2015) Proteins involved in nodulation competitiveness of two *Bradyrhizobium diazoefficiens* strains induced by soybean root exudates. Biol Fertil Soils 51:251–260

Liu YC, Qin XM, Xiao JX, Tang L, Wei CZ, Wei JJ, Zheng Y (2017) Intercropping influences component and content change of flavonoids in root exudates and nodulation of faba bean. J Plant Int 12:187–192

Lu Y, Zhou Y, Nakai S, Hosomi M, Zhang H, Kronzucker HJ, Shi W (2014) Stimulation of nitrogen removal in the rhizosphere of aquatic duckweed by root exudate components. Planta 239:591–603

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10

Maimaiti J, Zhang Y, Yang J, Cen YP, Layzell DB, Peoples M, Dong Z (2007) Isolation and characterization of hydrogen-oxidizing bacteria induced following exposure of soil to hydrogen gas and their impact on plant growth. Environ Microbiol 9:435–444

Marschner H (1995) Mineral nutrition of higher plants. Academic, London

McCully ME, Boyer JS (1997) The expansion of maize root-cap mucilage during hydration changes in water potential and water content. Physiol Plant 99:169–177

Moghaddam MMM, Emtiazi G, Salehi Z (2012) Enhanced auxin production by Azospirillum pure cultures from plant root exudates. J Agric Sci Technol 14:985–994

Moscatiello R, Squartini A, Mariani P, Navazio L (2010) Flavonoid-induced calcium signalling in *Rhizobium leguminosarum* bv. *viciae*. New Phytol 188:814–823

Mosier A, Kroeze C, Nevison C, Oenema O, Seitzinger S, Van Cleemput O (1998) Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. Nutr Cycl Agroecosyst 52:225–248

Naher K, Miwa H, Okazaki S, Yasuda M (2018) Effects of different sources of nitrogen on endophytic colonization of rice plants by *Azospirillum* sp. B510. Microbes Environ 2018:17186

Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375–396

Nie Y, Wang M, Zhang W, Ni Z, Hashidoko Y, Shen W (2018) Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment. Sci Environ 624:407–415

O'Neal L, Akhter S, Alexandre GA (2019) PilZ-containing chemotaxis receptor mediates oxygen and wheat root sensing in *Azospirillum* brasilense. Front Microbiol 10:312

Offre P, Spang A, Schleper C (2013) Archaea in biogeochemical cycles. Annu Rev Microbiol 67:437–457

Okazaki S, Zehner S, Hempel J, Lang K, Gottfert M (2009) Genetic organization and functional analysis of the type III secretion system of *Bradyrhizobium elkanii*. FEMS Microbiol Lett 295:88–95

Okazaki S, Okabe S, Higashi M, Shimoda Y, Sato S, Tabata S (2010) Identification and functional analysis of type III effector proteins in *Mesorhizobium loti*. Mol Plant Microbe Interact 23:223–234

Oldroyd GED (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

O'Neal L, Vo L, Alexandre G (2020) Specific root exudates compounds sensed by dedicated chemoreceptors shape *Azospirillum brasilense* chemotaxis in the rhizosphere. Appl Environ Microbiol 86(15):e01026–e01020. https://doi.org/10.1128/AEM.01026-20

Oshiki M, Ali M, Shinyako-Hata K, Satoh H, Okabe S (2016) Hydroxylamine-dependent anaerobic ammonium oxidation (anammox) by "*Candidatus* Brocadia sinica". Environ Microbiol 18:3133–3143

Pan H, Qin Y, Wang Y, Liu S, Yu B, Song Y, Wang X, Zhu G (2020) Dissimilatory nitrate/nitrite reduction to ammonium (DNRA) pathway dominates nitrate reduction processes in rhizosphere and non-rhizosphere of four fertilized farmland soil. Environ Res 1:109612

Pang Y, Ji G (2019) Biotic factors drive distinct DNRA potential rates and contributions in typical Chinese shallow lake sediments. Environ Pollut 254:112903

Pereira LC, Bertuzzi Pereira C, Correia LV, Matera TC, Santos RF, Carvalho CD, Osipi EA, Braccini AL (2020) Corn responsiveness to *Azospirillum*: accessing the effect of root exudates on the bacterial growth and its ability to fix nitrogen. Plan Theory 9:923

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799

Phillips DA, Joseph CM, Yang GP, Martinez-Romero E, Sanborn JR, Volpin H (1999) Identification of lumichrome as a *Sinorhizobium* enhancer of alfalfa root respiration and shoot growth. Proc Natl Acad Sci U S A 22:12275–12280

Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends Microbiol 20:523–531

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125

Read DB, Bengough AG, Gregory PJ, Crawford JW, Robinson D, Scrimgeour CM, Young IM, Zhang K, Zhang X (2003) Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. New Phytol 157:315–321

Robertson EK, Roberts KL, Burdorf LD, Cook P, Thamdrup B (2016) Dissimilatory nitrate reduction to ammonium coupled to Fe (II) oxidation in sediments of a periodically hypoxic estuary. Limnol Ocean 61:365–381

Roper MM, Ladha JK (1995) Biological N2 fixation by heterotrophic and phototrophic bacteria in association with straw. In: Management of biological nitrogen fixation for the development of more productive and sustainable agricultural systems 1995. Springer, Dordrecht, pp 211–224

Rütting T, Boeckx P, Müller C, Klemedtsson L (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. Biogeosciences 8:1779–1791

Ryan PR, Delhaize E (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Mol Biol 52:527–560

Schubert CJ, Durisch-Kaiser E, Wehrli B, Thamdrup B, Lam P, Kuypers MM (2006) Anaerobic ammonium oxidation in a tropical freshwater system (Lake Tanganyika). Environ Microbiol 8:1857–1863

Sealey LJ, McCully ME, Canny MJ (1995) The expansion of maize root-cap mucilage during hydration. Kinetics Physiol Plant 93:38–46

Shcherbak I, Millar N, Robertson GP (2014) Global metaanalysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. Proc Natl Acad Sci U S A 111:9199–9204

Sinha Roy S, Mittra B, Sharma S, Das TK, Roy BCR (2002) Detection of root mucilage using an antifucose antibody. Ann Bot 89:293–299

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ Pollut 100:179–196

Sonthiphand P, Hall MW, Neufeld JD (2014) Biogeography of anaerobic ammonia-oxidizing (anammox) bacteria. Front Microbiol 5:399

Srivastava P, Sharma PK, Dogra RC (1999) Inducers of *nod* genes of *Rhizobium ciceri*. Microbiol Res 154:49–55

Steenhoudt O, Vanderleyden J (2000) *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506

Stein LY, Klotz MG (2016) The nitrogen cycle. Curr Biol 26:94–98

Subbarao GV, Ishikawa T, Ito O, Nakahara K, Wang HY, Berry WL (2006) A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with Brachiaria humidicola. Plant Soil 288:101–112

Subbarao GV, Rondon M, Ito O, Ishikawa T, Rao IM, Nakahara K, Lascano C, Berry WL (2007) Biological nitrification inhibition (BNI)—is it a widespread phenomenon? Plant Soil 294:5–18

Subbarao GV, Nakahara K, Hurtado MP, Ono H, Moreta DE, Salcedo AF, Yoshihashi AT, Ishikawa T, Ishitani M, Ohnishi-Kameyama M, Yoshida M, Rondon M, Rao IM, Lascano CE, Berry WL, Ito O (2009) Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proc Natl Acad Sci U S A 106:17302–17307

Subbarao GV, Sahrawat KL, Nakahara K, Ishikawa T, Kishii M, Rao IM, Hash CT, George TS, Srinivasa Rao P, Nardi P, Bonnett D, Berry W, Suenaga K, Lata JC (2012) Biological nitrification inhibition—a novel strategy to regulate nitrification in agricultural systems. Adv Agron 114:249–302

Subbarao GV, Nakahara K, Ishikawa T, Ono H, Yoshida M, Yoshihashi T, Zhu Y, Zakir HAKM, Deshpande SP, Hash CT, Sahrawat KL (2013) Biological nitrification inhibition (BNI) activity in *Sorghum* and its characterization. Plant and Soil 366:243–259

Thornton B, Osborne SM, Paterson E, Cash P (2007) A proteomic and targeted metabolomic approach to investigate change in *Lolium perenne* roots when challenged with glycine. J Exp Bot 58:1581–1590

Usyskin-Tonne A, Hadar Y, Yermiyahu U, Minz D (2020) Elevated CO_2 has a significant impact on denitrifying bacterial community in wheat roots. Soil Biol Biochem 142:107697

van Kessel MA, Speth DR, Albertsen M, Nielsen PH, den Camp HJ, Kartal B, Jetten MS, Lücker S (2015) Complete nitrification by a single microorganism. Nature 528:555–559

van Niftrik L, Jetten MSM (2012) Anaerobic ammonium-oxidizing bacteria: unique microorganisms with exceptional properties. Microbiol Mol Biol Rev 76:585–596

Velmourougane K, Prasanna R, Singh S, Chawla G, Kumar A, Saxena AK (2017) Modulating rhizosphere colonisation, plant growth, soil nutrient availability and plant defense enzyme activity through *Trichoderma viride–Azotobacter chroococcum* biofilm inoculation in chickpea. Plant and Soil 421:157–174

Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. ISME J 5:1067–1071

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Vitousek PM, Menge DN, Reed SC, Cleveland CC (2013) Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philos Trans R Soc B 368:20130119

Vranova V, Rejsek K, Formanek P (2013) Aliphatic, cyclic, and aromatic organic acids, vitamins, and carbohydrates in soil: a review. Sci World J, Article ID-524239

Wang YF, Wang JF, Xu ZM, She SH, Yang JQ, Li QS (2020) L-glutamic acid induced the colonization of high-efficiency nitrogen-fixing strain Ac63 (*Azotobacter chroococcum*) in roots of *Amaranthus tricolor*. Plant and Soil 451:357–370

Welsh A, Chee-Sanford JC, Connor LM, Löffler FE, Sanford RA (2014) Refined NrfA phylogeny improves PCR-based nrfA gene detection. Appl Environ Microbiol 80:2110–2119

Zakir HAKM, Subbarao GV, Pearse SJ, Gopalakrishnan S, Ito O, Ishikawa T, Kawano N, Nakahara K, Yoshihashi T, Ono H, Yoshida M (2008) Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum bicolor*). New Phytol 180:442–451

Zhai X, Piwpuan N, Arias CA, Headley T, Brix H (2013) Can root exudates from emergent wetland plants fuel denitrification in subsurface flow constructed wetland systems? Ecol Eng 61:555–563

Zhang J, Subramanian S, Zhang Y, Yu O (2007) Flavone synthases from *Medicago truncatula* are flavanone-2-hydroxylases and are important for nodulation. Plant Physiol 144:741–751

Zhang Z, Qiao M, Li D, Yin H, Liu Q (2016) Do warming-induced changes in quantity and stoichiometry of root exudation promote soil N transformations via stimulation of soil nitrifiers, denitrifiers and ammonifiers? Eur J Soil Biol 74:60–68

Zhang CB, Liu WL, Han WJ, Guan M, Wang J, Liu SY, Ge Y, Chang J (2017) Responses of dissimilatory nitrate reduction to ammonium and denitrification to plant presence, plant species and species richness in simulated vertical flow constructed wetlands. Wetland 37:109–122

Zhou M, Butterbach-Bahl K, Vereecken H, Brüggemann N (2017) A meta-analysis of soil salinization effects on nitrogen pools, cycles and fluxes in coastal ecosystems. Glob Chang Biol 23:1338–1352

Zhu G, Jetten MS, Kuschk P, Ettwig KF, Yin C (2010) Potential roles of anaerobic ammonium and methane oxidation in the nitrogen cycle of wetland ecosystems. Appl Microbiol Biotechnol 86:1043–1055

Zhu B, Gutknecht JL, Herman DJ, Keck DC, Firestone MK, Cheng W (2014) Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biol Biochem 76:183–192

Zuanazzi JAS, Clergeot PH, Quirion JC, Husson HP, Kondorosi A, Ratet P (1998) Production of *Sinorhizobium meliloti nod* gene activator and repressor flavonoids from *Medicago sativa* roots. Mol Plant Microbe Interact 11:784–794

Part III
Techniques and Applications

Chapter 9
Techniques for Improving Nitrogen Use Efficiency in Rice

Sepideh Bagheri Novair, Babak Motesharezadeh, and Behnam Asgari Lajayer

Abstract Nitrogen (N) is an essential macroelement for rice growth and development in paddy fields. Nitrogen use efficiency (NUE) is an indicator of nitrogen utilization in rice plants. Increasing NUE reduces nitrogen fertilizer consumption, prevents N fertilizer loss and rice productivity. In the present study, fertilizer types, water irrigation management, and rice cultivars are the factors for improving nitrogen use efficiency in rice. The organic compound application with controlled-release N fertilizers in the soil is one of the effective techniques for NUE improvement. In this chapter, we have paid to N increase necessity, its loss of environmental consequences and increase plant yield.

Keywords Rice · Nitrogen fertilizer · Productivity

9.1 Introduction

Nitrogen is an essential element for the growth of rice plants (Spiertz 2010). To crop output increase, a lot of nitrogenous fertilizer is applied (Iqbal et al. 2020). Cereal plants have nitrogen use efficiency by nearly 33%. The rest of nitrogen fertilizer use efficiency is more than 65%, which causes a lot of financial loss annually (Fig. 9.1) (Raun and Johnson 1999), rise in the price of the crop, and to anthropological and ecological difficulties (Iqbal et al. 2020). However, it is possible to increase NUE in some ways (Fig. 9.2). The suitable methods are in the following (Iqbal et al. 2020):

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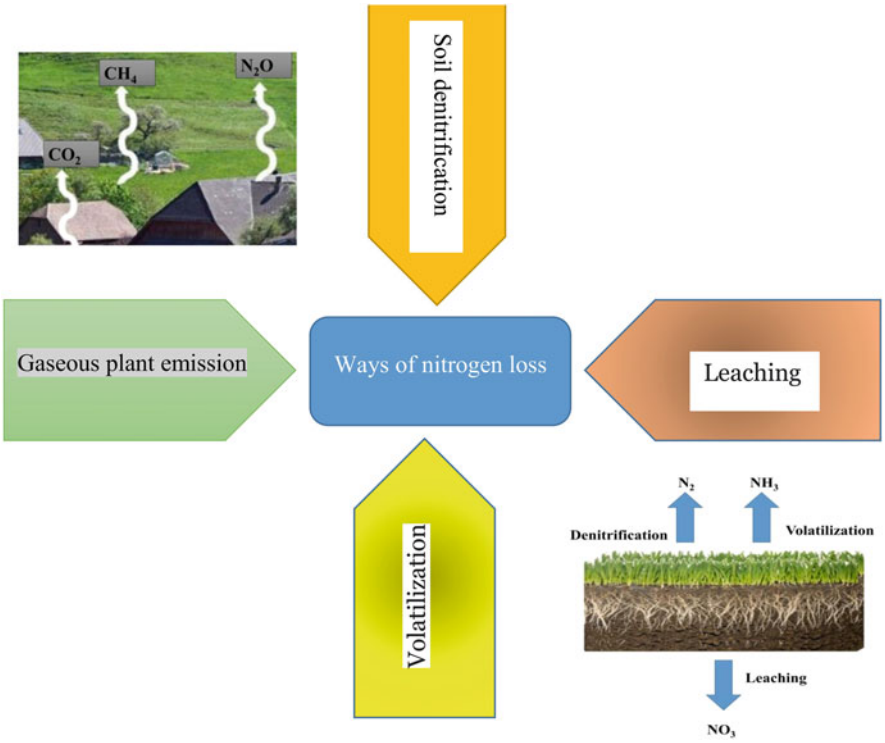

Fig. 9.1 The most important ways to fertilizer nitrogen loss

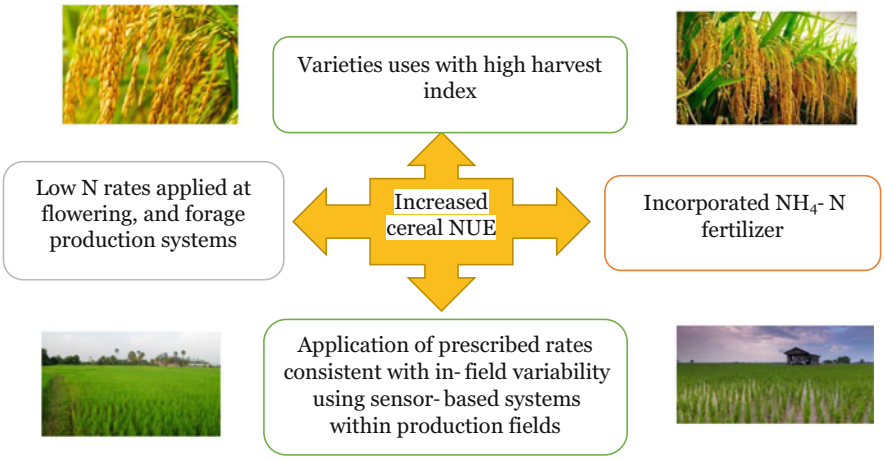

Fig. 9.2 The most important ways to increased cereal NUE

- Organization agronomic.
- Biotechnological.
- Breeding approaches.

Many studies about NUE are associated with irrigation, effective use of fertilizer, uptake of nitrogen fertilizer from the soil, and application of nitrogen fertilizer for plant yield (Cassman et al. 1993; Ye et al. 2013; Cabangon et al. 2011). Evaluation of each factor and its management has an effective role in determining it.

9.2 What Factors Does NUE Depend on?

If we want to summarize the effective detail factors on NUE in plant, we have to refer to Fig. 9.3. However, the following general factors can be described: These factors are two squares.

9.2.1 Application Fertilizer Types

Nitrogen recovery in rice is low, while it is significantly higher in the soil N pool (nearly 2 times) (Zistl-Schlingmann et al. 2020). Fertilizer nitrogen sources are used to play an important role in NUE changes, for example, controlled-release urea vs conventional urea (Guo et al. 2016) or organic and inorganic fertilizer (Behera et al. 2009) and N fertilizers with the new nitrification inhibitor (NI) 3,4-dimethyl pyrazole phosphate (Pasda et al. 2001).

9.2.2 Water Irrigation Management

Wetland rice systems maintained N uptake rice plants and increased N efficiency by improved management (Cassman et al. 1998). Other studies presented that

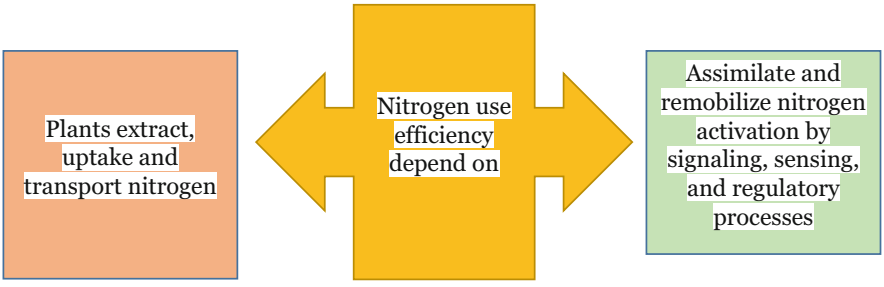

Fig. 9.3 The most important effective factors on NUE in plant

alternation irrigation and N application provide better rice growth, yield, and N utilization. Its reason is that increased glutamine synthetase and glutamate synthase activity in root rice (Sun et al. 2012). Alternate wetting and drying (AWD) are better than continuous flooding on plant NUE, while N fertilization as controlled-release nitrogen fertilizer application considerably increased NUE (Ye et al. 2013). Moderate AWD is an important practice in progressive integrative crop management to the increase in NUE rice (Zhang 2018).

In some studies, only AWD (without amendment) decreased water input and nitrogen fertilizer controlling without a negative impact on NUE rice. It can be stated that the alternative irrigation regime is convenient for low water areas (Cabangon et al. 2004).

9.2.3 Rice Cultivars

The choice of enhanced (NUE) genotypes is important to decrease N input. These plants can absorb, use, and the remobilization of N from in soils and help sustainable agriculture (Mauceri et al. 2020). Duan et al. (2007) stated that Nanguang is a high-NUE cultivar and Elio is a Low-NUE rice cultivar in chins. Change in ratio $N\text{-}NH_4$/$N\text{-}NO_3$ until to 3 increased N accumulation in the high-NUE cultivar more than the Low-NUE rice cultivar. Yang Dao had significantly greater NUE than Nong Ken for rice production. Application of a variety of nitrogenous compounds increased yield. Nitroxin and nano potassium consumption are used in two Iranian rice cultivars. Tarom Hashemi has a higher yield than Tarom Mahalli (Lemraski et al. 2017). Among to Hashemi, Ali-Kazemi, and Khazar as Iranian rice cultivars, Cultivar Khazar is the highest N physiological efficiency (Moosavi and Mohamadi 2014). Wuyunjing 23 (W23), Zhendao 11 (Z11), Wuyujing 3 (W3), and Aoyusi 386 (A386) are four cultivars of rice. W23 and Z11 are two high-NUE cultivars (Chen et al. 2020). *Japonica* and *indica-japonica* rice cultivar were grown in Progressive integrative crop management caused to improve NUE, grain yield agronomic, and physiological achievements (Zhang 2018).

Wuyunjing 7, Nanguang, and 4007 are high NUE and Elio is a low-NUE rice cultivar. Elio had fewer reproductive tillers due to a reduced amount of demand for N during rice middle growth (Zhang et al. 2009).

9.3 Some Methods to Increase Nitrogen Use Efficiency in Rice

The increase of NUE in the non-N2 fixing crops (for example rice) is done according to the growing population need of the world (via improved NUE cultivars and managing) (Raun and Johnson 1999). For this purpose, there is a need to recognize newer methods (Table 9.1).

Table 9.1 Some researches about nitrogen efficiency improvement

Methods and strategies	Results	reference
(Nitrogen–water management) distribution management (MNWD)	Grain yield maintenance Improved of N use efficiency (NUE) Improved of water-use efficiency (WUE) Reduction of input resources (20%)	Yang et al. (2020)
Controlled-release nitrogen fertilizer (CRNF)	Increased yield, N uptake, and N use efficiency compared with urea	Geng et al. (2015)
Progressive integrative crop management (ICM)	Increase grain yield, NUE, and irrigation water productivity in rice	Zhang (2018)
Omics technologies	The remobilization of N from leaves (a strategy to enhance N-utilization component)	Mauceri et al. (2020)
Use of urease inhibitor and nitrification inhibitor	Increase fertilizer use efficiency and to minimize its negative impact on the environment	Xiang et al. (2008)
Struvite	Nitrogen conservation	Kataki et al. (2016)
Swine production	Economic and environmental benefits	Monteiro et al. (2017)

Nitrogen and water irrigation management is a widespread technique to meet of rice production. Nitrogen–water management reduces N application and water irrigation by 20% and increases N uptake in rice plants (Yang et al. 2020).

Zeolite application (as soil amendment) with urea (as N fertilizer) improved NUE in rice (Kavoosi 2007).

Controlled-release urea applications could reduce N losses, yield increase, and N quantity in straw and grain (Guo et al. 2016).

9.4 Nitrogen Forms as a Particular Index

A nitrate is a nitrogen form for N uptake in rice plants. It is an indicator molecule for physiological developments in plant growth. Change of nitrate transporter and assimilation genes (Iqbal et al. 2020) may increase NUE in rice.

Bentonite hydro-char composites (BTHC) reduced archaeal amoA genes (AOA) that probably reserved nitrification and improved soil NH_4^+ maintenance (Chu et al. 2020). NUE and NH_3 volatilization relations have not been confirmed in paddy soils, but nitrogen losses are decreased by high-NUE rice cultivars farming (Chen et al. 2020). High-affinity transporter systems in high-NUE genotypes have an important role in the N efficiency of rice. Nitrate reductase and nitrite reductase activity are increased in low-NUE cultivars along with an increase in nitrogen fertilizer application (Hakeem et al. 2011).

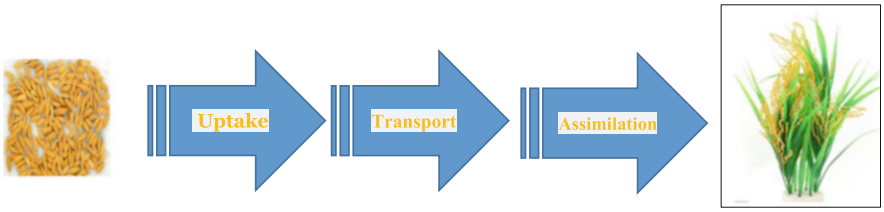

Fig. 9.4 N consumption process (three steps)

High-NUE rice meaningfully improved NH4 + cycle and tissue accumulation in the rhizosphere (Chen et al. 2013).

Li et al. (2017) stated N utilization includes three procedures (Fig. 9.4). Transport and assimilation management determine NUE in rice plants (enzymes, nitrate, and ammonium transporters).

9.5 Compounds Application and Their Effects

Many compounds are applied for increasing rice yield and growth (Fig. 9.5). These compounds cause more nitrogen uptake and increase NUE in different ways without environmental damage.

- In terms of N accumulation, conventional urea performed poorly than controlled-release urea in rice straws and grains (Guo et al. 2016).
- Ammonia volatilization is reduced by Bentonite hydro-char composites in flood-water and it subsequently leads to an increase of rice yield and NUE (by nearly 40%) (Chu et al. 2020) in paddy fields.
- Motesharezadeh et al. (2015) stated that zeolite and nitrifying bacteria application decreased ammonia loss and increased plant biomass.
- The application of organic amendments (rice straw and azolla compost) increased the concentration of total nitrogen in paddy fields (Novair et al. 2020).

9.6 Agricultural Managements

Agricultural management will change NUE in according to different points in agriculture and climate conditions. Some studies investigated that intensive and extensive management with slurry applications. The nitrogen harvest of intensive management is more than extensive management (Zistl-Schlingmann et al. 2020).

Another issue is soil tillage condition in paddy fields (tillage and no-till conditions and with and without cover). Continuous no-till and covering showed to increase NUE and WUE (Habbib et al. 2020).

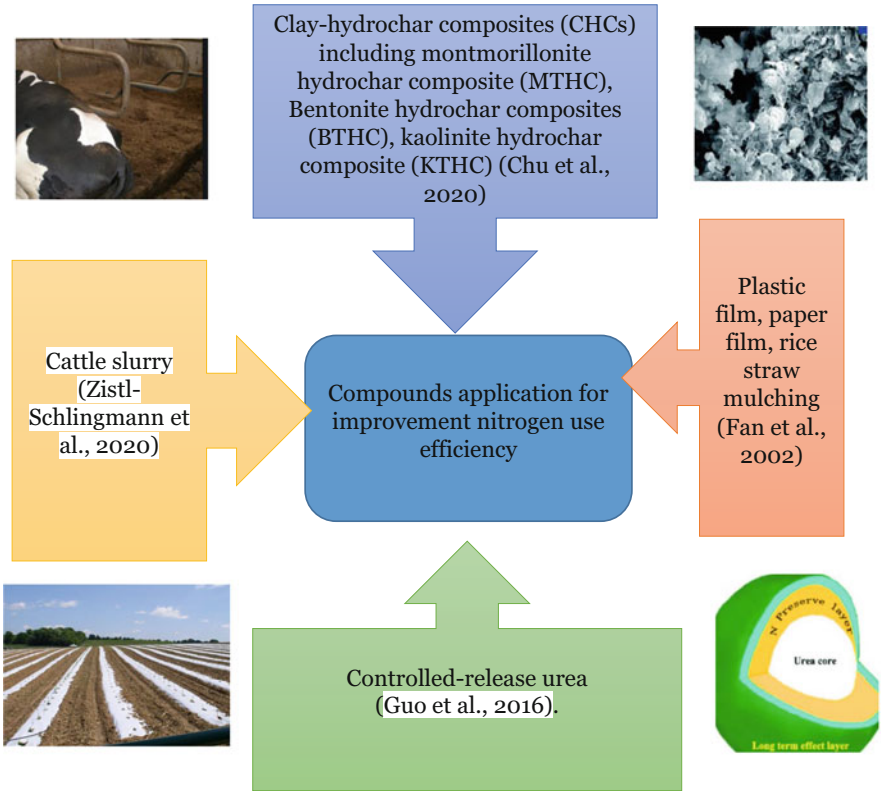

Fig. 9.5 Usual compounds application for increasing NUE

One of the most effective ways to reduce nitrogen loss, urea must be applied with a nitrification inhibitor (3,4-dimethyl pyrazole phosphate, DMPP) and urease inhibitor (n-butyl thiophosphoric triamide, NBPT). These applications increased recovery of N and reduced N loss in the soil (Wallace et al. 2020).

Integrative crop management along with moderate alternative drying-rewetting are techniques in higher NUE and productivity (Zhang 2018).

9.7 Laboratory Analysis Types

Indicators related to the measurement of NUE rice are photosynthesis parameters and water-use recovery. Leaf area, and chlorophyll are factors related to photosynthesis parameters. Transpiration rate and stomatal conductance are factors related to water-use recovery (Habbib et al., 2020).

Agronomic and physiological factors are evaluated leaf epidermal cell cytosolic nitrate activities and nitrate reductase activity (Fan et al. 2007).

15 N mass balance was evaluated to the determination of nitrogen fertilizer fate (Wallace et al., 2020).

Comparison of root morphology, NH4+ concentration, root oxygen consumption, and transmembrane NH4+ fluxes in the root meristem was done among two rice cultivars (high NUE and low NUE) (Chen et al. 2013).

Zhang (2018) estimated the number of other indicators with NUE:

- Spikelet.
- Tillers.
- Leaf characters.
- Rice growth.
- Carbohydrate accumulation and remobilization.
- Photosynthetic characters.
- Morphological characteristics.
- Redox properties of root.
- Growth regulating chemical compounds in roots and leaves.

9.8 Environmental Issues and Problems

One of the most important problems in the application of nitrogen fertilizers is their loss to the environment. The low rice NUE causes to N losses, ecological problems and degradation, floodwater pollution, and economic cost.

In summary, the main effects of increasing fertilizer nitrogen concentration on the environment can be described as follows (Fig. 9.6) (Kant et al. 2011; Bashir et al. 2013):

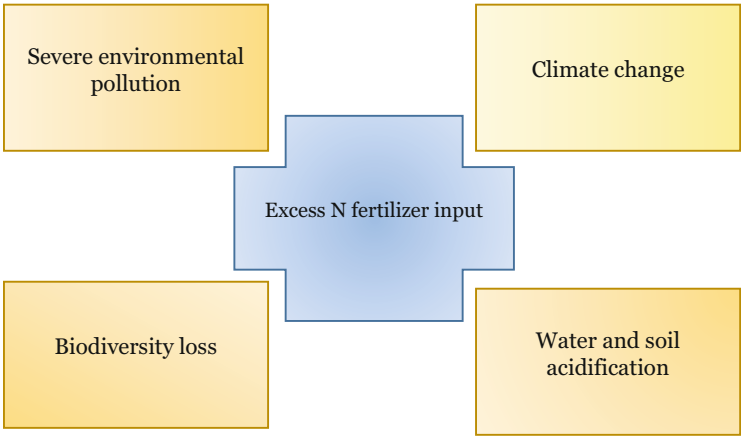

Fig. 9.6 Impact of excess N fertilizer input

However, different results were observed in this regard. Chen et al. (2020) observed that higher-NUE genotypes do not decrease ammonia volatilization. It can be done in other loss ways because low NUE causes excess N concentration in the soil condition (Johnson and Raun 2003).

Including different losses of nitrogen in ecological (−) and best N management (+):

(−) Zhu and Chen (2002) showed that increase the concentration of nitrogen in surface and subsurface waters, algal growth and greenhouse gas emissions.

(−) High concentration nitrogen fertilizer (NH4+) reserved the total root expansion (Chen et al. 2013).

(−) Leached ammonium is transferred to underground water and transformed to NH3 and it accelerated nitrification reactions rapidly in the soil pore spaces. Then, nitrates changed to gaseous (denitrification) upon drainage (nitrification-denitrification in paddy soil) (Aulakh 1996).

(+) The soil manure controlling system returns 50% of lost nitrogen to the plant nutrient cycle (Oenema et al. 2007).

(+) Organic matter addition to soil caused to aggregate stability and nitrogen sequestrated in macroaggregate (Guo et al. 2007).

9.9 Conclusion

It is important to improve the NUE today to reduce the negative environmental effects, increase crop yield, and decrease N fertilizer use in the twenty-first century. Increasing NUE plays an important role in improving rice growth and reducing the loss of different forms of nitrogen. It is possible with the application of N fertilizer compounds, field management, type rice cultivar (high NUE), and irrigation regime. According to the listed content, the organic application is the best way to increase nitrogen maintenance in the soil.

References

Aulakh MS (1996) Nitrogen losses and fertilizer N use efficiency in irrigated porous soils. Nutr Cycl Agroecosyst 47:197–212

Bashir MT, Ali S, Ghauri M, Adris A, Harun R (2013) Impact of excessive nitrogen fertilizers on the environment and associated mitigation strategies. Asian J Microbiol Biotechnol Environ Sci 15:213–221

Behera B, Sankar GRM, Mohanty SK, Mishra A, Chari GR (2009) Sustainable and effective fertilizer management for rice+ pigeonpea intercropping under sub-humid alfisols. E-planet 7 (1):20–25

Cabangon RJ, Tuong TP, Castillo EG, Bao LX, Lu G, Wang G, Cui Y, Bouman BA, Li Y, Chen C, Wang J (2004) Effect of irrigation method and N-fertilizer management on rice yield, water productivity and nutrient-use efficiencies in typical lowland rice conditions in China. Paddy Water Environ 2:195–206

Cabangon RJ, Castillo EG, Tuong TP (2011) Chlorophyll meter-based nitrogen management of rice grown under alternate wetting and drying irrigation. Field Crop Res 121:136–146

Cassman KG, Kropff MJ, Gaunt J, Peng S (1993) Nitrogen use efficiency of rice reconsidered: what are the key constraints? In: Plant nutrition—from genetic engineering to field practice. Springer, Dordrecht, pp 471–474

Cassman KG, Peng S, Olk DC, Ladha JK, Reichardt W, Dobermann A, Singh U (1998) Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. Field Crop Res 56:7–39

Chen G, Guo S, Kronzucker HJ, Shi W (2013) Nitrogen use efficiency (NUE) in rice links to NH 4+ toxicity and futile NH 4+ cycling in roots. Plant and Soil 369(1–2):351–363

Chen G, Zhao G, Cheng W, Zhang H, Lu C, Zhang H, Shen Y, Wang B, Shi W (2020) Rice nitrogen use efficiency does not link to ammonia volatilization in paddy fields. Sci Total Environ 741:140433

Chu Q, Xu S, Xue L, Liu Y, Feng Y, Yu S, Yang L, Xing B (2020) Bentonite hydrochar composites mitigate ammonia volatilization from paddy soil and improve nitrogen use efficiency. Sci Total Environ 718:137301

Duan YH, Zhang YL, Ye LT, Fan XR, Xu GH, Shen QR (2007) Responses of rice cultivars with different nitrogen use efficiency to partial nitrate nutrition. Ann Bot 99(6):1153–1160

Fan X, Zhang J, Wu P (2002) Water and nitrogen use efficiency of lowland rice in ground covering rice production system in South China. J Plant Nutr 25(9):1855–1862

Fan X, Jia L, Li Y, Smith SJ, Miller AJ, Shen Q (2007) Comparing nitrate storage and remobilization in two rice cultivars that differ in their nitrogen use efficiency. J Exp Bot 58:1729–1740

Geng J, Ma Q, Zhang M, Li C, Liu Z, Lyu X, Zheng W (2015) Synchronized relationships between nitrogen release of controlled release nitrogen fertilizers and nitrogen requirements of cotton. Field Crop Res 184:9–16

Guo JH, Chen XY, Liu MQ, Hu F, Li HX (2007) Effects of fertilizer management practice on distribution of aggregates and content of organic carbon and nitrogen in red paddy soil. Soil 39:787–793

Guo C, Li P, Lu J, Ren T, Cong R, Li X (2016) Application of controlled-release urea in rice: reducing environmental risk while increasing grain yield and improving nitrogen use efficiency. Commun Soil Sci Plant Anal 47:1176–1183

Habbib H, Hirel B, Spicher F, Dubois F, Tétu T (2020) In winter wheat (Triticum Aestivum L.), no-till improves photosynthetic nitrogen and water-use efficiency. J Crop Sci Biotechnol 23:39–46

Hakeem KR, Ahmad A, Iqbal M, Gucel S, Ozturk M (2011) Nitrogen-efficient rice cultivars can reduce nitrate pollution. Environ Sci Pollut Res 18:1184–1193

Iqbal A, Qiang D, Alamzeb M, Xiangru W, Huiping G, Hengheng Z, Meizhen S (2020) Untangling the molecular mechanisms and functions of nitrate to improve nitrogen use efficiency. J Sci Food Agric 100:904–914

Johnson GV, Raun WR (2003) Nitrogen response index as a guide to fertilizer management. J Plant Nutr 26:249–262

Kant S, Bi YM, Rothstein SJ (2011) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. J Exp Bot 62:1499–1509

Kataki S, West H, Clarke M, Baruah DC (2016) Phosphorus recovery as struvite: recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. Resour Conserv Recycl 107:142–156

Kavoosi M (2007) Effects of zeolite application on rice yield, nitrogen recovery, and nitrogen use efficiency. Commun Soil Sci Plant Anal 38:69–76

Lemraski MG, Normohamadi G, Madani H, Abad HHS, Mobasser HR (2017) Two Iranian rice cultivars' response to nitrogen and nano-fertilizer. Open J Ecol 7:591–603

Li H, Hu B, Chu C (2017) Nitrogen use efficiency in crops: lessons from Arabidopsis and rice. J Exp Bot 68:2477–2488

Mauceri A, Bassolino L, Lupini A, Badeck F, Rizza F, Schiavi M, Toppino L, Abenavoli MR, Rotino GL, Sunseri F (2020) Genetic variation in eggplant for nitrogen use efficiency under contrasting NO3-supply. J Integr Plant Biol 62:487–508

Monteiro ANTR, Bertol TM, Kessler ADM (2017) Applying precision feeding to improve the nitrogen efficiency of swine production: a review of possible practices and obstacles. Ciência Rural 47:7

Moosavi SG, Mohamadi O (2014) Response of seed yield and nitrogen use efficiencies of rice cultivars as affected by N fertilizer rates. Int J Biosci 4:8

Motesharezadeh B, Arasteh A, Pourbabaee AA, Rafiee GR (2015) The effect of zeolite and nitrifying bacteria on remediation of nitrogenous wastewater substances derived from carp breeding farm. Int J Environ Res 9:2

Novair SB, Hosseini HM, Etesami H, Razavipour T (2020) Rice straw and composted azolla alter carbon and nitrogen mineralization and microbial activity of a paddy soil under drying–rewetting cycles. Appl Soil Ecol 154:103638

Oenema O, Oudendag D, Velthof GL (2007) Nutrient losses from manure management in the European Union. Livest Sci 112:261–272

Pasda G, Hähndel R, Zerulla W (2001) Effect of fertilizers with the new nitrification inhibitor DMPP (3, 4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. Biol Fertil Soils 34:85–97

Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production. Agron J 91:357–363

Spiertz JHJ (2010) Nitrogen, sustainable agriculture and food security. Agron Sustain Dev 30:43–55

Sun Y, Ma J, Sun Y, Xu H, Yang Z, Liu S, Jia X, Zheng H (2012) The effects of different water and nitrogen managements on yield and nitrogen use efficiency in hybrid rice of China. Field Crop Res 127:85–98

Wallace AJ, Armstrong RD, Grace PR, Scheer C, Partington DL (2020) Nitrogen use efficiency of 15 N urea applied to wheat based on fertiliser timing and use of inhibitors. Nutr Cycl Agroecosyst 116:41–56

Xiang YAN, Jin JY, Ping HE, Liang MZ (2008) Recent advances on the technologies to increase fertilizer use efficiency. Agric Sci China 7:469–479

Yang Z, Li N, Ma P, Li Y, Zhang R, Song Q, Ma J (2020) Improving nitrogen and water use efficiencies of hybrid rice through methodical nitrogen–water distribution management. Field Crop Res 246:107698

Ye Y, Liang X, Chen Y, Liu J, Gu J, Guo R, Li L (2013) Alternate wetting and drying irrigation and controlled-release nitrogen fertilizer in late-season rice. Effects on dry matter accumulation, yield, water and nitrogen use. Field Crop Res 144:212–224

Zhang H (2018) Progressive integrative crop managements increase grain yield, nitrogen use efficiency and irrigation water productivity in rice. Field Crop Res 215:1–11

Zhang YL, Jian-Bo F, Dong-Sheng W, Qi-Rong S (2009) Genotypic differences in grain yield and physiological nitrogen use efficiency among rice cultivars. Pedosphere 19:681–691

Zhu ZL, Chen DL (2002) Nitrogen fertilizer use in China–contributions to food production, impacts on the environment and best management strategies. Nutr Cycl Agroecosyst 63:117–127

Zistl-Schlingmann M, Kwatcho Kengdo S, Kiese R, Dannenmann M (2020) Management intensity controls nitrogen-use-efficiency and flows in grasslands—a 15N tracing experiment. Agronomy 10:606

Chapter 10
Tools for Characterization of Nitrogen Fixing Microbes

Chitrakshi Shandilya, Nitin Kumar, Neeraj Shrivastava, Ajit Varma, and Kanchan Vishwakarma

Abstract The fundamental element of the nitrogen cycle is considered to be the biological nitrogen fixation which is naturally providing the fixed nitrogen to the Earth's ecosystem. The process of fixing nitrogen has been widely researched and the microorganisms carrying out nitrogen fixation process have been identifying and studied for their role in ecosystem. The identification and characterization of nitrogen fixers involve number of techniques and tools and some of these techniques have been discussed in the present chapter.

Keywords Nitrogen fixation · Microorganisms · Characterization · Molecular tools

10.1 Introduction

Earth atmosphere is formed of various gases out of which nitrogen (N) forms most part of it, 78% of total atmospheric gases thus considered as most abundant gas present. Concentration of other gases of earth's atmosphere is present as oxygen (21%), argon (0.9%), carbon dioxide (0.03%), and nitrogen (72%) being the most abundant of all. However, it is still not taken up directly in its atmospheric form by plants due to lack of such mechanism. Usually nitrogen is taken up or utilized by plants and various microbes in reduced or oxidized forms. Atmospheric nitrogen is converted into its various forms by several biological systems like reactive nitrogen, primarily nitrite and nitrate, and ammonium formed by utilization of high energy that

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lead to dissociation of triple bonded diatom dinitrogen (Kamp et al. 2016), which is incorporated into organic compounds mainly amino acids so that it could be available for uptake by plants. Nitrate is majorly utilized form of nitrogen as compared to ammonium by most agricultural soils (Crawford and Forde 2002) and even acts as a nutrient as well as is important as regulatory signal. Growth and productivity of aquatic as well as terrestrial plants are highly impacted by nitrogen thus it acts as a most common limiting factor for their growth, high crop productivity, and yield stability (Landrein et al. 2018). Along with enhancing optimal growth, nitrogen also affects alleviation of biotic as well as abiotic stress (Gupta et al. 2013) thus plays major role in influencing sustainable plant productivity under stress (Wang et al. 2003) and being a major component of various secondary metabolites, it also provides tolerance against biotic stresses (Wink 1999). Nitrogen composition is highly variable in soil depending on various properties and parameters like oxygen, temperature, moisture of soil, physiochemical activity, and activities of nitrifying, denitrifying microbes present in the soil (Schimel and Bennett 2004). Due to flexible metabolism of plants, they are highly capable of adapting their metabolism with fluctuating levels of nitrogen thus making these metabolisms sensitive and selective so that facilitative uptake and assimilation of nitrogen becomes easy to promote growth under limiting conditions of nitrogen. These adaptive mechanisms play important role in achieving nitrogen use efficiency (NUE).

10.2 Nitrogen Fixation

Nitrogen is considered as an essential element for growth of plants and important for all living organisms present on earth thus it is cycled in the ecosystem by the process of fixation. This fixation is carried out in various ways. Natural processes that fix atmospheric nitrogen into biosphere are lightening and biological nitrogen fixations. Net nitrogen which is to be fixed per year is fixed in the form of ammonia and 1% of ammonia from net nitrogen is fixed by lightening (Igarashi and Seefeldt 2003), whereas biological nitrogen fixation (BNF) is usually carried out by group of microbes which are usually distributed among archaeal and bacterial domains (Vitousek et al. 1997) termed as diazotrophs (Postgate 1982). Total of 50% nitrogen is fixed biologically per year (Igarashi and Seefeldt 2003). There is another process which helps in fixing the remaining 50% of atmospheric nitrogen available, Haber Bosch process. This process leads to reduction of atmospheric nitrogen into ammonia by reacting with hydrogen under high temperature and pressure by using iron as catalyst (Jennings 2013; Vojvodic et al. 2014). This process leads to revolution agricultural production as it is useful in fulfilling the immediate requirement of nitrogen as a fertilizer. Fixing of nitrogen by industrial means has harmful consequences which lead to emission of nitrogen oxides, water eutrophication, and soil acidification (Dixon and Kahn 2004). Ecological and economical effective measure adapted for sustainable agricultural production is biological nitrogen fixation (Bohlool et al. 1992).

Biological nitrogen fixation being a primary source of fixation by which various plants take up nitrogen for their growth from our ecosystem is usually carried out by a mass group of highly diverse prokaryotes and diazotrophes which may include freely living bacteria that belongs to genera such as *Burkholderia*, *Azotobacter*, *Azospirillum*, *Bacillus*, and *Clostridium*, bacteria that form symbioses with legumes like *Rhizobium*, with actinorhizal plants such as *Frankia*, and *Cyanobacteria* associated with cycads (Postgate 1982). Biological nitrogen fixation is considered as an expensive metabolic process as it utilizes 16 molecules of energy that is ATP for fixing a molecule of nitrogen into two molecules of ammonia (Simpson and Burris 1984). There are several molecules involved in uptake of fixed nitrogen in soil. The essential elements involved in this process are as follows:

- *Nitrogenase enzyme*: It is considered important for fixing nitrogen biologically and is an important step in nitrogen cycle globally (Zhao et al. 2006; Ribbe et al. 1997).
- *Molybdenum containing nitrogenase*: One of the broadly studied nitrogenase and is most widely distributed (Rees and Howard 2000).
- *Vanadium nitrogenase*: Usually considered as an alternative nitrogenase or backup system as it is expressed only in absence of molybdenum nitrogenase (Rehder 2000).
- *Iron only nitrogenase*: It is considered as a second alternative enzyme for fixation nifHDK strain of *A. vinelandii*. It was used to isolate this enzyme, and isolation was performed by Chisnell et al. (1988). This enzyme acts as an alternative enzyme as it is expressed only in the absence molybdenum and vanadium nitrogenases (Crans et al. 2004). It is encoded by *anf* genes. Being lowest in activity as compared to molybdenum and vanadium nitrogenases (Hinnemann and Norskov 2004), it is quite unstable as well as sensitive towards oxygen thus considered as a difficult enzyme to study out of all three nitrogenases (Chisnell et al. 1988; Crans et al. 2004).
- *Oxygenase insensitive Streptomyces thermoautotrophicus nitrogenase*: In 1992, an experiment was conducted on a chemolithotrophic *S. thermoautotrophicus* and it was found that this particular organism was capable of utilizing nitrogen when cultivated under aerobic conditions containing gases like carbon monoxide or hydrogen gas and carbon dioxide (Gadkari et al. 1992). Presence of two components makes it differentiable form the other three nitrogenases.
- *Dinitrogenases*: It consists of complex protein which is a heterotrimer and formed as MoFeS protein, whereas dinitrogenase reductase lacks Fe protein but contains manganese superoxide oxidoreductase (Zhao et al. 2006). It lacks the property of reducing acetylene into ethylene, has low requirement of MgATP and is insensitive to oxygen thus needs oxide ions for completing the process of reduction of atmospheric nitrogen into ammonia (Zhao et al. 2006).

Diazotrophes are important as they express complex of nitrogenase enzyme which has a role of carrying out nitrogen fixation. This complex formed of bacterial nitrogenase enzymes is considered as the only natural complex known to reduce nitrogen into ammonia (Seefeldt et al. 2009). There are several sets of nitrate

transporters that function in uptake of nitrate available in the soil. These transporters are functional on the basis of their affinity for nitrate available in soil. These transporters are classified and categorized under two groups: high affinity transporters HATs which include NRT2.1, NRT2.2, NRT2.4 and low affinity transporters LATs which include CLCa, NRT1.1, NPF1.2. These transporters are also an important part in metabolism of nitrogen. Mechanism followed by these transporters in uptake of nitrate includes several reactions. First reaction is that upon uptake of nitrate, reduction of nitrate to nitrite is catalyzed by cytosolic NR. This reaction leads to nitrite yield but requires NADPH for donation of electron. Second reaction is that nitrite formed by reduction is then further reduced to ammonium ions in plastids by use of enzyme called plastidial nitrite reductase. Activity of NR is influenced by various factors such as concentration of nitrate as well as photo conditions. Several other factors have been seen to influence NR activity usually induced under deficiency of oxygen commonly termed as hypoxia or anoxia which leads to decrease in cytosolic pH resulting in inhibition of reduction process of nitrite into ammonium ions. Once hindered, it causes accumulation and thus acts as a limiting factor.

10.3 Molecular Tools for Characterization of Nitrogen Fixing Microbes

Microbes play an integral part in solubilization, fixation, and uptake of nitrogen. Molecular techniques have prompted huge advances in our capacity of describing the diverse variety of bacteria specialized in oxidizing ammonia and their circulation as well as availability in natural habitats. A number of various discoveries have been made by researchers from the studies done on a scope of environmental conditions. For instance, the significance of *Nitrosospira* in both aquatic and terrestrial is presently perceived, the affectability and resistance to high ammonium fixations seem significant and contrasts have been appeared between networks surveyed by development based and development autonomous methodologies. Links between phylogenetic groups and physiological attributes of biological importance which are accessible on the basis of physiological attributes of pure cultures of oxidizers present in natural environment are capable of oxidizing ammonia in response to changes in the environment.

Challenging task for future would be to bind and closely link molecular to traditional approaches to study and analyze the changes occurring in communities which are in relation with varying environmental factors and physiochemical traits. Along with this, it is required to develop a strong relationship between structural community of ammonia oxidizing organisms or ammonia oxidizers and processes related to nitrification in our ecosystem, so that significance of diverse organisms occurring in natural environment could be determined. These study-based analyses on ammonia oxidizers could help in gathering information which is generically important. Diverse group of ammonia oxidizers are found in exclusive monophyletic

group, dominating natural populations. There are different techniques based on molecular and cultural basis used as molecular tools.

10.3.1 Molecular-Based Techniques

Various techniques have been adopted by researchers for their studies. 16S rRNA gene sequences are being used for analysis and study of basis required for phylogenetic classification of microorganisms. It was firstly applied to few limiting numbers of ammonia oxidizers in which 11 pure cultures were used in the experiment to analyze subsequent sequences (Head et al. 1993). By these analyses, categorization was done by placing *Nitrosococcus oceanus* in the γ-proteobacteria and other strains in two genera, *Nitrosomonas* and *Nitrosospira*, within the β-proteobacteria, *Nitrosospira* incorporating the genera *Nitrosovibrio* and *Nitrosolobus*. Construction of primers was done by use of these sequences (McCaig et al. 1994) which can be further used in amplification of 16S rRNA of ammonia oxidizers from DNA extracted from samples collected from natural environment. This helps in characterizing available natural population of ammonia oxidizers.

Amplification of 16S rRNA genes by process of cloning, sequencing, and analysis on the basis of phylogenes of members present in clone library is involved in this approach. Fingerprinting techniques like denaturing and temperature gradient gel electrophoresis (DGGE or TGGE) can also be used (Muyzer et al. 1993; Muyzer and Smalla 1998). Extension in molecular analysis has been observed by development and using of primers for encoding of genes of ammonia monooxygenase, particularly amoA analysis following cloning, sequencing and DGGE (Webster et al. 2002), or T-RFLP (Bothe et al. 2000a, b).

This molecular methodology has critical points of interest for investigation of communities of ammonia oxidizers. In particular, major problems can be discussed and resolved by analyzing pure cultures without isolation and enrichment. Important point that has to be noted here is that isolation processes and characterization on basis molecular data are slower than procurement of succession information but is considered more informative when compared to phenotypic characterization analysis. Moreover, in situ identification utilizing 16S rRNA approach is usually considered as more advantageous than traditional immunological techniques. In these techniques, it does not require any pure cultures for development of antibodies thus discarding the need for cultivation of target organisms before any experiment is conducted. In situ examination of ammonia oxidizers has been accomplished in tests from wastewater treatment forms in which high population has been observed, and has even shown discrete clusters existence (Schramm et al. 1999). Application of this approach is reduced due to low cell concentrations of ammonia oxidizers in aquatic environment while examination of soil tests is additionally entangled by foundation fluorescence and particulate material colonization. Discussion is specifically focused on natural terrestrial and aquatic environment population of ammonia oxidizers.

10.3.2 16S rRNA Gene Based Approach

An experiment conducted on banana plant and pineapple plant showed isolation of various nitrogen fixing bacteria, which were identified at molecular basis using molecular approaches. Some bacterial species such as *Azospirillum brasilense, Herbaspirillum seropedicae*, and *Acetobacter diazotrophicus* were known as associative nitrogen fixing bacteria. It was also supposed that these species could have several benefits for host plants in fixing nitrogen and promoting plant growth. First ever species suggested to be endophytic (Baldani et al. 1997; James and Olivares 1997) in nature were considered to be the latter two species mentioned above but still several new species have been classified as diazotrophic bacteria which have been isolated from plants particularly mono and dicotyledonous (Ferreira et al. 1995; Jimenez-Salgado et al. 1997) plants of economic importance considering banana and pineapple (Weber et al. 1999). In the experiment, 38 nitrogen fixing bacteria were analyzed following DNA sequencing and PCR restriction fragment length polymorphism analysis of 16S rRNA gene for defining their phylogenetic positions, which were isolated from stems, leaves, roots, and fruits of banana and pineapple and used as experimental material, collected from cultivators of Bahia (BA) and Rio de Janeiro (RJ) States, Brazil. The reference strains used by them belonged to their collection. Specific strains used were Z67, Z78, and M2 for *H. seropedicae*, M4 for *Herbaspirillum rubrisubalbicans*, M130 for *Burkholderia brasiliensis*, and Ppe8 for *Burkholderia tropicalis*. All the above-mentioned strains were left overnight for germination or growth in NFbHPN medium at 30 °C at 120 rpm (Machado et al. 1991). Solution was diluted to ratio 1:10 and boiled for 5 min under ice cooled conditions. By following these steps, DNA was amplified in an OmniGene thermocycler from Hybaid Ltd., Teddington, United Kingdom (Laguerre et al. 1994). The primers used were Y1 (59-TGGCTCAGAACGAACGCTGGC GGC39) (Young et al. 1991) (positions 20 to 43 of the *Escherichia coli* 16S rRNA gene) and Y3 (59-TACCTTGTTACGACTTCACCCCAGTC39) (positions 1482 to 1507 of the *E. coli* 16S rRNA gene). These genes were complementary to ends of 16S rDNA. Single band of approximately 1500 bp was observed to be formed from the DNA templates extracted from all of the strains. For the digestion of Y1-Y3 PCR products (10 mL), AluI, HaeIII, HinfI, or RsaI (5 U) were used specified (by Life Technologies), and the fragments were separated on a 2.5% agarose gel and stained with ethidium bromide (0.5 mg/mL). Patterns of 5–10 fragments along with three to seven fragments were produced by endonucleases. The 12 different patterns were formed also called as ARDA types. Few of the isolated strains shared same patterns which included Isolates AB7, BA10, BA11, BA12, BA14, BA15, BA16, BA17, BA134, BA149, and BA161 had the same pattern as *H. rubrisubalbicans* strain M4. Isolate BA124 showed the same pattern as *B. brasiliensis* strain M130. Finally, AB98 and AB147 had the same pattern as *B. tropicalis* strain Ppe8. Eight new ARDA pattern or 5–12 types were produced from remaining 22 isolates (Cruz et al. 2001). For allocation of strains into 12 different types, restriction cut with endonucleases AluI and HaeIII was found to be sufficient. The purification of the

Y1-Y3 PCR products was conducted by Nucleon QC followed by sequencing with dye terminator chemistry and an ABI PRISM 310 sequencer. Both strands of variable regions (300 bp approximately) which were found to be located at the 59 end present on 16S rRNA gene were sequenced by use of primers Y1 and Y2 (59-CCCACTGCTGCCTCC CGTAGGAGT-39) (Young et al. 1991). Different lengths of variable regions were reported for various bacteria according to number of bp and were allocated for different types (types I to XI), with each group consisting of isolates that were identical to sequence. No apparent polymorphism was observed as obtained sequence showed no difference from ARDA defined types. Although reference strains of *H. seropedicae* and *H. rubrisubalbicans* showed unique ARDRA (types 1 and 2) and sequence (types I and III) types, the 11 isolates still showed the same *H. rubrisubalbicans* ARDRA type while depicting 100% sequence identity to *H. seropedicae* in the Y1-Y2 region. Failure of hybridization was seen between obtained isolates and *H. seropedicae* 23S rDNA species-specific probe (Weber et al. 1999), whereas according to newly obtained date there is a possibility that it may constitute new *Herbaspirillum* cluster.

These type I to XI sequences plus 47 sequences of 16S rDNAs of alpha and beta Proteobacteria available in the GenBank database were used to construct a phylogenetic tree. Alignment of sequences with ClustalX program (Thompson et al. 1997) and phylogenetic tree was reconstructed with help of TreeCon program (Van de Peer and De Wachter 1994). Evidence of proximal relationship of types I, II, and III to the *Herbaspirillum* cluster was seen. From various studies about different isolates it was concluded that two isolates and the eight new isolates found which shared same pattern or ARDA types were associated with Comamonadaceae. The paper published had redefined 14 isolates which were described by Weber et al. (1999) and also found out 24 new isolates into genotypes. Discovery made by researchers of eight new types of genotypic bacteria capable of fixing nitrogen along with *H. seropedicae*, *H. rubrisubalbicans, B. brasiliensis*, and *B. tropicalis* in few bacterial isolates from experimental material that are banana and pineapple showed adverse diversity of bacteria associated with fruit crops, which are capable of fixing nitrogen.

10.3.3 Amplified Ribosomal DNA Restriction Analysis (ARDA)

16S rRNA fragments which are amplified using PCR technique are digested or cut out at specific sites by the help of restriction enzymes, are used for amplified ribosomal DNA restriction analysis. Resulting digested part is further separated by gel electrophoresis. Chopping off different DNA sequences at different locations will be done leading to profiling unique results of community analyzed. Type of restriction enzymes used during gel electrophoresis plays major role in divergence of community rRNA restriction pattern (Gich et al. 2000). Measurement of bacterial

community composition or screening of clones can be done using ARDA banding patterns (Kirk et al. 2004). ARDA properties of being simple, rapid, and cost effective lead to its use in microbial identification and studies related to microbial community (Vaneechoutte et al. 1992, 1995; Kita-Tsukamoto et al. 2006; Krizova et al. 2006). When assessment of microbial community composition and succession in an aquifer were made in response to phenol, chlorinated aliphatic hydrocarbons and toluene, ARDA revealed that dominant microbial community members were stable and could be used for fingerprinting bands formed on gel (Fries et al. 1997). Main aim behind assessment was to identify dominating microbial community involved in trichloroethene (TCE) biodegradation (Fries et al. 1997).

ARDA was used in another study for examination of differences in microbes present in activated sludge collected from treatment plants fed on industrial or domestic wastewater (Gich et al. 2000). From this study, it was found out that microbial community present in activated sludge was complete different from wastewater of industrial or domestic treatment plants. Domination of highly diverse community of *Dehalococcoides ethenogenes*-like microorganisms was observed over composition of microbial community found in well waters which were contaminated with TCE, this was achieved by using ARDA fingerprinting technique. Advantage of using ARDA is that it helps in discovering structural changes occurring in microbial community, whereas it has a disadvantage of not being able to measure diversity of microbes or unable to detect specific phylogenetic groups with a community fingerprinting profile (Liu et al. 1997). Requirement of optimizing with restriction enzymes especially when sequences are unknown is very difficult, resulting in further optimizing for production of fingerprinting patterns characterizing microbial community (Vaneechoutte et al. 1992; Spiegelman et al. 2005), adding up of complexity in banding patterns of diverse microbial communities, thus making analysis difficult using ARDA (Kirk et al. 2004).

In recent studies, combination of molecular techniques such as T-RFLP and DGGE with ARDA is done for characterizing communities of microbes from different contaminated sources (Watts et al. 2001; Haack et al. 2004). One more study was conducted on plants taken from Brazilian agriculture, using ARDA and amplification was done. Incubation of reaction mixtures was done in a MJ PTC-100 thermocycler for 2 min at 94 °C. This cycle was repeated 30 times at different temperatures: Independence, equivalence, and relatively intense bands were similar in get mobility during analyzing ARDA patterns. Bands that were between 120 bp and 900 bp were considered in investigation and further analyzed (NTSys-PC software package, Rohlf 1992).

10.3.4 The **nifH** *Gene Based Approach*

As we have already discussed that nitrogen being and important element for plant growth is cycled globally by fixation and converted into ammonia from atmospheric nitrogen. Biological fixation is natural and most effective way of fixation usually

done by several groups of microbes majorly bacteria and methanogenic archaea (Raymond et al. 2004a, b), catalyzed by enzyme nitrogenase. Components involved in formation of nitrogenase complex are a nitrogenase reductase component, i.e. Fe protein encoded by nifH and MoFe protein which is a nitrogenase component encoded by nifDK (Schindelin et al. 1997). These genes are responsible for formation of clusters in known prokaryotes that fix nitrogen (Fani et al. 2000; Raymond et al. 2004a, b). Fe protein is believed to be unreactive directly with nitrogen, but amino acid sequences of Fe proteins are considered highly conservative among nitrogen fixing prokaryotes. Furthermore, categorization of *nifH* gene is highly compatible with taxonomy of prokaryotes by 16S rRNA gene sequences (Hennecke et al. 1985; Normand and Bousquet 1989; Young 1992). Thus, *nifH* gene is taken up as a remarkable marker used in determination of possible nitrogen fixers in different environments like oligotrophic oceans (Man-Aharonovich et al. 2007; Zehr et al. 1998), marine microbe mats (Zehr et al. 1995), modern marine stromatolites (Falcon et al. 2007), tropical sea grass beds, rice roots (Demba Diallo et al. 2008; Lovell et al. 2000), salt marsh sediments (Lovell et al. 2008; Welsh et al. 2007), hot springs (Hall et al. 2008), marine sponges (Mohamed et al. 2008), and hydrothermal fields (Mehta et al. 2003, 2005).

The *nifH* gene is considered a convenient phylogenetic gene marker for studying and analyzing nitrogen fixers present in our natural ecosystems. For evaluating nitrogen fixing communities of different soil types, PCR analysis of *nifH* gene fragments is carried out. There is diversity in techniques of PCR fingerprinting like Enterobacterial Repetitive Intergenic Consensus (ERIC), Repetitive element sequence-based (REP) and BOX-A1R-based repetitive extragenic palindromic (BOX) based PCR used in amplifying repetitive DNA sequences found in genomes of several Gram positive bacteria and majorly in Gram negative bacteria. REP-PCR works on basis of targeting primers with repetitive extragenic palindromic sequences having 35–40 bp (Stern et al. 1984). Similarly, ERIC-PCR is technique which is based on primers targeting repetitive intergenic consensus which are highly conserved in enterobacterial organisms having bp from 124–127. Being a highly sensitive technique, it is useful in detection of microorganisms from any possible environment (Frye and Healy 2006; Hulton et al. 1991). BOX-PCR is based on primer targeting DNA sequences of BOXA subunit of BOX element that are highly conservative repetitive sequences having 59 bp (Martin et al. 1992). BOX-PCR is considered as a superior technique when compared to others in forming distinctive fingerprinting patterns. And the other two techniques are primarily used in various methods of genotyping. Sharing of any homologous sequences with either REP-PCR or ERIC-PCR is not any option in BOX-PCR (Das et al. 2014; Olive and Bean 1999). However, some of the advantages of repetitive element PCR (REP-PCR) are capability of distinguishing between closely related strains, simple technique, reliable, low cost, and rapid method of detection (Bilung et al. 2018). Therefore, in various studies, comparison of different PCR techniques including

REP-PCR, ERIC-PCR, and BOX-PCR was done with molecular differentiation of native *A. chroococcum* and *A. salinestris*, and their strain diversities were also evaluated. In evaluation of mentioned strains, nifD and nifH genes were used for molecular identification.

10.3.5 Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE called as denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) is used in separation of amplified segments of rDNA having same length but differ in composition of base pairs. Electrophoretic mobility of partially melted double stranded DNA molecules is main reason of dependence of separation of bands in both DGGE and TGGE, in polyacrylamide gels having a gradient of DNA denaturants acting linearly or linear temperature gradients (Muyzer and Smalla 1998). There is a limit of size that is up to 500 bp in PCR amplified DNA segments which are separated on basis of differences in sequences instead of variation in lengths. Band numbers formed during DGGE/TGGE are related in direct proportionality to dominant species numbers present in sample. DGGE/TGGE is a technique for decision when the ideal data does not need to be as phylogenetically thorough as that given by 16S rRNA quality clone libraries, yet is as yet exact to decide the prevailing individuals from microbial networks with medium phylogenetic goal. DGGE/TGGE provides a great opportunity of microbial population identification by excision and sequencing of bands for largely unknown microbial diversity from different environmental or contaminated sources (Lovell et al. 2008). Use of DGGE technique is carried out in many studies, for assessing structure of microbial community especially collected from contaminated soil and water sources (Watanabe et al. 2007). Besides assessment of structure of microbial community, DGGE is also used for examination of genetic clusters like as dissimilatory sulfite reductase beta-subunit (dsrB) genes in sulfate-reducing bacterial communities and benzene, toluene, ethylbenzene and xylene (BTEX) monooxygenase genes isolated from bacterial strains of community, usually collected from hydrocarbon-polluted aquifers. Along with the advantage of enabling observation of spatial/temporal changes occurring in microbial community morphology and providing a less complex view about dominance in microbial species within sample, there are limitations of using DGGE/TGGE technique in various studies related to microbial community, such as lack of specificity in sequence limited to 500 bp fragments of 16SA rRNA, which is desired for phylogenetic identification of some organisms. Occurrence of multiple bands for single species could be a possibility due to existence of multiple copies of 16S rRNA in various organisms (Nuble et al. 1996), whereas similarity in mobility can be seen in different 16S rRNA sequences. Calculating band intensity cannot be an accurate way of knowing exact

population of microbial community, even DGGE/TGGE analysis leads to production of complex profiles of microbial communities which can have a possibility of being sensitive towards spatial and temporal sampling variation (Nuble et al. 1996).

Several fingerprinting techniques of DNA and RNA based on different separations, which are most often applied these days in studying rhizosphere are denaturing gradient gel electrophoresis (DGGE), terminal restriction length polymorphism (T-RFLP), and single-strand conformation polymorphism (SSCP).

- DGGE—based on melting behavior of DNA which is double stranded and this is due to differential sequences in denaturing gradient during gel electrophoresis (Muyzer et al. 1993).
- T-RFLP—helps in separation of fragments on basis of their terminal lengths acquired due to restriction endonuclease site differences (as in RFLP and ARDRA) (Liu et al. 1997).
- SSCP—separation of fragments on basis of difference in mobility of single stranded DNA carried out in non-denaturing gels (Schwieger and Tebbe 1998).

Results acquired from these three methods have been seen to reveal same type of microbial clusters as found in soil samples. These three techniques of fingerprinting are appeared to be equally suitable for analysis of differences in communal patterns that are obtained by physio-chemical and biological differences within various sites of investigation but, despite equal of these three methods, several strengths and weaknesses have been observed on comparison. Isolation of specific genetic elements for sequencing subsequently is possible by using DGGE and SSCP, which acts as an advantage for both these techniques whereas a disadvantage for T-RFLP as isolation for subsequent sequences is not possible by using this technique. Meanwhile, despite the requirement of step which requires restriction digestion, T-RFLP is found suitable for high throughput analysis. Linking of obtained peaks with a specific organism is not possible by use of this technique still, output format obtained as an electropherogram of T-RFLP helps in comparatively analyzing of database at higher taxonomical level by digesting database sequences using silico digestions which is an exclusive characteristic involved in RDP for 16S rRNA gene. Re-annealing of single stranded DNA at a higher rate during electrophoresis acts as a major limitation if SSCP (Nocker et al. 2007). All these three methods have been applied in studying and researching about rhizosphere, even have shown a good success rate. Use of SSCP has given evidence about association of crenarcheal consortia with rhizosphere ranging from terrestrial plants and association of crenarcheal consortia from bulk soil is different from each other (Sliwinski and Goodman 2004) and that the determination of rhizospherical community is done by plant species and type of soil (Miethling et al. 2003). For studying structural diversity found in rhizosphere, extensive fingerprinting method called as DGGE can be used, which is also used for investigating scientific questions of diverse range about rhizospherical microbiology, for instance, dynamics of methanogenic archaeal

communities found in Japanese paddy soil (Watanabe et al. 2007), association of community structure of *Pseudomonas* spp. with antagonistic potential of rhizosphere (Costa et al. 2007) and effect on structural variety of microorganisms in grassland due to elevation in concentration of carbon dioxide (Drissner et al. 2007). For studying and analyzing differences appeared in profiles at DNA and RNA level, fingerprinting methods are increasingly used for inheriting knowledge about the links between functioning and communal diversities. Use of rRNA as a marker gene is mostly used in studies related to these subjects. For studying influential rice cultivator diversity on expression of *nifH* genes in rhizosphere, nitrogenase (*nifH*) genes and transcripts were investigated and analyzed using T-RFLP technique. Use of T-RFLP technique has been observed in studying various compositions of bacteria in soil and rhizosphere of arable field sites (Ulrich and Becker 2006). It ought to be noticed that fingerprinting strategies dependent on PCR intensification do not commonly give solid proportions of assorted variety boundaries like equity and wealth, halfway because of the issue of getting equivalent enhancement effectiveness of all pieces in the PCR response and the way that remotely related taxa can add to a similar sign in the investigation.

10.4 Conventional Tools

10.4.1 Culture Based Techniques

Ammonia oxidizing bacteria having autotrophic nature acquired from environmental samples are usually obtained from inorganic ammonium containing medium which is enriched (Allison and Prosser 1992). Oversight of natural carbon disheartens; however, does not wipe out heterotrophs, which develop on natural results of ammonia oxidizer growth and on unpredictable natural mixes of different salts forming volatile compounds or contaminants of media and culture vessels. End-point dilution method was used to obtain enriched liquid cultures which are considered as pure cultures. In the two cases, filtration is made troublesome by the moderate development and low yield of ammonia oxidizers on research facility media. For instance, for producing microbial colonies, incubation of several weeks on solid medium is required. A combination of end-point dilution and enumeration by most probable number method has been done in some studies with identification and isolation of various microorganisms from several cultures exhibiting enhancement in growth at higher dilutions (Belser and Schmidt 1978). An opportunity for determination of physical traits related to environment is been provided by pure cultures. But this approach has been limited due to difficulty in cultivation of ammonia oxidizers and restricted pure cultures availability. These are exacerbated by the slow growth of ammonia oxidizers in liquid culture and on solid media and by

the fact that pure cultures, once obtained, are difficult to keep free of contamination and often die out with repeated subculture. Despite these disadvantages, successful enrichment of a particular strain is necessary for unequivocal evidence of its presence and viability in an environment. In addition, laboratory cultures provide the potential for development of antibodies specific to particular groups, again allowing direct detection of target organisms in environmental samples. Characterization of pure cultures led to the traditional classification of autotrophic ammonia oxidizers into five genera, based on cell morphology and a limited number of additional phenotypic characters. Confirmation of taxonomic groupings, including species descriptions, has been achieved using DNA:DNA hybridization of pure cultures of these organisms but this approach is necessarily limited by the difficulties in obtaining pure cultures of ammonia oxidizers. Identification of cultures using the available phenotypic characters is also difficult and these problems have restricted studies of natural diversity of autotrophic ammonia oxidizers to those utilizing immunological techniques (Belser and Schmidt 1978; Ward and Carlucci 1985) and long-term isolation programs.

10.4.2 Acetylene Reduction Assay

Acetylene reduction assay (ARA) was performed with a single colony of bacteria grown in N-free WAT4C semisolid medium. The cultures were incubated for 72 h at 28 °C, and then acetylene enriched atmospheres to a final concentration of 1% and 10% were injected. Acetylene was produced by dissolving calcium carbide in tap water and injected to a final concentration of 1% (v/v) by replacement of an identical volume of air. The acetylene reduction activity was measured with a Varian 3300 (Walnut Creek, CA, USA) gas chromatograph with a flame ionization detector. *Klebsiella variicola* ATCC BAA-830 T and *Escherichia coli* DH10b were included as positive and negative controls, respectively.

Acetylene reduction assay (ARA) can be utilized for identifying nitrogen fixers. It has implemented for sorghum roots grown in fields at different growth stages (Schollhorn and Burris 1967). Random sampling of healthy plants was done to perform ARA with acetylene and one control without acetylene. 10% concentration of acetylene gas was maintained and injected followed by incubation for 1 day. Ethylene levels were estimated in collected gas samples with gas chromatography with flame ionization. The activity of nitrogen fixation was observed at different growth levels in 04 sorghum cell lines by ARA in closed bottles. Since, emission of ethylene is favored by sorghum plants, ARA of bacterial nitrogenase was measured as the difference in the rate of ethylene formation in the presence and absence of acetylene. It was found that at early stages no ARA was present in the seedlings. However, significant ARA values (KM1, 36.1 nmol per plant per h, KM2, 52.6 nmol

per plant per h) at later stages of growth (71 days after treatment) in the washed roots were observed. Roots of all the four sorghum lines showed significant rise in ARA values at 102 days after treatment in comparison to 71 days. Also, ARA of cell lines differed between the growth stages hence indicating that root ARA has dependency upon the sorghum genotype (Hara et al. 2019).

10.4.3 Detecting Nitrogen Concentration Using Nitrogen Isotope

The biological nitrogen fixation is an essential process for proving nitrogen in available form to the biosphere. Among nitrogen fixers, the free-living diazotrophs residing in soil act as the dominating source of fixed N in ecosystem; however, there is still requirement of studies to know about their ecological and evolutionary significance (Cleveland et al. 1999). The key property is the nifH nitrogenase gene. The microbial diversity that harbors nitrogenase sequence is largely unidentified diazotrophs, which can also be the dominant communities for nitrogen fixation (Hamelin et al. 2002; Tan et al. 2003). The identification of non-culturable diazotrophs can be done by detecting their nifH sequences; however, only limited amount of information can be gathered via this process because nigH gene phylogenetics is not consistent to the phylogeny of the organism (Raymond et al. 2004a, b). Hence, the use of stable isotope probing using $^{15}N_2$-DNA ($^{15}N_2$-DNA-SIP) can be done for linking particular 16 s rRNA genes with N2-fixing process. The SIP process enable finding the link with 16 s rRNA genes of non-culturable diazotrophs with respect to their nifH genes and can also give the genomic fragments to be used in characterizing diazotrophs along with their ecological importance (Buckley et al. 2007).

In a study, three distinct microbial groups have been identified by applying $^{15}N_2$-DNA-SIP. These groups have not been earlier associated with N_2-fixation. They belonged to the uncharacterized and unclassified Betaproteobacteria and Actinobacteria (Buckley et al. 2007). Roley et al. (2019) studied the atmospheric nitrogen fixation in switchgrass by enriching the N_2 with isotope ^{15} N under in vitro, greenhouse and field studies. It was observed that N_2 fixation took place in early stage greenhouse experiments and it took 3-month time in field trails. Further, the microbial diversity in the soil was shaped according to the nitrogen fertilization (Fig. 10.1).

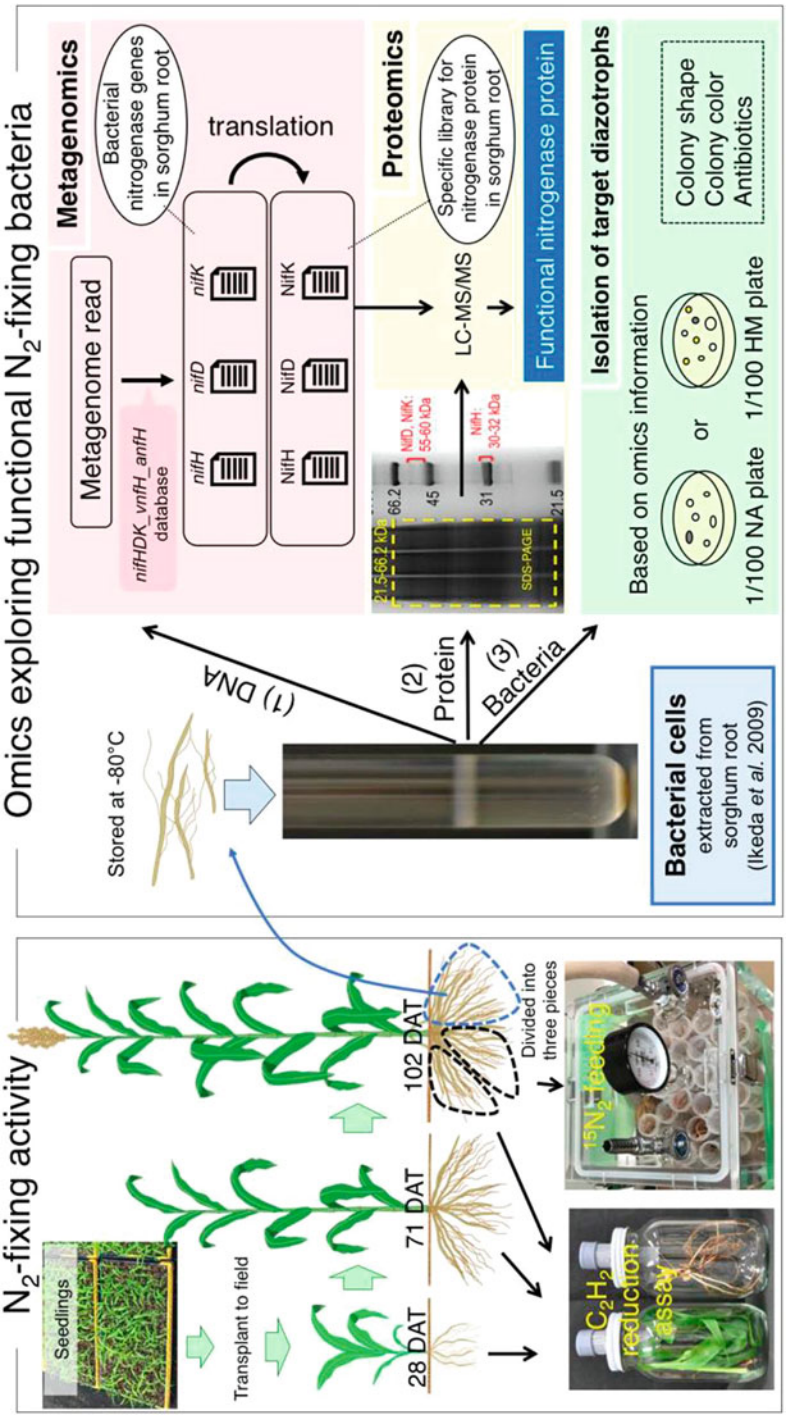

Fig. 10.1 Outline of "omics" strategy used to explore and identify functional N2-fixing bacteria associated with sorghum plants. N_2-fixing activities were monitored in tissues of sorghum at different growth stages by acetylene reduction assay. Bacteria were extracted from sorghum root tissues with higher N_2-fixing activities, and their metagenomes (1) and proteomes (2) were analyzed. Functional N_2-fixing bacteria were isolated from the extracted bacteria (3). DAT = days after transplant (Adapted with permission from Hara et al. (2019), Frontiers in Microbiology)

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References

Allison SM, Prosser JI (1992) Isolation and identification of autotrophic nitrifying bacteria. In: Board RG, Jones D (eds) Identification methods in applied and environmental microbiology. Blackwell Scientific Publications, Oxford, pp 87–102

Baldani JI, Caruso L, Baldani VLD, Goi SR, Dobereiner J (1997) Recent advances in BNF with non-legume plants. Soil Biol Biochem 29:911–922

Belser LW, Schmidt EL (1978) Serological diversity within a terrestrial ammonia-oxidising population. Appl Environ Microbiol 36:584–588

Bilung LM, Pui CF, Su'ut L, Apun K (2018) Evaluation of BOXPCR and ERIC-PCR as molecular typing tools for pathogenic Leptospira. Dis Markers 2018:1–9

Bohlool B, Ladha J, Garrity D, George T (1992) Biological nitrogen fixation for sustainable agriculture: a perspective. In: Biological nitrogen fixation for sustainable agriculture. Springer, Berlin, pp 1–11

Bothe H, Günter Jost B, Schloter M, Ward BB, Witzel K-P (2000a) Molecular analysis of ammonia oxidation and denitrification in natural environments. FEMS Microbiol Rev 24:673–690

Bothe H, Jost G, Schloter M, Ward BB, Witzel KP (2000b) Molecular analysis of ammonia oxidation and denitrification in natural environments. FEMS Microbiol Rev 24:673–690

Buckley DH, Huangyutitham V, Hsu SF, Nelson TA (2007) Stable isotope probing with 15N2 reveals novel noncultivated diazotrophs in soil. Appl Environ Microbiol 73:3196–3204

Chisnell JR, Premakumar R, Bishop PE (1988) Purification of a second alternative nitrogenase from a nifHDK deletion strain of Azotobacter vinelandii. J Bacteriol 170:27–33

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N-2) fixation in natural ecosystems. Global Biogeochem Cycle 13:623–645

Costa R, Gomes NCM, Krogerrecklenfort E, Opelt K, Berg G, Smalla K (2007) Pseudomonas community structure and antagonistic potential in the rhizosphere: insights gained by combining phylogenetic and functional gene-based analyses. Environ Microbiol 9:2260–2273. https://doi.org/10.1111/j.1462-2920.2007.01340.x

Crans DC, Smee JJ, Gaidamauskas E, Yang L (2004) The chemistry and biochemistry of vanadium and the biological activities exerted by vanadium compounds. Chem Rev 104:849–902

Crawford NM, Forde BG (2002) Molecular and developmental biology of inorganic nitrogen nutrition. Arabidopsis Book 1:e0011

Cruz LM, de Souza EM, Weber OB, Baldani JI, Döbereiner J, de Oliveira Pedrosa F (2001) 16S ribosomal DNA atterns of terrestrial biological nitrogebacteria isolated from banana (Musa spp.) and pineapple (Ananas comosus (L.) Merril). Appl Environ Microbiol 67:2375–2379

Das S, Dash HR, Mangwani N, Chakraborty J, Kumari S (2014) Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms. J Microbiol Methods 103:80–100

Demba Diallo M, Reinhold-Hurek B, Hurek T (2008) Evaluation of PCR primers for universal nifH gene targeting and for assessment of transcribed nifH pools in roots of Oryza longistaminata with and without low nitrogen input. FEMS Microbiol Ecol 65:220–228

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631

Drissner D, Blum H, Tscherko D, Kandeler E (2007) Nine years of enriched CO2 changes the function and structural diversity of soil microorganisms in a grassland. Eur J Soil Sci 58:260–269. https://doi.org/10.1111/j.1365-2389.2006.00838.x

Falcon LI, Cerritos R, Eguiarte LE, Souza V (2007) Nitrogen fixation in microbial mat and stromatolite communities from Cuatro Cienegas. Mexico Microb Ecol 54:363–373

Fani R, Gallo R, Lio P (2000) Molecular evolution of nitrogen fixation: the evolutionary history of the nifD, nifK, nifE, and nifN genes. J Mol Evol 51:1–11

Ferreira AC, Cozzolino K, Carvalho ARV, Dobereiner J (1995) Isolation and characterization of diazotrophic bacteria in oil palm trees. In: International symposium on sustainable agriculture for the tropics—the role of biological nitrogen fixation, Angra dos Reis, Rio de Janeirio, Brazil, p 210

Fries MR, Hopkins GD, McCarty PL, Forney LJ, Tiedje JM (1997) Microbial succession during a field evaluation of phenol and toluene as the primary substrates for trichloroethene cometabolism. Appl Environ Microbiol 63:1515–1522

Frye SR, Healy M (2006) Molecular strain typing using repetitive sequence-based PCR. In: Tang Y-W, Stratton CW (eds) Advanced techniques in diagnostic microbiology, 1st edn. Springer, New York, pp 444–471

Gadkari D, Mörsdorf G, Meyer O (1992) Chemolithoautotrophic assimilation of dinitrogen by Streptomyces thermoautotrophicus UBT1: identification of an unusual N2- fixing system. J Bacteriol 174:6840–6843

Gich FB, Amer E, Figueras JB, Abella CA, Balaguer MD, Poch M (2000) Assessment of microbial community structure changes by amplified ribosomal DNA restriction analysis (ARDRA). Int Microbiol 3:103–106

Gupta KJ, Brotman Y, Segu S, Zeier T, Zeier J, Persijn ST, Cristescu SM, Harren FJM, Bauwe H, Fernie AR (2013) The form of nitrogen nutrition affects resistance against pseudomonas syringae pv. Phaseolicola in tobacco. J Exp Bot 64:553–568

Haack SK, Fogarty LR, West TG, Alm EW, McGuire JT, Long DT (2004) Spatial and temporal changes in microbial community structure associated with recharge-influenced chemical gradients in a contaminated aquifer. Environ Microbiol 6:438–448

Hall JR, Mitchell KR, Jackson-Weaver O, Kooser AS, Cron BR, Crossey LJ, Takacs-Vesbach CD (2008) Molecular characterization of the diversity and distribution of a thermal spring microbial community by using rRNA and metabolic genes. Appl Environ Microbiol 74:4910–4922

Hamelin J, Fromin N, Tarnawski S, Teyssier-Cuvelle S, Aragno M (2002) nifH gene diversity in the bacterial community associated with the rhizosphere of Molinia coerulea, an oligonitrophilic perennial grass. Environ Microbiol 4:477–481

Hara S, Morikawa T, Wasai S, Kasahara Y, Koshiba T, Yamazaki K, Fujiwara T, Tokunaga T, Minamisawa K (2019) Identification of nitrogen-fixing Bradyrhizobium associated with roots of field-grown sorghum by metagenome and proteome analyses. Front Microbiol 10:407

Head IM, Hiorns WD, Embley TM, McCarthy AJ, Saunders JR (1993) The phylogeny of autotrophic ammonia-oxidizing bacteria as determined by analysis of 16S ribosomal RNA gene sequences. J Gen Microbiol 139:1147–1153

Hennecke H, Kaluza K, Thony B, Fuhrmann M, Ludwig W, Stackebrandt E (1985) Concurrent evolution of nitrogenase genes and 16S rRNA in rhizobium species and other nitrogen fixing bacteria. Arch Microbiol 142:342–348

Hinnemann B, Norskov JK (2004) Structure of the FeFe-cofactor of the iron-only nitrogenase and possible mechanism for dinitrogen reduction. Phys Chem Chem Phys 6:843–853

Hulton CSJ, Higgins CF, Sharp PM (1991) ERIC sequences: a novel family of repetitive elements in the genomes of Escherichia coli, Salmonella typhimurium and other enterobacteria. Mol Microbiol 5:825–834

Igarashi RY, Seefeldt LC (2003) Nitrogen fixation: the mechanism of the Mo-dependent nitrogenase. Crit Rev Biochem Mol Biol 38:351–384

James EK, Olivares FL (1997) Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. Crit Rev Plant Sci 17:77–119

Jennings J (2013) Catalytic ammonia synthesis: fundamentals and practice. Springer, Berlin

Jimenez-Salgado T, Fuentes-Ramirez LE, Tapia-Hernandez A, Mascarua-Esparza MA, Martinez-Romero E, Caballero-Mellado J (1997) Coffea arabica L., a new host plant for Acetobacter

diazotrophicus, and isolation of other nitrogen-fixing acetobacteria. Appl Environ Microbiol 63:3676–3683
Kamp A, Stief P, Bristow LA, Thamdrup B, Glud RN (2016) Intracellular nitrate of marine diatoms as a driver of anaerobic nitrogen cycling in sinking aggregates. Front Microbiol 7:1669
Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H (2004) Methods of studying soil microbial diversity. J Microbiol Methods 58:169–188
Kita-Tsukamoto K, Wada M, Yao K, Kamiya A, Yoshizawa S, Uchiyama N (2006) Rapid identification of marine bioluminescent bacteria by amplified 16S ribosomal RNA gene restriction analysis. FEMS Microbiol Lett 256:298–303
Krizova J, Spanova A, Rittich B (2006) Evaluation of amplified ribosomal DNA restriction analysis (ARDRA) and species-specific PCR for identification of Bifidobacterium species. Syst Appl Microbiol 29:36–44
Laguerre G, Allard M, Revoy F, Amarger N (1994) Rapid identification of rhizobia by restriction fragment length polymorphism analysis of PC-amplified 16S rRNA genes. Appl Environ Microbiol 60:56–63
Landrein B, Formosa-Jordan P, Malivert A, Schuster C, Melnyk CW, Yang W, Turnbull C, Meyerowitz EM, Locke JCW, Jonsson H (2018) Nitrate modulates stem cell dynamics in Arabidopsis shoot meristems through cytokinins. Proc Natl Acad Sci U S A 115:1382–1387
Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522
Lovell CR, Piceno YM, Quattro JM, Bagwell CE (2000) Molecular analysis of diazotroph diversity in the rhizosphere of the smooth cordgrass, Spartina alterniflora. Appl Environ Microbiol 66:3814–3822
Lovell CR, Decker PV, Bagwell CE, Thompson S, Matsui GY (2008) Analysis of a diverse assemblage of diazotrophic bacteria from Spartina alterniflora using DGGE and clone library screening. J Microbiol Meth 73:160–171
Machado HB, Funayama S, Rigo LU, Pedrosa FO (1991) Excretion of ammonium by Azospirillum brasilense mutants resistant to ethylenediamine. Can J Microbiol 37:549–553
Man-Aharonovich D, Kress N, Zeev EB, Berman-Frank I, Beia O (2007) Molecular ecology of nifH genes and transcripts in the eastern Mediterranean Sea. Environ Microbiol 9:2354–2363
Martin B, Humbert O, Camara M, Guenzi E, Walker J, Mitchell T, Andrew P, Prudhomme M, Alloing G, Hakenbeck R, Morrison DA (1992) A highly conserved repeated DNA element located in the chromosome of Streptococcus pneumoniae. Nucleic Acids Res 20:3479–3483
McCaig AE, Embley TM, Prosser JI (1994) Molecular analysis of enrichment cultures of marine ammonia oxidisers. FEMS Microbiol Lett 120:363–368
Mehta MP, Butterfield DA, Baross JA (2003) Phylogenetic diversity of nitrogenase (nifH) genes in deep-sea and hydrothermal vent environments of the Juan de Fuca ridge. Appl Environ Microbiol 69:960–970
Mehta MP, Huber JA, Baross JA (2005) Incidence of novel and potentially archaeal nitrogenase genes in the deep Northeast Pacific Ocean. Environ Microbiol 7:1525–1534
Miethling R, Ahrends K, Tebbe CC (2003) Structural differences in the rhizosphere communities of legumes are not equally reflected in community-level physiological profiles. Soil Biol Biochem 35:1405–1410. https://doi.org/10.1016/S0038-071700221-9
Mohamed NM, Colman AS, Tal Y, Hill RT (2008) Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. Environ Microbiol 10:2910–2921
Muyzer G, Smalla K (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek 73:127–141
Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Nocker A, Burr M, Camper AK (2007) Genotypic microbial community profiling: a critical technical review. Microb Ecol 54:276–289. https://doi.org/10.1007/s00248-006-9199-5
Normand P, Bousquet J (1989) Phylogeny of nitrogenase sequences in Frankia and other nitrogen-fixing microorganisms. J Mol Evol 29:436–447
Nuble U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, Ludwig W, Backhaus H (1996) Sequence heterogeneities of genes encoding 16S rRNAs in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. J Bacteriol 178:5336–5643
Olive DM, Bean P (1999) Principles and applications of methods for DNA-based typing of microbial organisms. J Clin Microbiol 37:1661–1669
Postgate JR (1982) The fundamentals of nitrogen fixation. Cambridge University Press, Cambridge
Raymond J, Siefert JL, Staples CR, Blankenship RE (2004a) The natural history of nitrogen fixation. Mol Biol Evol 21:541–554
Raymond J, Siefert JL, Staples CR, Blankenship RE (2004b) The natural history of nitrogen fixation. Mol Biol Evol 21:541–554
Rees DC, Howard JB (2000) Nitrogenase: standing at the crossroads. Curr Opin Chem Biol 4:559–566
Rehder D (2000) Vanadium nitrogenase. J Inorg Biochem 80:133–136
Ribbe M, Gadkari D, Meyer O (1997) N2 fixation by Streptomyces thermoautotrophicus involves a molybdenum-dinitrogenase and a manganese-superoxide oxidoreductase that couple N2Reduction to the oxidation of superoxide produced from O2by a molybdenum-CO dehydrogenase. J Biol Chem 272:26627–26633
Rohlf FJ (1992) NTSYS-pc numerical taxonomy and multivariate analysis system. Exeter Software, New York
Roley SS, Xue C, Hamilton SK, Tiedje JM, Robertson GP (2019) Isotopic evidence for episodic nitrogen fixation in switchgrass (Panicum virgatum L.). Soil Biol Biochem 129:90–98
Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602
Schindelin H, Kisker C, Schlessman JL, Howard JB, Rees DC (1997) Structure of ADP AIF4 -stabilized nitrogenase complex and its implications for signal transduction. Nature 387:370–376
Schollhorn R, Burris RH (1967) Acetylene as a competitive inhibitor of N2 fixation. Proc Natl Acad Sci USA 58:213–216. https://doi.org/10.1073/pnas.58.1.213
Schramm A, de Beer D, van den Heuvel JC, Ottengraf S, Amann R (1999) Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Appl Environ Microbiol 65:3690–3696
Schwieger F, Tebbe CC (1998) A new approach to utilize PCR single-strand-conformation polymorphism for 16s rRNA gene-based microbial community analysis. Appl Environ Microbiol 64:4870–4876
Seefeldt LC, Hoffman BM, Dean DR (2009) Mechanism of Mo-dependent nitrogenase. Annu Rev Biochem 78:701
Simpson FB, Burris RH (1984) A nitrogen pressure of 50 atmospheres does not prevent evolution of hydrogen by nitrogenase. Science 224:1095–1097
Sliwinski MK, Goodman RM (2004) Comparison of crenarchaeal consortia inhabiting the rhizosphere of diverse terrestrial plants with those in bulk soil in native environments. Appl Environ Microbiol 70:1821–1826. https://doi.org/10.1128/AEM.70.3.1821-1826.2004
Spiegelman D, Whissell G, Greer CW (2005) A survey of the methods for the characterization of microbial consortia and communities. Can J Microbiol 51:355–386
Stern MJ, Ames GFL, Smith NH, Robinson EC, Higgins CF (1984) Repetitive extragenic palindromic sequences: a major component of the bacterial genome. Cell 37:1015–1026
Tan XY, Hurek T, Reinhold-Hurek B (2003) Effect of N-fertilization, plant genotype and environmental conditions on nifH gene pools in roots of rice. Environ Microbiol 5:1009–1015

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Ulrich A, Becker R (2006) Soil parent material is a key determinant of the bacterial community structure in arable soils. FEMS Microbiol Ecol 56:430–443. https://doi.org/10.1111/j.1574-6941.2006.00085.x

Van de Peer Y, De Wachter R (1994) TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Appl Biosci 10:569–570

Vaneechoutte M, Rossau R, De Vos P, Gillis M, Janssens D, Paepe N (1992) Rapid identification of bacteria of the Comamonadaceae with amplified ribosomal DNA-restriction analysis (ARDRA). FEMS Microbiol Lett 72:227–233

Vaneechoutte M, Dijkshoorn L, Tjernberg I, Elaichouni A, de Vos P, Claeys G, Verschraegen G (1995) Identification of Acinetobacter genomic species by amplified ribosomal DNA restriction analysis. J Clin Microbiol 33:11–15

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Vojvodic A, Medford AJ, Studt F, Abild-Pedersen F, Khan TS, Bligaard T, Nørskov JK (2014) Exploring the limits: a low-pressure, low-temperature Haber– Bosch process. Chem Phys Lett 598:108–112

Wang WX, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14

Ward BB, Carlucci AF (1985) Marine ammonia- and nitrite-oxidizing bacteria: serological diversity determined by immunofluorescence in culture and in the environment. Appl Environ Microbiol 50:194–201

Watanabe T, Kimura M, Asakawa S (2007) Dynamics of methanogenic archaeal communities based on rRNA analysis and their relation to methanogenic activity in Japanese paddy field soils. Soil Biol Biochem 39:2877–2887. https://doi.org/10.1016/j.soilbio.2007.05.030

Watts JE, Wu Q, Schreier SB, May HD, Sowers KR (2001) Comparative analysis of polychlorinated biphenyl-dechlorinating communities in enrichment cultures using three different molecular screening techniques. Environ Microbiol 3:710–719

Weber OB, Baldani VLD, Teixeira KRS, Kirchhof G, Baldani JI, Dobereiner J (1999) Isolation and characterization of diazotrophic bacteria in banana and pineapple plants. Plant and Soil 210:103–113

Webster G, Embley TM, Prosser JI (2002) Grassland management regimes reduce small-scale heterogeneity and species diversity of β-proteobacterial ammonia oxidiser populations. Appl Environ Microbiol 68:20–30

Welsh A, Burke DJ, Hahn D (2007) Analysis of nitrogen-fixing members of the ε subclass of Proteobacteria in salt marsh sediments. Appl Environ Microbiol 73:7747–7752

Wink M (1999) Functions of plant secondary metabolites and their exploitation in biotechnology. In: Annual plant reviews, vol 3. CRC Press, Boca Raton

Young JPW (1992) Phylogenetic classification of nitrogen-fixing organisms. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman and Hall, New York, NY

Young JPW, Downer HL, Eardly BD (1991) Phylogeny of the phototrophic rhizobium strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment. J Bacteriol 173:2271–2277

Zehr JP, Mellon MT, Braun S, Litaker W, Steppe T, Paerl HW (1995) Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat. Appl Environ Microbiol 61:2527–2532

Zehr JP, Mellon MT, Zani S (1998) New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (nifH) genes. Appl Environ Microbiol 64:3444–3450

Zhao Y, Bian SM, Zhou HN, Huang JF (2006) Diversity of nitrogenase systems in diazotrophs. J Integr Plant Biol 48:745–755

Chapter 11
Biopriming is Emerging as a Supplemental Strategy for Improving Nitrogen Use Efficiency of Crop Species

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Abstract A recent review of worldwide data on nitrogen use efficiency warrants an immediate intervention of best management practices which will optimize best fertilizers option, farmer profitability and crop productivity. Nitrogen being a primary nutrient is critical for congenial plant growth. Additionally, the risk to human health due to indiscriminate use and concern due to energy intensive production of this input paves the way for use of bio-inoculants as the possible link for better utilization and use efficiency. Biopriming which involves seed priming in combination with low dosage of beneficial microbes has emerged as the most feasible eco-friendly supplement to the existing integrated plant nutrition system. In this chapter, we have presented an overview of the recent advances in biopriming with a special reference to nitrogen.

Keywords Nitrogen use efficiency · Bio-inoculants · Biopriming

11.1 Nitrogenous Fertilizer-Energy Intensive/ Non-renewable Energy

India is the second most populous country after china. India is primarily an agricultural country as 65% of its people depend on agriculture for their livelihood. Farmers of India mostly rely on organic manures for supplementing soil until middle of twentieth century. However, with the emergence of green revolution during early 1960s, demand and consumption of synthetic fertilizers increased per unit area

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Table 11.1 Various manufacturing processes of hydrogen

Process	Reaction	Approx relative energy consumption (%)
Water electrolysis	$2H_2O \rightarrow 2H_2 + O_2$	300
Coal gasification	$C + 2H_2O \rightarrow 2H_2 + CO_2$	170
Naphtha reforming	$CH_2 + 2H_2O \rightarrow 3H_2 + CO_2$	104
Nat gas reforming	$CH_4 + 2H_2O \rightarrow 4H_2 + CO_2$	100

Source: Pach (2007)

tremendously. Use of high yielding and developed irrigation facilities during the time of green revolution required higher input which was then fulfilled by fertilizers. As a consequence, Indian soils become less and less fertile. Out of many reports of nutrient deficiency, nitrogen (N) deficiency is the first one to be reported in Indian soil. As a result consumption of nitrogenous fertilizers was increased sharply from 0.06 million ton (Mt) in 1950–1951 to 10.8 Mt. in 2000–2001, about 190-fold increase in 50 years after independence (Pathak et al. 2010). Green revolution causes increase in consumption of all the fertilizers mainly phosphatic and potassic after nitrogenous fertilizers. This makes India third largest producer and consumer of fertilizers worldwide. Energy consumed in fertilizer sector per unit of output is 14.7% from 1996 to 2005. Coal, petroleum and natural gas are major sources of energy but non-renewable in nature. Production of nitrogenous fertilizers is highly energy intensive out of four nitrogen, phosphate, potash and complex type fertilizers. Ammonia (NH_3) production is a primary stage for nitrogenous fertilizers as it is basic chemical used in their production (Ray 2011). Production of NH_3 at a large scale is mainly carried out by Haber–Bosch process. Ammonia is produced using N_2 and H_2 from the atmosphere at a very high temperature and pressure. Harsh condition during the process results lower efficiency level than desirable for sustainable fertilizer production (Zhou et al. 2017). Hydrogen used in the process is chemically produced which then reacted with atmospheric N_2 and produce NH_3. Atmospheric nitrogen is used as a N_2 source which is present in adequate quantity but energy and feedstock required for the process are limited. Natural gas is one of the key input for NH_3 production which share 78.5% in total N production; external NH_3 and naphtha come after that with 16 and 5.3% share, respectively (Tewatia and Chanda 2017). Energy consumed during various process of hydrogen production was given in Table 11.1. The hydrogen produced from various processes is further reacted with atmospheric N_2 for final ammonia production.

According to Dawson and Hilton (2011), annual energy requirement for fertilizer requirement in world is approximately 1.1% of energy used globally in the year 2008. Out of the total energy input used in fertilizer production, N fertilizers alone account for more than 90% (Table 11.1).

Industries related to N fertilizers are not only energy intensive but also deals with non-renewable sources of energy which is of great concern. In developing countries like India where energy crisis is a major problem, the energy consumption of NH_3 plants is 12.48 giga calories metric ton^{-1} (Gcal MT^{-1}) and that of urea plants is 5.95 Gcal MT^{-1} in 2015 (Tewatia and Chanda 2017). This much energy consumption is

Table 11.2 World demand for fertilizer nutrient use, 2015–2020 (1000 tonnes)

Year	2015	2016	2017	2018	2019	2020
Nitrogen (N)	110,027	111,575	113,607	115,376	117,116	118,763
Phosphate (P_2O_5)	41,151	41,945	43,195	44,120	45,013	45,858
Potash (K_2O)	32,838	33,149	34,048	34,894	35,978	37,042
Total (N+ P_2O_5 + K_2O)	184,017	186,668	190,850	194,390	198,107	201,663

Source: FAO (2019)

of great concern, as most of the Indian soils are deficient in N and farmers are totally depend on nitrogenous fertilizers for crop production (Table 11.2).

11.2 Nitrogen-No Alternative Source; Supplementation and Improving Rhizosphere Efficiency Only Option

Nitrogen requirement for plant is more than any other nutrient and is a key component of chlorophyll responsible for photosynthesis, as well as amino acids, ATP and nucleic acids. Nitrogen plays major role in growth and development of plant and is a critical limiting element in soil. Though nitrogen quantity is ample in atmosphere, in Indian soils nitrogen is universally deficient element for profitable crop production and almost all soils respond to external application of nitrogen. Cost of fertilizers is still close to record highs which increase cost of cultivation and adverse effects on benefits of the farmer, also impacts environment through nitrogen losses. Also farmers may apply excess fertilizers that exceed agronomic recommendations. To get rid of this complication focus should be on management approaches and new interventions to supplement costly fertilizers, to meet crop requirement and increase efficiency especially for nitrogen. Management options are more effectual if it can target the efficiency of rhizosphere which is directly influenced by root secretions and associated microorganisms and is the seat of all complex interactions among soil, microorganisms and plant roots. To enhance nutrient availability and uptake, rhizosphere role is incredible. In soil system, 95–99% of nitrogen is in organic form which cannot be directly available to the plants, but will be mineralized into available forms by microorganisms. Rhizospheric microorganisms participate in this cycling of nitrogen and determine the plant availability. To minimize cost of cultivation and chemical fertilizer use the option in front of us is improving the rhizosphere efficiency through manipulating its microclimate which can be achieved by integration with organics and microorganisms. Bio-organic fertilizers, a combination of suitable substrate and functional microbes can effectively suppress soil-borne diseases and promote plant growth (Zhao et al. 2018). Plants shape microbiome structure through its root exudates in rhizosphere through which bio-availability of nutrients, hormonal activity and plant growth will get positively affected. Though the external application of microbes to the rhizosphere can be done through several means, but the best, effective and feasible method is seed

biopriming which is the combination of both seed hydration and addition of bio-agent. Biopriming intervention is eco-friendly, low cost intensive and a potent technology for altering microclimate of the rhizosphere. This technology not only enhances the availability of nutrients especially nitrogen, but also resist the plant in abiotic and biotic stress situations.

11.3 Possible Microbes as a Suitable Agent for Biopriming

As biopriming is being emerging as an advanced form for nutrient management in agricultural crop production, the main reason underlying is the use of native and indigenous microorganisms which benefits the crop plants not only in terms of fertilization but also providing other advantageous attributes like plant growth promotion, safeguarding against pathogenic diseases and also biotic and abiotic stress (Table 11.3). The biopriming procedure was first described by Callan and co-workers in 1990 for biological control of *Pythium* preemergence damping-off of sweet corn at an optimal temperature with protection by using a biocontrol agent. Other advantages of this new priming approach are that it incite such changes in the bioprimed seeds that enhances the seed germination and emergence (Bisen et al. 2015). The indigenous strains here are emphasized because it assures of the compatibility with the crops in the region and also gets acclimatized better in the cop-soil ecosystem in comparison to the one that has been imported from outside the region (Fig. 11.1). This is also an exemplary alternative for organic production where use of chemicals for disease and nutrient management is not advised.

To get the favourable results suitability of the biopriming agent is needed to be taken under consideration which depends on many factors as aeration, quality of seed, temperature, light and duration (Kumar et al. 2020).

It improves seed viability, germination, vigour indices, plant growth and subsequent protection against diseases and finally enhances crop yield. Biopriming is the advanced technique which can be employed to improve the plant growth for which different plant growth promoting microorganisms can be used. To address the diseases and pests in crops biopriming using biocontrol agents, viz. *Trichoderma* sp., *Aspergillus* sp., Mycorrhizal fungi, etc. and antagonistic microbes can be done by suppressing various seed and soil-borne diseases as well as foliar diseases through induced systemic resistance mechanisms. Management of nutrients in the crops and soils can be accomplished with the help of biopriming agents having the ability of nutrient solubilization and improving the nutrient availability towards plants, e.g. *Rhizobium leguminosarum*, *Pseudomonas fluorescens*, vesicular-arbuscular mycorrhiza (VAM), viz. *Acaulospora* sp., *Glomus* sp. Biopriming is also a tool to aid plants during stress using such microorganisms those who elicit so-called induced systemic tolerance (IST) against biotic and abiotic stresses and can with stand high temperature, pH and salt concentrations e.g. *Colletotrichum magna*, *Piriformospora indica*, *Alternaria* sp. (Prasad et al. 2016).

Table 11.3 Different biopriming agents and various functions performed by them

	Crop	Microbial Agent	Use	Advancement	Region	Reference
Cereals	Rice (*Oryza sativa*)	*Bacillus* sp.	Dirty panicle disease (*Bipolaris* sp. and *Curvularia* sp.)	Antifungal activity (nearly 100%)	Chiang Mai, Thailand	Rangjaroen et al. (2019)
	Maize (*Zea mays*)	*Trichoderma lixii*	Salt toxicity	Proline accumulation increased by 21.87%(roots), 35.71% (leaves)	Rize, Turkey	Pehlivan et al. (2017)
	Rice (*Oryza sativa*)	*Piriformospora indica*	Cadmium stress	Root ROS decreased by 18.5%	Noida, India	Dabral et al. (2019)
Pulses and legumes	Chickpea (*Cicer arietinum*) and rajma (*Phaseolus vulgaris*)	*Pseudomonas fluorescens, Trichoderma asperellum* and *Rhizobium*	Plant growth	Germination % increased by 24–27%, plant height increased by approx. 23.6%	Varanasi, India	Yadav et al. (2013)
	Faba bean (*Vicia faba*)	*Trichoderma* spp., *Bacillus* spp., *P. fluorescens*	Root rot incidence caused by *R. solani*, *F. solani* and *S. rolfsii*	Disease incidence decreased upto 6.9–14.8% (fungal agent),6.9–23.3% (bacterial agent)	Giza, Egypt	El-Mougy and Abdel-Kader (2008)
	Soybean (*Glycine max*)	*Trichoderma* spp., *Pseudomonas fluorescent*	Nutrition	Fe uptake increased to 77%Zn uptake (90 mg/kg), N (7.5%)	Tehran, Iran	Entesari et al. (2013)
Vegetables	Carrot (*Daucus carota*)	*Clonostachys rosea*	*Alternaria* spp	Alternaria spp. incidence reduction by ≥94%	Copenhagen, Denmark	Jensen et al. (2004)
	Okra (*Abelmoschus esculentus*)	*Alcaligenes faecalis*	Against *Sclerotium rolfsii*	Mortality reduced to 20%	Varanasi, India	Ray et al. (2016)
Spices	Cumin (*Cuminum cyminum* L.)	*Pseudomonas fluorescence, Trichoderma harzianum*	Against drought stress	Emergence percentage (52.23%), emergence rate (0.358 seedling d^{-1})	Yasuj, Iran	Piri et al. (2019)

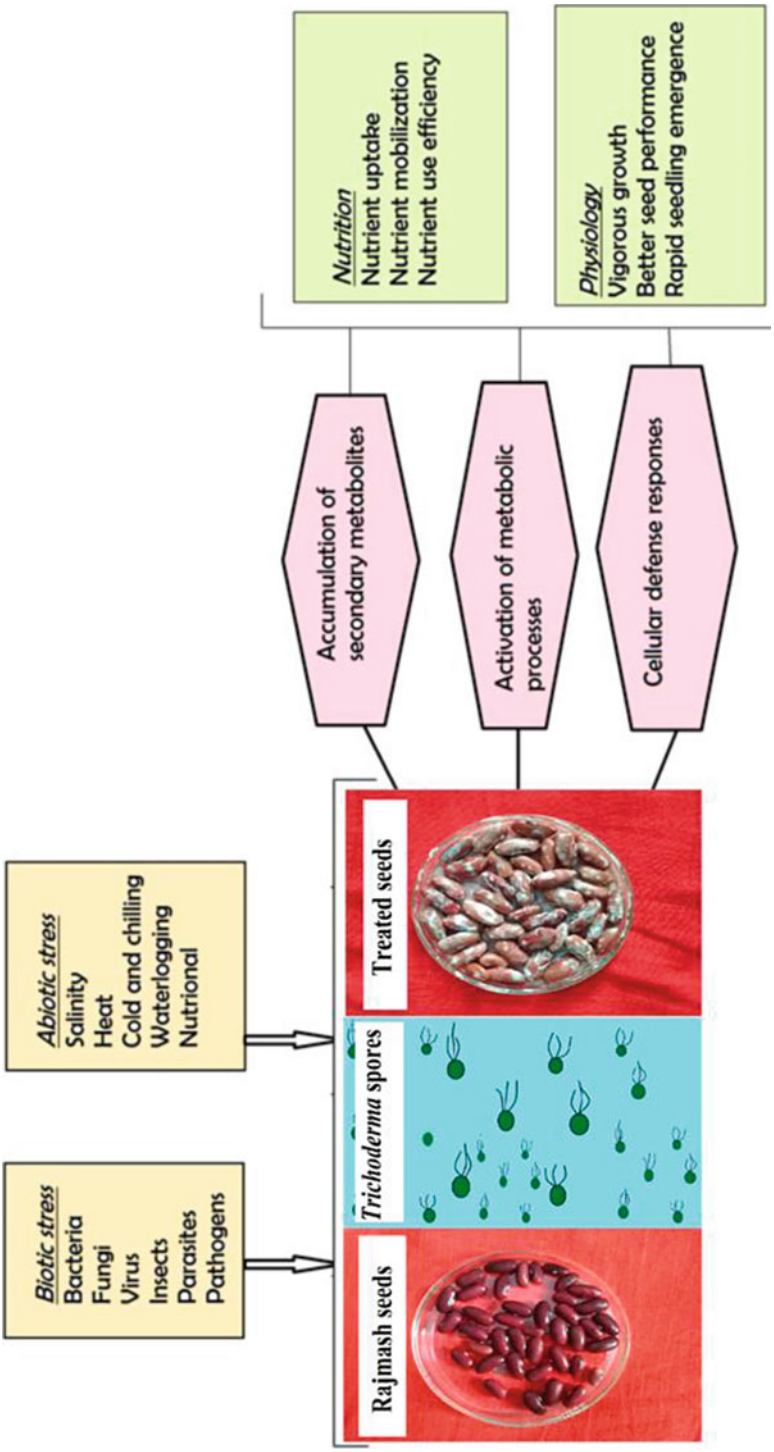

Fig. 11.1 Biopriming affecting plant growth via different mechanisms

11.4 Probable Mechanism Involved

Nitrogen is the second most limited nutrient for plants growth after water (Malik et al. 2001), despite its abundant concentration in atmosphere (78%). Nitrogen is present in the atmosphere as dinitrogen gas (N_2) and has triple bonded structure ($N \equiv N$) because of which very high energy is required to take that forms directly by plant (Shridhar 2012). Certain microbial species have the special ability to fix atmospheric N into ammonical form with the help of nitrogenase system (Halbleib and Ludden 2000). Use of synthetic nitrogenous fertilizer in agriculture is the most popular method of providing sufficient nutrient concentration to the plants. Long term and imbalance use of nitrogenous fertilizers lead to many environmental issues. Therefore, use of bio-inoculants can improve nitrogen use efficiency by various ways listed below:

1. Increase total surface area of root which improve N uptake either by increase in root growth, more branching and root hairs formation or by addition of extension in prevailing root system (e.g. mycorrhizal associations) (Saia et al. 2014).
2. Direct contribution of nutrient in soil solution pool either by biological N-fixation (BNF) or by changing the kinetics of rhizospheric processes (e.g. mineralization, nitrification inhibition, etc.) (Mohammadi and Sohrabi 2012).
3. Through microbial biomass turnover in the rhizosphere (Richardson et al. 2009).

Of all the mechanisms listed above, BNF is the most popular and efficient one. Biological nitrogen fixation is enzymatic conversion of dinitrogen into NH_3 catalysed by nitrogenase, an oxygen reactive enzyme complex (Bhat et al. 2015), by the following reaction:

$$N_2 + 8H + 8e^- + 16ATP \xrightarrow{\text{Nitrogenase}} 2NH_3 + H_2 + 16\ ADP + 16Pi$$

Nitrogenase consists of two separate metalloprotein called larger dinitrogenase (Mo-Fe protein) and smaller dinitrogenase reductase (Fe protein) (Pathak and kumar 2017). Firstly Fe protein interacts with ATP and Mg^{2+}, accepts electron from ferredoxin or flavodoxin and becomes reduced. Finally electron flow takes place from reduced Fe protein to oxidized Mo-Fe protein and it is reduced (Fig. 11.2). It is the reduced species of Mo-Fe protein which combine with N_2 and other substrates to yield NH_3.

Nitrogen fixation by bacterial species is carried out either by symbiotic, associative or free living relationship between plants and bacteria. In symbiotic association, bacterial strains penetrate inside cortical cells of root system and form a nodule (Bhattacharjee et al. 2008). Nodules have iron containing substance known as leghaemoglobin which impart pink colour to nodule (Ampomah et al. 2012). Nodule is the primary site of nitrogen fixation. In this type of association, bacterial species provide NH_3 to plants after fixation and in return plant provides protection, energy and photosynthates to microbial species. Bacterial species like *Azotobacter* fix nitrogen in free living condition (Fig. 11.3). They are present in rhizosphere and

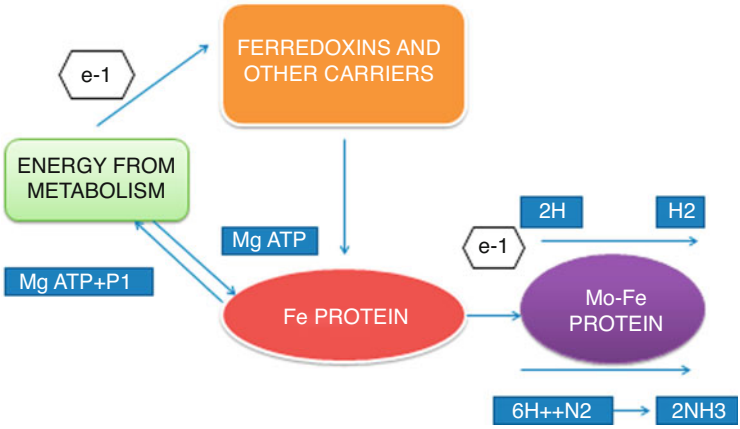

Fig. 11.2 Mechanism of N-fixation

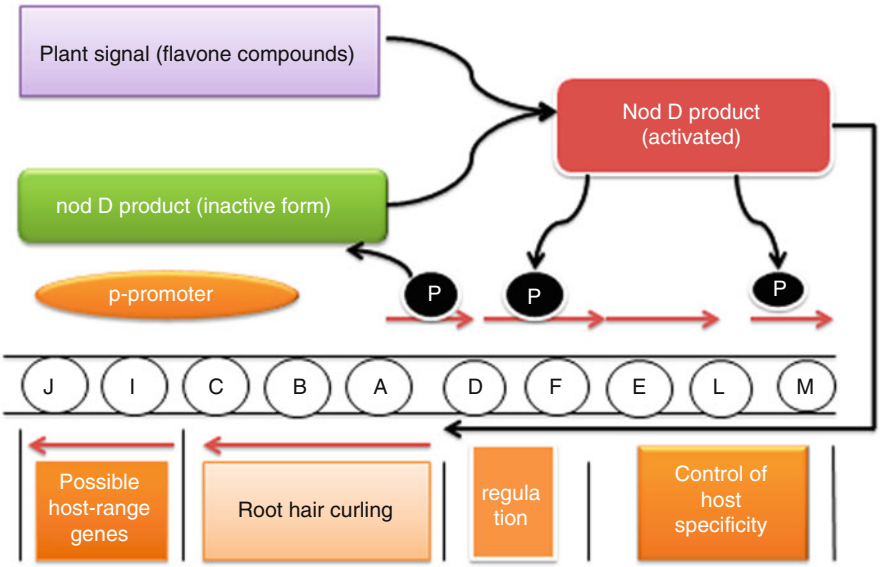

Fig. 11.3 Schematic diagram of events at molecular level in root region in Rhizobium–legume interaction of N- fixation

from there, they fix nitrogen. Plants assimilated the nitrogen from rhizosphere and in return provide energy and photosynthates through root exudates. While bacterial species like *Azospirillum* fix nitrogen by forming association with roots of Poaceae family (Duarte et al. 2020). Unlike bacteria, some fungi can also be used for improving N use efficiency through bio-priming. *Trichoderma spp.* were greatly studied for its role in augmenting N availability in soil. Some studies suggest that, its

Table 11.4 Effect of biopriming on nitrogen use efficiency

	Crops	Type of experimental	Biopriming agent	Mechanism	NUE	References
Cereals	Wheat (*Triticum aestivum* L.)	Pot experiment	*Trichoderma harzianum*	Root length and nitrogen uptake was improved	AUE of N alluvial soil (3.36%), blacksoil (0.67%), redsoil (0.18%)	Meena et al. (2016)
	Rice (*Oryza sativa*)	Pot experiment	*Trichoderma harzianum*	Growth promotion and uptake	AUE increased by 69.55%	Priya et al. (2018)
	Rice (*Oryza sativa*)	Pot experiment	*Thalassobacillus denorans* and *Oceanobacillus kapialis*	Increased uptake of nitrogen, synthesis of phytohormones and N-fixation	N content of plants increased by 20%	
	Rice (*Oryza sativa*)	Greenhouse experiment	*Azospirillum amazonense*	Nitrogen fixation and plant growth promoting activity	Nitrogen harvest index increased by 17%	Rodrigues et al. (2008)
	Maize (*Zea mays*)	Pot experiment	*T. Harzianum*	Phytohormone production, increase in photosynthetic features and root biomass	N content (root) was 8.8–9.76%	Akladious and Abbas (2012)
	Wheat (*Triticum aestivum* L.)	Greenhouse experiment	*Bacillus* spp.	Plant growth promotion and N-fixation, nitrogenase activity	N content (seeds) increased by 84.9%	Brahim et al. (2019)
	Maize (*Zea mays*)	Pot experiment	*Trichoderma* and *Bacillus*	Increased photosynthetic activity, the mobilization of photosynthates	N content (plant) increased by 25.2%	Mutetwa et al. (2019)
Pulses	Chickpea (*Cicer arietinum* L.)	Glass house pot and field experiment	*Rhizobium, Bacillus megaterium* and *Trichoderma* spp.	Growth promoting substances, N-fixation	N uptake increased by 100% in the shoots	Rudresha et al. (2004)
	Soybean (*Glycine max*)	Greenhouse conditions	*T. Harzianum, T. atroviride, pseudomonas fluorescent*	Increased root growth, enzymatic activity	Plant N content was (15.8%)	Entesari et al. (2013)
	Dry bean (*Phaseolus vulgaris*)	Shadehouse pot trial	*T. atroviride* and *Bacillus*	Increase in nodulation and dry biomass due to growth promotion activities	Plant N content was increased by 175.6%	Yobo et al. (2009)

(continued)

Table 11.4 (continued)

	Crops	Type of experimental	Biopriming agent	Mechanism	NUE	References
Vegetables and others	Amaranthus (*Amaranthus Hypochondriacus*)	Pot + field experiment	*Bacillus* spp.	PGP activity, improved uptake	ARE for N was 1.17%	Pandey et al. (2018)
	Broccoli (*Brassica oleracea* L.)	Pot experiment	*Trichoderma viride, Glomus mosseae; Acaulospora laevis* and *Pseudomonas fluorescens*	Plant growth promoting activity, production of secondary metabolites	N % increased by 142% in shoot	Tanwar et al. (2013)
	Tea (*Camellia sinensis*)	In-vivo	*Pseudomonas fluorescens, Azospirillum brasilense, Trichoderma harzianum*	Enhanced nitrogen fixation, improved plant water relations	NUE improved by 30.1%	Thomas et al. (2010)
	Native plants (*E. gamophylla, G. wickhamii* and *T. wiseana*)	Glasshouse experiment	Cyanobacteria: *Leptolyngbya* sp., *Microcoleus* sp., *Nostoc* sp. and *Scytonema* sp.	Metabolites production, N-fixation	Soil Total N increased by 14.25%	Chua et al. (2019)

potential to degrade cellulose can release huge amount of N from organic to inorganic pool of N in the rice rhizosphere (Doni et al. 2014).

11.5 Biopriming Mediated NUE in Different Crops

Several works have been done on biopriming particularly to improve nitrogen use efficiency. Increase in nitrogen use efficiency means the plant can efficiently utilize the nitrogen and is able to assimilate the nutrient into its chlorophyll, nucleic acids, protein content, etc. Different mechanisms have been explained by various investigators as the reason behind this increment in use efficiency of nitrogen. This increase is attributed to different functions of biopriming agents—N-fixation, nitrogenase activity, plant growth promoting activities, etc. (Table 11.4)

11.6 Conclusions and Way Forward

Green revolution led to the adoption of high yielding varieties whose seeds lay primarily on chemical fertilizers especially nitrogen which is produced through highly energy intensive process. Indian soils are poor in nitrogen status and almost all soils respond to the application of external nitrogen. As fossil fuels are expensive, cost of fertilizers is still at high which increase economic burden to farmer. In modern agriculture avoiding chemical fertilizers completely is not possible, but there is every need to focus on the management aspects and new interventions to supplement a part of the chemical nutrient source. The outcome of new technologies will be more effective if it is designed to increase the efficiency of rhizosphere. Rhizospheric microbes play a major role in complex interactions and nutrient cycling at root zone. The best intervention to supplement a part of energy and to manipulate rhizospheric microclimate is seed bio-priming which is an ecocentric and user friendly technique. Seed biopriming enables better plant performance even under adverse conditions, improves nutrient uptake and resists the plant against biotic and abiotic stress conditions. In current scenario, there is growing attention towards beneficial microorganisms to get higher quality and quantity of economic yields and has incredible scope in near future.

References

Akladious SA, Abbas SM (2012) Application of *Trichoderma harziunum* T22 as a biofertilizer supporting maize growth. Afr J Biotechnol 11:8672–8683

Ampomah OY, James EK, Iannetta PP, Kenicer G, Sprent JI, Huss-Danell K (2012) Nodulation and ecological significance of indigenous legumes in Scotland and Sweden. Symbiosis 57:133–148

Bhat TA, Ahmad L, Ganai MA, Khan OA (2015) Nitrogen fixing biofertilizers; mechanism and growth promotion: a review. J Pure Appl Microbiol 9:1675–1690

Bhattacharjee RB, Singh A, Mukhopadhyay SN (2008) Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. Appl Microbiol Biotechnol 80:199–209

Bisen K, Keswani C, Mishra S, Saxena A, Rakshit A, Singh HB (2015) Unrealized potential of seed biopriming for versatile agriculture. In: Rakshit A, Singh HB, Sen A (eds) Nutrient use efficiency: from basics to advances. Springer, New Delhi, pp 193–206

Brahim AH, Jlidi M, Daoud L (2019) Seed-biopriming of durum wheat with Diazotrophic plant growth promoting bacteria (PGPB) enhanced tolerance to fusarium head blight (FHB) and salinity stress. Res Square 12:12. https://doi.org/10.21203/rs.2.16636/v2

Chua M, Erickson TE, Merritt DJ, Chilton AM, Ooi MK, Muñoz-Rojas M (2019) Bio-priming seeds with cyanobacteria: effects on native plant growth and soil properties. Restor Ecol 28:168–176

Dabral S, Yashaswee Varma A, Choudhary DK, Bahuguna RN, Nath M (2019) Biopriming with *Piriformospora indica* ameliorates cadmium stress in rice by lowering oxidative stress and cell death in root cells. Ecotoxicol Environ Saf 186:109741

Dawson CJ, Hilton J (2011) Fertiliser availability in a resource-limited world: production and recycling of nitrogen and phosphorus. Food Policy 36:S14–S22

Doni F, Isahak A, Zain CRCM, Yusoff WMW (2014) Physiological and growth response of rice plants (Oryza sativa L.) to Trichoderma spp. inoculants. Amb Express 4:45

Duarte CFD, Cecato U, Biserra TT, Mamédio D, Galbeiro S (2020) Azospirillum spp. in grasses and forages. Review. Rev Mex Cienc Pecu 11:223–240

El-Mougy NS, Abdel-Kader MM (2008) Long-term activity of bio-priming seed treatment for biological control of faba bean root rot pathogens. Australas Plant Pathol 37:464–471

Entesari M, Sharifzadeh F, Ahmadzadeh M, Farhangfar M (2013) Seed biopriming with Trichoderma species and pseudomonas fluorescent on growth parameters, enzymes activity and nutritional status of soybean. Int J Agron Plant Prod 4:610–619

FAO (2019) World fertilizer trends and outlook to 2022. FAO, Rome

Halbleib CM, Ludden PW (2000) Regulation of biological nitrogen fixation. J Nutr 130:1081–1084

Jensen B, Knudsen IMB, Madsen M, Jensen DF (2004) Biopriming of infected carrot seed with an antagonist, *Clonostachys rosea*, selected for control of seedborne *Alternaria* spp. Phytopathology 94:551–560

Kumar A, Droby S, White JF, Singh VK, Singh SK, Zhimo VY, Biasi A (2020) Endophytes and seed priming: agricultural applications and future prospects. In: Microbial endophytes. Woodhead Publishing, New York, pp 107–124

Malik FR, Ahmed S, Rizki YM (2001) Utilization of lignocellulosic waste for the preparation of nitrogenous biofertilizer. Pak J Biol Sci 4:1217–1220

Meena SK, Rakshit A, Meena VS (2016) Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (*Triticum aestivum* L.) under greenhouse conditions. Biocatal Agric Biotechnol 6:68–75

Mohammadi K, Sohrabi Y (2012) Bacterial biofertilizers for sustainable crop production: a review. ARPN J Agric Biol Sci 7:307–316

Mutetwa M, Nyaera K, Masaka T, Mtaita TA (2019) Effect of bio priming seeds with microbial based bio fertilizers on growth of maize seedlings. Int J Res Rev 6:281–288

Pach JD (2007) Ammonia production: energy efficiency, CO_2 balances and environmental impact. Proc Int Fertil Soc 601:1–26

Pandey C, Negi YK, Maheshwari DK, Rawat D, Prabha D (2018) Potential of native cold tolerant plant growth promoting bacilli to enhance nutrient use efficiency and yield of Amaranthus hypochondriacus. Plant and Soil 428:307–320

Pathak H, Mohanty S, Jain N, Bhatia A (2010) Nitrogen, phosphorus, and potassium budgets in Indian agriculture. Nutr Cycl Agroecosyst 86:287–299

Pehlivan N, Abullah Muhammed Yesilyurt AM, Durmus N, Karaoglu SA (2017) *Trichoderma lixii* ID11D seed biopriming mitigates dose dependent salt toxicity in maize. Acta Physiol Plant 39:79

Piri R, Moradi A, Balouchi H, Salehi A (2019) Improvement of cumin (*Cuminum cyminum*) seed performance under drought stress by seed coating and biopriming. Sci Hortic 257:108667

Prasad SR, Kamble UR, Sripathy KV, Bhaskar KU, Singh DP (2016) Seed bio-priming for biotic and abiotic stress management. In: Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 211–228

Priya P, Bisen K, Rakshit A, Singh HB (2018) Seedling bio-priming with Trichoderma spp. enhances nitrogen use efficiency in rice. In: Advances in seed priming. Springer, Singapore, pp 297–307

Rangjaroen C, Lumyong S, Sloan WT, Sungthong R (2019) Herbicide-tolerant endophytic bacteria of rice plants as the biopriming agents for fertility recovery and disease suppression of unhealthy rice seeds. BMC Plant Biol 19:580

Ray S (2011) Measuring energy intensity in selected manufacturing industries in India. J Energy Resour Technol 1:31–44

Ray S, Singh V, Singh S, Sarma BK, Singh HB (2016) Biochemical and histochemical analyses revealing endophytic *Alcaligenes faecalis* mediated suppression of oxidative stress in *Abelmoschus esculentus* challenged with *Sclerotium rolfsii*. Plant Physiol Biochem 109:430–441. https://doi.org/10.1016/j.plaphy.2016.10.019

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil 321:305–339

Rodrigues EP, Rodrigues LS, de Oliveira ALM (2008) *Azospirillum amazonense* inoculation: effects on growth, yield and N2 fixation of rice (*Oryza sativa* L.). Plant and Soil 302:249–261

Rudresha DL, Shivaprakasha MK, Prasad RD (2004) Effect of combined application of *Rhizobium*, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer arietinum* L.). Appl Soil Ecol 28:139–146

Saia S, Benítez E, García-Garrido JM, Settanni L, Amato G, Giambalvo D (2014) The effect of arbuscular mycorrhizal fungi on total plant nitrogen uptake and nitrogen recovery from soil organic material. J Agric Sci 152:370–378

Shridhar BS (2012) Review: nitrogen fixing microorganisms. Int J Microbiol Res 3:46–52

Tanwar A, Aggarwal A, Kaushish S, Chauhan S (2013) Interactive effect of AM fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on growth and yield of broccoli. Plant Protect Sci 49:137–145

Tewatia RK, Chanda TK (2017) Trends in fertilizer nitrogen production and consumption in India. Elsevier, Amsterdam, pp 45–56

Thomas J, Ajay D, Raj Kumar R, Mandal AKA (2010) Influence of beneficial microorganisms during in vivo acclimatization of in vitro-derived tea (*Camellia sinensis*) plants. Plant Cell Tiss Org 101:365–370

Yadav SK, Dave A, Sarkar A, Singh HB, Sarma BK (2013) Co-inoculated biopriming with *Trichoderma*, *Pseudomonas* and *Rhizobium* improves crop growth in *Cicer arietinum* and *Phaseolus vulgaris*. Int J Agric Environ Biotechnol 6:255–259

Yobo KS, Laing MD, Hunter CH (2009) Effects of single and dual applications of selected *Trichoderma* and *Bacillus* isolates on performance of dry bean seedlings grown in composted pine bark growth medium under Shadehouse conditions. J Plant Nutr 32:1271–1289

Zhao J, Liu J, Liang H, Huang J, Chen Z, Nie Y, Wang Y (2018) Manipulation of the rhizosphere microbial community through application of a new bio-organic fertilizer improves watermelon quality and health. PLoS One 13:2

Zhou F, Azofra LM, Ali M, Kar M, Simonov AN, McDonnell-Worth C, Sun C, Zhang X, MacFarlane DR (2017) Electro-synthesis of ammonia from nitrogen at ambient temperature and pressure in ionic liquids. Energ Environ Sci 10:2516–2520

Chapter 12
Overview of the Role of Nitrogen in Copper Pollution and Bioremediation Mediated by Plant–Microbe Interactions

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Abstract Nitrogen is an essential and incredibly versatile element for living organisms. In agriculture, nitrogen is a key element to understand soil and aquatic pollution, very often associated with heavy metal pollution. In this chapter, we overview the correlations between nitrogen and copper pollution, mediated by antimicrobial compounds used in agriculture. Plant Growth-Promoting Microorganisms (PGPM) are a heterogeneous group of microorganisms ranging from Bacteria (PGPBs) to Archaea and Fungi, which can modulate plant growth by conferring direct and indirect benefits, such as phytohormone production and stress alleviation. Many of these microorganisms are tolerant to high concentrations of Copper, increasing plant

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productivity even under metallic stress. We suggest the use of bacterial and fungal species associated with plant species for the bioremediation of degraded soil and water bodies, alarm about the bioaugmentation of copper through economically relevant plants, and review plant and microbial species applied to bioremediation.

Keywords PGPB · Bioremediation · Cu · N · Phytoremediation · Bordeaux mixture · Burgundy mixture · Viçosa mixture

12.1 Plant Growth-Promoting Bacteria

Nitrogen is a fundamental element for all living beings, for its participation in the constitution of amino acids, nucleotides, and other compounds essential to life. For this reason, it can limit plant growth as much as water availability (Malavolta 2006). Bacteria can improve the acquisition of nutrients through *biofertilization*, which means providing nutrients for plants through natural processes, rather than chemical fertilizers. The *biological nitrogen fixation* (BNF) is a biofertilization process better understood in comparison to others, like nutrient solubilization (Weselowski et al. 2016; Schütz et al. 2018; Przemieniecki et al. 2019).

The first discoveries of nitrogen-fixing bacteria were reported at the end of the nineteenth century by Beijerinck (1888), who explained that the BNF process is carried out by bacteria, which he named *Bacillus radicicola*. Later, the genus *Rhizobium* was proposed by Frank (1889) for nitrogen-fixing bacteria present in the nodules of leguminous roots. In Brazil, Johanna Döbereiner started her career developing research to elucidate the ability of native pastures to remain green without nitrogen supplementation and still succeed in sugarcane fields. In the late 1950s, Dr. Döbereiner isolated the diazotrophic bacteria *Azotobacter paspali* (Döbereiner 1966) and *Beijerinckia fluminensis* (Döbereiner and Ruschel 1958), starting a legacy of biofertilizers in South America.

Nitrogen-fixing bacteria (diazotrophs) can transform atmospheric nitrogen (N2), which is not assimilated by plants, into ammonia (NH3) through the nitrogenase enzyme complex, not only making this nutrient available to plants (Newton 2000) but also keeping the biogeochemical nitrogen cycle afloat. When living inside plant tissues, some types of bacteria establish a symbiotic association with leguminous plants forming nodules (Gupta et al. 2015) that facilitate nitrogen assimilation through BNF. However, the richest niche for bacteria is the plant-soil system, in which they can form complex communities and dynamic interactions with the rhizosphere—the region of the soil where microbe-mediated processes are more heavily influenced by the root system, considering the release of a wide variety of compounds that attract organisms through the roots of plants (Rab et al. 2016).

Bacteria associated with plants are classified according to the proximity and intimacy with the root system, as *free-living* (while not attracted to the root exudates); rhizospheric, living in the zone around the surface of the root (benefiting from

the released exudates); *epiphytic* or *associative* bacteria, colonizing the rhizoplane; or finally as *endophytic* bacteria, which colonize tissues internally without causing damage, being symbiotic with the host plant or not (Figueiredo et al. 2010; Souza et al. 2015).

Besides improving plant nutrient uptake, beneficial bacteria might also be able to protect the host against biotic and abiotic stresses. Thus, beneficial bacteria might, directly and indirectly, promote plant growth, thus named *plant growth-promoting bacteria* (PGPBs) (Jian et al. 2019). PGPB can provide growth-promoting benefits directly, by synthesizing *phytohormones* (auxins, cytokinins, and gibberellins), *siderophores*, the enzyme ACC deaminase (which reduces ethylene levels in plants); and solubilize minerals such as phosphorus and zinc, making those nutrients more readily available for plant growth. They can also perform ecosystemic services such as providing nitrate, oxidation of sulfur, and increased permeability of the roots. The indirect promotion of plant growth by PGPB occurs mainly by antagonism to pathogenic microorganisms, in which the competition for space and nutrients induces the production of antibiotics, bacteriocins, lytic enzymes, and hydrocyanic acid, which might also induce systemic resistance in the plant (Glick 2012; dos Santos Silva et al. 2019). In the next paragraphs, we will further describe these ecosystemic services while drawing the role of nitrogen in those processes.

Phosphorus (P) is the second most required element in plant nutrition, second only to nitrogen (Raij 1991), being fundamental for practically all plant metabolism, participating in energy transfers, photosynthesis, respiration, synthesis of nucleic acids, among others (Santoyo et al. 2016). Phosphate solubilizing bacteria solubilize inorganic forms of phosphate as a consequence of the release of organic acids into the medium, changing the pH and increasing the availability of phosphorus for the host plant (Silva 2019a). Nitrogen sources preferred for phosphate solubilization in bacteria seem to be related to the stage of BNF for which that microorganism is specialized. For example, Pallavi and Gupta (2013) found nitrate to be a more efficient source of N in vitro for phosphate solubilization in *Pseudomonas lurida*. However, the authors mentioned that different studies point to ammonia compounds as a preferred source, likely by ammonia oxidizers. This pattern was described earlier in fungi, where nitrate is preferred over ammonium (Reyes et al. 1999; Seshadri et al. 2004). It is also important to note that phosphate solubilization and other growth-promotion traits do not exist with the *intention* of promoting plant growth, but prioritize the microbe's metabolism, with "leftover" soluble phosphate in the environment, which is then available to the plant (Yandigeri et al. 2011). Other traits which have no clear function in the microorganism metabolism can also be treated as a competitive advantage over other microorganisms, rather than causality.

Similarly, bacteria synthesize siderophores, which are molecules with a high affinity for Fe+3, as well as membrane receptors capable of binding to the Fe–siderophore complex, thus facilitating the absorption of iron by microorganisms and plants, bringing the direct benefits of siderophores to the bacterial-mediated growth of plants (Saha et al. 2016). Although iron is the fourth most abundant element on Earth, in aerobic soils, iron is not easily assimilated by bacteria or plants (Galvão 2010). The enzymes nitrogenase, leghemoglobin, ferredoxin, and hydrogenase,

which are required for BNF, are all dependent on iron. Thus, nodulated legumes have an increased demand for iron compared to plants that do not form nodules. Even though iron is not essential to nodulation, some studies reviewed by Khan et al. (2017) show that siderophore-producing bacteria have a better performance of BNF in nodules than siderophore-mutants.

Another mechanism for the promotion of plant growth is *phytostimulation*, resulting from the production of phytohormones by microorganisms. These are chemical regulators that trigger specific actions in varied functions in the development of plants (Gupta et al. 2015). Indoleacetic acid (a type of auxin) regulates cell division and elongation of the roots providing greater absorption of water and nutrients from the soil for the plant (Taiz and Zeiger 2013) and it is known to be positively associated with higher N fixation, even though this is still poorly understood (Keyeo et al. 2011). Gibberellins determine important physiological changes in plants, interfere with flowering, sexual expression, senescence, abscission, germination, breaking dormancy, while cytokinins stimulate cell division (cytokinesis) and the development of lateral buds (Glick 2012). The effect of N on production and regulation of phytohormones is better described in algae (valued for biofuel production), where the addition of phytohormones (in those studies, not produced by PGPB) stabilizes algal growth under N starvation or depletion, even though these exogenous phytohormones could not protect the algae against reactive oxygen species (ROS) (Yu et al. 2018; Renuka et al. 2018; Chokshi et al. 2017), which is one of the functions provided by PGPB. Nonetheless, it is also worth noting that in agricultural settings, low-nitrogen water bodies are rare. A much more common situation is the occurrence of hypoxic, high-nutrient water bodies, caused by the irresponsible use of fertilizers. Macrophytes thrive in low-oxygen aquatic environments over aerated setups (Wang et al. 2020), nonetheless, the nutrient discharge destabilizes the growth of those plants making shallow eutrophic water bodies more susceptible to algal blooms (Bakker et al. 2010).

Biocontrol is considered an indirect plant promotion mechanism consisting of the negative interaction between a biocontrol agent and a phytopathogenic microorganism, with the inhibition of the latter (Lacava et al. 2018; Chenniappan et al. 2019). Iron competition, for example, inhibits the growth of potentially harmful microorganisms when PGPB has better advantages for iron assimilation (Pérez-Montaño et al. 2014). Similarly, the competition for nitrogen is an important regulator of success for microbial colonization.

Several studies bring context to the plant–microbe interactions in different stages of the nitrogen cycle. To bring a few examples, Zheng et al. (2014) described the dynamics of methane-oxidizing and ammonia-oxidizing bacteria in paddy rice fields, in which the methane-oxidizing dominate when both urea and methane are plenty, while ammonia-oxidizing bacteria prefer environments rich only in urea. Other studies describe bacterial dynamics under high nitrogen concentrations, and the effects of those microorganisms on anammox (*anaerobic ammonium oxidation*) (Ni et al., 2012) and nitrite (Pérez et al. 2014) through denitrification and ammonization (Kraft et al. 2014). Wastewater treatment plants have been used as a model for this type of study since such processes occur naturally in this

environment. This relates to the plant–microbe interest of this topic considering wetlands wastewater treatment, in which the depuration is mainly carried out by plant and microbial processes (Vymazal 2011).

Studies on PGPB concerning aquatic environments are still little explored if compared to terrestrial environments. Macrophytes are plants that indicate water quality and are used for environmental monitoring (Sood et al. 2012), and the growth of some species potential can be used to remove or minimize the incidence of excess nutrients or control eutrophication in water (Kraft et al. 2014), while others are sensitive to eutrophication (Bakker et al. 2010). *Acinetobacter calcoaceticus* was one of the first isolates from aquatic plants showing the potential to promote growth, doubling the weekly growth of *Lemna minor* (Suzuki et al. 2014). The ubiquitous occurrence of PGPB in the tissues of *L. minor* has also been reported by Yamakawa et al. (2018). A strain of *Aquitalea magnusonii* improved the growth of *L. minor* by approximately 40% (Ishizawa et al. 2020). Some recent studies have brought bacterial isolation from aquatic plants and the characterization of PGPB. Gilbert et al. (2018) isolated 47 endophytic bacteria from *L. minor* tissues and evaluated the production of indole compounds. Ishizawa et al. (2019) isolated and characterized 22 bacteria from the *Lemna gibba* in terms of promoting growth. Shehzadi et al. (2016) obtained 41 endophytic bacteria isolated from three aquatic plants, having found eight isolates associated with *Eichhornia crassipes*, 24 with *Typha domingensis,* and nine with *Pistia stratiotes* and evaluated the production of indole compounds and photosynthesis. Those studies have associated the promotion of plant growth with the improvement of physiological conditions of plants and favoring of the processes of remediation of soil and water contaminants.

12.2 Copper Resistance in Bacteria

Another characteristic of some PGPB species is the ability to tolerate a high concentration of toxic metals and still maintain their growth-promotion traits. These bacteria are good candidates for the bioremediation of contaminants of soil and water bodies through their mutual interactions with plants, which increases plant productivity (de Andrade et al. 2019). Copper (Cu), when in excess, is one of these toxic metals. As an element necessary for correct metabolism functioning, it is classified as an essential micronutrient for organisms (in concentrations below 2.0 mg.L1), however high concentrations of copper in the environment are toxic to microorganisms and plants, affecting the dynamics in the soil-microorganism-plant system (de Angeli et al. 2019), and causing stress and toxicity in plant roots, interfering with the absorption of iron and other nutrients, in addition to decreasing soil fertility (Lamichhane et al. 2018).

Copper resistance is an important natural selection mechanism, influencing the evolution dynamics of soil bacterial communities. Since the concentration of copper has increased in several bacterial habitats due to anthropogenic processes, it is possible to find resistant bacteria in these sites. This ability allows bacteria with

Table 12.1 Copper-based antimicrobial solutions used in agriculture

Mixture name	Chemical components	Developed in
Bordeaux	$CuSO_4$, $Ca(OH)_2$	France
Burgundy	$CuSO_4$, Na_2CO_3	France
Viçosa	$CuSO_4$, $Ca(OH)_2$, $MgSO_4$, $ZnSO_4$, H_3BO_3	Brazil

different degrees of sensitivity or resistance to be used as indicators of pollution (Penha-Lopes et al. 2011).

Lamichhane et al. (2018) review various studies in which resistant bacterial isolates and communities are selected in the environment due to the presence of anthropic copper sources such as pesticide use through *copper-based antimicrobial compounds* (CBAC), animal supplementation, industrial activities, and copper mining. A widely studied bacterial genus is *Cupriavidus*, which possesses several mechanisms of resistance to toxic metals, thriving in environments with high concentrations of copper. *Cupriavidus metallidurans* (isolated from freshwater) and *Cupriavidus gilardii* (isolated from soils and plants contaminated by toxic metals) are resistant to high concentrations of copper, presenting high complexity in their homeostasis mechanisms (Huang et al. 2019).

Different studies also describe the effects of copper contamination on microbial communities in the field. The use of the aforementioned CBAC, such as the Bordeaux mixture, its Brazilian derivative Viçosa mixture (in Portuguese *Calda Viçosa*), and the Burgundy mixture is the greatest source of copper contamination in agricultural soil (Lamichhane et al., 2018) (Table 12.1). Even though those mixtures were initially thought of as fungicides in vineyards, they have been largely applied in other cultivations, using different application methods (Fig. 12.1).

Copper and other metals might reach the microbial cell through a chemiosmotic gradient of the cytoplasmic membrane (passive transport) or hydrolysis of ATP (active transport). Resistance mechanisms are mainly based on maintaining the intracellular level of the metal ion well controlled. The uptake and efflux of ions by the microbial cell occurring through active transport by ATPases prevent the excess and accumulation of ions (Boechat et al. 2017). As other micronutrients, copper is used as a catalytic and structural cofactor of biochemical processes in the formation of metalloenzymes such as superoxide dismutase (antioxidant defense) and cytochrome C oxidase (electron transport chain), however in excess, copper induces the production of reactive oxygen species (ROS), causing damage to nucleic acids, lipids, and proteins (Avanzi et al. 2017).

Bacteria such as *Pseudomonas fluorescens* and *Pseudomonas putida* are examples of PGPB that can act as bioremediators in contaminated environments and promote the concomitant growth of plants in places contaminated by copper (Gutiérrez-Barranquero et al. 2013; Xing et al. 2020). That is possible through the selection of resistance mechanisms, which is a long term effect of Copper pollution, including the selection of genes such as *copA*, encoding for a P-type ATPase (De la Iglesia et al. 2010), the Cus efflux system (Li et al. 2014a) which pumps copper ions out of bacterial cells, plus unknown mechanisms, yet to be described (Xing et al. 2020).

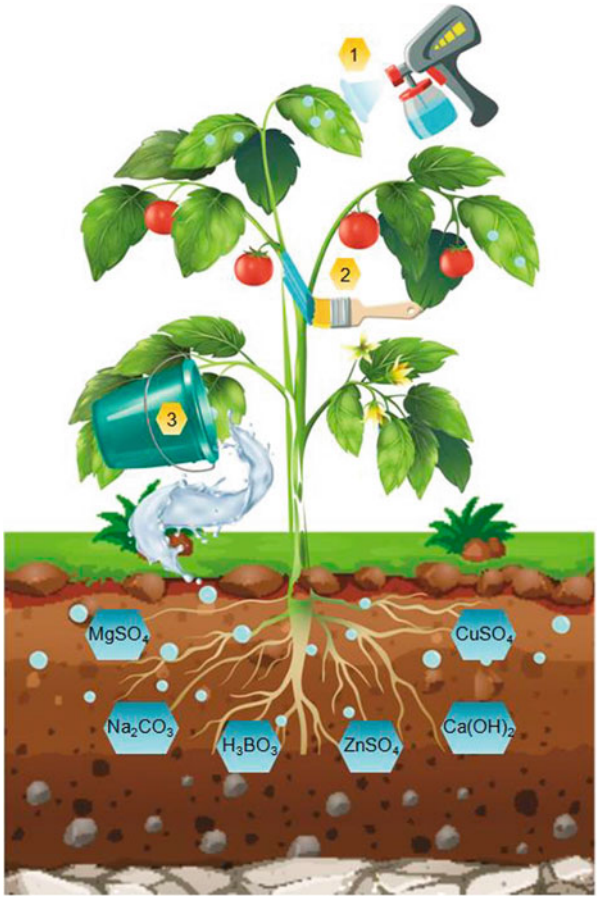

Fig. 12.1 Different methods for the application of copper-based antimicrobial compounds (CBACs) in agriculture. (1) with a hand mist sprayer for leaves, (2) with a brush for stem, or (3) with a bucket for root diseases, directly into the soil. In the soil, chemical compounds of any of the three CBACs are inserted in high concentrations

These homeostasis mechanisms work differently for Gram-positive and Gram-negative bacteria (e.g. *Escherichia coli*).

Basically, in Gram-positive bacteria, a cytoplasmic reductase reduces Cu2+ to Cu+, which can be absorbed by ATPase CopA (Fig. 12.2a). The excess copper binds to CopZ, and then is donated to the CopY, to stimulate transcription of the cop operon. In conditions of low copper content, CopY forms dimers and binds to zinc. When CopZ donates Cu + to CopY, each Zn2+ in each CopY monomer is replaced by two Cu + ions, with the concomitant release of CopY from the promoter allowing gene transcription to proceed. Copper efflux occurs through ATPase CopB. In Gram-negative bacteria, Cu2+ ions are exported to the periplasmic space through the porins and subsequently reduced by CueO to Cu+, which can be absorbed by CusS. The two-component CusRS system detects elevated intracellular Cu+ and finally activates the multi-component CusCFBA efflux system to expel the metal (Fig. 12.2b).

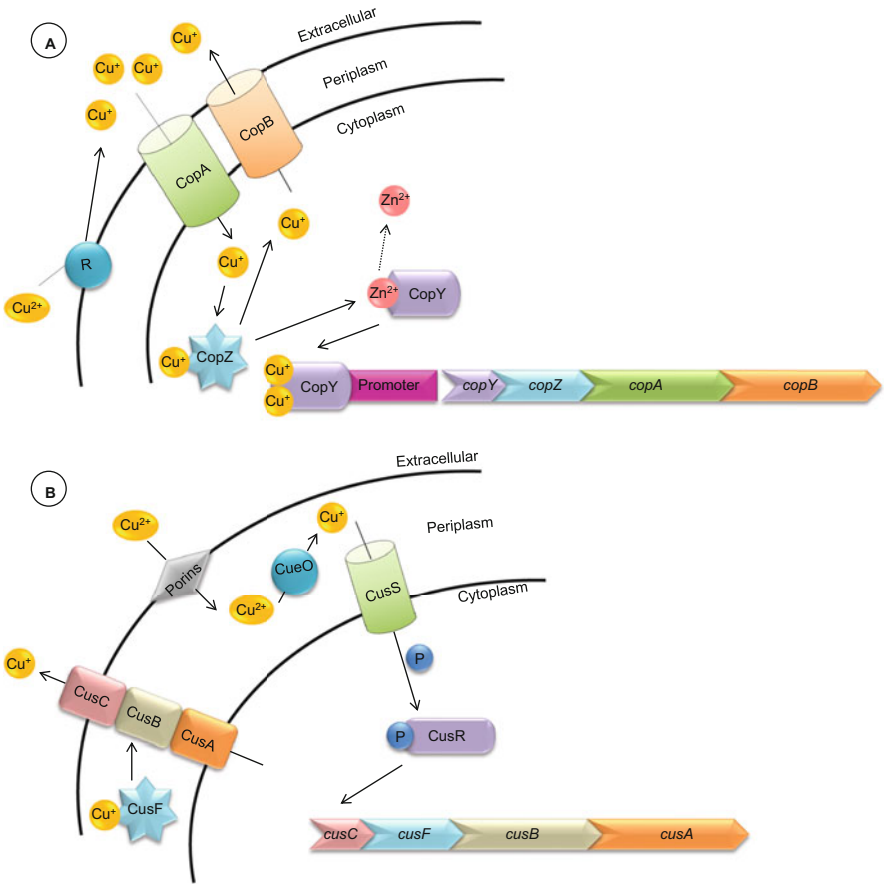

Fig. 12.2 Copper homeostasis model in (**a**) gram-positive bacteria (e.g. *Enterococcus hirae*) and (**b**) gram-negative bacteria (e.g. *Escherichia coli*). **A:** a cytoplasmic reductase (R, in blue) reduces Cu2 + to Cu +, which can be absorbed by ATPase CopA. The excess copper binds to CopZ and then is donated to the CopY, to stimulate transcription of the cop operon, shown in the lower part of the figure, in arrows with each color corresponding to the protein translated. In conditions of low copper content, CopY forms dimers and binds to zinc. When CopZ donates Cu + to CopY, each Zn2 + in each CopY monomer is replaced by two Cu + ions, with the concomitant release of CopY from the promoter allowing gene transcription to proceed. Copper efflux occurs through ATPase CopB. **B:** In Gram-negative bacteria, Cu2 + ions are exported to the periplasmic space through the porins and subsequently reduced by CueO to Cu +, which can be absorbed by CusS. The two-component CusRS system detects elevated intracellular Cu + and finally activates the multi-component CusCFBA efflux system to expel the metal

Copper pollution causes a consequential shifting of microbial communities composition favoring resistant species such as *Sphingomonas*, *Stenotrophomonas*, and *Arthrobacter* (Altimira et al. 2012). All of those genera have growth-promotion traits described in the literature (Berg et al. 2010; Aviles-Garcia et al. 2016; Khan et al. 2017).

12.3 Bioremediation of Copper and Promotion of Plant Growth

Pollution of soil and aquatic systems by copper is a factor that affects the quality of the environment and constitutes an imminent risk of poisoning to humans. Anthropic actions are responsible for adding 1.16 million tons of toxic metals per year to terrestrial and aquatic ecosystems worldwide (EPA 2007) including mining and mining waste areas, sewage sludge, pig slurry, industrial waste, and viniculture (Sharma et al. 2015; Srivastava et al. 2015) causing the availability of metals in the environment at high levels (Wu et al. 2016). In agriculture, the use of toxic metal-based antimicrobials for disease control in crops leads to the accumulation of metals in the soil (La Torre et al. 2018). For copper, those compounds, such as the Bordeaux and Burgundy mixtures are still widely applied, for their advantages such as relatively low toxicity to plants, low cost, and apparent low toxicity to mammals. It has led, however, to the widespread use of copper in the management of plant diseases since the last century (Lamichhane et al. 2018).

Considering the toxicity of copper to microorganisms, it is hypothesized that a high concentration of the metal would affect microbial metabolism, including the growth-promotion traits. Few studies, however, associate growth-promotion mechanisms and resistance to copper in bacteria. The sequestration of metals in bacteria and plants can be performed by different mechanisms, such as biosorption, biomineralization, bioassimilation, biosurfactants, and production of enzymes that act in the process of reducing or oxidizing the metal to less toxic forms (Andreazza et al. 2013). Forward, we will further analyze some of those properties, and how they relate to the biogeochemical cycle of nitrogen.

In general, copper in small amounts positively affects the nitrogen metabolism by increasing levels of ammonia and nitrate (Zhang et al. 2014), photosynthesis, respiration, and nutrient uptake (Laporte et al. 2020). On the other hand, higher concentrations of this metal induce modulation in enzymes related to nitrogen metabolisms, such as NADH-glutamate dehydrogenase, NAD-glutamate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase, to alleviate stress. Even though bacteria are also affected by copper stress (Ouyang et al. 2016), PGPB might help further reduce this stress when associated with plants (Li et al. 2014b).

The bioremediation via "biosorption" includes both *absorbing* (i.e. bringing into the tissues or cells) or *adsorbing* (immobilizing on the surface of tissues or cells), and can be performed both by plants and associative microbiota, including cases in which endophytes increased the plant tolerance to toxic metals (Shen et al. 2013). Nonetheless, it is worth noting that although in different levels for different plant species, toxic metal absorption is a common plant trait, and include species of economical relevance, such as tomato, maize, and cabbage (An et al. 2011), which highlights the importance of bioremediation in metal-polluted areas, considering the threat that metal accumulation in cultures pose to human and animal health. As for adsorption, plant-microbe-mediated remediation stands as a natural alternative to carbon nanotubes and graphene (Xu et al. 2018) and the biopolymer chitosan (Ngah

et al. 2011), both of which seek to adsorb pollutants on their surface, with posterior removal of carbon compounds with adsorbed metal from that environment.

Biomineralization is a common metabolism in many plant species, in which they create calcium oxalate, calcium carbonate, or silicon crystals in their tissues. Described functions for those crystals range from osmotic regulation to protection against herbivores, and also includes remediation of toxic metals. Even though the literature for copper biomineralization in plants is scarce, He et al. (2014) review studies describing the incorporation of this metal, and also aluminum, strontium, cadmium, and other metals into the plant's silicon crystals. The biomineralization of copper is better described, however, in microorganisms, including bacterial species *Rahnella* sp. (Zhao et al. 2019) and *Kocuria flava* (Kaur et al. 2015), and the fungus *Penicillium ochrochloron* (Crusberg 2013). Moreover, the effect of nitrogen sources in the biomineralization of copper is better described in fungi, as seen in Fomina et al. (2017), which described ammonia as a source of inorganic nitrogen that induced a seven-fold increase in copper immobilization in malachite crystals, in comparison to nitrate. Liu et al. (2019) describe the role of nitrogen in biomineralization through organic structures. According to his research, L-glutamic acid supplementation yielded larger copper carbonate particles and stabilized their structures in the early stages. Similarly, Zhang et al. (2014) described a stimulation in the activity of glutamine synthetase and glutamate synthetase in *Luffa cylindrica.* Table 12.2 shows these different examples of research for the remediation of copper.

Table 12.2 Examples of bioremediation of copper using plant and/or microbial species for the bioremediation of copper

Plant and/or microorganism	Site	Reference
Penicillium ochrochiloron	in vitro	Crusberg (2013)
Zea mays	Ta, China	An et al. (2011)
Lycopersicon esculentum		
Brassica chinensis		
Brassica oleracea		
Microbial community	Valparaiso, Chile	Altimira et al. (2012)
Zea mays, Pseudomonas sp. TLC 6-6.5-4	Michigan, USA	Li et al. (2014b)
Bidens pilosa Plantago lanceolata	Bento Gonçalves, Brazil	Andreazza et al. (2013)
Brachybacterium	India	Kaur et al. (2015)
Alcaligenes aquatilis		
Brevibacterium epidermidis		
Kocuria flava		
Brevibacterium epidermidis		
Pseudoalteromonas tetraodonis		
Microbial community	Hygum, Denmark	Nunes et al. (2016)
Aspergillus niger	in vitro	Fomina et al. (2017)
Rahnella sp. LRP3	Panshi, China	Zhao et al. (2019)
Neurospora crassa	in vitro	Liu et al. (2019)
Microbial community	Shijiazhuang, China	Xing et al. (2020)

The Cu-resistant species *Sinorhizobium melilot*, in association with *Medicago lupulina* and *Medicago sativa*, allowed the host plant to grow better and accumulate copper ions showing a decrease in Cu-induced growth inhibition and an increase in nitrogen concentration (Li et al. 2014b). The PGPB *Paenibacillus mucilaginosus* co-inoculated bacteria in Alfalfa showed a significant increase in nutrient content (N, P, and K) in plant tissues promoting plant growth in copper-contaminated soil (Ju et al. 2019). In similar studies Jian et al. (2019) showed that co-inoculation of the same bacteria resistant to copper and *Agrobacterium tumefaciens* increases the phytoextraction of Cu, increasing the antioxidant activities under stress by Cu in *Medicago lupulina*. In the work by Elias et al. (2018), bacterial isolates, capable of fixing nitrogen, isolated from *Brachiaria decumbens* grown in soil with high concentrations of metals with Pb, Co, and Zn showed resistance. Pérez-Portuondo et al. (2019), isolated bacteria from the rhizosphere of three herbaceous plants grown in soils contaminated with phenolic compounds and hydrocarbons, and verified the promotion of growth concerning phosphate solubilization, production of organic acids and indoles.

In the aquatic environment, *Aquitalea magnusonii* increased the promotion of growth when the plant was subjected to stresses of copper and zinc (Ishiwaza et al. 2020). In *Salvinia auriculata*, *Enterobacter* sp. was the most resistant PGPB to copper found in the work of Silva (2019b) presenting CIM of 9 mmol L-1 for copper and promoting plant growth. Irawati et al. (2017) isolated *Acinetobacter* spp. bacteria from industrial effluent and showed that the strains increased phytoremediation efficiency using *E. crassipes*.

12.4 Conclusions

PGPBs have several mechanisms for plant improvement and development, where microorganisms synthesize and stimulate plant growth through the production of plant growth regulators, BNF, phosphate solubilization, and the production of siderophores, in addition to promoting biological control for possible pathogens. Copper-resistant bacteria have mechanisms of resistance, to maintain levels of intracellular copper that do not interfere with their homeostasis and do not cause cellular damage, besides transforming available reactive copper to less toxic compounds. Bacteria have different copper influx and efflux mechanisms, controlled by different genes that are activated to maintain homeostasis. These genes may have been selected depending on the habitats of these bacteria for their survival. This premise can be used in the development of bioremediation strategies for the decontamination of environments contaminated by this metal. The potential for bioremediation of areas contaminated by copper using plant–microbe interactions is described in the literature. Nonetheless, few studies have evaluated the role of copper-resistant PGPB that can be used to establish new strategies for the removal of this metal from impacted environments, which may be a viable alternative for recovering the impacted environment using resistant and efficient organisms in

promoting plant growth. Considering this, the exploitation of PGPB with copper detoxifying characteristics, as well as several beneficial properties for plants, is a promising tool, competitive in terms of costs and bioremediation of metals, and favorable to the environment. It can have a positive effect on the biochemical responses of plants in soils or aquifers contaminated by copper and can provide an efficient strategy for the bioremediation of environments contaminated by this metal.

References

Altimira F, Yáñez C, Bravo G, González M, Rojas LA, Seeger M (2012) Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of Central Chile. BMC Microbiol 12:1–12

An L, Pan Y, Wang Z, Zhu C (2011) Heavy metal absorption status of five plant species in monoculture and intercropping. Plant Soil 345:237–245

Andreazza R, Camargo FADO, Antoniolli ZI, Quadro MS, Barcelos AA (2013) Biorremediação de áreas contaminadas com cobre. Rev Ciênc Agrárias 36:127–136

Avanzi IR, Gracioso LH, Baltazar MDPG, Karolski B, Perpetuo EA, do Nascimento CAO (2017) Rapid bacteria identification from environmental mining samples using MALDI-TOF MS analysis. Environ Sci Pollut Res 24:3717–3726

Aviles-Garcia ME, Flores-Cortez I, Hernández-Soberano C, Santoyo G, Valencia-Cantero E (2016) La rizobacteria promotora del crecimiento vegetal Arthrobacter agilis UMCV2 coloniza endofíticamente a Medicago truncatula. Rev Argent Microbiol 48:342–346

Bakker ES, Van Donk E, Declerck SAJ, Helmsing NR, Hidding B, Nolet BA (2010) Effect of macrophyte community composition and nutrient enrichment on plant biomass and algal blooms. Basic Appl Ecol 11:432–439

Beijerinck MW (1888) A bactéria do nódulo da raiz. Botanische Zeitung 46:725–804

Berg G, Egamberdieva D, Lugtenberg B, Hagemann M (2010) Symbiotic plant–microbe interactions: stress protection, plant growth promotion, and biocontrol by Stenotrophomonas. In: Symbioses and stress. Springer, Dordrecht, pp 445–460

Boechat CL, Giovanella P, Amorim MB, de Sá ELS, de Oliveira Camargo FA (2017) Metal-resistant rhizobacteria isolates improve Mucuna deeringiana phytoextraction capacity in multi-metal contaminated soils from a gold mining area. Environ Sci Pollut Res 24:3063–3073

Chenniappan C, Narayanasamy M, Daniel GM, Ramaraj GB, Ponnusamy P, Sekar J, Ramalingam PV (2019) Biocontrol efficiency of native plant growth promoting rhizobacteria against rhizome rot disease of turmeric. Biol Control 129:55–64

Chokshi K, Pancha I, Ghosh A, Mishra S (2017) Nitrogen starvation-induced cellular crosstalk of ROS-scavenging antioxidants and phytohormone enhanced the biofuel potential of green microalga Acutodesmus dimorphus. Biotechnol Biofuels 10:60

Crusberg TC (2013) Biomineralization of copper by a fungus revealed by SEM. Microsc Anal 18:11–13

de Andrade FM, de Assis Pereira T, Souza TP, Guimarães PHS, Martins AD, Schwan RF et al (2019) Beneficial effects of inoculation of growth-promoting bacteria in strawberry. Microbiol Res 223:120–128

de Angeli JTSK, de Oliveira Boina WL, Sigolo JB (2019) Disponibilidade de cobre em lodo de curtume: Potencial contaminante químico do solo. Colloquium Vitae 11(2):1–4

De la Iglesia R, Valenzuela-Heredia D, Pavissich JP, Freyhoffer S, Andrade S, Correa JA, González B (2010) Novel polymerase chain reaction primers for the specific detection of bacterial copper P-type ATPases gene sequences in environmental isolates and metagenomic DNA. Lett Appl Microbiol 50:552–562

Döbereiner J (1966) Azotobacter paspali sp. n., uma bactéria fixadora de nitrogênio na rizosfera de Paspalum. Pesq Agrop Brasileira 1:357–365

Döbereiner J, Ruschel AP (1958) Uma nova espécie de Beijerinckia. Rev Biol 1:261–272

dos Santos Silva C, de Araújo RGV, de Lima JRB, dos Santos TMC, da Silva Nascimento M, Montaldo Y, da Silva JM (2019) Resistence induction in Brassica oleracea var. acephala to xanthomonas campestris pv. Campestris and growth promotion by endophytic/bactéria. Brazilian J Dev 5:22401–22414

Elias, S., Baldani, V., & Berbara, R. (2018). Características morfológicas e resistência à metais pesados de bactérias diazotróficas isoladas de plantas de Brachiaria decumbens crescidas em solo contaminado. Embrapa Agrobiologia-Capítulo em livro técnico Infoteca-E

EPA, Environmental Protection Agency. (2007) Framework for metals risk assessment. EPA 1205/R-07/001. Office of Science advisor, risk assessment forum. http://www.epa.gov/osa/metalsframework

Figueiredo MDVB, Seldin L, de Araujo FF, Mariano RDLR (2010) Plant growth promoting rhizobacteria: fundamentals and applications. In: Plant growth and health promoting bacteria. Springer, Berlin, pp 21–43

Fomina M, Bowen AD, Charnock JM, Podgorsky VS, Gadd GM (2017) Biogeochemical spatio-temporal transformation of copper in Aspergillus niger colonies grown on malachite with different inorganic nitrogen sources. Environ Microbiol 19:1310–1321

Frank B (1889) Über die Pilzsymbiose der Leguminosen. In: Berichte Deutschen Botanischen Gesellschaft. Wiley, Hoboken, NJ

Galvão P (2010) Interação entre plantas e bactérias promotoras do crescimento vegetal. Embrapa Agrobiologia, Seropédica

Gilbert S, Xu J, Acosta K, Poulev A, Lebeis S, Lam E. (2018) Bacterial production of indole related compounds reveals their role in associationbetween duckweeds and endophytes. Frontiers in chemistry 6:265

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:1–15

Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V (2015) Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7:096–102

Gutiérrez-Barranquero JA, de Vicente A, Carrión VJ, Sundin GW, Cazorla FM (2013) Recruitment and rearrangement of three different genetic determinants into a conjugative plasmid increase copper resistance in Pseudomonas syringae. Appl Environ Microbiol 79:1028–1033

He H, Veneklaas EJ, Kuo J, Lambers H (2014) Physiological and ecological significance of biomineralization in plants. Trends Plant Sci 19:166–174

Huang N, Mao J, Zhao Y, Hu M, Wang X (2019) Multiple transcriptional mechanisms collectively mediate copper resistance in Cupriavidus gilardii CR3. Environ Sci Technol 53:4609–4618

Irawati W, Parhusip AJN, Sopiah N, Tnunay JA (2017) The role of heavy metals-resistant Bacteria Acinetobacter sp. in copper phytoremediation using Eichhornia crasippes [(Mart.) Solms]. KnE Life Sci 3:208–220

Ishizawa H, Kuroda M, Inoue K, Inoue D, Morikawa M, Ike M (2019) Colonization and competition dynamics of plant growth-promoting/inhibiting bacteria in the phytosphere of the duckweed lemna minor. Microb Ecol 77:440–450

Ishizawa H, Ogata Y, Hachiya Y, Tokura KI, Kuroda M, Inoue D, Ike M (2020) Enhanced biomass production and nutrient removal capacity of duckweed via two-step cultivation process with a plant growth-promoting bacterium, Acinetobacter calcoaceticus P23. Chemosphere 238:124682

Jian L, Bai X, Zhang H, Song X, Li Z (2019) Promotion of growth and metal accumulation of alfalfa by coinoculation with Sinorhizobium and agrobacterium under copper and zinc stress. PeerJ 7:e6875

Ju W, Liu L, Fang L, Cui Y, Duan C, Wu H (2019) Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. Ecotoxicol Environ Saf 167:218–226

Kaur H, Dolma K, Kaur N, Malhotra A, Kumar N, Dixit P, Choudhury AR (2015) Marine microbe as nano-factories for copper biomineralization. Biotechnol Bioprocess Eng 20:51–57

Keyeo F, Ai'shah ON, Amir HG (2011) The effects of nitrogen fixation activity and phytohormone production of diazotroph in promoting growth of rice seedlings. Biotechnology 10:267–273

Khan AL, Waqas M, Asaf S, Kamran M, Shahzad R, Bilal S, Al-Rawahi A (2017) Plant growth-promoting endophyte Sphingomonas sp. LK11 alleviates salinity stress in Solanum pimpinellifolium. Environ Exp Bot 133:58–69

Kraft B, Tegetmeyer HE, Sharma R, Klotz MG, Ferdelman TG, Hettich RL, Strous M (2014) The environmental controls that govern the end product of bacterial nitrate respiration. Science 345:676–679

La Torre A, Iovino V, Caradonia F (2018) Copper in plant protection: current situation and prospects. Phytopathol Mediterr 57:201–236

Lacava P, Melo I, Pereira J (2018) Controle biológico e simbiótico de insetos-pragas e doenças por micro-organismos endofíticos. Bioteconol Microb Ambiental 1:83–104

Lamichhane JR, Osdaghi E, Behlau F, Köhl J, Jones JB, Aubertot JN (2018) Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. Agron Sustain Dev 38:28

Laporte D, Rodríguez F, González A, Zúñiga A, Castro-Nallar E, Sáez CA, Moenne A (2020) Copper-induced concomitant increases in photosynthesis, respiration, and C, N and S assimilation revealed by transcriptomic analyses in Ulva compressa (Chlorophyta). BMC Plant Biol 20:1–16

Li K, Pidatala VR, Shaik R, Datta R, Ramakrishna W (2014a) Integrated metabolomic and proteomic approaches dissect the effect of metal-resistant bacteria on maize biomass and copper uptake. Environ Sci Technol 48:1184–1193

Li Z, Ma Z, Hao X, Rensing C, Wei G (2014b) Genes conferring copper resistance in Sinorhizobium meliloti CCNWSX0020 also promote the growth of Medicago lupulina in copper-contaminated soil. Appl Environ Microbiol 80:1961–1971

Liu F, Csetenyi L, Gadd GM (2019) Amino acid secretion influences the size and composition of copper carbonate nanoparticles synthesized by ureolytic fungi. Appl Microbiol Biotechnol 103:7217–7230

Malavolta E (2006) Manual de nutrição mineral de plantas. Agronômica Ceres, Sau Paulo, Brasil

Newton WE (2000) Nitrogen fixation in perspective. In: Nitrogen fixation: from molecules to crop productivity. Springer, Dordrecht, pp 3–8

Ngah WW, Teong LC, Hanafiah MM (2011) Adsorption of dyes and heavy metal ions by chitosan composites: a review. Carbohydr Polym 83:1446–1456

Ni SQ, Ni JY, Hu DL, Sung S (2012) Effect of organic matter on the performance of granular anammox process. Bioresour Technol 110:701–705

Nunes I, Jacquiod S, Brejnrod A, Holm PE, Johansen A, Brandt KK, Priemé A, Sørensen SJ (2016) Coping with copper: legacy effect of copper on potential activity of soil bacteria following a century of exposure. FEMS Microbiol Ecol 92:fiw175

Ouyang F, Zhai H, Ji M, Zhang H, Dong Z (2016) Physiological and transcriptional responses of nitrifying bacteria exposed to copper in activated sludge. J Hazard Mater 301:172–178

Pallavi KP, Gupta PC (2013) Effect of different carbon and nitrogen sources on solubilization of insoluble inorganic phosphate by psychrotolerant bacterial strains. Bioscan 8:1299–1302

Penha-Lopes G, Torres P, Cannicci S, Narciso L, Paula J (2011) Monitoring anthropogenic sewage pollution on mangrove creeks in southern Mozambique: a test of Palaemon concinnus Dana, 1852 (Palaemonidae) as a biological indicator. Environ Pollut 159:636–645

Pérez J, Lotti T, Kleerebezem R, Picioreanu C, van Loosdrecht MC (2014) Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: a model-based study. Water Res 66:208–218

Pérez-Montaño F, Alías-Villegas C, Bellogín RA, Del Cerro P, Espuny MR, Jiménez-Guerrero I, López-Baena FJ, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169:325–336

Pérez-Portuondo I, Meriño-Reyes L, Pérez-Silva RM, Abalos-Rodríguez A, Weyens N, Cuypers A (2019) Plantas herbáceas de ambientes contaminados como fuentes de bacterias degradadoras y promotoras del crecimiento vegetal. Cultivos Tropicales 40(2):e01

Przemieniecki SW, Kurowski TP, Kotlarz K, Krawczyk K, Damszel M, Pszczółkowska A, Kacprzak-Siuda K, Chareńska A, Mastalerz J (2019) Bacteria isolated from treated wastewater for biofertilization and crop protection against Fusarium spp pathogens. J Soil Sci Plant Nutr 19:1–11

Rab A, Khalil SK, Asim M, Mehmood N, Fayyaz H, Khan I, Zahid S, Nawaz H (2016) Response of sorghum (Sorghum bicolor L.) extract type, concentration and application time to weeds weight, grain and biomass yield of wheat. Pure and applied. Biology 5:1

Raij BV (1991) Fertilidade do solo e adubação (No. 631.42 R149f). Associaçao Brasileira para Pesquisa da Potassa e do Fosfato, Piracicaba, Brasil

Renuka N, Guldhe A, Singh P, Bux F (2018) Combined effect of exogenous phytohormones on biomass and lipid production in Acutodesmus obliquus under nitrogen limitation. Energy Convers Manag 168:522–528

Reyes I, Bernier L, Simard RR, Antoun H (1999) Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of Penicillium rugulosum and two UV-induced mutants. FEMS Microbiol Ecol 28:281–290

Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2016) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res 23:3984–3999

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99

Schütz L, Gattinger A, Meier M, Müller A, Boller T, Mäder P, Mathimaran N (2018) Improving crop yield and nutrient use efficiency via biofertilization—a global meta-analysis. Front Plant Sci 8:2204

Seshadri S, Ignacimuthu S, Lakshminarasimhan C (2004) Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different Aspergillus Niger strains. Chem Eng Commun 191:1043–1052

Sharma S, Singh B, Manchanda VK (2015) Phytoremediation: role of terrestrial plants and aquatic macrophytes in the remediation of radionuclides and heavy metal contaminated soil and water. Environ Sci Pollut Res 22:946–962

Shehzadi M, Fatima K, Imran A, Mirza MS, Khan QM, Afzal M (2016) Ecology of bacterial endophytes associated with wetland plants growing in textile effluent for pollutant-degradation and plant growth-promotion potentials. Plant Biosyst 150:1261–1270

Shen M, Liu L, Li DW, Zhou WN, Zhou ZP, Zhang CF et al (2013) The effect of endophytic Peyronellaea from heavy metal-contaminated and uncontaminated sites on maize growth, heavy metal absorption and accumulation. Fungal Ecol 6:539–545

Silva, J. M. M. D. (2019a) Bactérias solubilizadoras de fosfato inorgânico em solo preservado e antropizado da reserva biológica de Pedra Talhada-AL Bachelor's thesis, Brasil

Silva, Jussara Tamires De Souza. (2019b). Promoção do crescimento de Salvinia auriculata aublet mediada por Enterobacter sp. Sob Estresse Por Cobre. Dissertação mestrado. Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro

Sood A, Uniyal PL, Prasanna R, Ahluwalia AS (2012) Phytoremediation potential of aquatic macrophyte, Azolla. Ambio 41:122–137

Souza RD, Ambrosini A, Passaglia LM (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38:401–419

Srivastava S, Agrawal SB, Mondal MK (2015) A review on progress of heavy metal removal using adsorbents of microbial and plant origin. Environ Sci Pollut Res 22:15386–15415

Suzuki W, Sugawara M, Miwa K, Morikawa M (2014) Plant growth-promoting bacterium Acinetobacter calcoaceticus P23 increases the chlorophyll content of the monocot Lemna minor (duckweed) and the dicot Lactuca sativa (lettuce). J Biosci Bioeng 118:41–44

Taiz L, Zeiger E (2013) Fisiologia vegetal, 5th edn. Editora Artmed, Porto Alegre

Vymazal J (2011) Constructed wetlands for wastewater treatment: five decades of experience. Environ Sci Technol 45:61–69

Wang S, Liu Y, Li P, Wang Y, Yang J, Zhang W (2020) Micro-nanobubble aeration promotes senescence of submerged macrophytes with low total antioxidant capacity in urban landscape water. Environmental science: water research. Technology 6:523–531

Weselowski B, Nathoo N, Eastman AW, Mac Donald J, Yuan ZC (2016) Isolation, identification and characterization of Paenibacillus polymyxa CR1 with potentials for biopesticide, biofertilization biomass degradation and biofuel production. BMC Microbiol 16:1–10

Wu X, Cobbina SJ, Mao G, Xu H, Zhang Z, Yang L (2016) A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ Sci Pollut Res 23:8244–8259

Xing C, Chen J, Zheng X, Chen L, Chen M, Wang L, Li X (2020) Functional metagenomic exploration identifies novel prokaryotic copper resistance genes from the soil microbiome. Metallomics 12:387–395

Xu J, Cao Z, Zhang Y, Yuan Z, Lou Z, Xu X, Wang X (2018) A review of functionalized carbon nanotubes and graphene for heavy metal adsorption from water: preparation, application, and mechanism. Chemosphere 195:351–364

Yamakawa Y, Jog R, Morikawa M (2018) Effects of co-inoculation of two different plant growth-promoting bacteria on duckweed. Plant Growth Regul 86:287–296

Yandigeri MS, Meena KK, Srinivasan R, Pabbi S (2011) Effect of mineral phosphate solubilization on biological nitrogen fixation by diazotrophic cyanobacteria. Indian J Microbiol 51:48–53

Yu Z, Pei H, Jiang L, Hou Q, Nie C, Zhang L (2018) Phytohormone addition coupled with nitrogen depletion almost tripled the lipid productivities in two algae. Bioresour Technol 247:904–914

Zhang LL, He XJ, Chen M, An RD, An XL, Li J (2014) Responses of nitrogen metabolism to copper stress in Luffa cylindrica roots. J Soil Sci Plant Nutr 14:616–624

Zhao X, Do H, Zhou Y, Li Z, Zhang X, Zhao S, Wu D (2019) Rahnella sp. LRP3 induces phosphate precipitation of cu (II) and its role in copper-contaminated soil remediation. J Hazard Mater 368:133–140

Zheng Y, Huang R, Wang BZ, Bodelier PLE, Jia ZJ (2014) Competitive interactions between methane-and ammonia-oxidizing bacteria modulate carbon and nitrogen cycling in paddy soil. Biogeosciences 11:3353–3368

Part IV
Metagenomics

Chapter 13
Metagenomics for Improving Soil Fertility

Kalaivani Nadarajah

Abstract Soil is one of the most complex habitats with billions of fungi, bacteria, and other macro and microfauna. These microorganisms have their niche functions from nutrient cycling to stress management and as disease causing agent. Soil dynamics is constantly changing due to the changes above and below ground. The current practice of agriculture, which is intensive and is largely dependent on chemicals results in detrimental effects to soils biology as well as to its physico-chemical properties. These changes that are incurred by the agricultural practices on the soil structure and ecosystem are still poorly understood. Thus far, studies have shown that the soil microbial communities influence the nutrient cycles and the plant–soil interactions in most soil systems. Depending on the source of nutrients and controls provided to the agricultural system, the soil microbial community can thrive (organic, biofertilizers, biopesticides) or be inhibited (agrochemical fertilizers and pest controls). Therefore, the direction of today's agriculture is towards sustainability in which the soil ecosystems have to be maintained in a healthy and fertile manner through conditions that encourage the soil microbiota to thrive. Thus, the present book chapter highlights the importance of metagenomics tool to characterize microbial diversity and maintain the soil fertility.

Keywords Soil fertility · Microorganisms · Soil health · Metagenome

13.1 Introduction

Soil is one of the most complex habitats with billions of fungi, bacteria, and other macro and microfauna. These microorganisms have their niche functions from nutrient cycling to stress management and as disease causing agent (Mercado-Blanco et al. 2018; Shinwari et al. 2019). Soil dynamics is constantly changing

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due to the changes above and below ground. Human activity such as agriculture has had its effect on soil health and fertility. The current practice of agriculture, which is intensive and is largely dependent on chemicals results in detrimental effects to soils biology as well as to its physico-chemical properties. These changes that are incurred by the agricultural practices on the soil structure and ecosystem are still poorly understood. Thus far, studies have shown that the soil microbial communities influence the nutrient cycles and the plant–soil interactions in most soil systems (Bhowmik et al. 2017; Castellano-Hinojosa and Strauss 2020.). Depending on the source of nutrients and controls provided to the agricultural system, the soil microbial community can thrive (organic, biofertilizers, biopesticides) or be inhibited (agrochemical fertilizers and pest controls). Therefore, the direction of today's agriculture is towards sustainability in which the soil ecosystems have to be maintained in a healthy and fertile manner through conditions that encourage the soil microbiota to thrive (Bargaz et al. 2018; Schmidt et al. 2019).

The soil microbial communities are involved in many key functions that include nutrient cycling, and sustaining plant growth. These functions can be threatened by poor agricultural practices and climate change. Some key cycles that are crucial in maintaining soil well-being are the C and N cycles that can be easily affected by soil degradation and climate change (Amundson et al. 2015). However, the knowledge of ecosystem microbiomes is important in the manipulation of soil health and fertility (Calderon et al. 2017). By understanding how the soil microbiomes work we will be better equipped to manage bioprospecting and ecosystems services. However, exploring and managing the information derived from the soil microbiome remain a daunting task, as a large number of these microorganisms may not have been identified and therefore resulting in their characteristics, and function being cryptic.

In this chapter we will focus on the main issue soil microbiologists face, which is understanding the soil energy cycles that are fueled by soil microbes. Soil microorganisms together with the plants determine the below ground cycles and control the gasses released into the environment as well as the exudates released into the soil (Crowther et al. 2016). The microbial populations and the environment largely influence these cycles. The microbial communities in the soil are highly diverse and are subject to change from any disturbances to the chemistry and physical structure of the soil (Sollins et al. 1996). The heterogeneous soil environment opens up opportunities for us to identify the major microbial networks within the soil and to advance soil microbiome research to provide opportunities to commandeer metabolic interactions in the soil and for prospecting of organisms that may be utilized in industries in producing novel byproducts such as antibiotics, and biofuels and for use in the agricultural industry (Ling et al. 2015). From the various studies that have been conducted on soil microbial content, it has been reported that the rhizospheric region of the soil, especially is influenced by root exudates, which recruit microbes in the plant–microbe interaction. With the advent of high-throughput technologies it will make it a lot easier for these gross communities to be studied at a scientific level. These technologies will enable us to know what are the communities in the soil and to determine their functions in the soil in order to

understand the species and interspecies interactions. So here is where metagenome studies will be crucial in unlocking the wealth of information that is in the soil.

13.2 Importance of Studying Soil Microbiome

The global population will increase in the next few decades leading to an increase in demand for food. FAO has predicted that by the year 2050 that the population will be about 10 billion in size and our food demands will be up by 70%. Therefore, there is a need to move agriculture in the direction of sustainable agriculture with diminishing resources and the exasperating effects of climate change (Tian et al. 2016; Sergaki et al. 2018). The current anthropogenic activities influence the environment and the outcome of sustainable agriculture (Malla et al. 2018). In order to keep up with the demands for food it is important for us to adopt intensified and sustainable agriculture to produce more food with the limitations on resources and climate change (Chowdhary et al. 2018). Climate change has its impact on the macro and micropopulation (Sergaki et al. 2018). Climatic changes such as temperature fluctuations, flooding, and drought significantly affect the functional activity in the plant-soil-microbe equation (Bojko and Kabala 2017). These climatic changes result in variation in community structures, soil community taxa, relative abundance and the overall microbial density in response to the environment (Hashem et al. 2018). It has been postulated that the changes to soils environments such as temperature, oxygen, and water content affect the microbial population just as much as the interaction between the root exudates and the soil environment. These factors play a crucial role in shaping the microbial communities below ground, where these change to the ecosystems will result in structural and compositional changes that affects the soil health and fertility (Schimel and Schaeffer 2012). It is therefore important for us to acknowledge that soil microbial communities are important, especially in the agricultural front and therefore studies into the soil population dynamics need to be intensified to support the sustainable intensification of agriculture (Hameed et al. 2014).

13.3 The Difficulty in Studying Soil Microorganisms

Soil heterogeneity poses a challenge for microbiologist to study the community and microbial composition. Further, the soil chemical and physical structure varies in formation (Girvan et al. 2003). Beyond the variations observed in the soil matrix, the soil microbial communities vary based on environmental changes such as nutrient content, moisture, and temperature (Nunan et al. 2002). Therefore, it is hard for us to make comparisons between reports made by various researchers as the method of sampling used may vary giving very different representation of the soils population dynamics. We have to be aware that the microbial communities may be nested in

various levels or communities depending on soil properties, depth, and sampling techniques, among other parameters. This results in microsites that provide variation in data and results in gross misrepresentation of information when we neglect to take these variations into account when studying the soil dynamics. Hence it is important for us to determine the sampling technique and sampling size as both these parameters affect the identification of microbial diversity and function. Over the years various methods have been employed but in the late 1990s Klironomos suggested a combination of techniques, which would provide a better representation of the microbial population by reducing the error in sampling regime (Klironomos et al. 1999; Fujisawa et al. 2015).

Obstacles towards studying the soil microbial population are due to the inability to culture and study all these organisms. It has been postulated through mathematical predictions that the soil contains about 10^7 prokaryotic cells in a gram of soil (Gans et al. 2005). This of course does not take into account the several unculturability microorganisms, which cannot be studied due to the lack of understanding on their physiology and cultivable needs. Further, these inabilities to culture these organisms may also be a result in phenotypic and genetical differences that make these organisms different from the 1% population that is easily cultivated in the laboratory and may most likely be the minority of soil population as the unknown may form the larger population (Stewart 2012). The mathematical modeling to determine the population diversity in the soil is largely bias and a large number of soil samples will be required to provide sufficient information of the estimates (Curtis and Sloan 2005; Lombard et al. 2011).

As one major issue lies in the identification of non-culturable organisms, therefore methods that are culture-independent such as DNA- and RNA-based techniques have become a commonplace (Kowalchuk et al. 2004; Jacoby et al. 2017). Identification methods using 16S, 18S, and ITS regions of organisms have contributed significantly in identification of prokaryotes and eukaryotes and have defined organisms to taxonomic groups based on sequence information. These techniques have gone through vigorous modifications yielding towards metagenomics, which allows for the understanding of the microbiome in a holistic perspective. This technique has been extensively reviewed by Rincon-Florez et al. (2016) who included in their scope of information in sampling, purification, separation, and sequencing, and finally data analysis and interpretation techniques. In the past decade itself the sequencing technology has been evolving at a very rapid rate. The changes in the platforms have enabled a more in-depth view of metagenomics through the high-throughput sequencing (HTS) or next-generation sequencing (NGS). HTS would include platforms such as 454 Genome Sequencer, HiSeq, MiSeq, PacBio, and the AB SOLiD (Rincon-Florez et al. 2016; Yergeau et al. 2014). By utilizing these platforms it would be easier and achievable to identify the functional versatility, diversity of the microbiome, and diversity based on niches through the study of metagenomics and metatranscriptomics (Escobar-Zepeda et al. 2015; Oulas et al. 2015).

13.4 Microbes in Soil Health and Fertility

In the recent years, soil health has become an important area to study as any disturbances to surface and internal soil profile and content. One important component of soil health is the microbial biodiversity in the soil. In recent years the successful application of microbes to the soil has become crucial in maintaining soil health and fertility. In addition microbial content has been implicated in improving water-holding capacity, carbon storage, improved root growth, nutrient cycling, bioremediation, and conservation of soil biodiversity (Keesstra et al. 2012; Lal 2016; Jacoby et al. 2017). Many research groups have focused their studies on soil beneficial microbes that can contribute towards improved yield, biotic, and abiotic stress management. These beneficial microbes are able to provide long-term solutions in the area of improved growth, disease management, and also in the management of abiotic stresses (Bargaz et al. 2018).

The soil microbes form the organic component of the soil where it is involved in supplying nutrients and protection against biotic and abiotic stresses. However, our agricultural practices have resulted in the depletion of microbes in the soil through the extensive use of agro-chemicals (Amundson et al. 2015). These chemicals cause soil degradation, eutrophication, and greenhouse gas emissions (Steffen et al. 2015). This therefore requires an alternative that is not detrimental to soil health and fertility and reduces the reliance on agrochemical inputs (Foley et al. 2011). Therefore, we need microbes that are beneficial that not only improve and protect the plants but will also protect the environment against the detrimental effects of toxic compounds in the soil from our practices. These microbes also assist in the utilization of specific nutrients from the soil by the plants indicating a crucial role played by microbes in plants' well-being (Ahmad et al. 2018; Vyas et al. 2018).

Factors contributing to better soil health and well-being such as agro-ecological practices have brought about information on factors that increase the soil biodiversity. Research has been conducted to obtain information of soil microbiomes that have been identified in different agricultural practices where sequence data and pipelines were developed according to these needs (Hou et al. 2018; Dias and Antunes 2014). Supplementing the soil ecosystem with microbes that enrich the soil and benefit the plants has become an accepted remediation to our current agricultural practices. Enriching soil microbial communities must take into consideration the presence, abundance, and diversity of microorganisms and their proposed contribution to soil health and fertility and suitability as bioindicators (Lu et al. 2014). Various groups such as bacteria, fungi, and AM fungi play a crucial role in the nutrient management of plant–soil interactions (Cavagnaro et al. 2015; Kumar et al. 2015). These organisms are responsible for the management of energy and nutrient acquisition and utilization by plants. Their role in sustainable agricultural practices is crucial in ensuring better yields and protection from stresses. This would require a concerted effort from multidiscipline such as ecology, agronomy, soil science, genetics, economics, and social sciences (Kumar et al. 2016).

13.5 Emerging Technologies in Microbial Population Studies

There are various molecular techniques that have been used previously to identify the soil microbial diversity and structure. These include techniques such as Phospholipid Fatty Acid Analysis (PLFA), Terminal Restriction Fragment Length Polymorphism (TRFLP), Amplified Ribosomal DNA Restriction Analysis (ARISA), amplified rDNA restriction analysis (ARDRA), single strand conformation polymorphism (SSCP), denaturing/temperature gradient gel electrophoresis (DGGE/TGGE), Random Amplification of Polymorphic DNA (RAPD), Amplification Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), and various other genomic and postgenomic techniques (Nadarajah and Kumar 2019). While these techniques have provided some understanding of the microbial composition in any studied sample, it does not provide a very good representation of the shifts in the microbial population relative to changes in environment (Nemergut et al. 2013). With the advent of genomics, transcriptomics, proteomics, and metabolomics, researchers are able to understand the diversity and functional shift of the microorganisms in the soil. All this has been made possible through the high-throughput sequencing platforms that are available in the past decades (Hugerth and Andersson 2017; Malla et al. 2018). With more techniques becoming available, the researcher has to be selective of the method that best serves his or her research needs. The most commonly used technique to date to identify taxonomic groups in the soil is the amplicon sequencing technique that enables the characterization of the bacterial composition within a given ecosystem (Malla et al. 2018). While the amplicon sequencing technique provides a large amount of information, this technique provides no information on the functionality of the species or communities (Gupta et al. 2019). This shortfall in the amplicon strategy has resulted in the application of the shotgun metagenomics platform to better understand the composition, diversity and functionality of the given communities. While the shotgun sequencing technique provides the functional potential of the communities but it lacks the depth associated with amplicon sequencing (Zhou et al. 2015). While we choose which technology to use what seem most important and relevant to date is the sampling technique used to ensure that we get the best representation of the microbes within the soil aggregates studied (Lombard et al. 2011). In addition to what platforms to use to understand the microbial communities, it would be important for large datasets to be generated to ensure that a good representation of the site of study is obtained (Zhou et al. 2015). To support the sequencing technologies that have been developed several bioinformatics pipelines have been established to assist with the analyses of the large datasets that have been obtained. Pipelines such as MEGAN, MG-RAST, QIIME, and MOTHUR have been utilized to analyze the datasets in various organisms (Oulas et al. 2015; Plummer et al. 2015; Barthi and Grimm 2019; Gardner et al. 2019).

13.6 Metagenomics as a Means to Unlock the Unknown

As mentioned in earlier segments, soil microbes are able to contribute towards improved agricultural practices (Bender et al. 2016). Therefore it is important to build up microbial associations that are able to maximize benefits and environmental restoration in meeting global climate change challenges (Lau et al. 2017). In any ecosystem microbial communities contribute towards the energy, nutrient, and the gaseous emissions (Deemer et al. 2016). These microbial communities have been traditionally divided into decomposers, pathogens, and symbionts (Baldrian 2017). The details of these communities and the synergism between them are obscure and require further study. The metagenomic tool is a potent and influential approach towards revealing the evolutionary, ecological, and influential role of these organisms in a given ecosystem. Through the utilization of metagenomics we are able to obtain longitudinal and cross-sectional information on the microbial population which allows us a deeper exploration of the microbial frontiers to tap the untapped microbial population and provide substantial insight on diversity and genes and functionalities that will provide opportunities for advancement in various industries such as agriculture, industrial microbiology, and medicine (Sangwan et al. 2016; Shakya et al. 2019).

To begin the exploration of the soil microbiota it will require the extraction of the whole microbial DNA from the soil. This collective extraction of the DNA from the soil sample is referred to as the metagenome (Daniel, 2005). The concept of metagenomics involves cloning and analyzing of the microbial DNA from the environment. The stages of preparation involved in metagenome analyses are: (1) the isolation of the DNA, (2) cloning DNA fragments into vector, (3) transformation into suitable host cells, (4) establishment of metagenome library, and (5) screening of the established library. The most critical step in the establishment of the library is obtaining sufficient high quality DNA that represents the soil microbial community (Daniel 2005; Lam et al. 2015). The amount of DNA and the size of DNA obtained from a particular soil sample will depend on the physiological status of the microbial population. In an environment that is under stress where the microbes have been forced into a state of dormancy, the size and amount of DNA will be smaller in comparison to the DNA extracted from an environment where the cells are active. In such circumstances where the amount of DNA is little which may lead to certain bacterial taxa being overlooked in the analyses, it is suggested that an enrichment technique is employed to ensure an equitable coverage of the microbial population. There are kits that are available for this purpose and may be used with great success. For further details on soil DNA extraction strategies and enrichment processes, several kits and methods have been compared and information provided in detail through manuals and articles (Haruta and Kanno 2015; Emerson et al. 2017).

Once the DNA is obtained the next step would be to construct the soil libraries. This would require the cloning of the extracted DNA into suitable vectors. The choice of vector is strongly dependent on the objective of the experiment. For

instance, if the DNA is being used for PCR, then the DNA yield is more critical than the vector, but if the sample is to be deposited in the gene bank, then the selection of vector is crucial as you would have to select vectors that can take large DNA fragments to reduce the number of clones needed to provide complete coverage. Generally, most vectors are cloned into *Escherichia coli* host cells for construction of a metagenome library. When larger fragments are involved vectors such as cosmid, fosmid (for less than 40 kb), and BAC (more than 40 kb) have been utilized. In certain studies, where the soil samples were surveyed for bioactive compounds, other host strains have been utilized such as *Rhizobium leguminosarum*, *Streptomyces lividans*, and *Pseudomonas aeruginosa* (Singh et al. 2009).

Following the library construction, the clones will be screen based on the sequence information and the functional annotations. The sequence annotation will only provide information at the nucleotide level while the functional annotation will provide information that may be utilized to fish for genes of interest related to the aim of our study (Fierer et al. 2007). However, there have been issues related to identifying genes in *E. coli* host system. This has resulted in researchers looking for alternative host systems that will provide for better gene identification. Some examples of these systems are METREX and SIGEX, which are novel strains that were developed to improve high-throughput screenings in metagenomics (Yun and Ryu 2005; van der Helm et al. 2018). Sequencing of clones and indirect evaluation of shotgun sequencing will provide with large datasets that will facilitate the determination of microbial diversity (Kunin et al. 2008; Manichanh et al. 2008). The metagenome libraries can also be analyzed with the microarray technology to provide information of the composition and the complexity of the microbial communities. However, this technique has been shown to be less sensitive then the PCR based detection strategy and is rather costly (Sebat et al. 2003; Zhou and Thompson 2002). While we have technologies that are being constantly updated to provide access to enormous amounts of microbial sequence data, these technologies are still not able to cope with complex soil communities as these techniques still do not provide an in-depth coverage of the microbial communities within (Escobar-Zepeda et al. 2015; Nayfach and Pollard 2016; Malla et al. 2018).

Therefore, the greatest challenge in metagenome work is the number of libraries generated to cover the site of study and the economic cost of the procedure. The more complex the environment, the more samples are required that covers greater width and depth of the site studied (Thomas et al. 2012; Singer et al. 2019). Compared to other genome studies such as human, animals, and isolated organisms, the soil microbiota still remains rudimentary and constitutes a huge and ambitious challenge. Further, with the enormous amount of information obtained from the various genome sequencing projects worldwide, it is important that this information is shared publicly and among scientific communities. To ensure that the quality of information that is being fed into these online resources meet the standards, the open access information through international working bodies have established the Genomic Standards Consortium (GSC) (http://gensc.org/gc_wiki/index.php/GSC) that defines the international "minimum information about a genome sequence" (MIGS) and the "minimum information about metagenome sequence" (MIMS)

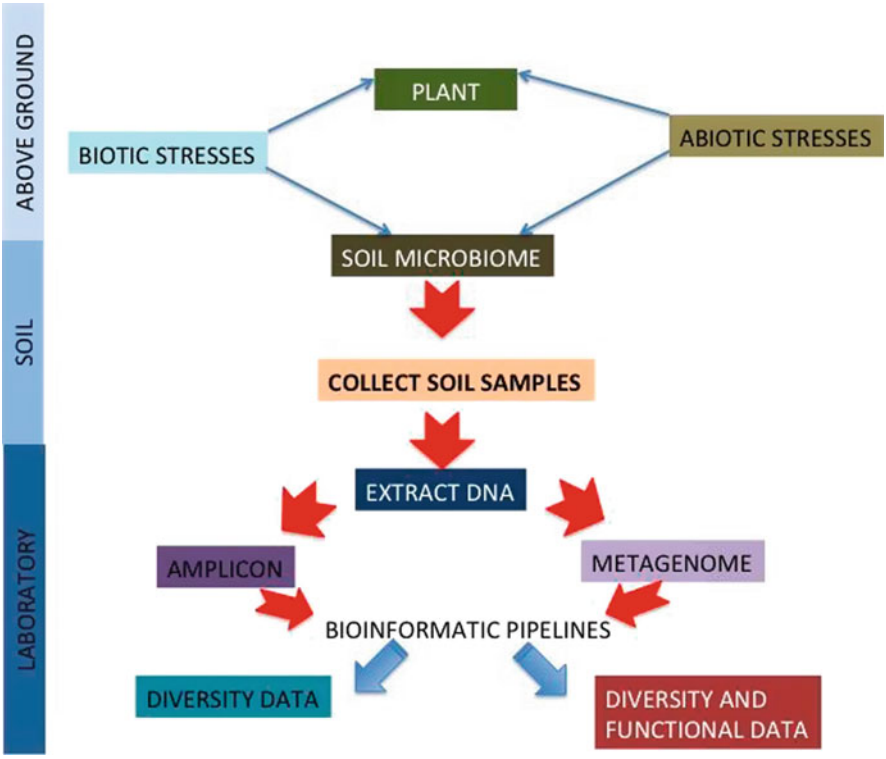

Fig. 13.1 Biotic and abiotic stresses influence the plant and the soil environment. The effect on the plant and the soil environment affects the soil microbiome. Depending on the aim of the study and the hyphothesized outcome of the research, sampling methods may be optimized to obtain best representation of the site of study. The soil is then subjected to DNA extraction of the soil microbiome. This may be utilized in amplicon sequencing technique to identify the community or we may utilize the metagenome techniques which provide both the diversity and functional data. It is advisable to utilize both methods to obtain better representation of the soil microbial diversity and functionality

specifications to ensure that this promotes quality, discussion, and good capture of metadata for use by the scientific communities (Field et al. 2008; Kettner et al. 2010). Figure 13.1 provides a simplistic overview of the steps in a metagenome analysis of soil samples.

13.7 Soil Metagenome Updates

Over the years the utilization of shotgun sequencing technique has allowed for the sequencing, assembly, and annotation of various genomes. There are currently over 1000 microbial genomes available in various databases such as NCBI (https://www.

ncbi.nlm.nih.gov), GOS (http://www.jcvi.org/cms/research/projects/gos), and GEBA (http://www.jgi.doe. gov/programs/GEBA). While the sequencing project has been made easier with the constantly improving sequencing platforms, but the one critical challenge lies in the assembly of the sequences into scaffolds. This is made harder when the organisms are heterokaryons (Holst-Jensen et al. 2016; Land et al. 2015; Reuter et al. 2015). Vogel et al. (2009) presented the TerraGenome, which elucidates soil microbial dynamic interpretation, and discusses the advantages and risk of using this technique in identifying new organisms with the potential to produce new useful molecules. The depth of analysis and the complexity of the soil environment can result in a poor knowledge of the environment, biases, spatial variation, and questionable interpretation of data from soil.

As soil microbiologist, we have to understand that although we may set out to identify a specific group of organisms in any given soil sample, for instance, if we were to set out to identify anti-phytopathogenic microorganisms, we may not end up isolating a large number of antimicrobial producing microorganism (van Elsas et al. 2008; Stewart 2012). This is in line with previous reports that state that it is difficult to obtain high expression levels of target which is hampered by sampling technique, soil environment, number of samples, and storage of sample (Chung et al. 2008). The current issue faced with most metagenomes is the identification of new and novel microorganisms that have not been cultured or sequenced before. This would require computational screening and development of new and efficient informatics tools that will assist with the identification or prediction of these organisms (Kunin et al. 2008; Meyer et al. 2008; Rosen et al. 2009).

Some of these tools that may be applied in analyzing the microbial diversity are multiple displacement amplification (MDA) that has enabled the identification of soil metagenomes in low biomass areas (Binga et al. 2008; Kallies et al. 2019). To address the genetic and functional diversity of uncultured microorganism and to link them to biogeochemical processes and functionalities, the GeoChips microarray platform has been utilized. This system has successfully identified microbial genes or populations and linked them to processes and functions (He et al. 2007; Neelakanta and Sultana 2013). Other than the GeoChip, the reactome array has also been utilized as a sequence independent system that identifies the metabolic phenotype of the communities (Beloqui et al. 2009; Batra et al. 2015). Further, through the use of information derived from various sites such as KEGG and PubMed we are able to identify possible metabolic pathways and utilize this information to compare and contrast communities (Kanehisa et al. 2014).

Further, a microfluid mechanism was designed to analyze multiple microbial uncultivated, unknown microorganisms in any mixed community. This system combines FACS and MDA technique (Marcy et al. 2007), where the MDA amplified fragment is identified through the 16S rRNA sequence of the population in the sample (Lasken and McLean 2014).

From various genomic initiatives that are ongoing worldwide and even in independent labs, more and more microbial databases are being made available which will facilitate the identification of microorganisms obtained from any soil. Currently GSC has a collection of genomes and metagenomes that covers communities from

research conducted worldwide creating mapping identifiers of genome databases to facilitate better understanding and consolidation of information and is labeled as "Genomic Rosetta Stone"(GRS) and aims to enrich our ever growing data collection of genomic and metagenomic sequences (van Brabant et al. 2008). International Nucleotide Sequence Database such as INSDC, DDBJ, EMBL-EBI, and GenBank contain public annotated, processed or raw sequence data from various researchers worldwide (Handelsman 2004).

13.8 Concluding Remarks

Soil microorganisms have an important role in maintaining soil health and fertility and therefore have become a key factor in sustainable agriculture. However, for microbial diversity to be studied and understood for it to be applied in agriculture and biotechnology we will first have to overcome the limitation of culture dependent and non-culturable organism. Metagenomic studies have been an enormous help in understanding soil microbial diversity and for use in agriculture and biotechnology. Through the advent of the genomics era, the ever-advancing high-throughput sequencing technologies and platforms have enable researchers to identify large number of soil microorganism. These platforms together with gene arrays, proteomics, transcriptomics, and various other molecular techniques have provided sufficient information for us to identify key players that may be utilized in addressing agricultural problems such as soil health and fertility. However, for continuous flow of good quality information into the soil microbial databases, the metagenome projects need to be provided with good sample source, good DNA extractions methods, good sequencing platforms and most importantly for the downstream analyzing bioinformatics pipelines that allows us to analyze and identify the members of the soil communities.

As mentioned in the previous sections, there are several international organizations that are working together to consolidate and share the information obtained for the mutual benefit of the scientific community. Open sourcing of bioinformatics tools through LinkOut or StrainInfo provides us with access to resources such as databases, publications, analytical tools, and more. This ensures that the large amount of data flooding the system is utilized well by the research community to make better sense as well as to better utilize the information for the benefit of mankind. All these efforts are designed to comply with the Convention on Biological Diversity (CBD) that is designed for conservation, sustainable, and equitable use of resources. We believe that it is with international cooperation and good working relationship between consortium and organizations that the metagenome information derived from various laboratories may be provided in useful form for open access and common use. Therefore, the purpose of metagenome data is for us to provide information on microbial communities in the soil for us to exploit not just to improve agricultural practices but for it to be utilized in other biotechnological research for wealth generation.

References

Ahmad M, Pataczek L, Hilger TH, Zahir ZA, Hussain A, Rasche F, Schafleitner R, Solberg SØ (2018) Perspectives of microbial inoculation for sustainable development and environmental management. Front Microbiol 9:2992

Amundson R, Berhe AA, Hopmans JW, Olson C, Sztein AE, Sparks DL (2015) Soil and human security in the 21st century. Science 348(6235):1261071. https://doi.org/10.1126/science.1261071

Baldrian P (2017) Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol Rev 41(2):109–130. https://doi.org/10.1093/femsre/fuw040

Bargaz A, Lyamlouli K, Chtouki M, Zeroual Y, Dhiba D (2018) Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Front Microbiol 9:1606. https://doi.org/10.3389/fmicb.2018.01606

Batra N, Bhatia S, Behal A, Singh J, Joshi A (2015) Metagenomic research: methods and ecological applications. In: Nelson KE (ed) Encyclopedia of metagenomics. Springer, Boston, MA

Beloqui A, Guazzaroni ME, Pazos F, Vieites JV, Godoy M, Golyshina OV, Chernikova TN, Waliczek A (2009) Reactome array: forging a link between metabolome and genome. Science 326:252–257

Bender SF, Wagg C, van der Heijden MGA (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol 31(6):440–452. https://doi.org/10.1016/j.tree.2016.02.016

Bharti R, Grimm DG (2019) Current challenges and best-practice protocols for microbiome analysis. Brief Bioinform 22(1):178–193. https://doi.org/10.1093/bib/bbz155

Bhowmik A, Cloutier M, Ball E, Bruns MA (2017) Underexplored microbial metabolisms for enhanced nutrient recycling in agricultural soils. AIMS Microbiol 3:826–845. https://doi.org/10.3934/microbiol.2017.4.826

Binga EK, Lasken RS, Neufeld JD (2008) Something from (almost) nothing: the impact of multiple displacement amplification on microbial ecology. ISME J 2:233–241

Bojko O, Kabala C (2017) Organic carbon pools in mountain soils—sources of variability and predicted changes in relation to climate and land use changes. Catena 149:209–220. https://doi.org/10.1016/j.catena.2016.09.022

Calderon K, Spor A, Breuil MC, Bru D, Bizouard F, Violle C, Barnard RL, Philippot L (2017) Effectiveness of ecological rescue for altered soil microbial communities and functions. ISME J 11:1–12

Castellano-Hinojosa A, Strauss SL (2020) Impact of cover crops on the soil microbiome of tree crops. Microorganisms 8:328. https://doi.org/10.3390/microorganisms8030328

Cavagnaro TR, Bender SF, Asghari HR, van der Heijden MGA (2015) The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends Plant Science 20:283–290. https://doi.org/10.1016/j.tplants.2015.03.004

Chowdhary K, Kumar A, Sharma S, Pathak R, Jangir M (2018) Ocimum sp.: source of biorational pesticides. Ind Crop Prod 122:686–701. https://doi.org/10.1016/j.indcrop.2018.05.068

Chung EJ, Lim HK, Kim JC, Choi GJ, Park EJ, Lee MH, Chung YR, Lee SW (2008) Forest soil metagenome gene cluster involved in antifungal activity expression in *Escherichia coli*. Appl Environ Microbiol 74:723–730

Crowther TW, Todd-Brown KEO, Rowe CW, Wieder WR, Carey JC, MAchmuller MB, Snoek BL, Fang S, Zhou G, Allison SD (2016) Quantifying global soil carbon losses in response to warming. Nature 540:104–108

Curtis TP, Sloan WT (2005) Exploring microbial diversityea vast below. Science 309:1331–1333

Daniel R (2005) The metagenomics of soil. Nat Rev Microbiol 3:470–478

Deemer BR, Harrison JA, Li S (2016) Greenhouse gas emissions from reservoir water surfaces: a new global synthesis. Bioscience 66:949–964. https://doi.org/10.1093/biosci/biw117

Dias T, Antunes PM (2014) Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. J Sci Food Agric 95(3):447–454. https://doi.org/10.1002/jsfa.6565

Emerson JB, Adams RI, Román CMB (2017) Schrödinger's microbes: tools for distinguishing the living from the dead in microbial ecosystems. Microbiome 5:86. https://doi.org/10.1186/s40168-017-0285-3

Escobar-Zepeda A, Vera-Ponce de León A, Sanchez-Flores A (2015) The road to Metagenomics: from microbiology to DNA sequencing technologies and bioinformatics. Front Genet 6:348. https://doi.org/10.3389/fgene.2015.00348

Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N (2008) The minimum information about a genome sequence (MIGS) specification. Nat Biotechnol 26:541–547

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364

Foley JA, Ramankutty N, Brauman KA (2011) Solutions for a cultivated planet. Nature 478:337–342. https://doi.org/10.1038/nature10452

Fujisawa T, Vogler AP, Barraclough TG (2015) Ecology has contrasting effects on genetic variation within species versus rates of molecular evolution across species in water beetles. Proc Biol Sci 282:20142476. https://doi.org/10.1098/rspb.2014.2476

Gans J, Wolinsky M, Dunbar J (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309:1387–1390

Gardner PP, Watson RJ, Morgan XC, Draper JL, Finn RD, Morales SE, Stott MB (2019) Identifying accurate metagenome and amplicon software via a meta-analysis of sequence to taxonomy benchmarking studies. PeerJ 7:e6160. https://doi.org/10.7717/peerj.6160

Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol 69:1800–1809

Gupta S, Mortensen MS, Schjørring S (2019) Amplicon sequencing provides more accurate microbiome information in healthy children compared to culturing. Commun Biol 2:291. https://doi.org/10.1038/s42003-019-0540-1

Hameed A, Wu QS, Abd-Allah EF, Hashem A, Kumar A, Lone HA, Ahmad P (2014) Role of AM fungi in alleviating drought stress in plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses. Springer, New York

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68(4):669–685. https://doi.org/10.1128/MMBR.68.4.669-685.2004

Haruta S, Kanno N (2015) Survivability of microbes in natural environments and their ecological impacts. Microbes Environ 30:123–125. https://doi.org/10.1264/jsme2.ME3002rh

Hashem A, Kumar A, Al-Dbass AM, Alqarawi AA, Al-Arjani ABF, Singh G, Farooq M, Abd-Allah EF (2018) Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea. Saudi J Biol Sci 26:614–624. https://doi.org/10.1016/j.sjbs.2018.11.005

He Z, Gentry TJ, Schadt CW, Wu L, Liebich J, Chong SC, Huang Z, Wu W (2007) GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. ISME J 1:67–77

Holst-Jensen A, Spilsberg B, Arulandhu AJ, Kok E, Shi J, Zel J (2016) Application of whole genome shotgun sequencing for detection and characterization of genetically modified organisms and derived products. Anal Bioanal Chem 408:4595–4614. https://doi.org/10.1007/s00216-016-9549-1

Hou PF, Chien CH, Chiang-Hsieh YF (2018) Paddy-upland rotation for sustainable agriculture with regards to diverse soil microbial community. Sci Rep 8:7966. https://doi.org/10.1038/s41598-018-26181-2

Hugerth LW, Andersson AF (2017) Analysing microbial community composition through amplicon sequencing: from sampling to hypothesis testing. Front Microbiol 8:1561. https://doi.org/10.3389/fmicb.2017.01561

Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S (2017) The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. Front Plant Sci 8:1617. https://doi.org/10.3389/fpls.2017.01617

Kallies R, Hölzer M, Brizola Toscan R, Nunes da Rocha U, Anders J, Marz M, Chatzinotas A (2019) Evaluation of sequencing library preparation protocols for viral metagenomic analysis from pristine aquifer groundwaters. Viruses 11:484. https://doi.org/10.3390/v11060484

Kanehisa M, Goto S, Sato Y, Kawashima M, Furumichi M, Tanabe M (2014) Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res 42:D199–D205. https://doi.org/10.1093/nar/gkt1076

Keesstra SD, Geissen V, Mosse K (2012) Soil as a filter for groundwater quality. Curr Opin Environ Sustain 4:507–516. https://doi.org/10.1016/j.cosust.2012.10.007

Kettner C, Field D, Sansone SA (2010) Meeting report from the second "minimum information for biological and biomedical investigations" (MIBBI) workshop. Stand Genom Sci 3:259–266. https://doi.org/10.4056/sigs.147362

Klironomos JN, Bednarczuk EM, Neville J (1999) Reproductive significance of feeding on saprobic and arbuscular mycorrhizal fungi by the collembolan, *Folsomia candida*. Funct Ecol 13:756–761

Kowalchuk GA, de Bruijn FJ, Head IM, Akkermans AD, van Elsas JD (2004) Molecular microbial ecology manual, 2nd edn. Kluwer Academic Publisher, Amsterdam

Kumar A, Sharma S, Mishra S (2016) Evaluating effect of arbuscular mycorrhizal fungal consortia and *Azotobacter chroococcum* in improving biomass yield of *Jatropha curcas*. Plant Biosyst 150:1056–1064. https://doi.org/10.1080/11263504.2014.1001001

Kumar A, Sharma S, Mishra S, Dames JF (2015) Arbuscular mycorrhizal inoculation improves growth and antioxidative response of *Jatropha curcas* (L.) under Na_2SO_4 salt stress. Plant Biosyst 149(2):260–269. https://doi.org/10.1080/11263504.2013.845268

Kunin V, Copeland A, Lapidus A, Mavromatis K, Hugenholtz P (2008) A bioinformatician's guide to metagenomics. Microbiol Mol Biol Rev 72:557–578. https://doi.org/10.1128/MMBR.00009-08

Lal R (2016) Soil health and carbon management. Food Energy Secur 5:212–222. https://doi.org/10.1002/fes3.96

Lam KN, Cheng J, Engel K, Neufeld JD, Charles TC (2015) Current and future resources for functional metagenomics. Front Microbiol 6:1196. https://doi.org/10.3389/fmicb.2015.01196

Land M, Hauser L, Jun SR, Nookaew I, Leuze MR, Ahn TH, Karpinets T, Lund O, Kora G, Wassenaar T, Poudel S, Ussery DW (2015) Insights from 20 years of bacterial genome sequencing. Funct Integr Genomics 15:141–161. https://doi.org/10.1007/s10142-015-0433-4

Lasken RS, McLean JS (2014) Recent advances in genomic DNA sequencing of microbial species from single cells. Nat Rev Genet 15:577–584. https://doi.org/10.1038/nrg3785

Lau JA, Lennon JT, Heath KD (2017) Trees harness the power of microbes to survive climate change. Proc Natl Acad Sci USA 114(42):11009–11011. https://doi.org/10.1073/pnas.1715417114

Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schaberle TF, Hughes DE, Epstein S (2015) A new antibiotic kills pathogens without detectable resistance. Nature 22:455–459

Lombard N, Prestat E, van Elsas JD, Simonet P (2011) Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiol Ecol 78:31–49

Lu JX, Burton SD, Xu YS (2014) The flexible structure of the K24S28 region of Leucine-rich Amelo- genin protein (LRAP) bound to apatites as a function of surface type, calcium, mutation, and ionic strength. Front Physiol 100:101. https://doi.org/10.3389/fphys.2014.00254

Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd-Allah EF (2018) Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 9:1132

Manichanh C, Chapple CE, Frangeul L, Gloux K, Guigo R, Dore J (2008) A comparison of random sequence reads versus 16S rDNA sequences for estimating the biodiversity of a metagenomic library. Nucleic Acids Res 36:5180–5188

Marcy Y, Ouverney C, Bik EM, Lo ?sekann T, Ivanova N, Garcia Martin H, Szeto E, Platt D (2007) Dissecting biological "dark matter" with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth. PNAS 104:11889–11894

Mercado-Blanco J, Abrantes I, Barra Caracciolo A, Bevivino A, Ciancio A, Grenni P, Hrynkiewicz K, Kredics L, Proença DN (2018) Belowground microbiota and the health of tree crops. Front Microbiol 9:1006. https://doi.org/10.3389/fmicb.2018.01006

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A (2008) The Metagenomics RAST server: a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinform 9:386

Nadarajah K, Kumar I (2019) Molecular microbial biodiversity assessment in the Mycorrhizosphere. In: Varma A, Choudhary DK (eds) Mycorrhizosphere and pedogenesis. Springer, Singapore. https://doi.org/10.1007/978-981-13-6480-8_23

Nayfach S, Pollard KS (2016) Toward accurate and quantitative comparative metagenomics. Cell 166:1103–1116. https://doi.org/10.1016/j.cell.2016.08.007

Neelakanta G, Sultana H (2013) The use of metagenomic approaches to analyze changes in microbial communities. Microbiol Insights 6:37–48. https://doi.org/10.4137/mbi.s10819

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, Ferrenberg S (2013) Patterns and processes of microbial community assembly. Microbiol Mol Biol Rev 77:342–356. https://doi.org/10.1128/MMBR.00051-12

Nunan N, Wu K, Young IM, Crawford JW, Ritz K (2002) In situ spatial patterns of soil bacterial populations, mapped at multiple scales, in an arable soil. Microb Ecol 44:296–305

Oulas A, Pavloudi C, Polymenakou P (2015) Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinform Biol Insights 9:75–88. https://doi.org/10.4137/BBI.S12462

Plummer E, Twin J, Bulach DM, Garland SM, Tabrizi SN (2015) A comparison of three bioinformatics pipelines for the analysis of preterm gut microbiota using 16S rRNA gene sequencing data. J Proteomics Bioinform 8:283–291. https://doi.org/10.4172/0974-276X.1000381

Reuter JA, Spacek DV, Snyder MP (2015) High-throughput sequencing technologies. Molecular Cell 58:586–597. https://doi.org/10.1016/j.molcel.2015.05.004

Rincon-Florez VA, Dang YP, Crawford MH (2016) Occasional tillage has no effect on soil microbial biomass, activity and composition in Vertisols under long-term no-till. Biol Fertil Soils 52:191–202

Rosen GL, Sokhansanj BA, Polikar R, Bruns MA, Russell J, Garbarine E, Essinger S, Yok N (2009) Signal processing for metagenomics: extracting information from the soup. Curr Genomics 10:493–510. https://doi.org/10.2174/138920209789208255

Sangwan N, Xia F, Gilbert JA (2016) Recovering complete and draft population genomes from metagenome datasets. Microbiome 4:8. https://doi.org/10.1186/s40168-016-0154-5

Schimel JP, Schaefer SM (2012) Microbial control over carbon cycling in soil. Front Microbiol 3:348. https://doi.org/10.3389/fmicb.2012.00348

Schmidt JE, Kent AD, Brisson VL (2019) Agricultural management and plant selection interactively affect rhizosphere microbial community structure and nitrogen cycling. Microbiome 7:146

Sebat JL, Colwell FS, Crawford RL (2003) Metagenomic profiling: microarray analysis of an environmental genomic library. Appl Environ Microbiol 69:4927–4934. https://doi.org/10.1128/aem.69.8.4927-4934.2003

Sergaki C, Lagunas B, Lidbury I, Gifford ML, Schäfer P (2018) Challenges and approaches in microbiome research: from fundamental to applied. Front Plant Sci 9:1205. https://doi.org/10.3389/fpls.2018.01205

Shakya M, Lo CC, Chain PSG (2019) Advances and challenges in metatranscriptomic analysis. Front Genet 10:904. https://doi.org/10.3389/fgene.2019.00904

Shinwari Z, Tanveer F, Iqrar I (2019) Role of microbes in plant health, disease management, and abiotic stress management. In: Kumar V, Prasad R, Kumar M, Choudhary D (eds) Microbiome in plant health and disease. Springer, Singapore. https://doi.org/10.1007/978-981-13-8495-0_11

Singer GAC, Fahner NA, Barnes JG (2019) Comprehensive biodiversity analysis via ultra-deep patterned flow cell technology: a case study of eDNA metabarcoding seawater. Sci Rep 9:5991. https://doi.org/10.1038/s41598-019-42455-9

Singh J, Behal A, Singla N, Joshi A, Birbian N, Singh S, Bali V, Batra N (2009) Metagenomics: concept, methodology, ecological inference and recent advances. Biotechnol J 4:480–494

Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74:65–105

Steffen W, Richardson K, Rockström J (2015) Supplementary materials, planetary boundaries: guiding human development on a changing planet. Science 347(6223):1259855. https://doi.org/10.1126/science.1259855

Stewart EJ (2012) Growing unculturable bacteria. J Bacteriol 194:4151–4160. https://doi.org/10.1128/JB.00345-12

Thomas T, Gilbert J, Meyer F (2012) Metagenomics - a guide from sampling to data analysis. Microb Inform Exp 2:3. https://doi.org/10.1186/2042-5783-2-3

Tian J, Bryksa BC, Yada RY (2016) Feeding the world into the future – food and nutrition security: the role of food science and technology. Front Life Sci 9:155–166

Van Brabant B, Gray T, Verslyppe B, Kyrpides N, Dietrich K, Glo ckner FO, Cole J, Farris R (2008) Laying the foundation for a genomic rosetta stone: creating information hubs through the use of consensus identifiers. OMICS 12:123–127

Van der HE, Genee HJ, Sommer MOA (2018) The evolving interface between synthetic biology and functional metagenomics. Nat Chem Biol 14:752–759. https://doi.org/10.1038/s41589-018-0100-x

Van Elsas JD, Jansson CR, Sjo ling S, Bailey M, Nalin R, Vogel TM, van Overbeek L (2008) The metagenomics of disease-suppressive soils-experiences from the METACONTROL project. Trends Biotechnol 26:591–601

Vogel TM, Simonet P, Jansson JK, Hirsch PR, Tiedje JM, van Elsas JD, Bailey MJ, Nalin R (2009) TerraGenome: a consortium for the sequencing of a soil metagenome. Nat Rev Microbiol 7:252

Vyas P, Kumar D, Dubey A, Kumar A (2018) Screening and characterization of *Achromobacter xylosoxidans* isolated from rhizosphere of *Jatropha curcas* L. (energy crop) for plant-growth-promoting traits. J Adv Res Biotechnol 3(1):1–8. https://doi.org/10.15226/2475-4714/3/1/00134

Yergeau E, Sanschagrin S, Maynard C, St-Arnaud M, Greer CW (2014) Microbial expression profiles in the rhizosphere of willows depend on soil contamination. ISME J 8:344–358

Yun J, Ryu S (2005) Screening for novel enzymes from metagenome and SIGEX, as a way to improve it. Microbial Cell Factory 4:8. https://doi.org/10.1186/1475-2859-4-8

Zhou J, He Z, Yang Y (2015) High-throughput metagenomic technologies for complex microbial com- munity analysis: open and closed formats. MBio 6(1):e02288. https://doi.org/10.1128/mBio.02288-14

Zhou J, Thompson DK (2002) Challenges in applying microarrays to environmental studies. Curr Opin Biotechnol 13:204–207

Chapter 14
Soil Microbial Diversity and Metagenomics

Sandeep Sharma and Sukhjinder Kaur

Abstract The management of soil fertility for sustainable and productive agriculture embroils understanding of chemical, physical, and primarily biological components of soil. The soil microbiome ability to predict noticeable changes in soil properties as they are involved in nutrient cycling, soil structure formation, decomposition of organic matter, and plant growth promotion. The microbial diversity prevailing in the soil can be explored either through culture-based or recently through novel genomic approaches that proved to be powerful tool in microbe-centric studies and delivers more comprehensive assessment of microbial functions. Soil metagenomics holds unusual potential to enhance crop production and to discover several unexploited soil microorganisms, their functions and genes for diverse applications. In this book chapter, special emphasis has been highlighted on the role of metagenomics for unlocking the soil microbiome and its processes in different management practices.

Keywords Metagenomics · Management practices · Soil microbiome · Soil enzymes · Soil fertility

14.1 Introduction

Environmental soil degradation with long-term continuous cropping involving utilization of chemical fertilizers leads to the imbalance or reduction in nutrient availability and fertility of soil (Dong et al. 2012). The management of soil to ensure its long-term productivity, stability, and fertility is of paramount importance for plant growth. The maintenance of the physical and chemical soil fertility is driven by the metabolic repertoire of the soil microorganisms (Sabale et al. 2019). The soil biological fertility relies on the microbial community, which is termed as the

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indicators of soil health that directly impacts the functioning of soil ecosystem. Thus, the soil health comprises biological, chemical, and physical properties of soil but is mostly dependent on the activity of microorganisms. The biological measurement of soil health can be inferred from certain robust indicators (e.g. microbial diversity, enzyme activity, and soil organic matter content) that can provide instant information about the current status of soil (Rincon-Florez et al. 2013). Among different soil health indicators, there is increased concern in studying soil microorganisms in their specific environments, as microbial diversity is closely linked to soil structure and function. Moreover, soil microorganisms rapidly respond to any kind perturbations (Jacoby et al. 2017).

14.2 Soil Microbiome

The soil microbiome is indispensable as it performs key soil services including organic matter decomposition, biogeochemical cycling, aggregates formation, gaseous exchange, and plant growth promotion (Christopher 2017; Naylor et al. 2020). The soil represents the most diverse habitats consisting complex assemblages of bacteria, archaea, viruses, fungi, and other microbial eukaryotes which are collectively referred as the "soil microbiome" (Fierer 2017; Jansson and Hofmockel 2020). The estimate suggests 1000–10,000 bacterial species in per gram of agricultural soils as inferred from the 16S rRNA gene phylotypes (Attwood et al. 2019). The reservoir of microbial communities in soil improves plant growth by affecting nutrients availability, aids in crop residue recycling along with determination of agroecosystems productivity (Van-Der Heijden et al. 2008). The sustainable agriculture depends on the diversity of soil microbes that influences soil fertility. Therefore, the present day research focuses more in managing soil microbiome (Dubey et al. 2019).

The characterization and classification of soil microbiome by typical cultivation approaches (plate count and most probable number) have underestimated the microbial diversity as largest proportion of soil bacteria still remain uncharacterized (Dupont et al. 2016). The majority of the soil isolated microbes belonged to the phyla, namely *Proteobacteria*, *Actinobacteria*, *Firmicutes,* and *Bacteroidetes*, as these are cultivated easily under laboratory conditions (Hirsch et al. 2010). Due to severe constraints in isolation methods, there is need for switching to the molecular and genetic level approaches that will unearth more comprehensive picture of soil microbiome by discovering new microbial players through in-depth characterization (Agrawal et al. 2015; Sabale et al. 2019). During the last few years, significant improvement has been seen in the development of certain biomarkers and macromolecular probes, rapid and reliable measurements of soil microbial communities (Arias et al. 2005). The measurement of microbial diversity can be classified into phenotypic and molecular based approaches. The determination of true microbial diversity using phenotypic techniques is difficult due to lesser accuracy of the extraction or detection methods (Agrawal et al. 2011). Thus, soil microbiologists

have attempted to ameliorate molecular methods. This book chapter emphasized on the recent methods adopted for evaluating soil fertility with focus on strategies for identifying microbial communities via metagenomics.

14.3 Molecular Approaches for Measuring Soil Microbiome

The molecular approaches to analyze soil microbiome are DNA-based methods, microscopic observation of root colonizing labeled microbes, and labeled nutrient substrates. These new molecular, enzymatic, and organism-based methods have complimented the existing physico-chemical properties and possess ability to evaluate the soil diversity and composition. All the techniques are properly evaluated for their potential to differentiate among various types of soils and their significance in the ecosystem. The current molecular strategies have led to the discovery of unusual microbial diversity, majority of which was uncharacterized so far because of non-culturable nature (Agrawal et al. 2015). Molecular techniques to determine microbial diversity in soil can be categorized into PCR-dependent and PCR-independent techniques. Nucleic acid re-association/hybridization, carbon source utilization profile/community level, physiological profile (CLPP)/BIOLOG, fatty acid methyl ester (FAME) analysis, phospholipids fatty acid (PLFA) analysis are PCR-independent approaches used for measuring microbial communities. Some of the limitations of the aforementioned techniques include dominance of culturable community and preferring microbes that can utilize the available carbon sources. These methods mainly signify metabolic diversity rather than microbial diversity (Fakruddin and Mannan 2013).

14.4 PCR-Based Approaches

The initial molecular approach for investigating biological community depends on the cloning of target genes isolated from environmental samples (DeSantis et al. 2007). Majority of the genetic fingerprinting techniques relies on PCR amplification which provides information regarding the genetic makeup of microbes. The prokaryotic diversity, identification, and phylogenetic relationships are provided by PCR-based 16S rDNA profile. PCR-based fingerprinting methods of microbial communities involves the extraction of DNA from a culture, a bioreactor, or an environmental sample, followed by the amplification of rRNA/rDNA using the Polymerase Chain Reaction (PCR), and finally an analysis of the DNA amplification products (Ngom and Liu 2014). The PCR-based approaches are distributed into two groups depending on the differential electrophoretic migration on agarose or polyacrylamide gels: (1) size-dependent migration, viz. T-RFLP, ARISA/RISA, RAPD,

SSCP, LH-PCR and (2) sequence-dependent migration which includes denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE). In all the above-mentioned techniques, community structure of microbial populations can be evaluated from the amplified fragments generated by selected primers. Thus, 16S rDNA-based PCR techniques such as DGGE, TGGE, single-strand conformation polymorphisms (SSCPs), amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphisms (T-RFLPs), and ribosomal intergenic spacer analysis (RISA) offer comprehensive information regarding community richness, evenness, and composition present in a sample (Rawat and Johri 2014). All the PCR-based techniques are suitable for tracing the dominant members of the community in complex soil environment with selective amplification of shorter fragments comprising weaker secondary structures (Rincon-Florez et al. 2013). Moreover, these methods are time-consuming with low-throughput, PCR biased and prefer easily extractable DNA that usually leads to confusing and unsuitable results. Further, microarrays have also been developed with already known gene sequences from public databases with regular updating of new gene and genome sequences. But, the application of this technology for reviewing environmental sample still presents numerous limitations (He et al. 2012; Ngom and Liu 2014). Thus, to avoid the hindrance in evaluation of soil microbial communities, metagenomics combined with bioinformatics have been recently used and these new methods are more reliable in soil microbial diversity studies (Liu et al. 2006).

14.5 Concept of Metagenomics

Although several molecular approaches have been proposed but recently, the exploration of entire genomes existing in a soil sample, i.e. metagenomics, has provided a new strategy for studying microbial diversity bypassing the isolation and cultivation methods of individual species (Mocali and Benedetti 2010). The outgrowth of genomics and metagenomics demonstrated promising strategies that possess the ability to discover the hidden diversity of microbes along with their function in a well-defined manner. Further, advanced sequencing technologies recognized as the Next-Generation Sequencing (NGS) performs the analysis of soil-extracted microbial community DNA directly. The NGS resulted in the production of vast volume of data in a rapid and cost-effective manner. The ability to group the entire genome of any related organisms has permitted evolutionary and comparative studies on large scale that were impossible earlier (Weinstock 2012). The sequencing of soil by metagenomics offers understanding of microbial ecology that is beneficial or detrimental to crop production with the aim to improve agricultural sustainability (Petrosino et al. 2009). The concept of metagenomics and other associated strategies have become the prime technology in many research areas attributed to its efficiency for sequencing large volume of data. This technological advancement has generated a new direction for sequencing large-scale projects (Petrosino et al. 2009).

Metagenomics is basically community genomics which provides access to the genetic makeup of whole communities of organisms present in different ecosystems. It involves the isolation of soil DNA, fragmentation, and insertion of DNA into appropriate vectors followed by DNA cloning and transformation of suitable host cells and then delivering a metagenomic library and further screening of the clone library (Mocali and Benedetti 2010). In metagenomics, the combined genome is randomly sampled from simultaneously existing microbial communities and then sequenced (Ghazanfar et al. 2010). Through the direct assessment of collective genome, metagenomics possesses the potential to provide detailed insight about genetic diversity, species composition, development, and interactions with the microbial communities prevailing naturally in the environment (Fakruddin and Mannan 2013). Mass genome sequencing based shotgun analysis, genomic activity-driven studies aimed to find exact microbial functions, phylogenetic or functional gene expression analysis of genomic sequences, and next-generation sequencing strategies for evaluating entire gene content in environmental samples are the four sub-categories of unselective/untargeted and targeted metagenomics based on the various screening methods. The unselective/untargeted metagenomics involves shotgun analysis and next-generation sequencing, whereas targeted metagenomics includes activity and sequence-driven studies. Due to the cost-effectiveness and ease in DNA sequencing techniques, unselective metagenomic approach has been preferred widely (Neelakanta and Sultana 2013). Targeted metagenomics commonly sequences in parallel and extremely target genes, serving ribosomal RNA (rRNA) as evolutionary clocks. This biomarker relied on the massive databank of rRNA gene sequences (more than 200,000) collected for the reconstruction of the universal Tree of Life which increased exponentially due to targeted and untargeted sequencing. The rRNAs of all the organisms are sufficiently related to each other that they can be recognized as the same molecule but different enough that the differences are a good measure of evolutionary distance (Perito and Cavalieri 2018).

In sequence-based metagenomics, the researcher's emphasis on finding the complete genetic sequence, i.e., the arrangement of all the nucleotide bases (A, C, G, and T) found in the DNA strands of a sample. The sequence obtained can then be analyzed in several ways which includes utilization of community's sequence in determination of entire genome of a specific organism or this sequence can also be used to analyze the genome of the community as a whole that offers insight about evolution and population ecology. Further, the function-based metagenomics involves screening of metagenomic libraries for several functions/products, such as genes involved in nutrients cycling and metabolic pathways, vitamins or antibiotics produced by microbes in a community. Scientists can recognize various functions through this method that was known in microbes. Recently through advances in function-based metagenomics technology, researchers can also directly extract novel proteins from a microbial community and identify their metabolites involved in cellular processes. Therefore, the study of soil fertility indicators through metagenomic approach will enhance the soil biological system, which in turn promotes soil fertility and improved productivity.

14.5.1 Metagenomic-Based Studies on Soil Microbiome

The advancement in high-throughput molecular biology methods over the last decades has resulted in significant increase in the understanding of the soil microbiome (Nannipieri et al. 2019). The metagenomic approach showed enormous potential in unlocking myriad of functions which include identification of uncultivated or new phyla possessing novel traits, understanding metabolic and biochemical activity of microbial players, functional diversity of microbes, finding shifts in microbial diversity associated with stress and disease tolerant plants (Köhl et al. 2014; Dubey et al. 2019). The additional target of metagenomic-based studies is to gain insights into biochemical cycling of nutrients (C, N, P, S, and other elements) summarized in Fig. 14.1 (Myrold et al. 2013). One international effort focusing on sequencing and interpreting the soil metagenome was proposed by combining the abilities of the global scientific community (Vogel et al. 2009) and named the project as the Terra Genome. This international sequencing consortium possesses primary objective of complete sequencing of a reference soil metagenome. The soil system selected for research is Park Grass, an internationally recognized agroecology field experiment that has been running for more than 150 years at the UK agricultural sciences institute, Rothamsted Research (Fujii et al. 2009).

The rhizospheric and phyllospheric bacterial population of Basmati rice in Pakistan were studied using metagenomic approach by Rasul et al. (2020). The results described the dominance of phylum *Proteobacteria*, *Chloroflexi*, *Actinobacteria,* and *Firmicutes* at different sites in the rhizosphere than

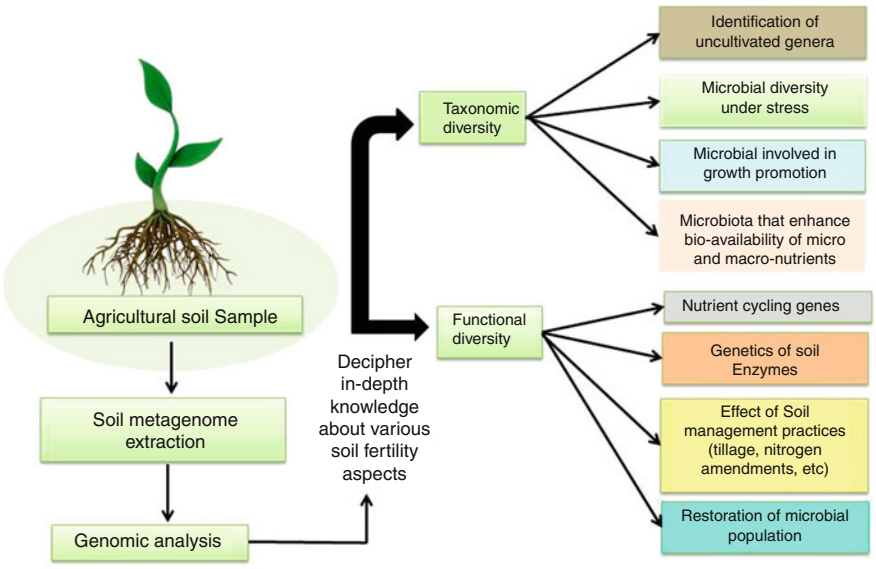

Fig. 14.1 Summary of the various soil aspects studied by metagenomics

phyllosphere. The plant growth promoting genera, *Azospirillum*, *Bacillus*, *Brevibacillus*, *Mesorhizobium*, *Paenibacillus*, *Streptomyces,* and *Sphingomonas* were also abundant in rhizosphere. Woźniak et al. (2019) compared rhizospheric and endophytic microbiome of *Paulownia* trees by Illumina MiSeq sequencing and described higher bacteria and fungi in endosphere samples. The abundant bacterial phyla reported were *Actinobacteria* and *Proteobacteria*. The rhizospheric fungal diversity includes *Ascomycota*, *Mortierellomycota*, and *Basidiomycota*, whereas the endophytic diversity involves *Olpidiomycota*, *Oomycota*, *Ascomycota,* and *Basidiomycota*. Hara et al. (2019) identified functional N_2-fixing bacteria associated with sorghum through omics approaches. Here, the roots extracted bacterial cells were studied by metagenome and proteome. Majority of the sequences were assigned to nif HDK of *Bradyrhizobium* species.

Ahmed et al. (2018) assessed the microbial diversity in the two rhizospheric saline soil samples through metagenomic approach and observed the dominance of halophilic/halotolerant phylotypes affiliated to *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Bacteroidetes, Firmicutes*, and *Acidobacteria*. Identification of osmotolerant clones SSR1, SSR4, SSR6, SSR2 harboring *BCAA_ABCtp, GSDH, STK_Pknb*, and *duf3445* genes confirmed their function in osmotolerance. The soil metagenomic libraries also reported the abundance and diversity of phosphatase genes using functional metagenome analysis. Similarly, Molina-Montenegro et al. (2018) compared the rhizospheric microbiome using shotgun metagenomic technology and found abundance of bacterial species (98%) followed by eukaryota (1.77%) and archaea (0.22%). The major genera reported in the rhizospheric soil were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria,* and *Firmicutes*. Metagenomic shotgun sequencing and functional annotation by means of eggNOG functional categories showed that metabolism was the highest represented category, followed by cellular process and signaling, and information storage and processing. In the category metabolism, the highest characterized terms were amino acid transport and metabolism, energy production and conversion, carbohydrate transport and metabolism, and inorganic ion transport and metabolism. Baeza et al. (2017) evaluated fungal sequences from Antarctica by amplicon metagenomic analysis and found 87 genera and 123 species, from which 37 genera were not reported previously. Lecanoromycetes and Eurotiomycetes were dominant the fungal classes.

The metagenomic DNA from bulk soil of tomato, vegetables, and native forest extracted by Val-Moraes et al. (2013) represented uncultured fungi. The individual amplified sequences matched with Glomeromycota, Fungi incertae sedis, and Neocallimastigomycota. The tropical mangrove soil microbial diversity was characterized by Ismail et al. (2012) through the metagenome of a Malaysian mangrove soil sample and its microbial ecological roles via next-generation sequencing (NGS). Shotgun NGS data analysis revealed high diversity of ecologically essential microbes from bacteria and archaea domains. Also, an unusually high number of archaea was observed along with abundance of *Deltaproteobacteria*.

14.5.2 Metagenomic Insight of Soil Management Practices

Agricultural intensification for increased production resulted in severe food security and adverse impacts on soil fertility, nutrient leaching, and increased greenhouse gas emissions (Hartman et al. 2018; Souza et al. 2018). These conventional agriculture based strategies have affected the biodiversity and functionality of soil microbiome through curtailment of functions performed by microbes and reduction in their species (Souza et al. 2018). Moreover, these practices alter soil physico-chemical as well as biological properties which act as valuable indicators of soil quality and health (Carbonetto et al. 2014). Thus, the adoption of soil conservation practices is required to prevent soil degradation and to maintain active soil biota (Souza et al. 2013). Numerous conservation practices such as tillage, organic fertilization, crop rotations/successions and crop residues retention have resulted in improved sustainability and have promoted beneficial ecosystem services (Srour et al. 2020). Soil metagenomics also unravels the understanding of different soil management approaches (such as tillage, organic fertilizer amendments) aimed for enhancing plant productivity and nutrient acquisition (Attwood et al. 2019). Furthermore, the understanding of aforementioned functions associated with soil microbiome will play an essential role in management of soil fertility. Some of the recent studies highlighting diversity analysis among various soil management practices through metagenomics are summarized in Table 14.1.

14.5.3 Functional Metagenomic-Based Insight of Soil Enzymes

The soil enzyme activities (β-glucosidase, cellulose, protease, urease, and phosphatase) are directly involved in the nutrient cycling (such as carbon, nitrogen, and phosphorus) and reflect the metabolic requirements of soil microorganisms, which are important in the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter (Burns et al. 2013; Yang et al. 2017). Soil enzymes are crucial for the functioning of soil because of their role in decomposition and transformation processes (Jesus et al. 2016). The activity of soil enzymes is directly related to the metabolic requirements of the soil community and the available nutrients present in soil. The soil enzymes are categorized into hydrolases and oxidases that decompose substrates and release nutrients to the soil. Another enzyme, urease is associated with microbial N acquisition, as it catalysis the urea decomposition. Microbially produced hydrolytic enzyme, β-1,4-glucosidase decomposes polysaccharides whereas acidic and alkaline phosphatase are associated with P-acquisition (Hai-Ming et al. 2014). The most studied enzymes from the soil metagenome are esterase and lipase attributed to wide potential in industry (Lee and Lee 2013). The molecular methods deliver valuable information on expression and potential of enzymes targeting the abundance of enzyme-encoding genes or

Table 14.1 Metagenomic-based studies on diversity and function analysis of soil in different management practices

Cropping system/ experimental design	Location	Duration of experiment	Soil type	Genomic technique	Results	References
Tillage and fertilizer amendment in corn-soybean	USA	Long-term (1990–2015)	Mesic Udollic Endoaqualf	Metagenome Illumina sequencing	• Metagenome analysis revealed higher population of arbuscular mycorrhizae, mycoparasites and nematophagous fungi in no-till soils. • Fungal saprotrophs and plant pathogens dominated in tilled soils. • Copiotrophic bacteria and *Fusarium* species were favored under conventional tillage. • Higher abundance of pathways associated with energy metabolism, translation, metabolism of cofactors and vitamins, glycan biosynthesis and nucleotide metabolism in no-till	Srour et al. (2020)
Crop rotation	Germany	Long-term (1992–2015)	Chernozem	Whole metagenome shotgun sequencing	• Dominant genera were *Streptomyces, Bradyrhizobium, Mycobacterium, Nitrososphaera*, and *Nocardioides*. • Metagenomically assembled genomes revealed two important taxa present in large numbers, *Thaumarchaeota* and *Rhizobiales*. • Methylotrophic bacterium of unclassified Rhizobiales is involved in conversion of C1-components (carbon cycle). • Thaumarchaeota members contribute to ammonium oxidation and carbon dioxide fixation	Nelkner et al. (2019)

(continued)

Table 14.1 (continued)

Cropping system/ experimental design	Location	Duration of experiment	Soil type	Genomic technique	Results	References
Napier grass amended with biochar	USA	2 years	Acidic oxisol	Shotgun metagenomics	• Biochar-amended soil microbiome exhibited enrichment in key metabolic pathways related to carbon turnover, such as the utilization of plant-derived carbohydrates and denitrification. • Increased soil carbon (labile and aromatic carbon compounds), available nutrients	Yu et al. (2016)
Tobacco (application of fertilizers incorporated with plant residues)	China	One year (2015)	Not specified	Metagenomic sequencing	• Functional annotation of metagenomic sequences revealed abundance of genes involved in metabolic pathways. • Predominant phyla were Proteobacteria, Actinobacteria, and Verrucomicrobia in 300 kg/mu straw. • Cyanobacteria, Basidiomycota, and Chlorophyta were abundant in soil samples with 200 kg/mu straw	Yang et al. (2017)
Nitrogen fertilization	USA	Long term	Sandy and silt loam	Short-gun Metagenomics	• Functionally assembled metagenomes revealed 6 deep-branching *Thaumarchaeota* and 3 ammonia oxidizer *Nitrospira.* • Also genomic analysis predicted its fivefold abundance in N fertilizer	Orellana et al. (2018)
Straw mulching in tobacco-rice rotation	China	1 year	Sandy loam	Illumina sequencing	• Abundance of Proteobacteria and Actinobacteria. • *Nitrospirae* was highest in the rice straw returning.	Lei et al. (2017)

					• Rice straw returning fire + quicklime and reduced fertilizer had the highest abundance of Firmicutes	
Forest and vineyards soils	Chilean Mediterranean	1 year	Not specified	Short-gun Metagenomics	• *Candidatus Solibacter*, *Bradyrhizobium* and the fungus *Gibberella* were most abundant. • Genes present in microbial diversity pertain to metabolism of amino acids, fatty acids, nucleotides as well as secondary metabolism	Castañeda and Barbosa (2017)
Tillage-crop residue management	Mexico	–	Hyposodic vertisol	Short-gun Metagenomics	• Population of degrading genera (*Promicromonospora, Bacillus, Agromyces, Streptomyces, Sinorhizobium*, and *Lysobacter*) was higher in retained treatments	Chávez-Romero et al. (2016)
Tillage effect on cellulose-degrading microbes	Germany	Long term (1992-2012)	Luvisol	Shotgun sequencing	• Abundance of cellulolytic enzymes and cellulolytic gene composition in reduced tillage. • Proteobacteria, Actinobacteria, and Bacteroidetes dominated in reduced tillage	DeVries et al. (2015)
Bacterial diversity under tillage	USA	Long term (52 years)	Typic Fragiudalf	Pyrosequencing	• No-till exhibited higher number of reads, bacterial richness, and five unique phyla. • Four unique phyla were observed in adjacent plow-till	Sengupta and Dick (2015)
No-till crop rotation in cultivated and uncultivated land	Argentina	15 years	Typic Argiudolls	Short-gun metagenomesequencing	• Phyla *Verrucomicrobia, Plactomycetes, Actinobacteria,* and *Chloroflexi* were more in non-cultivated soils while *Gemmatimonadetes, Nitrospirae* were abundant in cultivated soils. • The abundance of genes assigned to	Carbonetto et al. (2014)

(continued)

Table 14.1 (continued)

Cropping system/ experimental design	Location	Duration of experiment	Soil type	Genomic technique	Results	References
					transcription, protein modification, nucleotide transport and metabolism, wall and membrane biogenesis and intracellular trafficking and secretion were observed in cultivated fertilized soils	
Tillage practices+ crop rotation	Brazil	13 year	Oxisol	Short-gun sequencing	• Majority of the sequences were attributed to Bacteria (54%), and 0.3% and 0.2% to Archaea and Eucarya domains, respectively. • Significantly higher microorganisms associated with residue decomposition, carbon and nitrogen cycling, and xenobiosis were observed in conventional tillage (CT). • Eucarya were also abundant in CT, with possible relation in higher tolerance of environmental stresses. • No-till showed higher abundance of nitrogen-fixing Rhizobiales and Archaea	Souza et al. (2013)

transcribed sequences (Baldrian 2009). The soil microbiome harbors numerous novel enzymes which are identified by various metagenosmic studies and summarized in Table 14.2.

The application of metagenomic approaches for evaluating soil microbiomes and related functions has facilitated the better understanding of taxonomic, genetic, and functional characteristics of soil microbial community (Fierer et al. 2012). Still there are challenges that need to overcome by combining application of metatranscriptomics, metaproteomics, and metabolomics that are helpful to fill knowledge gaps about genes/protein expression and metabolic interactions (Jansson and Hofmockel 2018). Metatranscriptomics involves study of microbial RNA transcripts produced in a particular ecological sample (Baldrian et al. 2012). Metatranscriptomics approach immediately deciphers gene regulatory response as majority of bacteria exhibit transcriptional gene control that permits quick adaption to change altered environmental conditions at the sampling time (Moran 2009). The steps performed in this technique comprise extraction followed by reverse transcription, amplification, and lastly sequencing of transcripts. The transcript obtained is highly unstable and has shorter life span which is a major bottleneck to this technology (Cabellos-Ruiz et al. 2010). Meta-transcriptomic approach is widely preferred for unfolding microbial nutrient cycling (Barua et al. 2017).

Next, metaproteomics is the characterization of the microbial proteins (Ngom and Liu 2014) extracted from a sample, followed by fractionation, separation using liquid chromatography or two dimension polyacrylamide gel and then detection with tandem mass spectrometry (Zhang et al. 2010). Lin et al. (2013) conducted the metaproteomic profile of rhizospheric soil for elucidation of mechanism involved in yield decline of ratoon sugarcane. The results revealed 143 protein spots with high resolution and repeatability including 38 differentially expressed proteins involved in carbohydrate/energy, amino acid, protein, nucleotide, auxin and secondary metabolisms, membrane transport, signal transduction and resistance, etc. Thus, metagenomics is the predictive of community potential and combining it with the metaphenome will reveal the functional potential of soil communities and links between community genes and functions.

14.6 Conclusion

Soil microbiome plays an imperious role in cycling of nutrients, mineralization, enzymes production, and improvement of indispensable soil processes that impacts soil fertility. Owing to drawbacks of traditional plate count techniques, molecular methods have offered a desired alternative for exploring soil microbiome. The taxonomists have developed various molecular techniques that permit rapid analysis of desired traits within microbial communities. Several PCR and non-PCR based techniques have been developed to explain functional profiling of natural microbial communities. The advancement in sequencing tools has resulted in the advent of novel and rapid molecular method known as integrated omic approaches that

Table 14.2 Soil enzymes studies performed by metagenomic strategies

Cropping system/soil sample	Location	Enzyme studied	Genomic technique	Results	References
Forest soil	Germany	Phosphatases/ phytases	Functional genomics	• Metagenome analysis revealed largest number and diversity of phosphatase genes. • two of the gene products carry domains which have never been associated with phosphatase activity before. • Also found previously unreported phytase activity of alkaline phosphatase and sulphatase superfamily and purple acid phosphatases from non-vegetal origin	Villamizar et al. (2019)
Solanum phureja soil	Columbia	_	Functional genomics	• Functional metagenome revealed the abundance of oxidoreductase activity (18%). • Also identified a protease and lipase/esterase domain	Calderon et al. (2019)
Crop succession (soybean/wheat), or crop rotation (soybean/maize/wheat/lupine/ oat) + tillage practices	Brazil	Hydrolases	Shotgun metagenomes	• The abundance order was lipases> laccases > cellulases > proteases >amylases>pectinases. • No-till showed five times more hydrolases than conventional tillage. • Majority of enzyme sequences belonged to fungi (*Verticillium* and *Colletotrichum* for lipases, laccases, and *Aspergillus* for proteases), and the archaea, *Sulfolobus acidocaldarius* for amylases	Souza et al. (2018)

Cabbage soil sample	Sweden	Chitinase	Functional metagenomics	• Bacterial chitinase, Chi18H8, with antifungal activity was identified. • Sequence analysis showed chi18H8 gene encodes a 425-amino acid protein of 46 kDa with an N-terminal signal peptide. • A catalytic domain with conserved active site and a chitinase insertion domain were also observed	Hjort et al. (2014)
Grassland soil	Germany	Cellulase and Xylanase	Functional metagenomics	• Novel cellulase-encoding gene (*cel01*) and two xylanase-encoding genes (*xyn01* and *xyn02*) were identified. • From sequence analysis, Cel01 (831 amino acids) belongs to glycoside hydrolase family 9 • Xyn01 (170 amino acids) and Xyn02 (255 amino acids) are members of glycoside hydrolase family 11	Nacke et al. (2012)
Forest soil	Korea	Lipolytic enzymes	Metagenomics	• Seven lipolytic enzymes were identified comprising lipase families II, IV, and V	Hong et al. (2007)
Soil and compost sample	Germany	Hydrolytic enzymes	Expression Metagenomics	• Active clones of lipolytic enzymes, amylases, phosphatases, and dioxygenases were identified. • Three genes encoding phosphatase or dioxygenase activity were also identified	Lammle et al. (2007)

constitutes metagenomics, transcriptomics, metaproteomics, and metabolomics. Metagenomics techniques are based on the direct analysis of DNA extracted from environmental samples and have circumvent the steps of isolation and culturing of microbes. No single technique till date can measure the whole microbial diversity. Biases are introduced at each treatment step as all of these techniques present advantages as well as drawbacks. Advanced screening approaches involving function-driven and sequence-dependent metagenomics will provide deeper insights of soil metagenome that will aid in sustaining crop management and soil fertility.

References

Agrawal PK, Agrawal S, Verma SK (2011) Methods for studying microbial diversity from soyabean rhizosphere by phenotypic and molecular approach. Adv Appl Sci Res 2:538–545

Agrawal PK, Agrawal S, Shrivastava R (2015) Modern molecular approaches for analyzing microbial diversity from mushroom compost ecosystem. 3 Biotech 5:853–866

Ahmed V, Verma MK, Gupta S, Mandhan V, Chauhan NS (2018) Metagenomic profiling of soil microbes to mine salt stress tolerance genes. Front Microbiol 9:159. https://doi.org/10.3389/fmicb.2018.00159

Arias ME, González-Pérez JS, González-Vila FJ et al (2005) Soil health-a new challenge for microbiologists and chemists. Int Microbiol 8:13–21

Attwood GT, Wakelin SA, Leahy SC et al (2019) Applications of the soil, plant and rumen microbiomes in pastoral agriculture. Front Nutr 6:107. https://doi.org/10.3389/fnut.2019.00107

Baeza M, Barahona S, Alcaíno J et al (2017) Amplicon-metagenomic analysis of fungi from antarctic terrestrial habitats. Front Microbiol 8:2235. https://doi.org/10.3389/fmicb.2017.02235

Baldrian P (2009) Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ 55:370–378

Baldrian P, Kolařík M, Stursová M et al (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISMEJ 6:248–258

Barua S, Hait A, Barua S (2017) Metatranscriptomic study of microbes in environment: a community function based approach. J Bacteriol Mycol 4:12–15

Burns RG, De Forest JL, Marxsen J et al (2013) Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol Biochem 58:216–234

Cabellos-Ruiz A, Janca H, Baena S et al (2010) Beyond metagenomics: integration of complementary approaches for the study of microbial communities. Metagenomics: theory, methods and applications. Caister Academic Press, Norfolk, UK, pp 15–37

Calderon D, Peña L, Suarez A et al (2019) Recovery and functional validation of hidden soil enzymes in metagenomic libraries. MicrobiologyOpen 8:e572. https://doi.org/10.1002/mbo3.572

Carbonetto B, Rascovan N, Álvarez R et al (2014) Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in argentine pampas. PLoS One 9(6):e99949. https://doi.org/10.1371/journal.pone.0099949

Castañeda LE, Barbosa O (2017) Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. PeerJ 5:e3098. https://doi.org/10.7717/peerj.3098

Chávez-Romero Y, Navarro-Noyab YE, Reynoso-Martíneza SC et al (2016) 16S metagenomics reveals changes in the soil bacterial community driven by soil organic C, N-fertilizer and tillage-crop residue management. Soil Tillage Res 159. https://doi.org/10.1016/j.still.2016.01.007

Christopher J (2017) Living soils: the role of microorganisms in soil health. In: Strategic analysis paper. Future Directions International, Nedlands. Available via. https://www.futuredirections.

org.au/wp-content/uploads/2017/06/Living-Soils-the-Role-of-Microorganisms-in-Soil-Health.pdf

DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol 3:371–383. https://doi.org/10.1007/s00248-006-9134-9

DeVries M, Schöler A, Ertl J et al (2015) Metagenomic analyses reveal no differences in genes involved in cellulose degradation under different tillage treatments. FEMS Microbiol Ecol 91:7. https://doi.org/10.1093/femsec/fiv069

Dong W, Zhang X, Wang H et al (2012) Effect of different fertilizer application on the soil fertility of paddy soils in red soil region of southern China. PLoS One 7:e44504. https://doi.org/10.1371/journal.pone.0044504

Dubey A, Malla MA, Khan F et al (2019) Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 28:2405–2429

Dupont AÖC, Griffiths RI, Bell T et al (2016) Differences in soil micro-eukaryotic communities over soil pH gradients are strongly driven by parasites and saprotrophs. Environ Microbiol 18:2010–2024

Fakruddin M, Mannan KSB (2013) Methods for analyzing diversity of microbial communities in natural environments. Ceylon J Sci (Bio Sc) 42:19–33

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15:579–590. https://doi.org/10.1038/nrmicro.2017.87

Fierer N, Lauber CL, Ramirez KS et al (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J. https://doi.org/10.1038/ismej.2011.159

Fujii T, Morimoto S, Hoshino YT, Okada H, Wang Y, Chu H, Zhihua B, Yuko M, Tsushima S (2009) Metagenomic approach to soil microbial diversity and functions. Marco symposium 2009, Workshop 5: 1–9

Ghazanfar S, Azim A, Ghazanfar MA, Anjum MI, Begum I (2010) Metagenomics and its application in soil microbial community studies: biotechnological prospects. J Animal Plant Sci 6:611–622

Hai-Ming T, Xiao-Ping X, Wen-Guang T, Ye-Chun L, Ke W, Guang-Li Y (2014) Effects of winter cover crops residue returning on soil enzyme activities and soil microbial community in double-cropping rice fields. PLoS One 9:e100443. https://doi.org/10.1371/journal.pone.0100443

Hara S, Morikawa T, Wasai S et al (2019) Identification of nitrogen-fixing Bradyrhizobium associated with roots of field-grown sorghum by metagenome and proteome analyses. Front Microbiol 10:407. https://doi.org/10.3389/fmicb.2019.00407

Hartman K, van der Heijden MGA, Wittwer RA et al (2018) Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6:14. https://doi.org/10.1186/s40168-017-0389-9

He Z, Deng Y, Zhou J (2012) Development of functional gene microarrays for microbial community analysis. Curr Opin Biotechnol 23:49–55

Hirsch PR, Mauchline TH, Clark IM (2010) Culture-independent molecular techniques for soil microbial ecology. Soil Biol Biochem 42(6):878–887

Hjort K, Presti I, Elväng A et al (2014) Bacterial chitinase with phytopathogen control capacity from suppressive soil revealed by functional metagenomics. Appl Microbiol Biotechnol 98:2819–2828

Hong KS, Lim HK, Chung EG et al (2007) Selection and characterization of forest soil metagenome genes encodinglipolytic enzymes. J Microbiol Biotechnol 17(10):1655–1660

Ismail Z, Sam CK, Yin WF, Chan KG (2012) Tropical mangrove swamp metagenome reveals unusual abundance of ecologically important microbes. Int Microbiol 8:13–21

Jacoby R, Peukert M, Succurro A et al (2017) The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. Front Plant Sci 8. https://doi.org/10.3389/fpls.2017.01617

Jansson JK, Hofmockel KS (2018) The soil microbiome from metagenomics to metaphenomics. Curr Opin Microbiol 43:162–168

Jansson JK, Hofmockel KS (2020) Soil microbiomes and climate change. Nat Rev Microbiol 18:35–46. https://doi.org/10.1038/s41579-019-0265-7

Jesus EDC, Liang C, Quensen JF, Susilawati E, Jackson RD, Balser TC, Tiedje JM (2016) Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. GCB Bioenergy 8:481–494

Köhl L, Oehl F, Van Der Heijden MGA (2014) Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecol Appl 24:1842–1853

Lammle K, Zippera H, Breuer M et al (2007) Identification of novel enzymes with different hydrolytic activities by metagenome expression cloning. J Biotechnol 127:575–592

Lee MH, Lee SW (2013) Bioprospecting potential of the soil metagenome: novel enzymes and bioactivities. Genomics Inform 11(3):114–120

Lei Y, Xiao Y, Li L (2017) Impact of tillage practices on soil bacterial diversity and composition under the tobacco-rice rotation in China. J Microbiol 55:349–356

Lin W, Wu L, Lin S, Zhang A, Zhou M, Lin R, Wang H, Chen J, Zhang Z, Lin R (2013) Metaproteomic analysis of ratoon sugarcane rhizospheric soil. BMC Microbiol 13:135. https://doi.org/10.1186/1471-2180-13-135

Liu B, Jia G, Chen J, Wang G (2006) A review of methods for studying microbial diversity in soils. Pedosphere l6:18–24

Mocali S, Benedetti A (2010) Exploring research frontiers in microbiology: the challenge of metagenomics in soil microbiology. Res Microbiol 161(6):497–505. https://doi.org/10.1016/j.resmic.2010.04.010

Molina-Montenegro MA, Ballesteros GI, Castro-Nallar E, Meneses C, Torres-Díaz C, Gallardo-Cerda J (2018) Metagenomic exploration of soils microbial communities associated to Antarctic vascular plants. Peer J Preprints 6:e26508v1. https://doi.org/10.7287/peerj.preprints.26508v1

Moran MA (2009) Meta transcriptomics: eavesdropping on complex microbial communities–large–scale sequencing of mRNAs retrieved from natural communities provides insights into microbial activities and how they are regulated. Microbe 4:329

Myrold D, Zeglin L, Jansson J (2013) The potential of metagenomic approaches for understanding soil microbial processes. Soil Sci Soc Am J 78:3. https://doi.org/10.2136/sssaj2013.07.0287dgs

Nacke H, Engelhaupt M, Brady S et al (2012) Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes. Biotechnol Lett 34:663–675

Nannipieri P, Penton CR, Purahong W (2019) Recommendations for soil microbiome analyses. Biol Fertil Soils 55:765–766. https://doi.org/10.1007/s00374-019-01409-z

Naylor D, Fansler S, Brislawn C et al (2020) Deconstructing the soil microbiome into reduced complexity functional modules. mBio 11. https://doi.org/10.1128/mBio.01349-20

Neelakanta G, Sultana H (2013) The use of metagenomic approaches to analyze changes in microbial communities. Microbiol Insights 6:37–48

Nelkner J, Henke C, Lin TW et al (2019) Effect of long-term farming practices on agricultural soil microbiome members represented by metagenomically assembled genomes (mags) and their predicted plant-beneficial genes. Gen Dent 10(6):424. https://doi.org/10.3390/genes10060424

Ngom B, Liu X (2014) Techniques for tracking microbial community structure and function in natural environment and engineered systems. Int J Sci Res 3:800–807

Orellana LH, Chee-Sanford JC, Sanford RA (2018) Year-round shotgun metagenomes reveal stable microbial communities in agricultural soils and novel ammonia oxidizers responding to fertilization. Appl Environ Microbiol 84(2):e01646–e01617. https://doi.org/10.1128/AEM.01646-17

Perito B, Cavalieri D (2018) Innovative metagenomic approaches for detection of microbial communities involved in biodeteriorattion of cultural heritage. IOP Conf Series: Mater Sci Eng 364:012074. https://doi.org/10.1088/1757-899X/364/1/012074

Petrosino JF, Highlander S, Luna RA, Gibbs RA, Versalovic J (2009) Metagenomic pyrosequencing and microbial identification. Clin Chem 55:856–866

Rasul M, Yasmin S, Hakim S et al (2020) Metagenomic analysis of bacterial community associated with rhizosphere and phyllosphere of basmati rice. bioRxiv:4. https://doi.org/10.1101/2020.04.09.034009

Rawat S, Johri BN (2014) Thermophilic fungi: diversity and significance in composting. KAVAKA 42:52–68

Rincon-Florez VA, Carvalhais LC, Schenk PM (2013) Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity 5:581–612

Sabale SN, Suryawanshi PP, Krishnaraj PU (2019) Soil metagenomics: concepts and applications. In: Hozzein WN (ed) Metagenomics – basics, methods and applications. IntechOpen. https://doi.org/10.5772/intechopen.88958

Sengupta A, Dick WA (2015) Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. Microb Ecol 70:853–859

Souza RC, Cantão ME, Vasconcelos ATR et al (2013) Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. Appl Soil Ecol 72:49–61

Souza RC, Cantãoc ME, Nogueira MA, Vasconcelo ATR, Hungria M (2018) Outstanding impact of soil tillage on the abundance of soil hydrolases revealed by a metagenomic approach. Braz J Microbiol 49:723–730

Srour AY, Ammar HA, Subedi A et al (2020) Microbial communities associated with long-term tillage and fertility treatments in a corn-soybean cropping system. Front Microbiol 11:1363. https://doi.org/10.3389/fmicb.2020.01363

Val-Moraes SP, Pedrinho EPN, Lemos EGM et al (2013) Molecular identification of fungal communities in a soil cultivated with vegetables and soil suppressiveness to Rhizoctonia solani. Appl Environ Soil Sci. https://doi.org/10.1155/2013/268768

Van-Der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

Villamizar GAC, Nacke H, Boehning M et al (2019) Functional metagenomics reveals an overlooked diversity and novel features of soil-derived bacterial phosphatases and phytases. mBio 10(1):e01966-18. https://doi.org/10.1128/mBio.01966-18

Vogel TM, Simonet JJK, Hirsch PR, Tiedje JM, JElsas JD, Bailey MJ, Nalin R (2009) TerraGenome: a consortium for the sequencing of a soil metagenome. Nat Rev Microbiol 7:252

Weinstock GM (2012) Genomic approaches to studying the human microbiota. Nature 489:250–256

Woźniak M, Grządziel J, Gałązka A et al (2019) Metagenomic analysis of bacterial and fungal community composition associated with Paulownia elongata × Paulownia fortune. BioRes 14(4):8511–8529

Yang Y, Dong M, Cao Y, Wang J, Tang M, Ban Y (2017) Comparisons of soil properties, enzyme activities and microbial communities in heavy metal contaminated bulk and rhizosphere soils of Robinia pseudoacacia L. in the northern foot of Qinling Mountain. Forests 8:430. https://doi.org/10.3390/f8110430

Yu H, Si P, Shao W, Qiao X, Yang X, Gao D, Wang Z (2016) Response of enzyme activities and microbial communities to soil amendment with sugar alcohols. Microbiol Open 5:604–615

Zhang W, Li F, Nie L (2010) Integrating multiple omics analysis for microbial biology: application and methodologies. Microbiology 156:287–301

Part V
Bacteria in Nitrogen Fixation

Chapter 15
Beneficial Effects of Nitrogen-Fixing Bacteria for Agriculture of the Future

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Abstract Nitrogen is one of the essential macronutrients (among N, P, and K) for plant growth, and constitutes one of the most abundant elements in surface/crust and earth's atmosphere, but still this nitrogen is not available for immediate uptake by plants and crops are always facing nitrogen deficiency as a crop nutrient in all part of the globe. Nitrogen fixation is necessary for life sustenance**.** In soil microbes that carry nitrogen fixation are termed as diazotrophs. Nitrogen-fixing bacteria forms symbiotic association with plants and fungi. In some plants there is formation of nodules (in legume and actinorhizal, which fix most of the nitrogen) and in some nonnodulated (endosymbiont with cyanobacteria). In this chapter we have focused upon the beneficial effects of nitrogen-fixing bacteria for agriculture.

Keywords Cyanobacteria · Biological nitrogen fixation · Bacterial nitrogen fixation · Atmospheric nitrogen · Haber process · Nitrogen fixers

15.1 Introduction

Biological nitrogen fixation is one of the vital processes for agricultural productivity in most of the cropping systems as its inputs are the direct source for atmospheric nitrogen and its rotational effects like disease and pest control (Vitousek et al. 2002, Vats et al. 2019). Other recent advances in molecular biology techniques may issue further new opportunities regarding the study of ecology of the root nodule bacteria.

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It may also help in the improvement of the selection of elite strains for inoculation process. A basic understanding of the genetic basis of nodulation in pasture legumes including grains helps in the improvement of inoculation technologies (Kumar et al. 2020a; Kumar et al. 2020b, Bhargava et al. 2020; Bhargava et al. 2019a; Bhargava et al. 2019b).

Tropical and temperate pastures can also be improved with the use of other effective measurements such as removal of nutritional constraints, inoculations, and by using some alternative legume species. When nitrogen fixation in any crop increases it may result into locating difficulties in the legumes host, the microsymbiont, and the environment. The plant growth promoting bacteria is used to improve the productivity of one of the most important cereals crops like maize and wheat (Ijaz et al. 2019). Many other researches in this field have also enhanced the understanding on the physiology of bacteria as well as the mechanism of bacteria–plant interaction. Among all other bacteria, *Azospirillum brasilense* is one of the most promising and important one. Other field experiments have also shown increase in productivity consistently. Moreover, *Azospirillum* is vital to genetic studies and its importance in nitrogen metabolism is also known with its regulation of nitrogen fixation (Coniglio et al. 2019). The knowledge accumulated leads to its future genetic manipulation which leads in the maximization of the pros of plant growth promoting bacteria. Environmental issues arising from the manufacturing units of chemial fertilizers and their indiscriminate use has led to an increase in the reactive nitrogen in the atmosphere, hence there is an urgent need to focus upon biological nitrogen fixation (Torabian et al. 2019). Bacterial nitrogen fixation (BNF) is expected to contribute 105 Tg $yr.^{-1}$ worldwide (Staccone et al. 2020). Fixation of nitrogen via the nitrogenase enzyme is catalyzed. Advances include discovering alternate nitrogenases, clarifying information on mechanism of action, finding new nitrogenases, presence of nitrogenase partners in eukaryotes, etc. New attempts are being made to develop cereal plants to nodulate and fix nitrogen (Bennett et al. 2020; Gupta et al. 2018; Goel et al. 2017). The discovery by mycorrhization and nodulation of specific signaling mechanisms, cereal endophytes discovery, and advancement in plants physiology provided these approaches with a new impetus. Indeed, more rigorous evidence is still needed for the contribution of agronomically significant N quantities through endophytes. Molecular biological advancing involves better understanding of the native rhizoidal diversity and provides an enticing opportunity to improve BNF for breeding high-nodulation genotypes, combined with advances in plant breeding. Recent development has included the discovery of new rhizoidal organisms, focuses on climate change research and adaption to various abiotic stresses, such as high temperatures and drought. The population of the Planet is expected to rise exponentially and to almost double its present status to 10 billion by 2035 (Bockman 1990). Human population, 90% is projected to live in emerging Asia, Africa, and Latin America's tropical and subtropical regions (Chapman and Dutt 2019, Vats and Bhargava 2017; Bhargava et al. 2017). Plant products already supply 80% of the tropical countries caloric and dietary protein needs, and it is not supposed to change in the immediate future. Around 10% of the total photosynthesized carbon was used in 1910 (Spaink et al. 2012). Human beings

actually use 40% of this energy, and humans are expected to need 80% by 2030. At Global level, individual protein and caloric intake is going to increase. At present scenario, 70 g protein and 2400 calories per day will rise to 38–125 g protein and 1800–3500 calories per day for developing countries (Legocki et al. 1997). The predicted doubling of the world's population is likely to intensify global food disparities. Apparently, the current protein and caloric intake rates would require exponential increases in cultivation over the next 40 years. Despite a substantial decline in most of the prime agricultural property, this increased production must be done and vast areas which are now considered marginal will have to be used. Nitrogen is one of the limiting nutrients for all crops and plants, and its assimilation and acquisition is second most important after the photosynthesis (Legocki et al. 1997).

The input of organic products and biological nitrogen fixation in Indian agriculture must be improved urgently. The production of competitive rhizobium strains, which are resistant to high temperature, drought, nitrate, acidity and other abiotic stresses is very important (Bhattacharjee et al. 2008). The most rapids in Indian agriculture are the methods of management of soil, including soil recuperation, nutrient deficiency correction, organic application, and screening of segregation materials in legumes in low-N soils, where the collection/breeding of high-nodulation cultivars is not feasible. Inoculation may be a choice. The production of transgenic inoculants, nitrogen-independent cereals, and entophytic N fixation requires a very detailed description and stronger financing justification (Sutton et al. 2017).

15.2 Why Nitrogen Fixation is Important to Agriculture?

Nitrogen is found in abundance in the atmosphere and it is one of the most abundant elements on earth (Kumar et al. 2020a, 2020b; Saxena et al. 2019). 78% of the earth's atmosphere is in the form of N_2. N_2 is the most important nutrient required for plant growth as well as for their metabolic processes. The atmospheric nitrogen is converted into usable form of nitrogen compound by nitrogen fixation. There are three ways through which atmospheric nitrogen can be converted into nitrate/ammonium which is found in the soil (Saxena et al. 2019; Anastasopoulou et al. 2016). The first one is through atmospheric fixation events like lightning strikes, rain, snow which depends upon climatic changes. Another way is through man-made fertilizer which utilizes the Haber process which helps in the production of ammonium. Finally, there are some plants that had a symbiotic relationship with diazotrophic bacteria that fix atmospheric nitrogen into ammonium (Oses et al. 2018). Diazotrophs are those bacteria which contains enzymes, i.e. nitrogenase which is responsible for the process of biological nitrogen fixation (BNF). Diazotrophs include azotobacteraceae, cyanobacteria, frankia, green sulfur bacteria, and rhizobia. The wide range of plants which have symbiotic relationship with diazotrophs comes from the legume family, i.e. known as Fabaceae (Feng et al.

2018). This family includes plants like alfalfa, clovers, lupines, peanuts, soybeans, and rooibos. Legumes have certain root nodules that harbor the diazotrophs, provide them with the certain anaerobic conditions necessary to fix nitrogen. After the plant death the nitrogen which is fixed is released and acts as a natural fertilizer for the soil and it also helps in providing usable nitrogen to other non-legumes plants (Martin 2017; Painuly et al. 2019; Vats and Kumar 2015).

The use of Haber process in the agriculture is mainly for the production of fertilizers that is chemically efficient which requires boiling, cooling and sustain to very high pressure throughout the entire process (Pfromm, 2017). However, this also requires the use of fossil fuels which results into the high costs to farmers. Around 2% of the total world's energy goes into the production of fertilizers alone. By far, fertilizer is the most common method to provide ammonium to crops but many farmers are already using the alternative method also which includes nitrogen-fixing bacteria. Different farmers use different techniques regarding the process. Like, some farmers use crop rotation techniques in this they plant leguminous crops so that they can fertilize the soil for future harvesting (Rao and Balachandar 2017).

15.3 How Useful is Nitrogen-Fixing Bacteria in Agriculture

Bacteria supporting nitrogen fixation and plant growth are undoubtedly the most promising biotechnological resource in the short term for increasing productivity in agriculture (Rosenblueth et al. 2018). For the plant growth and development, nitrogen fixation and the development of phytohormone by these bacteria is the most important factor. Nonetheless, it is not clear what fundamental mechanisms are responsible for that efficiency by associative bacteria. Therefore, the duration of the promotion of plant growth depends on the efficient interaction of plant and bacterial genotype with the transfer of fixed nitrogen from bacteria to plant. Bacterium-sustaining plant growth (PGPB) are used in Brazil, Argentina, Mexico, India, and Europe, while Azospirillum brasilense is one of the most commonly used source of PGPB. Field experiments showed success rates of Azospirillum inoculation ranging from 60% to 70%, with statistically important increase in yields from 5% to 30%.

15.4 Why *Azospirillum Brasilense*?

Azospirillum brasilense is the well-studied, genetically tractable, nitrogen-fixing (diazotrophic), Gram-negative, alpha proteobacterial bacterium. *Azospirillum brasilense* colonizes the numerous grasses and cereals throughout the rhizosphere (Quatrin et al. 2019). This species is one of the most extensive genomes in the Proteobacteria phylum. Each cell has two sets of flagellar systems: a polar flagellum and a lateral flagella, and expresses themselves according to the requirements of development. The size of the genome is 7.530.241 bp, one of the largest genomes of

the bacteria. It is strongly glycosylated with flagellin. A freshly discovered bushy stuff appears on the hook. A variety of genes are expected to maintain signal peptides (Moure et al. 2019).

15.4.1 Regulation of Nitrogen Fixation in A. brasilense

NIF promoters are dependent on the NifA protein to start transcription. It is possible to control the NifA role at two levels: the gene transcription and the protein production of nifA, while oxygen and/or fixed nitrogen regulation occurs at both levels (Li et al. 2019). The first isolation and the characterization of a mutant nifA strain (FP10) were achieved by Pedrosa and Yates in the year 1984. NifA gene expression in A.*brasilense* has been studied by the researcher for understanding the nitrogen fixation process. Only ammonium and oxygen are partially removed and are maximized under conditions of nitrogen fixation. Oxygen levels have little effect on their expression if ammonium is absent, but ammonium is partially repressed under oxygen-restricting conditions and repressed almost entirely under high ammonium and high oxygen conditions. Two sequences identical to the Fnr consensus sequence downstream from the likely start of the transcription showed the nifA promoter region review, but their reliability was not proven. In terms of oxygen substitution and *Azorhizobium caulinodans* for ammonium control of promoter activities, the nifA expression regulation looks close to that of *Sinorhizobium meliloti*. Indeed, the involvement of FixJ- FixL and FNR- protein FixK with A. *brasilense* are the two major involvement systems. In Tasking of *A.brasilense* NifA protein is regulated by concentrations of oxygen and ammonium. NifA's oxygen sensitivity is likely linked to a connecting area between the central and carboxyl terminal domains containing a preserved Cys-X4-Cys, which together with 2 cysteine residue contained in the central domain, forms the CysX11-Cys-X19-Cys-X4-Cys cysteine motif allegedly involved in forming an oxygen sensitive FeS clusters (Souza et al. 2014). Analysis of mutagenesis showed the cysteine motif to be important for the B. *Japonicum* & H. *Seropedicae* In vivo NifA operation. For A.*brasilense,* a functional GlnB gene is required. GlnB's regulatory role of NIFA activity presumably includes his N-terminal domain, as a truncated NifA protein is functioning irrespective of the GlnB and is unstable against ammonium concentrations. In addition, A.*brasilense*, NifA is inactive in E.Coli; however A.*brasilense* GlnB expression is able in this context to activate the NifA. In contrast, refined GlnB and the GAF N-terminal can associate in vitro. A *brasilense's* nitrogenase operation is reversibly managed by the ADP dinitrogenase reductase (NifH) ribosylation in response to rising concentrations of ammonium or energy loss. Such mechanism is considered as nitrogenase turn-off and catalyzed by the dinitrogenase reductase ADP—ribosyltransferase (DraT) (Souza et al. 2014). When an additional ammonium is supplied or energy levels restored, the ADP-ribose group is separated from NifH by the dinitrogenase reductase.

It is important to note that, dinitrogenase reductase activating glycohydrolase (DraG) can be triggered by the removal of ADP-ribose group and which further play an important role in activation of nitrogenase. The two enzymes are regulated in reaction to the stimulus contrast: DraG is active and DraT is inactive in the nitrogen fixation setting, while ammonium addition leads to DraT activation and DraG inactivation. A concept has been developed for DraT and DraG ammonium management activities in A.*brasilense* (Souza et al. 2014). The PII proteins GlnB and GlnZ are uridylylated and soluble in cytosol under nitrogen fixation conditions. DraG is active and DraT is inactive under this situation, so NifH does not alter or function. With the rise in ammonium levels, the uridlyly-removing enzyme GlnD deuridylizes the PII proteins. Deuridylylated GlnB stimulates the DraT transmission activity which triggers NifH ADP ribosylation by means of the subsequent nitrogenase activity. DraG/GlnZ complexes that interact with the membrane protein AmtB are formed by deuridylylation of the GlnZ, eliminating the DraG from cytoplasm and inhibiting the active occurrence of ADP-ribosyl glycohydrolase (Souza et al. 2014). This model can be able to justify nitrogenase turn off in the presence of ammonium ions, but the anaerobic NifH modify attributes in A brasilense does not depend on GlnZ, GlnB, or AmtB, so an independent mechanism must take place.

15.4.2 Nitrogen-Fixing Bacteria as Biofertilizer

Organic fertilizers are product(s) containing cells of assorted microorganisms that, when applied to seeds, plant surfaces, or soils, colonize the plant or interior, and promote growth through the conversion from unavailable type to accessible nutrient essential components (nitrogen, phosphorus) through biological processes such as nitrogen attachment and rock solubilization (Ko Latt et al. 2018; Vats 2017; Vats and Bhargava 2017). In most environments, fixed nitrogen is a nutrient with molecular nitrogen from the atmosphere, as primary source for biosphere nitrogen. It is difficult to assimilate molecular nitrogen directly with plants but only prokaryotic cells can produce it via biological nitrogen fixation process. A few bacterial species were considered nitrogen fixers for many years, but in the last 30 years nitrogen fixation proved to be expressed in most bacterial phyla and methanogenic archaea. In two main groups of non-related bacteria, rhizobia (alpha-) which primarily associate with leguminous plants belonging to a super family of angiosperms (Fabaceae) and Frankia (in Actinobacteria) that are associated with a broader plant spectrum from eight families are the property of symbiotically fixing nitrogen within nodules of vascular plants. Biological fixation of nitrogen is one way to transform elementary nitrogen into plant accepted form. Bacteria which fix nitrogen (NFB) convert inert atmospheric N_2 into organic compounds. A living fertilizer consisting of microbial inoculation agents or groups of microorganisms is able to fix atmospheric nitrogen in biofertilizer or a N fixing agent is used for biofertilization (Kyaw et al. 2018). They are grouped together into free living bacteria (*Azotobacter, Azospirillum*), blue green algae,

Rhizobium, Frankia, and Azolla symbionts. On the other hand N_2-fixing bacterium related to non-legume are *Achromobacter, Alcaligenes, Arthrobacter, Acetobacter, Azomonas, Beijerinckia, Batillus, Clostridium, Enterobacterium, Erwinia, Derxia, Disulfovibrio, Corynebacterium, Campylobacterium, Herbaspirillum, Klebsiella, and Rhodopseudobacterium.*

15.4.2.1 Rhizobium

Belongs to the Rhizobiaceae family, which have a symbiotic relationship, only hold nitrogen with legumes 50–100 kg/ha. It colonizes tumor roots such as growths known as root nodules that are used as ammonia processing plants for different vegetables. In a symbiotic association of pulses and certain non-legumes such as parasponia, the rhizobium fixes atmospheric nitrogen (Andrews and Andrews 2017).

15.4.2.2 Azospirillum

A heterotrophic and associative existence belongs to the family Spirilaceae. They also develop growth-regulating substances as well as their nitrogen-fixing capacities of around 20–40 kg/ha. Azospirillum forms an associative symbiosis with many plants, particularly those with the C4-dicarboxyliac pathway of photosynthesis (Hatch and Slack pathway), since they grow and fix nitrogen in organic acid salts such as malic, aspartic acid. Therefore it is recommended primarily for maize, sugarcane, sorghum, pearl millet, etc. Not only does the Azotobacter colonizing the roots remain on the root surface, but a large proportion of them also penetrate the root tissues and live in harmony with the plants. On root tissue, however, they do not develop any noticeable nodules or growth out (Fukami et al. 2018).

15.4.2.3 Azotobacter

Belongs to this Azotobacteriaceae family, aerobic, free living or in nature heterotrophic. Azotobacters are found in acidic and alkaline soils with A. Chroococcum are by far the most prevalent species found in arable soils. Given the lack of organic matter and the presence of antagonistic microorganisms in the soil, the amount of Azotobacter rarely reaches 104–105 g^{-1} of soil. The bacterium produces anti-fungal antibiotics that inhibit the growth of several pathogenic fungi in the root area, thereby preventing some scale of seedling mortality. A variety of crop plants such as rice, maize, sugarcane, bajra, vegetables, and plantation crops have recorded the occurrence of this organism from the rhizosphere (Rodrigues et al. 2018).

15.5 Blue Green Algae (BGA) and Azolla

BGA belongs to eight different families, and is phototrophic in nature, contains Auxin, Indole acetic acid, and Gibberllic acid, BGA fixes 20–30 kg N/ha in submerged rice fields. BGA are abundant in paddy, because of which BGA is also called "paddy species". N is the main input needed for lowland rice production in large quantities. Soil N and BNF are major sources of N for low land rice by associated species (Dobrojan et al. 2016). The requirement of 50–60% N is met through the combination of organic soil N and BNF mineralization by free living and associated bacteria from rice plants. BNF will increasingly fulfill the need for fixed nitrogen, rather than industrial nitrogen fixation, to achieve food security through sustainable agriculture. Some N fixing BGA is filamentous, consisting of a chain of vegetative cells like specialized cells called heterocyst, acting as a synthesis micronodule and N fixing unit. BGA forms a symbiotic association capable of fixing nitrogen with fungi, liverworts, ferns, and flowering plants, but the most common symbiotic association has been found between Azolla and Anabaena azollae (BGA), a free-floating aquatic fern. The key factor in using Azolla as a biofertilizer for rice crops is its rapid soil decomposition and its effective nitrogen availability to rice plants. Such biofertilizers or biomanures in addition to N-fixation often contribute significant amounts of P, K, S, Zn, Fe, Mb, and other micronutrients. Azolla can be used as green manure by adding it before rice planting in the fields. On a commercial scale pinnata and the same can be propagated by vegetative means (Akhtar et al. 2020).

15.6 Nitrogen-Fixing Endophytes

A growing number of cases identify the presence of nitrogen-fixing bacteria within a host plant's plant tissues that do not show symptoms of diseases, with the most studied genera being *Azoarcus sp, Gluconacetobacter,* and *Herbaspirillum*. Endophytes multiplying and spreading inside plant tissues without causing damage. Early infection steps may be similar to those recorded with rhizosphere bacteria, initially involving surface colonization at the site of root hair emergence (Puri et al. 2020). Type IV pili has been found to be important for this process in the case of *Azoarcus*, and hydrolytic enzymes or endoglucanases are involved in tissue penetration. The concentration of bacteria recovered after root system sterilization can reach up to 108 CFU per g of dry mass. Another characteristic is the systemic spread of bacteria found in plant xylem vessels and shoots as defined in the case of *G.diazotrophicus* sugar cane infection and in the case of C4-gramineous plant *Miscanthus sinensis* by H infection.

15.6.1 Nitrogen Fixation by Free Living Heterotrophs

Most heterotrophic microbes reside in the soil and cure massive nitrogen amounts despite direct communication with the other species. Sources for this form of nitrogen-fixing bacteria included *Azotobacter, Bacillus, Clostridium, Klebsiella* organisms. Some free living organisms have chemolithotrophic capacity, and can therefore use inorganic compounds as a source of energy. As oxygen can inhibit nitrogenase, free living organisms function as anaerobic or a microaerophilic during the attachment of nitrogen. Giving them a significant share in the overall fixing levels of nitrogen is usually regarded because of the lack of sufficient carbon and energy resources (Perakis et al. 2017).

15.7 Associative Nitrogen Fixation

Azospirillum species may create close relationships with many of the Poaceae (grass) groups, including cereals of agricultural importance such as rice, wheat, maize, avenue, and barley. These bacteria repair significant nitrogen levels in the host plant rhizosphere. Several factors, including soil temperature (Azospirillum species are thriving in more temperate and/or tropical environments), the plant's ability to provide an environment with rhizosphere that is low in oxygen pressure, the availability of host photosynthesis for bacteria, bacterial competitiveness, and the efficiency of nitrogenase, are assessed (Wagner 2011).

15.8 Symbiotic Nitrogen Fixation

Several microorganisms symbiotically bind nitrogen to a parent plant by partnering. The plant provides sugar from photosynthesis that is used for the resources it needs to fix nitrogen by the nitrogen-fixing microorganism. The microbe supports the host plants with sufficient nitrogen for their development in return for this carbon inputs. The symbiosis of Azolla fern with the cyanobacterium, for example Azolla fern with Anabaena azollae is a this kind of nitrogen fixation. At the base of Azolla fronds, Anabaena colonizes cavities. In complex cells known as heterocyst, cyanobacteria release essential quantities of nitrogen. This symbiosis has been used in wetland paddies in Southeast Asia for at least 1000 years as a biofertilizer. Rice paddies are generally covered by Azolla flowering, which in the planting season fix to 600 kg N ha^{-1} $yr.^{-1}$ (Farooq et al. 2019; Braghiere et al. 2019; Singh and Vats 2019; Sharma et al. 2018; Sharma et al. 2014).

Sticking to the growth and development of plants nitrogen is extremely important but it cannot be used as atmosphere nitrogen in its most prevalent form. Instead, plants rely on nitrogen forms such as ammonia and nitrate combined or set. The crop

systems in the form of industrial nitrogen fertilizers obtain a good deal of this nitrogen. Using these fertilizers has spurred to ecological problems throughout the world, including the creation of dead coastal regions. On the other hand, biological nitrogen fixation provides a natural means of providing plants with nitrogen. It is a key component of many marine and terrestrial ecosystems in our biosphere (Farooq et al. 2019; Braghiere et al. 2019).

15.9 Recent Advances in Biological Nitrogen Fixation in Agricultural System

The availability of nitrogenous fertilizers worldwide has increased crop yields and food (Vats et al. 2013a; Vats and Mishra 2016; Vats and Bhargava 2017; Negi and Vats 2013; Maurya et al. 2014; Maurya et al. 2013). The Haber–Bosch method of ammonia processing has made a difference for our survival from all the technical marvels of the last century, and is considered the most precious innovation of the intervening millennium. It has, however, come at substantial economic and environmental costs. Industrial nitrogen fixation by the world industry has environmental impacts of nitrogen pollution at $100 billion per annum. Intensive crops utilizing highly analytical fertilizers, together with a concomitant decline in recycling of organics or waste, contributed to a decrease in soil organic carbon, weakened soil physical properties, decreased soil biodiversity, and increased demand for non-applied nutrients, which led to poor yields, lower productivity, and soil health problems. Not unexpectedly, biological innovations such as composting, biological nitrogen fastening of legume (BNF), biofertilizer, integrated nutrient management, biopesticides, etc. are now updated. It is also understood to the world that dependence on chemical inputs based farming alone is unacceptable over the long term, and it is important to support cultivation, soil health, and soils biodiversity by integrating plant nutrient systems (IPNS) with a combination of fertilizer, organics, and microbial inoculants (Farooq et al. 2019; Vats and Kumar 2015; Ojha et al. 2013; Vats et al. 2019; Vats et al. 2011).

15.10 Global Estimates of BNF

An increased reactive nitrogen form (Nr) in the atmosphere is a matter of concern. Since 1970, the global population has increased by 78% and the production of reactive nitrogen by 120%. Efforts for N budgeting in 2050 AD demonstrate that, internationally and regionally, human activities dominate the N budget. Furthermore, land and open ocean N budgets are largely isolated, leading to a build-up of fixed types of N in most environmental reservoirs. The main uncertainties in our perception of the budget at maximum scales are naturally biological nitrogen fixation

levels, Nr accumulation in most environmental reservoirs and denitrifying production rates of N_2.The second main cycle that influences primary production is nitrogen fixation after photosynthesis, which is the basis of life on Earth. Around 2.5 × 1011 kg of NH_3 from the atmosphere is collected annually via the ammonia industry by BNF (legumes and cyanobacteria), and about 8 × 1010 kg NH_3 are made. In addition, lightning will contribute about 1 × 1010 kg of NH_3/year worldwide. As fertilizer for cropping production, approximately 2 tons of industrially fixed nitrogen is currently required to balance the effects of 1 ton of biologically fixed nitrogen on the legume crop. Biologically stationary nitrogen thus has far less effect on the global nitrogen cycle than industrially stationary nitrogen (Rao 2014). Nitrogen fertilizers are widely used by the world population to meet food demands. It is expected that demand for total nutrients in fertilizers had risen to 2% per annum from 2011 to 2015. Nitrogen demand is projected to grow at 1.7% annually worldwide and by 2.6% in South Asia. Global consumption of fertilizer like nitrogen (N), potassium expressed as potash (K_2O), and phosphorus expressed as phosphate (P_2O_5) is 186.67 million tons in combined in 2016. The demand for N, P_2O_5, and K_2O is forecast to grow annually on average by 1.5, 2.2, and 2.4 percent, respectively, from 2015 to 2020 as per the report published by Food And Agriculture Organization Of The United Nations - Rome, 2017as world fertilizer trend and outlook to 2020 (FAO 2017).

Overall, BNF contributes about 107 Tg of nitrogen (1 Tg = 1 million tons) for natural terrestrial habitats, while marine N fixation contributes around 121 Tg of nitrogen annually. In farm crops and fields, cultivation-induced BNF contributes 33 Tg annually. The breakdown consists of BNF of symbiotic grade with the seed legume associated with the range: 10 Tg(range 8–12 Tg); legume plant (food and green manure)-12 Tg/yr.; non umbium Rhizobium N of seed species-4 Tg/yr., cyanobial N of wet rice fields 4–6 Tg; and endophytic N of sugar cane-1-4 Tg of organisms. Therefore, the average terrestrial fixation of nitrogen is 140 Tg N/yr. The projected increase from soybean and meat output in cultivation inducted BNF could be up to 40 Tg/yr.

Table: Report published by World Fertilizer Trends and outlook 2020[a]

(Thousand Tonnes)	2015	2016	2017	2018	2019	2020
Demand for non-fertilizer nutrient use	33,616	34,506	35,308	36,207	36,786	37,521
Total world nutrient capacity of Ammonia (NH3) as N	174,781	181,228	185,222	186,804	186,920	188,310
World supply of ammonia, Ammonia (NH3) as N	154,773	158,850	166,402	168,987	169,693	170,761
World demand for fertilizer nutrient nitrogen (N)	110,027	111,575	113,607	115,376	117,116	118,763
Potential world balance of nitrogen Nitrogen (N)	11,130	12,769	17,487	17,404	15,792	14,477

[a]Food and Agriculture Organization of the United Nations (2017)

In 1995 the Haber–Bosch cycle, approximately 100 Tg N per year of Ammonia, of which about 86 percent was used to make fertilizers, fixed approximately three times as much N as Ammonia induced by cultivating BNF. With the establishment of more plants, the industrial fixation of nitrogen rises each year and by 2006 it reached 121 Tg with the worldwide manufacture of 105 Tg of nitrogen fertilizer. While the conclusions are drawn that concerning the depletion of soil and its organic nitrogen content by synthetic nitrogen fertilizers, there can be no disagreement with that a transition to agricultural diversification may be needed and applicable solution. It can be achieved through legume-based crop rotations, which provide a valuable means of reducing the intensity of ammonia fertilization with the estimated fixed N annual inputs to be 2.95 Tg for the pulses and 18.5 Tg for the legumes of the oilseeds. Soybean is the dominant crop legume contributing 50% of the world's crop legume area and 68% of world growth. The soybean was measured at 16.4 Tg N per year, representing 77% of the N of the vegetables. Annual N2 fixation production was measured at 12–25 Tg (pastures, legumes), 5 Tg (rice), 0.5 Tg (sugar canes), <4 Tg (non-leguminous cropland), and <14 Tg (extensive savannas), totaling 50–70 Tg N fixed in farming systems biologically. The average BNF values in legumes, cereals, olive, seed, vegetables, and fodder crops in India are more than 190 million hectares, based on the response to the experiments carried out by the All India coordinated Biological Nitrogen Specification Researches Project (AICRP-BNF), cultivated in Indian farming, are 3.68 Tg per year.

15.10.1 Nitrogenase Biotechnology

The biological fixation of nitrogen is an anaerobe cycle catalyzed by the nitrogenase enzyme and involves machinery that assimilates reduction, ATP, and ammonia. Throughout the past century, and in particular over the last two decades in genetics and biochemistry, tremendous progress was made in almost all aspects of biological nitrogen attachment. Nitrogenase is encrypted by a variety of operons, including regulatory genes such as nif LA, structural genes such as nif HDK and additional genes. The Klebsiella pneumonia was the E. BNF coli and the regulation, synthesis, and assembly of nitrogenase have been well studied. The whole K pneumonia nif cluster contains 20 genes in a 24 kb base pair DNA region (Burns and Hardy 2012).

15.10.2 Endophytes

It is long known that certain endophytic bacteria, which fix nitrogen, are nodule-independent associated with cereal crops. In order to pursue this strategy, new cereal endophytes that fix nitrogen at high levels would also need screening. These endophytes may be used in improved microbial inoculants, once they are identified and cultivated (Beatty and Good 2011).

15.10.3 Advances in Non-Legume BNF

In strictly terms of science and BNF calculation methods, we need to study the work of the past and make significant brain storms in the future. This applies particularly to research on the contribution of associative symbiotic and endophytes. Three well-known species are Azotobacter, Azospirillum, and Gluconacetobacter in combination with non-legume BNF.

Originally, they were all thought to encourage plant growth by fixing nitrogen but later it was realized that bacteria, including producing indole acetic acid and other mechanisms could have growth-stimulating effects (Iannetta et al. 2016).

15.10.4 Heterotrophic N Fixation

Acetylene reduction methods for calculating N2 fixation were widely and indiscriminately used, but there are serious errors when applied to soil rhizospheric or non-living N2 fixation calculation. Several workers have been challenging the possibility to repair non-symbiotic or associative or endophytic nitrogen in an agronomically relevant way (~20 kg N ha^{-1}). The final test of N2-mounted contribution is the calculation of N2-mounted net inputs in long-term experiments (> 10 years); the complexity of control and calculation is well-known for all procedures. The inputs from N2-fixation with cyanobacteria were due to positive N balancing over long periods in the U.K region. Treatment-dependent sources of N have confounded findings in controlled experiments (Perakis et al. 2017; Herridge et al. 2008).

15.10.5 Endophytic N Fixation

Cereal rhizosphere, such as rice, is especially abundant in Azospirillum and Pseudomonas species, and in Enterobacteriaceae members. In addition, various members of the genera *Alcaligenes, Azotobacter, Burkholderia, Clostridium, Flavobacterium,* and *Xanthobacter* have also been isolated with regard to *endophytic diazotrophs* from paddy field soils or wetland rice. Rice inoculation of *Serratia marcescens* resulted in large numbers of this bacterium in intercellular spaces, senescent cortical root cells, aerenchyma, and xylem vessels, but they are not identified in intact host cells. While endophytes are considered to play an important part for BNF in some grasses in current thought, rice is only a relatively small sub-population of a significantly greater rhizosphere diazotrophic population and therefore may have a minor effect (Chalk and Craswell 2018). Studies have shown that, for a comparison, in Philippine, there is only 106–107 total number of culturally isolated diazotrophic endophytes from rice wetland, compared to 1011 rhizobic bacteroids, in soybeans

(Glycine max), on the basis of 109 nodule^{-1}. There are no question about the function of N_2-fixing endophytes, although many studies demonstrate their existence within grasses and cereals, because the C distribution of roots, rhizosphere, and endophytic bacteria is not fully understood. To properly evaluate the potential of N2-fixation in these plants, a detailed and comprehensive quantitative understanding of both the grass C and N resources, their correspondence rhizosphere and endophytic bacteria is required (Moyes et al. 2016).

15.11 Advances in Legume BNF

The amount of nitrogen used in the world grain and oil saw production was estimated at 20 percent for legumes. This is able to correct about 80% of its own N requirements and also contribute to the yield of subsequent crops (Rubiales et al. 2018). Nevertheless, only under certain circumstances can all these possible advantages be utilized. The fact that the legume is included in the production method does not guarantee high BNF. Two approaches can be taken to exploit BNF: first, enhanced crop management, land management, and water management to achieve optimum productivity of BNF and, second, inoculating rhizobium or selecting host genotypes in order to ensure a greater percentage of the nitrogen fixation in the programmer (Rao 2014). Among these, the first technique has been well established and still plays the appropriate position for almost 50 years. In host plant selection, the second method is more recent. The discovery of new species has also resulted in a major revision of the rhizoidal taxonomy. There are some interesting disputes about indigenous against exotic rhizobia strains, but most of them seem to be resolved, as most of the inoculants come out of indigenous adapted rhizobia. The diversity, phylogeny, and rhizobial biogeography are of great concern today (Rubiales et al. 2018; Junior et al. 2020; Meena et al. 2018).

15.12 Climate Change and BNF

A rhizobial inoculants system aims to select an inoculants strain which is highly efficient for N2 fixation, as well as to encourage plant growth and resistance to adverse humidity and temperature conditions (Lett and Michelsen 2014). This will be an important aspect of the coping strategies for predicted warmer and drier future climate scenarios. Symbiotic N2 fixing plants seem to have greater growth reactivity than those of other functional groups. The capacity of N2 plants to respond to high CO_2 under soil conditions N enhances their competitiveness with non-fixing plants (Kantar et al. 2010). A review of 165 studies analyzing weed responses and rangelands to global change shows that legume rates increase in grass-legume swards by about 10% and atmospheric CO_2 rates double. In the absence of environmental restrictions including nutrient deficiency, low temperature, or drought, higher

atmospheric CO_2 can overall stimulate the growth and N2 fixation of most symbiotic N2 fixation plants. And increased CO_2, which will rise with predicted changes in precipitation intensity and frequency expected to follow the rise in CO_2, will provide some defense against drought due to the decrease in N2.It greatly limits the characterization of atmospheric environments under which N2 fixation at high carbon levels can or cannot be induced. Feedback on the N2 fixation and photosynthesis was not quantified by the nutrient limiting results (Thomas et al. 2006; Vats et al. 2014; Vats et al. 2017; Vats et al. 2011).

15.12.1 Temperature Stress

N2 fixing plants and their related N2 strains—bacterial fixation has remarkable resistance to cold and warm temperature conditions. The major causes of nodulation failures are high temperature and humidity deficit; the upper limits of rhizobium growth were 32-47 °C; the upper limits of tropical legumes were lower with N2 attachment at 27–40 °C.Temperature can also change the result of competition among strains. Several experiments have explored the relationship between high CO_2 and low N_2-fixing temperatures and this usually supports the idea that high CO_2 effects are stronger at high temperatures (Zahran 1999; Kajic et al. 2016; Gao et al. 2019; Ferguson et al. 2019; Vats et al. 2012; Vats et al. 2013b; Vats and Miglani 2011; Vats and Negi 2013; Tandon and Vats 2016).

15.12.2 Drought Stress

The efficient survival of rhizobia even under unfavorable environmental conditions relies on optimum symbiosis. Water stress has been well studied and is a major environmental factor that inhibits growth and symbiotic N_2 fixation. Arid areas which survive and adapt to these adverse environmental conditions may trigger effective inoculants strains for crops that are grown under drought pressure. Temperature and drying stress were less tolerant than field isolates to commercial strains. Desiccation decreases the activity of water and enhances the capacity of some microorganisms to remain drying due to osmotic or salt stresses, and some have argued that desiccated microorganisms are required for use as dry seed inoculations. The capacity of microorganisms, including rhizobia, to survive drying depends on their ability to cope with diverse stresses, including solvents and extremes in temperature (Zahran 1999; Arora et al. 2017).

Dry stress is very susceptible to modulation, and sudden drought dramatically reduces the output of nodules already produced. Drying stress also significantly decreases the amount of rhizobia. The potential of rhizobia to thrive in humidity-limited conditions depends on the degree of temperature tolerance between the

species and the strains of each plant. Growth and nitrogen fixation are impaired in tropical areas with a matrix water capacity of −0.5 to −1.5 m Pa.

15.12.3 Nutrient and Metal Stress

Since the fixation of nitrogen is significantly restricted by available soil phosphorus, Soil P is likely to greatly reduce symbiotic plants' responses to carbon dioxide enrichment and experimental evidence indicates the need for sufficient soil P for elevated CO_2 to have a positive impact on the symbiosis of N_2. Soya plants fertilized adequately with P were receptive to higher CO_2 masses of nodule, unique activity of nodules, and total plant N, but this was removed in soil P deficiency. Fixing soil restrictions and relieving nutrient deficiencies significantly improve BNF production, as demonstrated in a number of studies, particularly in acidic soils. The adverse effects of heavy metals on rhizobia as a result of soil irrigation and the negative effects on nodulation parameters have been identified (Zahran 1999; Abd-Alla et al. 2014; Lebrazi and Benbrahim 2014; Jain et al. 2011; Kaur et al. 2010; Ajmani et al. 2019; Vats et al. 2019).

15.12.4 Salinity Stress

Soil salinity problems will increase in the future water-limited environment. In addition, the agriculture must progressively rely on recycled waters due to the demand for limited quantities of water in response to industry and infrastructure needs. Legumes are more prone to salinity and alkalinity than cereals in general. Most soybean rhizobia, particularly bradyrhizobia, are inhibited by 100 mm salt.

The rapidly growing strains have increased at salt levels of over 300 mm. It has been established that salinity prevents the infection of root hair and symbiotic processes that reduce legume nodulation and nitrogen fastening.

Stress-tolerant strains are helpful for use in biofertilizers both to increase the viability of the inoculants during their storage and to enhance survival after inoculation. Very small percentages (5%) of strains that are tolerant to all three individual stresses (40 °C, 40% PEG, 3% NaCl) and therefore require a careful selection of rhizobial strains that are effective, compatible, and stress-tolerant for superior biofertilizer preparation (Zahran 1999; Hessini et al. 2019; Fernandes et al. 1993).

15.13 Conclusion

This review concludes that for a sustainable agrosystem, the use of nitrogen fixing bacteria can enhance the agricultural productivity. The interaction of plant based on its genotype and bacteria based on its species has significant role in efficiency of nitrogen fixation. The production level in maize, rice, and wheat has been increased by the use of plant growth promoting bacteria. Both free living and symbiotic bacteria have their role to play. Nitrogen fixation by bacteria offers a sustainable approach and an alternative strategy to chemical based nitrogen fixation/supplementation. The need of the hour is use of biotechnological tool to make bacteria based nitrogen fixation more productive and competitive to chemical based nitrogen supply.

References

Abd-Alla MH, Issa AA, Ohyama T (2014) Impact of harsh environmental conditions on nodule formation and dinitrogen fixation of legumes. In: Advances in biology and ecology of nitrogen fixation, vol 9. Intechopen, New York

Ajmani A, Kumar R, Bhargava P, Vats S (2019) Mathematically optimized production purification and characterization of penicillin G Acylase from AA17A and AA17B: an industrial biocatalyst for production of aminopenicillanic acid a core moiety of different penicillins. Indian J Biotechnol 18:260–268

Akhtar M, Sarwar N, Ashraf A, Ejaz A, Ali S, Rizwan M (2020) Beneficial role of Azolla sp. in paddy soils and their use as bioremediators in polluted aqueous environments: implications and future perspectives. Arch Agron Soil Sci 2020:1–14

Anastasopoulou A, Butala S, Lang J, Hessel V, Wang Q (2016) Life cycle assessment of the nitrogen fixation process assisted by plasma technology and incorporating renewable energy. Ind Eng Chem Res 55:8141–8153

Andrews M, Andrews ME (2017) Specificity in legume-rhizobia symbioses. Int J Mol Sci 18:705

Arora NK, Verma M, Mishra J (2017) Rhizobial bioformulations: past, present and future. In: Rhizotrophs: plant growth promotion to bioremediation. Springer, Singapore, pp 69–99

Beatty PH, Good AG (2011) Future prospects for cereals that fix nitrogen. science 333 (6041):416–417

Bennett AB, Pankievicz VC, Ané JM (2020) A model for nitrogen fixation in cereal crops. Trends Plant Sci 25:226–235

Bhargava P, Gupta N, Kumar R, Vats S (2020) Plants and microbes: bioresources for sustainable development and biocontrol. In: Plant microbe symbiosis. Springer, Cham, pp 153–176

Bhargava P, Gupta N, Vats S, Goel R (2017) Health issues and heavy metals. Austin J Environ Toxicol 3:3018

Bhargava P, Khan M, Verma A, Singh A, Singh S, Vats S, Goel R (2019a) Metagenomics as a tool to explore new insights from plant-microbe Interface. In: Plant microbe Interface. Springer, Cham, pp 271–289

Bhargava P, Vats S, Gupta N (2019b) Metagenomics as a tool to explore Mycorrhizal fungal communities. In: Mycorrhizosphere and Pedogenesis. Springer, Singapore, pp 207–219

Bhattacharjee RB, Singh A, Mukhopadhyay SN (2008) Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. Appl Microbiol Biotechnol 80:199–209

Bøckman OC (1990) Agriculture and fertilizers: fertilizers in perspective, their role in feeding the world, environmental challenges, are there alternatives? Agricultural Group, Norsk Hydro, Oslo, Norway

Braghiere RK, Fisher J, Shi M, Yang X, Sulman BN, Soudzilovskaia N, Phillips R (2019) Considering carbon costs of plant phosphorus acquisition in earth system models. American Geophysical Union, Washington, DC

Burns RC, Hardy RW (2012) Nitrogen fixation in bacteria and higher plants, vol 21. Springer, Cham

Chalk PM, Craswell ET (2018) An overview of the role and significance of 15 N methodologies in quantifying biological N 2 fixation (BNF) and BNF dynamics in agro-ecosystems. Symbiosis 75:1–16

Chapman GP, Dutt AK (2019) Urban growth and development in Asia: volume I: making the cities. Routledge, Milton Park, UK

Coniglio A, Mora V, Puente M, Cassán F (2019) Azospirillum as biofertilizer for sustainable agriculture: Azospirillum brasilense AZ39 as a model of PGPR and Field traceability. In: Microbial probiotics for agricultural systems: advances in agronomic use. Springer, Cham, pp 45–70

Dobrojan S, Şalaru V, Stratulat I, Dobrojan G (2016) Influence of soil moisture on biological nitrogen fixation by blue-green algae of genus nostoc. In: Microbial biotechnology. CRC Press, Boca Raton, FL, pp 130–130

FAO (2017) Food and agriculture organization of the united nations - Rome, 2017 as world fertilizer trend and outlook http://www.fao.org/3/a-i6895e.pdf

Farooq M, Rehman A, Pisante M (2019) Sustainable agriculture and food security. In: Innovations in sustainable agriculture. Springer, Cham, pp 3–24

Feng M, Adams JM, Fan K, Shi Y, Sun R, Wang D, Chu H (2018) Long-term fertilization influences community assembly processes of soil diazotrophs. Soil Biol Biochem 126:151–158

Ferguson BJ, Mens C, Hastwell AH, Zhang M, Su H, Jones CH, Gresshoff PM (2019) Legume nodulation: the host controls the party. Plant Cell Environ 42:41–51

Fernandes TA, Iyer V, Apte SK (1993) Differential responses of nitrogen-fixing cyanobacteria to salinity and osmotic stresses. Appl Environ Microbiol 59:899–904

Fukami J, Cerezini P, Hungria M (2018) Azospirillum: benefits that go far beyond biological nitrogen fixation. AMB Express 8:73

Gao F, Wang X, Zhu Y (2019) Enhancement of nodulation and nitrogen fixation of vegetable soybean under high temperature stress by Overexpression of StP5CS gene. J Hebei Agric Univ 42(3):51–56

Goel R, Bhargava P, Gupta N, Vats S (2017) Health issues and heavy metals. Austin J Environ Toxicol 3:1018

Gupta N, Vats S, Bhargava P (2018) Sustainable agriculture: role of Metagenomics and metabolomics in exploring the soil microbiota. In: In Silico approach for sustainable agriculture. Springer, Singapore, pp 183–199

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Hessini K, Issaoui K, Ferchichi S, Saif T, Abdelly C, Siddique KH, Cruz C (2019) Interactive effects of salinity and nitrogen forms on plant growth, photosynthesis and osmotic adjustment in maize. Plant Physiol Biochem 139:171–178

Iannetta PP, Young M, Bachinger J, Bergkvist G, Doltra J, Lopez-Bellido RJ, Walker RL (2016) A comparative nitrogen balance and productivity analysis of legume and non-legume supported cropping systems: the potential role of biological nitrogen fixation. Front Plant Sci 7:1700

Ijaz M, Tahir M, Shahid M, Ul-Allah S, Sattar A, Sher A, Hussain M (2019) Combined application of biochar and PGPR consortia for sustainable production of wheat under semiarid conditions with a reduced dose of synthetic fertilizer. Braz J Microbiol 50:449–458

Jain P, Miglani K, Vats S (2011) Aptamers-potential applications in diagnostics and therapeutics. Everyman's Sci XLV(6):361

Junior EMP, Lobato EMSG, Lima BM, Quadros BR, da Silva Lobato AK, Andrade IP, Abreu LF (2020) Advancement of nitrogen fertilization on tropical environmental. In: Nitrogen fixation. IntechOpen, New York

Kajić S, Hulak N, Sikora S (2016) Environmental stress response and adaptation mechanisms in rhizobia. Agric Conspec Sci 81:15–19

Kantar F, Shivakumar BG, Arrese-Igor C, Hafeez FY, González EM, Imran A, Larrainzar E (2010) Efficient biological nitrogen fixation under warming climates. In: Climate change and Management of Cool Season Grain legume crops. Springer, Dordrecht, pp 283–306

Kaur A, Vats S, Rekhi S, Bhardwaj A, Goel J, Tanwar RS, Gaur KK (2010) Physico-chemical analysis of the industrial effluents and their impact on the soil microflora. Procedia Environ Sci 2:595–599

Ko Latt Z, San Yu S, Kyaw EP, Mar Lynn T, Thet Nwe M, Mon WW (2018) Isolation, evaluation and characterization of free living nitrogen fixing bacteria from agricultural soils in Myanmar for biofertilizer formulation. Int J Plant Biol Res 6(3):1092

Kumar N, Balamurugan A, Shafreen MM, Rahim A, Vats S, Vishwakarma K (2020a) Nanomaterials: emerging trends and future prospects for economical agricultural system. In: Biogenic nano-particles and their use in agro-ecosystems. Springer, Singapore, pp 281–305

Kumar N, Srivastava P, Vishwakarma K, Kumar R, Kuppala H, Maheshwari SK, Vats S (2020b) The rhizobium–plant Symbiosis: state of the art. In: Plant microbe symbiosis. Springer, Cham, pp 1–20

Kyaw EP, Lynn TM, Ko Latt Z, San Yu S (2018) Isolation, characterization and rapid composting of cellulolytic nitrogen-fixing Bacteria for biofertilizer preparation. Int J Plant Biol Res 6 (4):1095

Lebrazi S, Benbrahim KF (2014) Environmental stress conditions affecting the N2 fixing rhizobium-legume symbiosis and adaptation mechanisms. Afr J Microbiol Res 8:4053–4061

Legocki AB, Bothe H, Bothe B, Pühler A (eds) (1997) Biological fixation of nitrogen for ecology and sustainable agriculture, vol 39. Springer, Cham

Lett S, Michelsen A (2014) Seasonal variation in nitrogen fixation and effects of climate change in a subarctic heath. Plant Soil 379:193–204

Li Q, Liu X, Zhang H, Chen S (2019) Evolution and functional analysis of orf1 within nif gene cluster from Paenibacillus graminis RSA19. Int J Mol Sci 20:1145

Martin B (2017) The nitrogen cycle. The Rosen Publishing Group, New York

Maurya DP, Singh D, Vats S (2014) In: Jian MSA (ed) Cellulase production and utilization. Category-Chemical Technology. Publishing House-Lap Lambert Academic Publishing, Cambridge, MA

Maurya DP, Vats S, Rai S, Negi S (2013) Optimization of enzymatic saccharification of microwave pretreated sugarcane tops through response surface methodology for biofuel. Indian J Exp Biol 51:992–996

Meena BL, Fagodiya RK, Prajapat K, Dotaniya ML, Kaledhonkar MJ, Sharma PC et al (2018) Legume green manuring: an option for soil sustainability. In: Legumes for soil health and sustainable management. Springer, Singapore, pp 387–408

Moure VR, Siöberg CL, Valdameri G, Nji E, Oliveira MAS, Gerdhardt EC et al (2019) The ammonium transporter AmtB and the PII signal transduction protein GlnZ are required to inhibit DraG in Azospirillum brasilense. FEBS J 286:1214–1229

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC (2016) Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. New Phytol 210:657–668

Negi S, Vats S (2013) Pine forest litter based bio-refinery for biofuels and value-added phytochemicals. In: Singh RS, Pandey A, Larroche C (eds) Advances in industrial biotechnology. IK International Publishing House, Bengaluru, India, pp 98–116

Ojha AK, Forster S, Kumar S, Vats S, Negi S, Fischer I (2013) Synthesis of well–dispersed silver nanorods of different aspect ratios and their antimicrobial properties against gram positive and negative bacterial strains. J Nanobiotechnol 11:42

Oses R, Frank AC, Valenzuela S, Rodríguez J (2018) Nitrogen fixing endophytes in forest trees. In: Endophytes of forest trees. Springer, Cham, pp 191–204

Painuly AS, Gupta R, Vats S (2019) Bio-accumulation of arsenic (III) using Nelumbo Nucifera Gaertn. J Health Pollut 9:190902

Perakis SS, Pett-Ridge JC, Catricala CE (2017) Nutrient feedbacks to soil heterotrophic nitrogen fixation in forests. Biogeochemistry 134:41–55

Pfromm PH (2017) Towards sustainable agriculture: fossil-free ammonia. J Renewable Sustain Energy 9:034702

Puri A, Padda KP, Chanway CP (2020) Can naturally-occurring endophytic nitrogen-fixing bacteria of hybrid white spruce sustain boreal forest tree growth on extremely nutrient-poor soils? Soil Biol Biochem 140:107642

Quatrin MP, Olivo CJ, Simonetti GD, Bratz VF, De Godoy GL, Casagrande LG (2019) Response of dual-purpose wheat to nitrogen fertilization and seed inoculation with Azospirillum brasilense. Ciênc Agrotec 43(3):1–10

Rao DLN (2014) Recent advances in biological nitrogen fixation in agricultural systems. Proc Indian Natl Sci Acad 80(2):359–378

Rao DLN, Balachandar D (2017) Nitrogen inputs from biological nitrogen fixation in Indian agriculture. In: The Indian Nitrogen Assessment. Elsevier, Amsterdam, pp 117–132

Rodrigues MÂ, Ladeira LC, Arrobas M (2018) Azotobacter-enriched organic manures to increase nitrogen fixation and crop productivity. Eur J Agron 93:88–94

Rosenblueth M, Ormeño-Orrillo E, López-López A, Rogel MA, Reyes-Hernández BJ, Martínez-Romero JC, Martínez-Romero E (2018) Nitrogen fixation in cereals. Front Microbiol 9:1794

Rubiales D, Araújo SS, Vaz Patto MC, Rispail N, Valdés-López O (2018) Advances in legume research. Front Plant Sci 9:501

Saxena P, Srivastava J, Pandey S, Srivastava S, Maurya N, Kaushik NC, Mishra S, Asthana G, Bhargava P, Kumar R, Vats S (2019) Plants for biocontrol and biological control of plant pathogens. In: Plant biotic interactions. Springer, Cham, pp 147–179

Sharma D, Javed S, Arshilekha PS, Babbar P, Shukla D, Srivastava P, Vats S (2018) Food additives and their effects: a mini review. Int J Curr Res 10(06):69999–70002

Sharma KM, Kumar R, Vats S, Gupta A (2014) Production partial purification and characterization of alkaline protease from Bacillus aryabhattai K3. Int J Adv Pharm Biol Chem 3:290–298

Singh M, Vats S (2019) Mathematically designed bioprocess for release of value added products with pharmaceutical applications from wastes generated from spices industries. Int J Pharm Sci Res 10:130–138

Souza EM, Chubatsu LS, Huergo LF, Monteiro R, Camilios-Neto D, Wassem R, de Oliveira Pedrosa F (2014) Use of nitrogen-fixing bacteria to improve agricultural productivity. BMC Proc 8(S4):O23

Spaink HP, Kondorosi A, Hooykaas PJ (eds) (2012) The Rhizobiaceae: molecular biology of model plant-associated bacteria. Springer, Cham

Staccone A, Liao W, Perakis S, Compton J, Clark CM, Menge D (2020) A spatially explicit, empirical estimate of tree-based biological nitrogen fixation in forests of the United States. Global Biogeochem Cycles 34:e2019GB006241

Sutton MA, Drewer J, Moring A, Adhya TK, Ahmed A, Bhatia A, Hooda S (2017) The Indian nitrogen challenge in a global perspective. In: The Indian Nitrogen Assessment. Elsevier, Amsterdam, pp 9–28

Tandon S, Vats S (2016) Microbial biosynthesis of cadmium sulfide (Cds) nanoparticles and their characterization. Eur J Pharm Med Res 3(9):545–550

Thomas RB, Van Bloem SJ, Schlesinger WH (2006) Climate change and symbiotic nitrogen fixation in agroecosystems. In: Agroecosystems in a changing climate. CRC Press, Boca Raton, FL, pp 85–116

Torabian S, Farhangi-Abriz S, Denton MD (2019) Do tillage systems influence nitrogen fixation in legumes? A review. Soil Tillage Res 185:113–121

Vats S (2017) Methods for extractions of value-added nutraceuticals from lignocellulosic wastes and their health application. In: Ingredients extraction by physicochemical methods in food. Elsevier, Amsterdam, pp 1–64

Vats S, Bhargava P (2017) Alternate energy: fuel for "Modi's India" and "smart cities". Int J Curr Res 9:49090–49097

Vats S, Gupta N, Bhargava P (2019) Vulnerability of soil micro biota towards natural and anthropogenic induced changes and loss of Pedospheric functionality. In: Mycorrhizosphere and Pedogenesis. Springer, Singapore, pp 191–205

Vats S, Kumar R (2015) Amylolytic-extremoenzymes: saviour of environments. Eur J Biomed Pharm Sci 2:694–702

Vats S, Kumar R, Miglani AK (2011) Isolation, characterization and identification of high salinity tolerant, heavy metal contaminant and antibiotics resistant amylolytic-thermophilic pseudomonas Sp. Int J Pharm Sci Rev Res 10:125–129

Vats S, Kumar R, Negi S (2012) Natural food that meet antibiotics resistance challenge: In vitro synergistic antimicrobial activity of Azadirachta indica, Terminalia chebula, Piper nigrum and photoactivated cow urine. Asian J Pharm Biol Res (AJPBR) 2:84–87

Vats S, Kumar R, Maurya DP (2014) Alkaline amylase from multi resistant microbes and its applications. In: Alexei E (ed) Category-Microbiology. Publishing House-LAP LAMBERT Academic Publishing, Cambridge, UK

Vats S, Maurya DP, Jain A, Mall V, Negi S (2013b) Mathematical model-based optimization of physico-enzymatic hydrolysis of Pinus roxburghii needles for the production of reducing sugars. Indian J Exp Biol 51:944–953

Vats S, Maurya DP, agarwal A, Shamoon M, Negi S (2013a) Development of a microbial consortium for the production of blend of enzymes for the hydrolysis of agricultural wastes into sugars. J Sci Ind Res 75:585–790

Vats S, Miglani K (2011) Synergistic antimicrobial effect of cow urine and Azadirachta indica on infectious microbes. Int J Pharm Sci Res 2:1781

Vats S, Mishra A (2016) Soil agro-ecological management by Vermicompost a potential organic nutrient source for the state of Uttar Pradesh. Eur J Pharm Med Res 3(9):604–609

Vats S, Negi S (2013) Use of artificial neural network (ANN) for the development of bioprocess using Pinus roxburghii fallen foliages for the release of polyphenols and reducing sugars. Bioresour Technol 140:392–398

Vats S, Singh M, Siraj S, Singh H, Tandon S (2017) Role of nanotechnology in theranostics and personalized medicines. J Health Res Rev 4:1

Vitousek PM, Cassman KEN, Cleveland C, Crews T, Field CB, Grimm NB, Sprent JI (2002) Towards an ecological understanding of biological nitrogen fixation. In: The nitrogen cycle at regional to global scales. Springer, Dordrecht, pp 1–45

Wagner SC (2011) Biological nitrogen fixation. Nat Educ Knowl 3:15

Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989

Chapter 16
Functional Diversity of Nitrogen-Fixing Plant Growth-Promoting Rhizobacteria: The Story So Far

Mohd. Musheer Altaf

Abstract The concept of functional diversity assists in comprehension of natural intricacy during the widespread cooperation that microorganisms depict with other populations and ecological systems as how they communicate. Principally, microorganisms consist of several traits that describe their function inside ecological systems. Plant root associated bacteria that inhabit the plant root as ectophytes or endophytes and be capable of precisely boosting plant development through enhanced nourishment, production of plant hormones, mitigation of deleterious effect of different plant associated pathogens, and fighting for colonization sites on plants are referred as plant growth-promoting rhizobacteria (PGPR). Therefore, the application of specific nitrogen-fixing plant growth-promoting rhizobacteria in the form of microbial inoculants (single or mixed) can minimize the addiction of chemically synthesized fertilizers without compromising the crop yield. Despite their potential to enhance crop productivity and improved crop protection, nitrogen-fixing PGPR still have to cover a long distance to compete as effective bioinoculants. Therefore, there is urgent need to learn the functional diversity of nitrogen-fixing PGPR for sustainable crop production. Keeping in view the author make effort to evaluate the current development related with functional diversity about nitrogen-fixing PGPR along with their mode of action.

Keywords Nitrogen · Nitrogen-fixing PGPR · Functional diversity · Rhizobacteria · Plant interactions · Bioremediation

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16.1 Introduction

One of the key tests of the twenty-first century is to provide and produce sufficient quantity of food for the rising population. The United Nations Population Fund (UNFPA) projected that the global community will swell to 11.2 billion by 2100 (Alexandratos and Bruinsma 2012; Rana et al. 2020). The developed countries maintained their high agricultural productivity through high yielding varieties which are directly dependent on the application of chemical fertilizers, agrochemicals, and several other synthetic chemicals. Whereas, inside growing nations, forest has been swung into agricultural soil, creating danger of extinction to world biological diversity (Angus and Hirsch 2013). Similarly, India's population is projected to increase to 1.45 billion by 2028 (https://www.unfpa.org›data›transparency-portal›unfpa-india, Accessed 20 June 2020). In agriculture productivity, nitrogen works as an important component, however most of the profit crops are not capable of absorbing dinitrogen (Nitrogen) from the atmosphere (Fox et al. 2016). Since decades, high amount of chemically synthesized nitrogen manure have been applied to enhance crop yield in order to satisfy the hunger of growing earth population. On the other hand, merely 30–50% of the used synthetic manure is taken up by the crop plants, and at the same time remaining half amount is disappeared into the atmosphere through the process of volatilization (ammonia-NH_3) and leaching (Anderson et al. 2018). Injudicious application of synthetic manure severely impacts the soil quality and microorganisms population composition. Overdose of chemical manure helps in reducing the soil organic matter, fertility and increases the acidification of farming soil, which finally lowers the crop production (Li et al. 2017a; Wang et al. 2020a, 2020b). It has been proposed that nearly 65% of the total nitrogen used in farming is supplied through biological nitrogen fixation (BNF) which is probably one of the leading different resources of nitrogen for crop yield (Kuan et al. 2016; Wang et al. 2020a, 2020b).

Nitrogen-fixing plant growth-promoting rhizobacteria consist together of symbiotic and asymbiotic/associative microorganisms that have been routinely applied to crops to enhance their growth and yield. Among these, microbes that are able to form root nodules in contact with leguminous plants and convert atmospheric nitrogen (N) into working shape of nitrogen are usually referred as rhizobia (a universal word employed to designate every rhizobial genera jointly) (Lindstrom and Martinez-Romero 2005). *Bacillus radicicola* was the first nodule bacterium isolated by Beijerinck (1888). Frank (1889) renamed this bacterium as *Rhizobium leguminosarum* and recognized new species related to the similar faction. The name "rhizobia" was initially employed to label bacteria fitting to the genus *Rhizobium,* however, these days rhizobia also comprise new genera, such as *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium, Sinorhizobium* (Graham 2008). The name rhizobia at present comprise over 70 species scattered more than 13 genera together with some *Betaproteobacteria* such as *Burkholderia* and *Cupriavidus* (Chen et al. 2007). Numerous factions of soil and root connected nitrogen-fixing bacteria, for example, *Azotobacter vinelandii, Azospirillum brasilense, Azospirillum*

zeae, Pseudomonas stutzeri, Acetobacter diazotrophicus, Achromobacter insolitus, Bacillus megaterium, Bacillus rhizosphaerae, B. cereus, B. circulans, B. firmus, B. pumilus, B. licheniformis, B. subterraneous, B. aquimaris, B. vietnamensis, B. aerophilus, *Burkholderia tropica, Burkholderia xenovorans, Burkholderia silvatlantica, Burkholderia caballeronis, Bradyrhizobium Japonicum, Bradyrhizobium elkanii, Delftia tsuruhatensis, Enterobacter sacchari, Gluconacetobacter diazotrophicus, Stenotrophomonas maltophilia, Pseudomonas koreensis,* and *Pseudomonas entomophila* persistently establish to inhabit diverse crops and encourage plant development both precisely and circumlocutorily. However, their actions are affected through plant species, soil nature, and environment (Ehrmann and Ritz 2014; Ding et al. 2015; Yousuf et al. 2017; Singh et al. 2020). Conventionally, nitrogen fixers have been employed to provide nitrogen to crop plants. In view of that, soil microbes particularly PGPR turn out to be significant in farming techniques as they are economical and prevent soil contamination. In a group, nitrogen-fixing PGPR together with rhizobia are accounted to hold numerous attractive plant growth-promoting characters alone from their regular nitrogen fixation capability (Ghosh et al. 2015). When used appropriately, they continue to apply different positive developments on several key crop plants (García-Fraile et al. 2012). Unreasonably, nitrogen-fixing PGPR are capable of enhancing the development of crop plants through secreting composites, for instance, the plant hormone indole acetic acid or the enzyme ACC deaminase implicated in the assimilation of 1-aminocyclopropane-1-carboxylic acid (ACC), an originator of ethylene. They are capable of activating definite key nutrients to the plants like phosphorous through solubilization of soil insoluble phosphates (Lugtenberg and Kamilova 2009; Bhattacharyya and Jha 2012; Gouda et al. 2018). Nitrogen-fixing PGPR expliciting various plant growth-promoting characters are capable of precisely and circumlocutorily encouraging plant development. Moreover, several nitrogen-fixing PGPR produce antimicrobial complex substances such as siderophores, low-molecular weight iron-chelating compounds, which hamper the development of plant pathogens in soils and small amount of this ion encourage plant development circumlocutorily (Lugtenberg and Kamilova 2009). Seeing the significance of nitrogen-fixing PGPR in agriculture, the author in this chapter endeavor to evaluate the current development connected with functional diversity of nitrogen-fixing PGPR that could help in creating a bioinoculant expressing multiple plant growth-promoting traits which can be applied in dissimilar farming conditions for sustainable agriculture.

16.2 Biological Nitrogen Fixation (BNF)

The process of nitrogen fixation is a vibrant and requires huge amount of power (Rosenblueth et al. 2018). The track for the biological devaluation of inert nitrogen into the reactive complex ammonia (NH_3) under micro-aerobic situations is as pursues:

$$\text{Nitrogen} + 8H^{+} + 8e^{-} + 16\,\text{Mg} - \text{ATP} \rightarrow 2NH_3 + H_2 + 16\,\text{Mg} - \text{ADP} + 16\,\text{P}$$

Free-living diazotrophs conform to a little portion of the plant root ecological system; moreover, they are part of *Rhizobia*, *Bradyrhizobia*, *Rhodobacteria* (alphaproteobacteria), *Burkholderia*, *Nitrosospira* (betaproteobacteria), *Pseudomonas*, *Xanthomonas* (gammaproteobacteria), firmicutes, along with cyanobacteria (Morris and Schniter 2018). Conversely, their existence, role, along with significance might be elucidated through the "black queen" theory that projects that among free-living microorganisms populations, merely a small number of "helpers" that bear the considerable burden of different tasks, for instance, great power-demanding nitrogen fixation, sustain the remaining flora and fauna inhabitants or the "recipient" which depends upon the "helpers" for nitrogen requirements (Peix et al. 2015). The synergetic association among soil bacteria jointly identified as rhizobia and legume rhizosphere produces nodules that install atmospheric nitrogen through nitrogenase enzyme. Biological Nitrogen Fixation (BNF) through plants along with their bacterial relatives corresponds to a significant biological organization for trapping atmospheric nitrogen and converting it into useful nitrogen by means of enzymatic reduction. BNF is supposed to be a highly susceptible method affected with source of nourishment along with ecological situations and facilitates a plant to provide nearly every part or fraction of their necessities by communications among endosymbiotic, associative, and endophytic symbionts, therefore providing a combative benefit compared with non-nitrogen-fixing plants (Graham 1992; Mahmud et al. 2020). The nitrogenase enzyme complex is highly protected among free-living and symbiotic diazotrophs that capable them to take part among different kind of communications with its anchor plants. BNF through plant–rhizobia symbiotic organization is reconciled by means of endosymbiotic communication as soon as plants build up root nodules. Among legumes and rhizobia, Gram-negative alpha proteobacteria are one of the common bacterial species that connect (endosymbiotic relations) with legumes of the Fabaceae (Papilionaceae) family (Schultze and Kondorosi 1998; Desbrosses and Stougaard 2011). Actinomycetes, for example, the *Parasponia* species (family Cannabaceae) and *Frankia* sp. that connect by a wide range of actinorhizal plants are adequately recognized during nitrogen fixation (Santi et al. 2013). Cyanobacteria (mainly *Nostoc* sp.) are also capable to inhabit dissimilar plant parts, either within a cell like in the family Gunneraceae or outside a cell like in *Azolla*, *Cycadaceae*, liverworts, and hornworts. Associative nitrogen fixation (ANF) and/or endophytic collaboration are frequently noticed amongst diazotrophs, like *Azospirillum* spp., *Azoarcus* spp., and *Herbaspirillum*, along with a broad diversity of plant roots together with cereals. The nitrogenase protein, in addition to the connected proteins and non-proteins creating nitrogenase enzyme, is susceptible to oxygen. Due to this susceptibility, strict anaerobes like *Clostridium pasteurianum* are suitable applicant intended for nitrogen fixation. However, facultative anaerobes, for example, *Klebsiella oxytoca* are also competent of fixing nitrogen anaerobically only. Strict aerobes, for instance, *Azotobacter vinelandii* are capable of protecting

nitrogenase from oxygen and carry out nitrogen fixation by eating up oxygen through cytochrome oxidases (Poole and Hill 1997; Mahmud et al. 2020).

16.3 Symbiotic Nitrogen Fixation Among Legumes

Legumes provide a major portion of nitrogen under natural and farming ecological systems. Moreover the amounts of nitrogen fixed by persistent fodder legumes are also similar to the amount of synthetic nitrogen based fertilizers employed in traditional agriculture practices (McNeill and Fillery 2008; Unkovich et al. 2010). In addition, rhizosphere discharge by legumes is an extra significant resource of accessible nitrogen along with new vital plant nutriment (Khan et al. 2002). Some of the legumes frequently used by humans as food are dry bean, chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* L.), lentil (*Lens esculenta* L.), pigeon pea (*Cajanus cajan* L.), and peanut (*Arachis hypogaea* L.). Every one of these legumes are able to fix nitrogen and they are mostly cultivated as intercrops. Nitrogen fixation through the process of symbiotic relationship by soybean roots with *Rhizobia* plays a vital role in the development and maturation of plants (Fig. 16.1). The rise in the capacity of nitrogen fixation is related to the enhanced yield. Fodder legumes are routinely cultivated under wide range of climatic conditions and these crops possess the capability to provide increased crop yield together

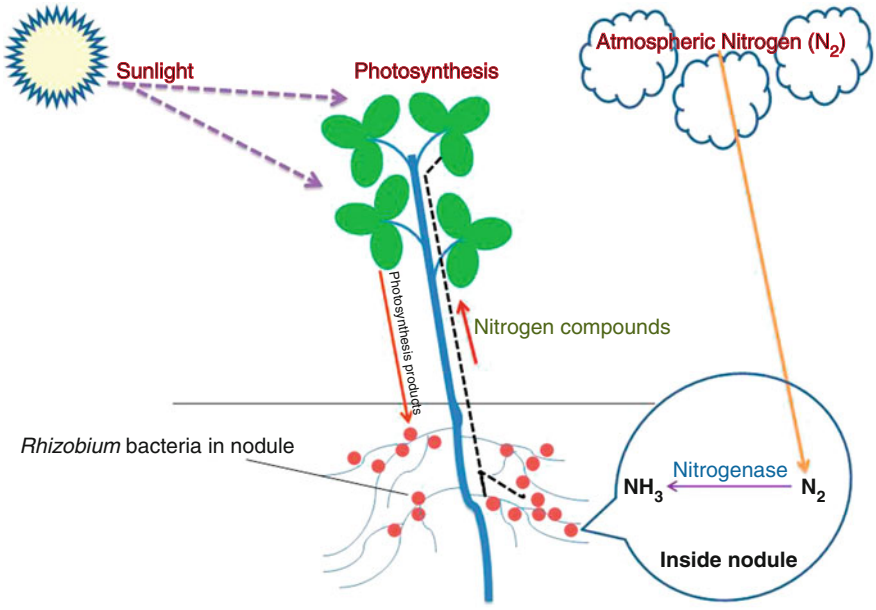

Fig. 16.1 Schematic diagram showing biological nitrogen fixation process in legumes (Partly adapted from Lindström and Mousavi 2019)

with necessary nitrogen to the agricultural soil. The four main fodder legume crops are alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), subterranean clover (*T. subterraneum* L.), and white clover (*T. repens* L.) jointly consist of the majority of the warm and dry regions of world's meadows. Alfalfa (*Medicago sativa* L.) is a cold climate yearly fodder legume that achieves nitrogen through the soil and the BNF via symbiotic relationship of its root nodules with soil microorganisms. Due to its capability to supply fixed nitrogen, alfalfa is progressively acquiring recognition as a friendly fodder crop in grasslands (Adhikari and Missaoui 2017). The fixed nitrogen through alfalfa is used by both alfalfa and following crop plants, which is called as a "niche complementarity effect." For instance, the transport of nitrogen fixed via alfalfa to diverse grass species like timothy (*Phleum pratense* L.) and bromegrass (*Bromus inermis* Leyss) was established by means of the 15 N dilution method (Mahmud et al. 2020). Besides, giving financial significance to fodder legumes and other crops, significant concentration has been focused to numerous plant species that can generate biofuel at the same time fixing nitrogen. Lone significant case is *Pongamia pinnata*, which in addition to considered as medicinal and green manure crop plant is capable of nodulating in the company of numerous strains of *Bradyrhizobium* and *Rhizobium*. However, preferred inocula were *B. japonicum* strains CB1809 and USDA110 (Kesari et al. 2013).

16.4 Nitrogen Fixation Within Nonlegumes

Inside a free-living organization like rhizosphere related nitrogen fixation, nitrogen-fixing bacteria install the nitrogen by means of employing carbon and energy resource acquired from the surroundings; in addition the bacteria discharge installed nitrogen most likely subsequent to disintegration of the bacterial cells (James 2000). On the contrary, the legume–rhizobia synergetic arrangement within nodules, the relations of plants and microorganisms inside rhizosphere and plant root core structure accustomed or modified nitrogen-fixing structure under anatomically nitrogen-lacking yet power-satisfactory situations. During last five decades, nitrogen fixations within nonleguminous crop plants and microbial cooperation are examined in detail intended for their agricultural importance. For instance, closely related nitrogen fixation among sugarcane (*Saccharum* spp.), sweet potato (*Ipomoea batatas* L.), and paddy rice (*Oryza sativa* L.) is agriculturally important. Dynamic interpretation from the di-nitrogenase reductase encrypted gene (*nifH*) phylogenetically analogous to the indicated *Bradyrhizobium* spp. and *Azorhizobium* sp. was plentifully reported within the nitrogen-fixing sugarcane, sweet potato stems, and storage tubers. *Setaria viridis*, with *Setaria italica* (foxtail millet), is able to capture a considerable portion of fixed nitrogen through cooperations in the company of *Azospirillum brasilense* (Pankievicz et al. 2015; Mahmud et al. 2020). Additional potential relations comprise *Azoarcus* sp. strain BH72, Kallar grass, *Klebsiella pneumoniae*, and wheat (Iniguez et al. 2004). A rhizosphere-connected nitrogen fixation takes place in three methods. Initially, rhizobia utilize "crack entry" (a lack

of Nod ABC genes, which results in a Nod factor-separate infection method) and march into xylem parenchyma tissues by means of cortical cells in incise sugarcane stems and sweet potato tuber. Subsequently, within inadequate oxygen concentration or micro-aerobic situations, rhizobia might demonstrate free-living nitrogen fixation; such as *Bradyrhizobium* spp., nodulates *Aeschynomene* and *Parasponia*, *Azorhizobium caulinodans*, nodulates *Sesbania rostrata*, and *Burkholderia*, nodulates *Mimosa*, and all these rhizobia are competent enough to fix nitrogen exclusive of an anchor plant within inadequate oxygen environments. Finally, rhizobia based hormones which enhance the development of the anchor plant in agreement with fixed nitrogen acquirement; such as endophytic rhizobia encouraging plant development (Mus et al. 2016; Mahmud et al. 2020).

16.4.1 Rhizobacterial Nitrogen Fixation Among Some Cash Crops

Beijerinckia sp. commencing the rhizosphere of sugarcane was primarily segregated and noticed within EMBRAPA Agrobiologia, Brazil (Dobereiner 1961). The interpretation of nitrogenase *nifH* genes was scrutinized via cultivating sugarcane cut-stems in Japan soils for 50 and 100 days as a result of reverse transcription-polymerase chain reaction (RT-PCR) and the sequencing of *nifH* (encrypting nitrogenase iron protein) nucleotides (Thaweenut et al. 2011). In that experiment, *nifH* sequences demonstrated resemblance by *Bradyrhizobium* spp. and *Azorhizobium caulinodans*, which recommended that the proliferation of these *nifH* transporting *Bradyrhizobium* spp. might be an important aspect in the endophytic nitrogen fixation in the asymbiotic situation (Mahmud et al. 2020). In case of cultivating sweet potato (*Ipomoea batatas* L.), crop growers employ comparatively unproductive soil and use a little amount of synthetic manure. *Azospirillum* sp. was primarily recognized within fibrous root system and storage root skins of sweet potato, and in the similar experiment, supplementary study also points to the contribution of huge amount of full nitrogen in sweet potato stems and tubers by endophytic *Bradyrhizobium* spp.. In current years, a segregated endophytic diazotroph, *Bradyrhizobium* sp. strain AT1 demonstrated a *nifH* sequence resemblance with *Aeschynomene* stem-nodulating *Bradyrhizobium* sp. ORS391 (Mahmud et al. 2020).

Among rice, high amount of nitrogenase behavior in a rice–soil organization were noticed contrast to the paddy soil with no rice plants and in an upland rice–soil scheme. In water logged soil, the root–soil crossing point has been anticipated as the nitrogen-fixing spot and the bacteria supporting this nitrogen fixation action in dark underwater logged situations were considered to be heterotrophic diazotrophs, for instance, *Azotobacter* and *Clostridia*. Afterward, under continuing repetitive container trials at the IRRI (International Rice Research Institute), nitrogen fixation through not only photosynthetic cyanobacteria but also constantly via the

heterotrophic diazotrophs employing root discharge of carbonaceous source in the rhizosphere was noticed (Yoneyama et al. 2017). Additionally, an encouraging nitrogen equilibrium was considered, signifying the importance of atmospheric nitrogen contribution in paddy rice cultivated land. Beginning *nifD* (a nitrogenase protein-encrypted gene) sections originating via basic root DNA, cloned *nifD* genes comparable to those of γ-proteobacteria (*Azotobacter vinelandii*) and α-proteobacteria (*Bradyrhizobium japonicum*) were noticed (Ueda et al. 1995; Mahmud et al. 2020). A fresh investigation accomplished in nitrogen exhausted cultivated land of Oaxaca, Mexico, confirmed that the mucilage linked by the airborne roots of Sierra Mixe maize be capable of assisting a composite diazotrophic microorganisms which might encrypt live nitrogenase, and the captured nitrogen (29% to 82% of the plant nitrogen was obtained via atmosphere) be able to competently pass through nitrogen-fixing microbes to anchor plants. Within maize, airborne root system is recognized for encouraging nourishment and moisture absorption in addition to a well-organized gaseous swap among plant tissue and the ambiance (Van Deynze et al. 2018; Mahmud et al. 2020).

16.4.2 Rhizobacterial Nitrogen Fixation and Growth Improvement Cases

Crop plants serve as one of the significant merchandises which possess a key position in human diet. Cultivation of new, clean, and superior class food items such as fruits, vegetables, cereals, etc., is necessary to accomplish the requirements of food in the whole world. Therefore, nitrogen-fixing plant growth-promoting rhizobacteria play a vital function in the development of crop plants.

Wang et al. (2020a, 2020b) reported that genetically manipulated nitrogen-fixing bacterium *Pseudomonas protegens* CHA0-ΔretS-nif considerably enhanced the development and productivity of land cultivated garlic, although the contribution of nitrogen-fertilizers was decreased by quarter, along with plants treated by CHA0-ΔretS-nif exhibited prevention of garlic root rot infection.

Wang et al. (2020a, 2020b) explained that the bacterial combination including nitrogen-fixer is better contender for biofertilizers that might decrease synthetic fertilizer use without harming the regular development of wheat.

El-Serafy and El-Sheshtawy (2020) reported that equally nitrogen-fixing bacteria (NFB) and moringa leaf extract (MLE) use might augment the vegetative development, flower initiation, umbels amount/plant, photosynthesis speed, oil productivity, along with oil constitution of fennel plant. Nitrogen-fixing bacteria decreased estragole concentration (the cancer causing constituent) in fennel oil. Estragole concentration was also enhanced by MLE use.

Ryu et al. (2020) advocated the use of genetically engineered strains that can efficiently deliver fixed nitrogen to cereals. They manipulated inductive nitrogenase function in two cereal endophytes (*Azorhizobium caulinodans* ORS571 and

Rhizobium sp. IRBG74) and the fully identified plant epiphyte *Pseudomonas protegens* Pf-5, a maize seed inoculant. For each microorganism, diverse approaches were selected to eradicate ammonium suppression and place nitrogenase appearance in the command of agriculturally appropriate indicators, together with root discharges, biological control mediators, and plant hormones. They exhibited that *R.* sp. IRBG74 is capable of manipulating to express nitrogenase function in asymbiotic situations via transporting a nif group from both *Rhodobacter sphaeroides* and *Klebsiella oxytoca*. Considering, *Pseudomonas protegens* Pf-5, the shifting of an inducible group from *Pseudomonas stutzeri* and *Azotobacter vinelandii* provides ammonium acceptance and elevated oxygen resistance of nitrogenase function than that from *Klebsiella oxytoca.*

16.5 Nitrogen-Fixing Rhizobacteria Plant Interactions

Nitrogen-fixing rhizobacteria have been extensively utilized as biofertilizers in order to provide nitrogen to legumes and other related crop plants. Within nitrogen-fixing rhizobacteria, associate belonging to the family *Rhizobiaceae* has also been reported to establish vague cooperation with root system of other plants with no nodule formation (Reyes and Schimidt 1979). Asymbiotic relationship attributes to a broad diversity of nitrogen-fixing species that inhabit the roots of nonleguminous plants with no special architecture; furthermore, these bacteria have capability to encourage the development of nonlegumes via working as PGPR (Elmerich and Newton 2007; Santi et al. 2013). Actually, rhizobia is capable of connecting to the area of monocots similarly as they connect to dicot anchor plant (Terouchi and Syono 1990). In addition, rhizobia develop quickly in the company of sprouting seeds and growing roots similarly among legumes and nonlegumes (Pena-Cabriales and Alexander 1983). Usually, the nitrogen-fixing rhizobacteria, such as rhizobia, go within nonlegume plant tissues primarily via fractures within epidermal cells of the root structure and in crevices wherever lateral root system has come out (Dazzo and Yanni 2006). Instantly, the rhizobial endophytic organization starts through root migration that is pursued through fracture access into the root core via alienated epidermal cells (Sprent and Faria 1988). Subsequently, endophytes constantly move to the stem base, leaf cover, and leaves where they develop quickly to elevated inhabitant's thickness (Chi et al. 2005). After that they might affect plant development through diverse PGPR methods. Both rhizobia and *Azotobacter* species not only provide nitrogen to their anchor plants but also discharge many compounds that enhance the growth and development of nonlegume plants.

16.6 Mechanism of Plant Growth Promotion by Nitrogen-Fixing Rhizobacteria

Nitrogen-Fixing Rhizobacteria just like other traditional asymbiotic PGPR might influence the crop plant growth and development through direct and indirect methods (Fig. 16.2). The explicit methods through which nitrogen fixers prop up the development of nonlegumes comprise the solubilization of insoluble phosphate through rhizobia, *Azospirillum* and *Burkholderia* that change insoluble phosphorous into soluble structure by the process of acidification, discharge of organic acids or protons and chelation, thus serving to develop phosphate nourishment in the connected plant species (Abd-Alla 1994; Richardson et al. 2009). Symbiotic rhizobia have many benefits compared to asymbiotic PGPR in phosphate solubilization since these bacteria are secured within the nodule tissues and confront almost no or little antagonism from native soil microorganisms. One more significant development controller which unswervingly encourages the expansion of plants is the manufacturing of auxin IAA in conjunction with cytokinin discharge via *Rhizobia, Azospirillum* and *Azotobacter* (Spaepen et al. 2008; Kumar and Ram 2012; Kumar et al. 2014). The above-mentioned plant hormones function as vital elements in controlling plant growth, together with different aspects of root developments (Kramer and Bennett 2006). Indole acetic acid works as an indicator compounds that is implicated in plant signal alteration, movement or fastening of microbes to the root system that support in symbiotic relationship of legume with *Rhizobium* (Spaepen et al. 2009). In contrast, the circumlocutory methods related to plant development through *Azotobacter* comprise the discharge of molecules which diminishes the harmful outcome of one or other plant pathogenic microorganisms (Gandhi Pragash et al. 2009). Manufacturing of siderophores, a low-molecular

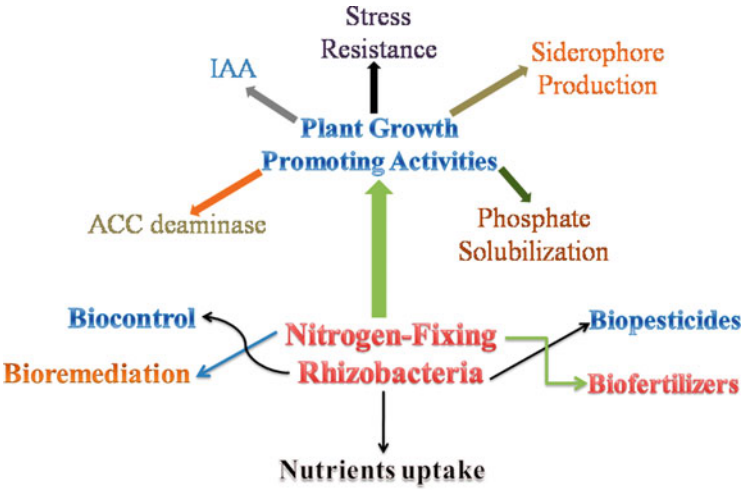

Fig. 16.2 Nitrogen-fixing plant growth-promoting rhizobacteria growth model

weight (≈1 kDa) iron-chelating compounds through *Azotobacter* (Altaf and Ahmad 2017) or rhizobia (Datta and Chakrabartty 2014), could be measured as an explicit aspect, because siderophores dissolve and segregate iron from soil in addition to supplying this to plant cells. However it could also be treated a circumlocutory aspect, given that it is connected through inhibition of plant disease causing bacteria through prevention of iron absorption. Furthermore, siderophore generating capability facilitates in the survival of rhizobia within iron insufficient soils (Lesueur et al. 1995). Plant growth-promoting elements secreted by few nitrogen-fixing rhizobacterial isolates are compiled in Table 16.1.

16.7 Bioremediation of Pollutants Through Nitrogen-Fixing Rhizobacteria

Important earth's activities like agriculture, weather, water, and different biogeochemical course together with preservation of biological heterogeneity remain harshly destabilized through human actions. Roughly 30% of the global surroundings are anticipated to be despoiled or polluted, intimidating farm yield, and the atmosphere (Valentín et al. 2013). Intensive research have been carried out related to the physico-chemical methods and enhanced biodegradation for their utilization as pollution removal tools. Even though physico-chemical methods such as manual elimination of polluted soils, chemical removal, and the utilization of chemical compounds are even the highly efficient approaches to quickly decontaminate highly contaminated pollution places, they are frequently power demanding as well as invasive for the atmosphere (Segura and Ramos 2013). On the contrary, the low power-demanding pollution removal methods employ live microorganisms along with their compounds for cleaning otherwise alleviating the inorganic/organic pollutants from the pollution site. As a result, bioremediation is a potential substitute due to its comparatively minimal interruption to polluted location, inexpensive along with large-scale civilian recognition in contrast with usual pollution removal techniques (Teng et al. 2015).

It is well known that the nitrogen-fixing rhizobia are broadly found in the plant root and soil ecological architecture, which can augment the growth and development of legumes through providing nitrogen that reduces the dependence on chemically synthesized fertilizers. Rhizobia in addition have the biochemical and environmental capability to deteriorate natural contaminants and are tolerant to heavy metals, shaping them as a beneficial tool for restoration of polluted sites. Further, rhizobia motivate the endurance and activities of different microorganism that are involved in breakdown of organic matter, which are helpful in decreasing the level of contaminants. The symbiotic activities of different rhizobia act in dual manner through increasing the plant development and accessibility of contaminants for bioremediation. Meanwhile, different microorganisms that are powerful within the rhizosphere do not exhibit or have only partial ability to deteriorate organic

Table 16.1 Plant growth-promoting agents secreted by various nitrogen-fixing rhizobacterial isolates

Nitrogen-fixing PGPR	Family	Isolation Source	PGP traits	References
Azotobacter vinelandii AZCH6	*Azotobacteraceae*	Rhizosphere soil	IAA, P solubilization siderophores, biofilm, ACC deaminase hydrogen cyanide, ammonia	Altaf and Ahmad (2017)
Azospirillum brasilense	*Rhodospirillaceae*	_	Nitrogen fixation, enhanced root growth, nutrients uptake, phytohormones	Fukami et al. (2016)
Bacillus megaterium (CY5); *Bacillus mycoides* (CA1)	Bacillaceae	Rhizosphere soil	IAA, HCN, nitrogen fixation, P solubilization, ammonia, siderophore, ACC deaminase, antifungal activity	Singh et al. (2020)
Brevibacterium epidermidis RS15; *Micrococcus yunnanensis* RS222; *Bacillus aryabhattai* RS341	Brevibacteriaceae; Micrococcacae; Bacillaceae	Rhizosphere soil	IAA, nitrogen fixation, phosphate and zinc solubilization, hydrolytic enzymes	Siddikee et al. (2015)
Pseudomonas sp. (N8); *Azotobacter chroococcum* (N9)	Pseudomonadaceae; *Azotobacteraceae*	Rhizosphere soil	IAA, nitrogen fixation	Wang et al. (2020a, 2020b)
Pseudomonas protegens CHA0-ΔretS-nif	Pseudomonadaceae	Rhizosphere soil	Nitrogen fixation, biocontrol activity	Wang et al. (2020a, 2020b)
Pseudomonas koreensis (CY4); *Pseudomonas entomophila* (CN11); *Pseudomonas spp.*	Pseudomonadaceae	Rhizosphere soil	IAA, nitrogen fixation, phosphate solubilization, siderophore, ACC deaminase, biocontrol capability	Li et al. (2017b)
Bacillus pumilus S1r1; *Bacillus subtilis* UPMB10; *Klebsiella pneumoniae* Fr1; *Acinetobacter* sp. S3r2	Bacillaceae; Enterobacteriaceae; Moraxellaceae	Rhizosphere soil	IAA, P solubilisation, nitrogen fixation	Kuan et al. (2016)
Providencia rettgeri P2; *Serratia plymuthica* P35;	*Enterobacteriaceae;* Alcaligenaceae; Moraxellaceae	Rhizosphere soil	IAA, nitrogen fixation, P	Li et al. (2020)

(continued)

Table 16.1 (continued)

Nitrogen-fixing PGPR	Family	Isolation Source	PGP traits	References
Advenella incenata P4, *Acinetobacter calcoaceticus* P19;			solubilization, biocontrol activity	
Brevibacterium sediminis A6	Brevibacteriaceae	Rhizosphere soil	IAA, nitrogen fixation, P solubilization, HCN, biofilm ammonia, siderophore, biocontrol activity,	Chopra et al. (2020)

pollutants. With the help of molecular biology and genetic manipulation different rhizobacteria having the contaminant deteriorating gene can be produced to complete the task of rhizosphere bioremediation (Ojuederie and Babalola 2017; Altaf et al. 2019).

16.8 Mediation of Stress Tolerance in Plants Through Nitrogen-Fixing Rhizobacteria

Crop plants are universally subjected to numerous ecological adverse conditions, for example, elevated and subzero temperatures, dryness, salinity, alkalinity, UV-rays. It is projected that nearly 30% of the world crop yield is destroyed due to these adverse conditions (Sharma et al. 2012; Goswami et al. 2016). The nitrogen-fixing rhizobacteria can rescue the world from these adverse environmental conditions through triggering a number of physiological and biochemical resistance methods within crop plants such as induced systemic tolerance (IST) (Lugtenberg and Kamilova 2009; Fukami et al. 2018). The methods associated with IST comprise antioxidant protection, osmotic modification, secretion of phytohormones, for instance, indole-3-aceticacid (IAA) (Spaepen and Vanderleyden 2015), protection approaches like the interpretation of PR-genes (Kim et al. 2014), and the initiation of heat-shock proteins (HSP) (Lim and Kim 2013). A number of researchers examined and established the task associated with nitrogen-fixing rhizobacteria for mitigation of adverse environmental conditions.

Abdel Latef et al. (2020) conducted a pot study to examine the effect of *Azospirillum lipoferum* and *Azotobacter chroococcum.* They demonstrated that adverse impact of salinity amplified Sodium and malondialdehyde (MDA) concentration and reduced majority of the biochemical, morphological, and physiological characters in the investigated maize variety. The treatment with *Azospirillum lipoferum* and *Azotobacter chroococcum* has revealed considerable reduction in adverse impact due to the saline situations on all the examined crop plant features via enhancement all of the characters, increase in the ROS foraging method in

addition to increase in the precision of Na^+ and K^+ ions for assimilation through the plant, thus restricting Na^+ assimilation and enhance K^+ absorption.

Chen et al. (2020) founded that *Kosakonia radicincitans* GXGL-4A, asymbiotic nitrogen-fixing bacterial strain segregated from maize (*Zea mays* L.) roots was establish posse's capability to disintegrate aromatic hydrocarbons. In his experiment, he explains that GC–MS examination demonstrated *K. radicincitans* GXGL-4A contains high potential to deteriorate toluene, ethylbenzene, and xylene (TEX).

Abd-Allah et al. (2019) showed that the application of *Rhizobium* (MK358859) along with mycorrhizal fungi and *Stenotrophomonas maltophilia* improved the nodulation, nitrogen fixation capability, nourishment condition and development of chickpea grown under saline soils.

Igiehon et al. (2019) showed that *Rhizobium* sp. strain R1, *Rhizobium tropici* strain R2, *Rhizobium cellulosilyticum* strain R3, *Rhizobium taibaishanense* strain R4, and *Ensifer meliloti* strain R5 were reported to contain the complete plant growth-promoting traits such as, these rhizobial strains were capable of solubilizing phosphate, producing exopolysaccharide (EPS), 1-aminocyclopropane-1-carboxylate (ACC), siderophore, and indole acetic acid (IAA). These strains in addition stay alive and develop at a temperature of 45 °C and in an acidic situation with a pH 4. As a result, every *Rhizobium* strains improved the propagation of soybean seeds (PAN 1532 R) in arid situation created by means of 4% poly-ethylene glycol (PEG). On the other hand, *Rhizobium* sp. strain R1 and *R. cellulosilyticum* strain R3 treatments were capable to perk up seeds propagation over R2, R4, and R5 strains. Consequently, genomic understanding of *Rhizobium* sp. strain R1 and *R. cellulosilyticum* strain R3 exposed the occurrence of several genes with their particular proteins implicated in symbiotic organization, nitrogen fixation, aridity acceptance, and plant development. Above all, *exoX, htrA, Nif, nodA, eptA*, *IAA,* and siderophore generating genes were established in the two rhizobial strains. Van Oosten et al. (2018) confirmed that the root treatment of tomato by *Azotobacter chroococcum* 76A encourages plant development, resistant to adverse conditions along with nourishment absorption effectiveness in modest and harsh saline situations.

16.9 Climate Change and Nitrogen-Fixing Rhizobacteria

World 61% agricultural land areas are occupied by crop plants (Ewert et al. 2005). The world two third cultivated land is used to grow different crops like wheat, maize, barley, rice, rye, millet, sorghum, cassava, potatoes, sugar beets, sugarcane, pulses, soybeans, groundnuts, rapeseed, sunflower, oil palm fruit, and cotton (FAOSTAT 2012; Dhir 2018). Besides, the growing requirement of food to feed the ever increasing inhabitants of the world also puts severe strain to increase the yield and superiority of existing crop plants in inadequate assets. Worldwide climate alteration brings about aridness and salinity, with >6% of world area and > 30% of watered agriculture being affected by salinity. World carbon dioxide level contain to rise from a before industrialization concentration of 285 μmol mol^{-1} to the present

concentration of 384μmol mol^{-1}, and they have been forecasted to attain 936 μmol mol^{-1} by the year 2100, with enhancement in universal ambiance temperature of about 6.4 °C (IPCC 2014; Yadav et al. 2020). Within stress farming, the rhizospheric bacteria as biofertilizers can be employed both as inoculants, plant booster, plant growth stimulator or biological pesticide relying upon their mechanisms and effectiveness (Sharma et al. 2014). The nitrogen-fixing rhizospheric bacteria related to the genera *Azotobacter, Bacillus, Paenibacillus*, *Pseudomonas, Rhizobium,* etc., are implicated in improved growth in diverse crops. However, different situations confronted via plants result in the buildup of different microorganisms. Conversely, only some microbes related to the plant microbiome can be studied through microbial culture methods, while others need metagenomics tool for their evaluation (Hirsch and Mauchline 2012). On the other hand, the function of unculturable microorganisms cannot be contradicted in plant growth (Wintermans et al. 2016). Finally, the plant microbiome available both within soil and in plant could be the most excellent approach to conquer adverse conditions associated with climatic change and might also be employed for its alleviation (Bisht and Chauhan 2020).

16.10 Nitrogen-Fixing Rhizobacteria and Biofilm

Rhizobacterial biofilms are closely associated populations enclosed within extracellular polymeric substances (EPS) that are attached to biological and non-biological area. Extracellular polymeric substances usually generated thought the amalgamation of polysaccharides, extracellular proteins and enzymes, DNA, along with extra materials. Biofilm provides a state of protection against drought to rhizobacteria. The root surface and its surrounding region are dynamic ecological region that work as a living micro habitat and it is extensively studied in order to apprehend the microorganism's diversity and its management. Polyphasic pathways such as genotypic, chemotaxonomic, and phenotypic are used to become familiar with these environments (Hinsinger et al. 2009; Bogino et al. 2013). Root and their related discharge openly influence the rhizosphere ambience. Prosperous inhabitation of useful rhizospheric microbes is regulated through clamping along with micro niche formation. The growth of biofilm plays a key function in the survival and working of microbes. The rhizobacteria need assistance through several microorganisms and should fix personally as multispecies biofilm within the rhizosphere (Compant et al. 2010). The interactions of biofilm forming rhizobacteria by plants might be helpful or destructive. Biofilm also helps in nutrient productivity and mitigation in different adverse conditions (Angus and Hirsch 2013).

The potential to build up biofilm through nitrogen-fixing rhizobacteria equally by symbiotic like *Rhizobium alamii*, *R. leguminosarum* bv. *Viciae* 3841, *R. leguminosarum*, *Rhizobium* sp. NGR234, *Rhizobium*, *Sinorhizobium* and asymbiotic such as *Azospirillum brasilense*, *Azorhizobium caulinodans*, *Azotobacter chroococcum,* and *Azotobacter vinelandii* has been recognized by several workers

(Shelud'ko et al. 2010; Krysciak et al. 2011; Robledo et al. 2012; Altaf and Ahmad 2017; Altaf et al. 2019). Biofilms that are found in nature could be used as biofilm based bioinoculants. These types of future generation invention accurately reverberate with normal ecological units. Despite the fact that this technique possesses the capability in increasing the crop productivity together with maintaining soil feature and fecundity, use of such biofilm based bioinoculants stays behind globally because of its inception problems within the rhizosphere. The biofilm based bioinoculants will thrive better in the rhizosphere as useful invention if it consists of native microorganisms because they will sustain site specific characters efficiently (Altaf 2016; Pandit et al. 2020).

16.11 Nitrogen-Fixing Rhizobacteria and Nanoparticles

Nanotechnology unlocks a broad horizon of new task related to the area of farming in order to accomplish the food requirements of ever increasing inhabitants of the world. Nanoparticles (NPs) possess distinguishing physico-chemical characters, i.e., good surface region, tunable pore size, high reactivity along with particle morphology. Administration of highly appropriate source of nourishment for feasible crop production is a leading topic of research in farming. The use of nanotechnology has been extensively exploited in different farming area and abundant information is presented on the relevance of nanomaterials (NMs) in the area of plant protection (Servin et al. 2015; Vishwakarma et al. 2018). The nanomaterials are favored above traditional manures owing to improved source use effectiveness, least fertilizer utilization, gradual and continued discharge of nutrients, better plant development and productivity with nominal soil disturbance. In addition they also work as magical dose for a well-organized discharge of dynamic compounds and genes intended for enhancement of crop well-being (Pérez-de-Luque and Rubiales 2009). An illustration of this style of release method is accounted via Torney et al. (2007) with the growth of mesoporous silica NPs that have been created with chemical compounds and work as a gene transporter for the transmittance of genes to tobacco and corn plants. The cell walls then take up the NPs and the marked genes are efficiently moved to the anchor plant in an environmental friendly method. NMs not only work in the growth of new and efficient biologically active products containing one or more beneficial microbial strains like insecticides or pesticides to increase a viable crop health methods (Gajbhiye et al. 2009). The exploitation of silica NPs has been found earlier for gene transport within bacterial cells and this system could be used for the growth of numerous new biological control means (Torney 2009). In addition, the utilization of nanoemulsions was also demonstrated for the growth of pesticides in opposition to plant disease causing microbes (Wang et al. 2007). NPs of zinc oxide, copper oxide, and cerium oxide have also been used in sweet potato for their bioaccessibility (Bradfield et al. 2017). Conversely, there is also information of harmful impact of nanoparticles on bacterial passageways. Like

McGee (2020) accounted that silver nanoparticles (AgNPs) probably create considerable danger for microbial nitrogen cycling species.

16.12 Conclusion

Nitrogen-fixing rhizobacteria are acknowledged for their constructive impact developing from the synergetic and nonsymbiotic nitrogen fixation process. Currently, the information associated with complex environment of the plant root system, functional diversity within nitrogen-fixing plant growth-promoting rhizobacteria**,** mechanisms of their work, processes of biofertilizer improvement, along with their discharge system has enhanced considerably. In contrast, there are several problems associated with their large-scale utilization, such as their uncertainty under field conditions. This problem can be alleviated through the use of nitrogen-fixing plant growth-promoting rhizobacteria in biofilm mode. Nitrogen-fixing rhizobacteria assist crop plants in coping with adverse environmental effects, for example, it provide resistance against low, high temperature, aridity, saline conditions, heavy metals, etc. Considering the growth in the availability of information associated with comprehension of diverse methods of functions of nitrogen-fixing plant growth-promoting rhizobacteria, it turn out to be simpler to genetically alter the nitrogen-fixing PGPR to increase the potential in the direction of improving the plant growth under nitrogen poor situations. These kinds of genetically tailored microbes with several plant growth-promoting traits can advance inhabitation and development efficacy, resulting in superior agricultural productivity. At the same time preserving farming soil fecundity, alleviating adverse environmental conditions and decreasing the obsession of synthetic manure and pesticides, that could be regarded as an ecologically feasible approach toward augmenting food yield along with fighting climate change in future.

References

Abd-Alla MH (1994) Use of organic phosphorus by *Rhizobium leguminosarum* biovar. Viceae phosphatases. Biol Fertil Soils 18:216–218

Abd-Allah MH, Nafady NA, Bashandy SR et al (2019) Mitigation of effect of salt stress on the nodulation, nitrogen fixation and growth of chickpea (*Cicer arietinum* L.) by triple microbial inoculation. Rhizosphere 10:100148

Abdel Latef AAH, Abu Alhmad MF, Kordrostami M et al (2020) Inoculation with *Azospirillum lipoferum* or *Azotobacter chroococcum* reinforces maize growth by improving physiological activities under saline conditions. J Plant Growth Regul 39:1–14

Adhikari L, Missaoui AM (2017) Nodulation response to molybdenum supplementation in alfalfa and its correlation with root and shoot growth in low pH soil. J Plant Nutr 40:2290–2302

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA working paper FAO, Rome

Altaf MM (2016) In vitro screening for biofilm forming ability of rhizospheric bacteria and their plant growth promoting potential. Ph.D Thesis. Aligarh Muslim University
Altaf MM, Ahmad I (2017) In vitro and in vivo biofilm formation by *Azotobacter* isolates and its relevance to rhizospere colonization. Rhizosphere 3:138–142
Altaf MM, Khan MSA, Ahmad I (2019) Functional diversity of plant growth-promoting rhizobacteria: recent progress and future prospects. In: Singh D, Gupta V, Prabha R (eds) Microbial interventions in agriculture and environment. Springer, Singapore, pp 229–253
Anderson KR, Moore PA, Miller DM (2018) Phosphorus leaching from soil cores from a twenty-year study evaluating alum treatment of poultry litter. J Environ Qual 47:530–537
Angus AA, Hirsch AM (2013) Biofilm formation in the rhizosphere: multispecies interactions and implications for plant growth. In: de Bruijn FJ (ed) Molecular microbial ecology of the rhizosphere. Wiley, New Jersey, pp 701–712
Beijerinck MW (1888) Culture des Bacillus radicicola aus den Knollchen. Bot Ztg 46:740–750
Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350
Bisht N, Chauhan PS (2020) Microorganisms in maintaining food and energy security in a world of shifting climatic conditions. In: Singh P, Singh R, Srivastava V (eds) Contemporary environmental issues and challenges in era of climate change. Springer, Singapore, pp 171–181
Bogino P, Abod A, Nievas F et al (2013) Water-limiting conditions alter the structure and biofilm forming ability of bacterial multispecies communities in the alfalfa rhizosphere. PLoS One 8: e79614
Bradfield SJ, Kumar P, White JC et al (2017) Zinc, copper, or cerium accumulation from metal oxide nanoparticles or ions in sweet potato: yield effects and projected dietary intake from consumption. Plant Physiol Biochem 110:128–137
Chen WM, de Faria SM, James EK et al (2007) *Burkholderia nodosa* sp. nov., isolated from root nodules of the woody Brazilian legumes *Mimosa bimucronata* and *Mimosa scabrella*. Int J Syst Evol Microbiol 57:1055–1059
Chen Y, Huang Z, Li J et al (2020) Complete genome sequence of Kosakonia radicincitans GXGL-4A, a nitrogen-fixing bacterium with capability to degrade TEX. Curr Microbiol. https://doi.org/10.1007/s00284-020-01942-3
Chi F, Shen SH, Cheng HP et al (2005) Ascending migration of endophytic rhizobia from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71:7271–7278
Chopra A, KumarVandana U, Rahi P et al (2020) Plant growth promoting potential of *Brevibacterium sediminis* A6 isolated from the tea rhizosphere of Assam, India. Biocatal Agric Biotechnol:101610
Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678
Datta B, Chakrabartty PK (2014) Siderophore biosynthesis genes of rhizobium sp. isolated from Cicer arietinum L. 3 Biotech 4:391–401
Dazzo FB, Yanni YG (2006) The natural rhizobium-cereal crop association as an example of plant bacterial interaction. In: Uphoff N, Ball AS, Fernandes E et al (eds) Biological approaches to sustainable soil systems. CRC Press, Boca Raton, pp 109–127
Desbrosses GJ, Stougaard J (2011) Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. Cell Host Microbe 10:348–358
Dhir B (2018) Crop productivity in changing climate. In: Lichtfouse E (ed) Sustainable agriculture reviews. Springer, Cham, pp 213–241
Ding X, Peng XJ, Jin BS (2015) Spatial distribution of bacterial communities driven by multiple environmental factors in a beach wetland of the largest freshwater lake in China. Front Microbiol 6:129
Dobereiner J (1961) Nitrogen-fixing bacteria of the genus *Beijerinckia* Derx in the rhizosphere of sugar cane. Plant Soil 15:211–216

Ehrmann J, Ritz K (2014) Plant: soil interactions in temperate multi-cropping production systems. Plant Soil 376:1–29
Elmerich C, Newton WE (2007) Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Springer, Dordrecht, p 323
El-Serafy RS, El-Sheshtawy AA (2020) Effect of nitrogen fixing bacteria and moringa leaf extract on fruit yield, estragole content and total phenols of organic fennel. Sci Hortic:265
Ewert F, Rounsevell MDA, Reginster I et al (2005) Future scenarios of European agricultural land use I. estimating changes in crop productivity. Agric Ecosyst Environ 107:101–116
FAOSTAT (2012) Online database. http://faostat.fao.org/
Fox AR, Soto G, Valverde C et al (2016) Major cereal crops benefit from biological nitrogen fixation when inoculated with the nitrogen fixing bacterium *Pseudomonas protegens* Pf-5 X940. Environ Microbiol 18:3522–3534
Frank B (1889) Uber die Pilzsymbiose der leguminosen. Berichte der Deutschen Beru Deut Bot Ges 7:332–346
Fukami J, Nogueira MA, Araujo RS, Hungria M (2016) Accessing inoculation methods of maize and wheat with Azospirillum brasilense. AMB Express 6:1. https://doi.org/10.1186/s13568-015-0171-y
Fukami J, Cerezini P, Hungria M (2018) *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express 8:73
Gajbhiye M, Kesharwani J, Ingle A (2009) Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine 5:382–386
Gandhi Pragash M, Narayanan KB, Naik PR (2009) Characterization of *Chryseobacterium aquaticum* strain PUPC1 producing a novel antifungal protease from rice rhizosphere soil. J Microbiol Biotechnol 19:99–107
García-Fraile P, Carro L, Robledo M (2012) *Rhizobium* promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One 7:38122
Ghosh PK, De TK, Maiti TK (2015) Production and metabolism of indole acetic acid in root nodules and symbiont (*Rhizobium undicola*) isolated from root nodule of aquatic medicinal legume *Neptunia oleracea* Lour. J Bot 11:575067
Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric 2:1127500
Gouda S, Kerry RG, Das G (2018) Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res (Pavia) 206:131–140
Graham PH (1992) Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Can J Microbiol 38:475–484
Graham PH (2008) Ecology of the root-nodule bacteria of legumes. In: Dilworth MJ, James EK, Spent JI et al (eds) Nitrogen-fixing leguminous symbioses. Springer, Dordrecht, pp 23–58
Hinsinger P, Bengough AG, Vetterlein D (2009) Rhizosphere: biophysics, biogeochemistry, and ecological relevance. Plant Soil 321:117–152
Hirsch PR, Mauchline TH (2012) Who's who in the plant root microbiome? Nat Biotechnol 30:961–962
Igiehon NO, Babalola OO, Aremu BR (2019) Genomic insights into plant growth promoting rhizobia capable of enhancing soybean germination under drought stress. BMC Microbiol 19:159
Iniguez AL, Dong Y, Triplett EW (2004) Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. Mol Plant-Microbe Interact 17:1078–1085
IPCC (2014) Climate change 2014: synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge
James EJ (2000) Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res 65:197–209

Kesari V, Ramesh AM, Rangan LJ (2013) *Rhizobium pongamiae* sp. nov. from root nodules of *Pongamia pinnata*. Bio Med Res Int:165198

Khan WDF, Peoples MB, Herridge DF (2002) Quantifying below-ground nitrogen of legumes. Plant Soil 245:327–334

Kim K, Jang YJ, Lee SM (2014) Alleviation of salt stress by *Enterobacter* sp. EJ01 in tomato and *Arabidopsis* is accompanied by up-regulation of conserved salinity responsive factors in plants. Mol Cells 37:109–117

Kramer EM, Bennett MJ (2006) Auxin transport: a field in flux. Trends Plant Sci 11:382–386

Krysciak D, Schmeisser C, Preußß S (2011) Involvement of multiple loci in quorum quenching of autoinducer I molecules in the nitrogen-fxing symbiont *Rhizobium* (*Sinorhizobium*) sp. strain NGR234. Appl Environ Microbiol 77:5089–5099

Kuan KB, Othman R, Abdul Rahim K, Shamsuddin ZH (2016) Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PLoS One 11(3):e0152478. https://doi.org/10.1371/journal.pone.0152478

Kumar PR, Ram MR (2012) Production of indole acetic acid by *Rhizobium* isolates from *Vigna trilobata* (L) Verdc. Afr J Microbiol Res 6:5536–5541

Kumar A, Kumar K, Kumar P (2014) Production of indole acetic acid by *Azotobacter* strains associated with mungbean. Plant Arch 14:41–42

Lesueur D, Carr Del Rio M, Dien HG (1995) Modification of the growth and the competitiveness of *Bradyrhizobium* strain obtained through affecting its siderophore-producing ability. In: Abadia J (ed) Iron nutrition in soil and plants. Kluwer Academic Publishers, PaysBas, pp 59–66

Li XG, Jia B, Lv J, Ma Q, Kuzyakov Y, Li F-M (2017a) Nitrogen fertilization decreases the decomposition of soil organic matter and plant residues in planted soils. Soil Biol Biochem 112:47–55

Li HB, Singh RK, Singh P, Song QQ, Xing YX, Yang LT, Li YR (2017b) Genetic diversity of nitrogen-fixing and plant growth promoting *pseudomonas* species isolated from sugarcane rhizosphere. Front Microbiol 8:1268

Li H, Qiu Y, Yao T (2020) Effects of PGPR microbial inoculants on the growth and soil properties of *Avena sativa*, *Medicago sativa*, and *Cucumis sativus* seedlings. Soil Till Res 199:104577

Lim JH, Kim SD (2013) Induction of drought stress resistance by multifunctional PGPR *Bacillus licheniformis* K11 in pepper. Plant Pathol J 29:201–208

Lindstrom K, Martinez-Romero ME (2005) International committee on systematics of prokaryotes; subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium*: minutes of the meeting, 26 July 2004, Toulouse, France. Int J Syst Evol Microbiol 55:1383

Lindström K, Mousavi SA (2019) Effectiveness of nitrogen fixation in rhizobia. Microb Biotechnol. https://doi.org/10.1111/1751-7915.13517

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Mahmud K, Makaju S, Ibrahim R (2020) Current progress in nitrogen fixing plants and microbiome research. Plants (Basel) 9:97

McGee CF (2020) The effects of silver nanoparticles on the microbial nitrogen cycle: a review of the known risks. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-020-09548-9

McNeill A, Fillery IJP (2008) Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate. Plant Soil 302:297–316

Morris JJ, Schniter EJ (2018) Black queen markets: commensalism, dependency, and the evolution of cooperative specialization in human society. J Bioecon 20:69–105

Mus F, Crook MB, Garcia K (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Appl Environ Microbiol 82:3698–3710

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14:1–26

Pandit A, Adholeya A, Cahill D (2020) Microbial biofilms in nature: unlocking their potential for agricultural applications. J App Microbiol. https://doi.org/10.1111/jam.14609
Pankievicz VCS, Amaral FPD, Santos KFDN (2015) Robust biological nitrogen fixation in a model grass-bacterial association. Plant J 81:907–919
Peix A, Ramírez-Bahena MH, Velázquez E (2015) Bacterial associations with legumes. Crit Rev Plant Sci 34:17–42
Pena-Cabriales JJ, Alexander M (1983) Growth of *Rhizobium* in unamended soil. Soil Sci Soc Am J 47:81–84
Pérez-de-Luque A, Rubiales D (2009) Nanotechnology for parasitic plant control. Pest Manag Sci 65:540–545
Poole RK, Hill S (1997) Respiratory protection of nitrogenase activity in *Azotobacter vinelandii*-roles of the terminal oxidases. Biosci Rep 17:303–317
Rana KL, Kour D, Yadav AN (2020) Agriculturally important microbial biofilms: biodiversity, ecological significances, and biotechnological applications. In: Yadav MK, Singh BP (eds) New and future developments in microbial biotechnology and bioengineering: microbial biofilms: current research and future trends. Elsevier, Amsterdam, pp 221–265
Reyes VG, Schimidt EL (1979) Population densities of *Rhizobium japonicum* strain 123 estimated directly in soil and rhizosphere. Appl Environ Microbiol 37:854–858
Richardson AE, Barea JM, McNeill AM (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:111–117
Robledo M, Rivera L, Jiménez-Zurdo JI (2012) Role of *Rhizobium* endoglucanase CelC2 in cellulose biosynthesis and biofilm formation on plant roots and abiotic surfaces. Microb Cell Factories 11:125
Rosenblueth M, Ormeño-Orrillo E, López-López A (2018) Nitrogen fixation in cereals. Front Microbiol 9:9
Ryu M, Zhang J, Toth T (2020) Control of nitrogen fixation in bacteria that associate with cereals. Nat Microbiol 5:314–330
Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot 111:743–767
Schultze M, Kondorosi AJ (1998) Regulation of symbiotic root nodule development. Annu Rev Genet 32:33–57
Segura A, Ramos JL (2013) Plant–bacteria interactions in the removal of pollutants. Curr Opin Biotech 24:467–473
Servin A, Elmer W, Mukherjee A (2015) A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J Nanopart Res 17:1–21
Sharma P, Jha AB, Dubey RS (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012:1–26
Sharma AK, Bhattacharyya PN, Rajkhowa DJ et al (2014) Impact of global climate change on beneficial plant-microbe association. Ann Biol Res 5:36–37
Shelud'ko AV, Shirokov AA, Sokolova MK (2010) Wheat root colonization by *Azospirillum brasilense* strains with different motility. Microbiology 9:688–695
Siddikee MA, Sundaram S, Chandrasekaran M (2015) Halotolerant bacteria with ACC deaminase activity alleviate salt stress effect in canola seed germination. J Kor Soc Appl Biol Chem 58:237–224
Singh RK, Singh P, Li H (2020) Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: a comprehensive study of plant-microbe interactions for growth enhancement in *Saccharum* spp. BMC Plant Biol 20:220
Spaepen S, Vanderleyden J (2015) Auxin signaling in *Azospirillum brasilense*: a proteome analysis. In: de Bruijn FJ (ed) Biological nitrogen fxation. Wiley, Hoboken, pp 937–940
Spaepen S, Dobbelaere S, Croonenborghs A (2008) Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. Plant Soil 312:15–23
Spaepen S, Das F, Luyten E (2009) Indole-3-acetic acid-regulated genes in *Rhizobium etli* CNPAF512: research letter. FEMS Microbiol Lett 291:195–200

Sprent JI, Faria SM (1988) Mechanisms of infection of plants by nitrogen fixing organisms. Plant Soil 110:157–165

Teng Y, Wang X, Li L (2015) Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. Front Plant Sci 6:32

Terouchi N, Syono K (1990) *Rhizobium* attachment and curing in asparagus, rice and oat plants. Plant Cell Physiol 31:119–127

Thaweenut N, Hachisuka Y, Ando S (2011) Two seasons study on *nifH* gene expression and nitrogen fixation by diazotrophic endophytes in sugarcane (*Saccharum* spp. hybrids): expression of *nifH* genes similar to those of rhizobia. Plant Soil 338:435–449

Torney F (2009) Nanoparticle mediated plant transformation: emerging technologies in plant science research. In: Proceedings of the interdepartmental plant physiology major fall seminar series, (IPPM'09). Iowa State University, p 696

Torney F, Trewyn BG, Lin VS (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants *Triticum aestivum*. Nat Nanotechnol 2:295–300

Ueda T, Suga Y, Yahiro N (1995) Genetic diversity of N_2-fixing bacteria associated with rice roots by molecular evolutionary analysis of a nifD library. Can J Microbiol 41:235–240

Unkovich MJ, Baldock J, Peoples MB (2010) Prospects and problems of simple linear models for estimating symbiotic N_2 fixation by crop and pasture legumes. Plant Soil 329:75–89

Valentín L, Nousiainen A, Mikkonen A (2013) Introduction to organic contaminants in soil: concepts and risks. In: Kostianoy AG, Barceló D (eds) Emerging organic contaminants in sludges: the handbook of environmental chemistry. Springer, Heidelberg, pp 1–29

Van Deynze A, Zamora P, Delaux P-M (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLoS Boil 16:e2006352

Van Oosten MJ, Di Stasio E, Cirillo V (2018) Root inoculation with *Azotobacter chroococcum* 76A enhances tomato plants adaptation to salt stress under low. N conditions BMC Plant Biol 18:205

Vishwakarma K, Upadhyay N, Kumar N (2018) Potential applications and avenues of nanotechnology in sustainable agriculture. In: Tripathi DK, Ahmad P, Sharma S et al (eds) Nanomaterials in plants, algae and microorganisms: concepts and controversies, vol 1. Academic Press, pp 473–500

Wang L, Li X, Zhang G (2007) Oil-in-water nanoemulsions for pesticide formulations. J Colloid Interface Sci 314:230–235

Wang J, Li R, Zhang H (2020a) Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. BMC Microbiol 20:38

Wang M, Bian Z, Shi J (2020b) Effect of the nitrogen-fixing bacterium *Pseudomonas protegens* CHA0-ΔretS-nif on garlic growth under different field conditions. Ind Crop Prod 145:111982

Wintermans PC, Bakker PA, Pieterse CM (2016) Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting rhizobacteria. Plant Mol Biol 90:623–634

Yadav S, Rathor MS, Mishra A (2020) The pyruvate-phosphate dikinase (C_4-SmPPDK) gene from suaeda monoica enhances photosynthesis, carbon assimilation, and abiotic stress tolerance in a C_3 plant under elevated CO_2 conditions. Front Plant Sci 11:345

Yoneyama T, Terakado-Tonooka J, Minamisawa K (2017) Exploration of bacterial N_2-fixation systems in association with soil-grown sugarcane, sweet potato, and paddy rice: a review and synthesis. Soil Sci Plant Nutr 63:578–590

Yousuf J, Thajudeen J, Rahiman M (2017) Nitrogen fixing potential of various heterotrophic Bacillus strains from a tropical estuary and adjacent coastal regions. J Basic Microbiol 57:922–932

Chapter 17
Bacterial Mutants for Enhanced Nitrogen Fixation

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Abstract Biological nitrogen fixation (BNF) is a sustainable approach to reduce negative effects of chemical N fertilizers on ecosystem. Therefore, BNF has gained special attention in researchers for enhancing its effectiveness among different crop plants. The genetic mutation approach in bacterial spp. towards efficient symbiosis and plant growth promotion is an attractive strategy nowadays. In addition, positive mutants of plant growth promoting rhizobacterial (PGPR) strains could survive under harsh conditions and promote plant growth also. The objectives of the present chapter are to highlight the basic mechanisms of induced mutation in bacteria and applied aspects of these mutated bacteria for improving nitrogen-fixing abilities in plants.

Keywords Bacteria · Mutation · Nitrogen fixation · Plant growth promoting rhizobacteria

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17.1 Introduction

Rhizosphere encompasses the soil volume that is under the influence of plant root. As compared to bulk soil, rhizospheric soil is a hot spot of microbial diversity (more than 10 to 100 times) due to the presence of variety of nutrients secreted by root exudates (amino acids, sugars, enzymes, and organic acids) (Gray and Smith 2005). The secreted compounds also act as chemo-attractant for a diverse range of plant beneficial microbes, thus involved in symbiotic plant–microbe interaction, growth promotion, and disease suppression. The pattern of these compounds varies from one plant species to other and their holobiont (Kang et al. 2010). Therefore, rhizospheric region has been mostly focused in agricultural research for many years. The rhizospheric engineering including microbial or metabolic changes for sustainable plant growth has gained attention recently.

17.2 Plant–Microbe Interactions in the Rhizosphere

The rhizospheric interaction between plant and microbes is essential for uptake of water and nutrients (Ryan et al. 2009). The rhizospheric microbes are involved in biogeochemical cycling of various essential nutrients and also helpful for soil remediation (Choudhary et al. 2015). These microbes depend on each other as well as plant exudates for nutrient source. Hence, rhizosphere is a very dynamic ecological unit of plant holobiont interaction. The beneficial effect of rhizospheric microbes can vary upon their colonization with host plants. These microbes can colonize in rhizospheric soil, root surface, and intracellular region of plants (McCully 2001). In addition, microbial colonization with host plants also depends on their genetic traits, root exudates, and abiotic and biotic factors (Benizri et al. 2001). Most of the microbial cells first colonized in the rhizospheric soil and then some of the species attached on root surface and subsequently entered inside the root cells (Compant et al. 2010). The colonization in root not occurs in a uniform manner, different microbial populations are reported for diverse root zones. For instance, a gram negative motile bacterium *Kluyvera ascorbata* is able to colonize at upper surface of canola roots but not detected around the root tips (Vessey 2003).

In many rhizospheric relationships, the plant growth promoting rhizobacteria (PGPR) are known to colonize the plant root and stimulate plant growth. PGPR can exert their beneficial effects on plants by two ways, i.e. direct or indirect. In direct mechanism, PGPR increase nutrient availability in soil through solubilizing inorganic and organic complex compounds, nitrogen fixation, and siderophore production and synthesize plant hormones. In indirect mechanism, PGPR control phytopathogen growth by secreting antimicrobial compounds in the soil (Ahmad et al. 2008) (Fig. 17.1). PGPR mainly belong to Firmicutes and Proteobacteria including *Azospirillum*, *Bacillus*, *Pseudomonas, Arthrobacter, Rhizobium, Burkholderia, Proteus, Enterobacter, Serratia, Xanthomonas*, etc. genera

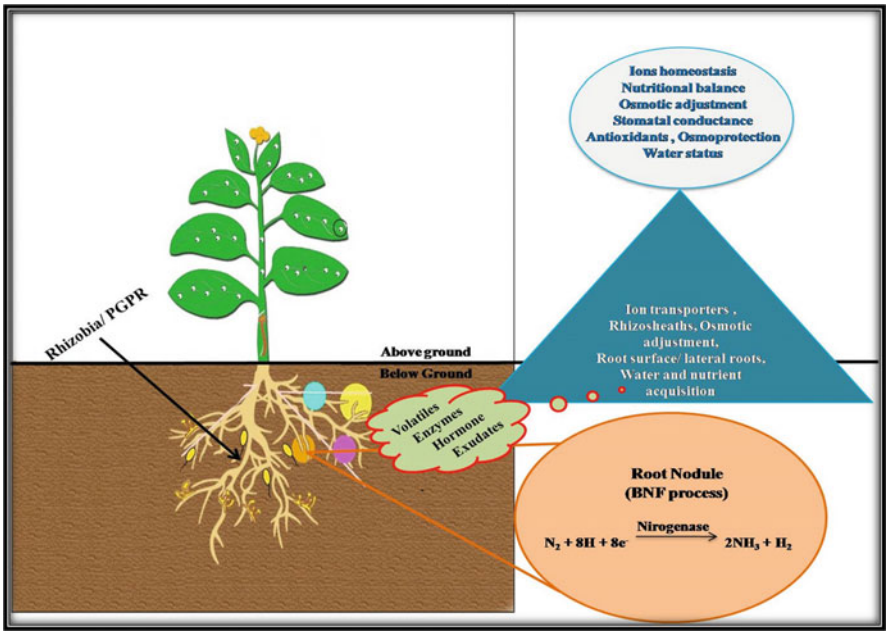

Fig. 17.1 Different mechanisms of PGPR mediated plant growth promotion

(Steenhoudt and Vanderleyden 2000; Trivedi et al. 2005). On the basis of interaction with host plants, PGPR are divided into two groups: symbiotic bacteria (iPGPR) and free-living rhizobacteria (ePGPR) (Gray and Smith 2005). The use of PGPR is steadily increasing in agriculture practices as biofertilizers and biopesticides.

17.3 Biological Nitrogen Fixation

Nitrogen is an essential nutrient in plant development and productivity, accounts approx. 2% of total dry weight that enters into food chain. The available sources of nitrogen for plants are ammonium and nitrates present in the soil. The limitation of nitrogen sources and their requirement for plants has generated a large number of chemical based N fertilizers (Westhoff 2009). About 60% of chemical N-fertilizers are used for cereals cultivation, even 10% in rice growth. The irregular use of chemical N fertilizer causes nitrate contamination in soil and ground water, leading to negative impact on health and agricultural sustainability. Moreover, manufacturing process for N fertilizer requires six times more energy than other fertilizers (Da Silva et al. 1978). Another process is biological N_2 fixation (BNF) by which plant can uptake atmospheric N_2 (approx. 2/3rd) with the help of symbiotic microorganisms. This process is economically and eco-friendly alternative of chemical fertilizers. The symbiotic microorganisms are able to fix nitrogen or ammonia in

plants through nitrogenase enzyme complex. These microbes are generally distributed in nature and work at mild temperatures. Nitrogen-fixing organisms are generally categorized in symbiotic and non-symbiotic organisms. Symbiotic organisms make association with leguminous plants and non-leguminous tree, i.e. *Rhizobium* and *Frankia* (Ahemad and Khan 2011). In non-symbiotic association, free-living microbes and endophytes fix nitrogen, i.e. Cyanobacteria (*Anabaena, Nostoc*), *Azospirillum, Azotobacter, Gluconoacetobacter diazotrophicus*, *Azoarcus*, etc. (Antoun et al. 1978; Riggs et al. 2001). Non-symbiotic microbes fix a less amount of nitrogen as compared to symbiotic microorganism (Glick 1995). In addition, plant growth promoting rhizobacteria (PGPR) are also able to fix N_2 in non-leguminous plants through a non-obligate interaction with the host plants (Glick et al. 1999).

17.4 Bacterial Strain Improvement

A growing demand of food for burgeoning population requires sustainable agriculture practices (Tilman et al. 2011). Food security of such a huge population will require increased agricultural production with increased productivities in the same land area. Development of overproducing PGPR mutants or strain improvement could be a more beneficial alternative to increase the productivity with the conventional approaches. Mutation is an instant change in genes character that is different from wild type. Mutations allowing new versions of DNA combinations can be harmful, benign, or beneficial. If mutations are harmful, they tend to be self-eliminating, unless they only show up after breeding age. Mutations which are benign may not influence the species. Mutations which are beneficial improve varieties of the organisms resulting in greater diversity and therefore greater adaptability in the species. Only mutations which are beneficial are of great use in the process of development of new organisms, individual, or variety, etc. Strain improvement for PGP activity can be approached by two types, i.e. direct or combinational. In direct approach, genetic sequence is known, while in combination sequence is unknown and two approaches used together for screen out.

17.5 Mechanism of Mutation

Mutation is a random event and occurs in spontaneous rate in nature. Mutation can also be induced artificially with the help of some physical or chemical agents (mutagens). Mutagenesis is described as a process to develop mutation in living cells either through chemical or physical approach. The first reported case of artificial induced mutations was back in 1920 with work on *Drosophila*, maize, and barley. On the basis of mutagens the mutagenesis can be broadly classified as:

17.6 Physical Mutagenesis

Physical mutagenesis is induced by physical mutagens including radiations (gamma rays, X-rays, UV rays, and neutron particles). Such radiations fall in the wavelength range below 340 nm and photon energy above 1 electro-volt (eV). These radiations can damage DNA by directly through ionization/excitation or indirectly through reactive free radicals. Y-rays have a lower effectiveness for mutations, hence a higher dose is required to obtain positive results (Van Harten 1998).

17.7 Chemical Mutagenesis

A variety of chemical mutagens induce mutations in living cell. The first report of mutagenic action of a chemical was in 1942 by Charlotte Auerbach, who showed that nitrogen mustard could cause mutations in cells (Auerbach 1949). Most chemical mutagens are alkylating agents, azides, and base analogs (Andrea and Afza 2003). Both, in vitro and in vivo, methyl is most reactive with nucleic acids fallowed by ethyl and higher homologues. Some of the most used alkalizing agents to induce mutation are N-methyl-N′-nitro-N-nitrosoguanidine (NTG), dimethyl sulfate (DMS), ethyl methane sulfonate (EMS), ethyl ethane sulfonate (EES). N-Methyl-N′-nitro-N-nitrosoguanidine (NTG), one of the most potent chemical mutagen has been widely used to induce mutations in bacteria for a long time (D Moore 1969). NTG creates changes in nucleic acid sequences by mutations including substitution of nucleotide base-pairs and insertions and deletions of one or more nucleotides in DNA sequences (Mishra and Goel 1999; Rasila et al. 2009; Harper and Lee 2012). Azides are another class of chemical mutagens which are frequently used in mutagenesis. Sodium azide (NAN3) is a well established chemical mutagen for base transition mutation. An experiment conducted by Berger et al. (1953) revealed that sodium azide increased the occurrence of mutants resistant to penicillin. Nilan et al. (1973) found high reversion frequencies through sodium azide in *Salmonella typhimurium* mutant TA1530 (Nilan et al. 1973). The azido derivatives of ethidium bromide were found highly mutagenic for yeast and in *Salmonella typhimurium* (Morita and Yielding 1977; Yielding et al. 1976). In an experiment, azide analogs of proflavine and acriflavine were found to produce mutation in excision-repair deficient *Salmonella strains* (Owais et al. 1979). 5-Bromouracil (5-BU) and 2-aminopurine (2-AP) are two analogues to induce mutation. Anologue 5-bromouracil (5-BU) resembles thymine (T) and can incorporate into DNA and pairs with A or G (Fig. 17.2). Likewise, 2-aminopurine (2-AP) induces mutation through two mechanisms: directly, by mispairing with cytosine, and indirectly, by saturation of mismatch repair in *E. coli* (Photini et al. 2004).

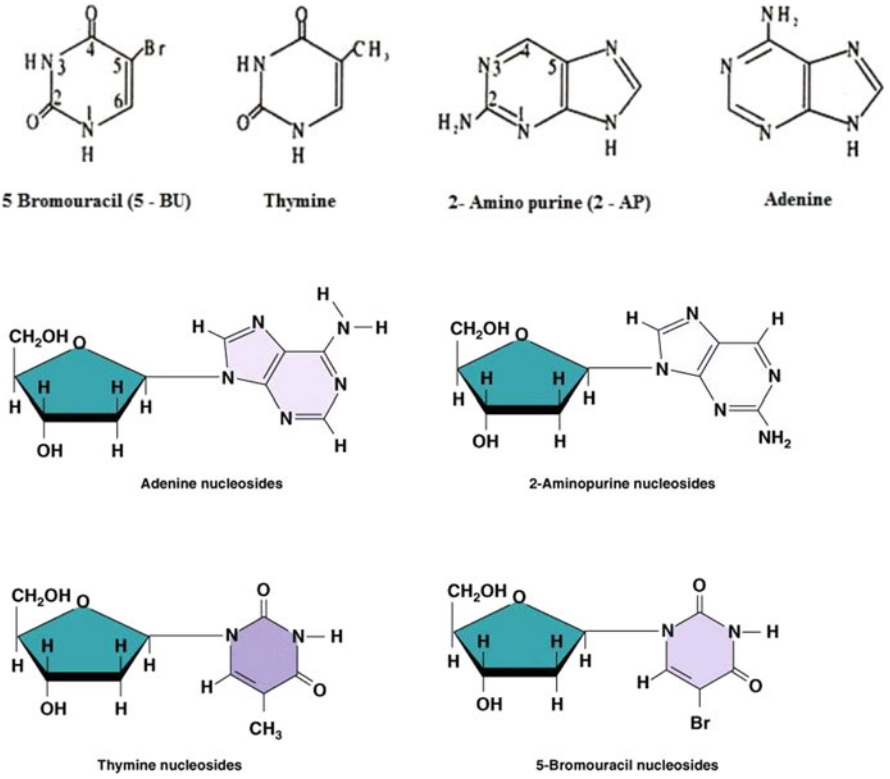

Fig. 17.2 Base analog of thymine, 5-bromouracil (5-BU), and 2 amino purine (2-AP)

17.8 Site-Directed Mutagenesis

Site-directed mutagenesis (SDM), also known as directed mutagenesis, is method for creating a specific mutation in a known sequence. It is an in vitro technique where mutations are generated by PCR using a pair of oligonucleotide primers designed with mismatching nucleotides at the center of the primers (Oded et al. 2009). In this approach, one part of targeted gene is amplified by PCR primer and then the PCR product is used as mega primer for amplifying whole gene. To screen out mutation, a restriction enzyme site is introduced into target gene during PCR process. Through this process, we can generate single and multiple mutations in a gene. In a study, *R. meliloti* genome was mutated with *Escherichia coli* transposon Tn*5* for symbiotic nitrogen fixation gene through site-directed mutagenesis (Ausubel and Ruvkun 1981).

17.9 Bacterial Mutation for Enhanced Nitrogen Fixation

The bacterial nitrogenase enzymes play important role in conversion of atmospheric nitrogen into ammonia. It is expected that approx. 100 million metric tonnes of N_2 are converted by the nitrogenase activity per annum (Downie 2014). In this context, biological nitrogen fixation (BNF) process covers major (about 2/3) portion in fixing atmospheric nitrogen, while the rest part is performed by Haber–Bosch process. BNF is firstly discovered in prokaryotes by Beijerinck in 1901 (Wagner 2012). However, this process supplies only 20–25% of total nitrogen requirements in cereals like rice and maize (Montanez et al. 2012). Although an enormous progress has been developed in nitrogen-fixing symbiosis between rhizobia and legumes, utilization of this knowledge to induce BNF in cereals is still less explored (Dixon and Arntzen 1997). In this context, genetic engineering techniques and mutagenesis approach can be employed for enhancing BNF process in cereals. The transformation of bacterial *nif* genes into cereal plant genomes is still a difficult task. There are few examples of study, which performed BNF in cereals through mutagenesis approach. For instance, Christiansen and Van Veen (1991) found that NH_4^+-excreting *Azospirillum brasilense* mutants enhanced the nitrogen supply in wheat plant. The mutant strain possessed two to three times higher nitrogenase activity as compared to the wild type. Furthermore, a point mutation in glutamine synthetase enzyme in *Azospirillum* leads to higher ammonium fixation for better plant growth in wheat as compared to wild type strain inoculation (Van et al. 2009).

Beside cereals, several attempts have been made with *Rhizobium* spp. to develop mutants for promoting N-fixation in legumes. Paau (1989) performed a mutagenesis experiment with *R. Japonicum* isolates by using N-methyl-N′-nitrosoguanidine and found higher level occupancy of nodules in soybean fields than the wild types. In a consortium approach, mutant strain of *Rhizobium trifolii* ANU794 was found from nitrogen-fixing nodules on white clover in the presence of added EPS producing bacterium *R. trifolii* ANU 843 (Steven et al. 1987). *Rhizobium tropici* induces nitrogen-fixing nodules on several unrelated tropical legume plants, including species of *Phaseolus*, *Leucaena*, and *Macroptilium*. Tn5-induced *Rhizobium tropici* mutant enhanced respiration and symbiotic nitrogen fixation (Marroqui et al. 2001). A study conducted by Shashi et al. (1997) revealed that mutations conferring azide resistance enhanced symbiotic nitrogen fixation in *Rhizobium loti*. Eric Wendell Triplett patented an efficient nitrogen-fixing mutant *Klebsiella* Kp342 in year 2009 (Triplett 2009). Pankievicz et al. (2015) developed an ammonium-excreting mutant strain of *A. brasilense,* strain HM053, which enhanced N-fixation many folds in association with *Setaria viridis. A* mutant of *Anabaena variabilis,* SA-1, increased the ammonia output (Knoche 2017). A frameshift mutation in zinc finger regulator (MucR1) enhanced nitrogen-fixing ability of *Sinorhizobium fredii* CCBAU45436 in soybean (Jiao et al. 2016).

Some attempts have also been made for developing mutants of plant growth promoting rhizobacteria (PGPR). Mutagenesis in PGPR for overproducing PGP traits is most effective strategy under stressed environment to increase plant growth

and productivity (Ali et al. 2014). Mutants of *Pseudomonas* spp. have been developed for overproducing ACC-deaminase and exopolysaccharide activity, which enhanced plant growth and tolerance capacity under stress conditions (Mishra and Goel 1999; Ali et al. 2014; Kasotia and Choudhary 2014; Kumari et al. 2016). In addition, Trivedi and Sa (2008) produced positive mutants of *P. corrugate* (NRRL B-30409) for Pi-solubilization activity. Similarly, Mohamed and Ibrahim Ebaid (2011) developed *Bacillus polymyxa* mutant strains for enhancing Pi-solubilization and Fe uptake activity, which increased plant biomass content. Hartmann et al. (1983) reported a mutant strain of *Azospirillum brasilense* sp. Cd, resistant to 5-fluorotryptophan (FT) that produces 10 times more IAA content than the wild type.

17.10 Conclusion

Development of mutant strains is a promising approach for applying PGPR strains in harsh conditions or enhancing their plant growth promotion ability. Efficient mutant strains should be generated for nitrogen fixation in cereals. In past, many mutant strains of *Anabaena*, *Rhizobium, Azospirillum, Klebsiella*, etc. have been developed as efficient N-fixer. Further progress in this direction required more intensive and internationally coordinated research efforts.

References

Ahemad M, Khan MS (2011) Effect of pesticides on plant growth promoting traits of green gram-symbiont, *Bradyrhizobium* sp. strain MRM6. Bull Environ Contam Toxicol 86:384–388

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Ali SZ, Vardhrajula S, Rao VL (2014) Isolation and characterization of drought tolerant ACC deaminase and exopolysaccharide producing fluorescent *Pseudomonas* sp. Ann Microbiol 64:493–502

Andrea K, Afza R (2003) Physical and chemical mutagenesis. Plant Funct Genom:189–203

Antoun H, Bordeleau LM, Gagnon C (1978) Antagonisme entre Rhizobium meliloti et Fusarium oxysporum en relation avec l'efficacité symbiotique, Can. J Plant Sci 58:75–78

Auerbach C (1949) Chemical mutagenesis. Biol Rev 24:355–391

Ausubel FM, Ruvkun GB (1981) Molecular cloning of nitrogen fixation genes from Rhizobium meliloti. In: Genetic engineering of symbiotic nitrogen fixation and conservation of fixed nitrogen. Springer, New York, pp 15–30

Benizri E, Baudoin E, Guckert A (2001) Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Sci Tech 11:557–574

Choudhary DK, Kasotia A, Jain S, Vaishnav A, Kumari S, Sharma KP, Varma A (2015) Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stresses. J Plant Growth Regul 35:276–300

Christiansen CW, Van Veen JA (1991) NH4+ excreting *Azospirillum brasilense* mutants enhance the nitrogen supply of a wheat host. Appl Environ Microbiol 57:3006–3012

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Da Silva JG, Serra GE, Moreira JR, Goncalves JC, Goldenberg J (1978) Energy balance for ethyl alcohol production for crops. Science 210:903–906

Dixon RA, Arntzen CJ (1997) Transgenic plant technology is entering the era of metabolic engineering. Trends Biotechnol 15:441–444

Downie JA (2014) Legume nodulation. Curr Biol 24:184–190

Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–117

Glick BR, Patten CL, Holguin G, Penrose GM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol Biochem 37:395–412

Hartmann A, Singh M, Klingmiller W (1983) Isolation and characterization of *Azospirillum* mutants, excreting high amounts of indoleacetic acid. Can J Microbiol 29:916–923

Jiao J, Wu LJ, Zhang B, Hu Y, Li Y, Zhang XX, Tian CF (2016) MucR is required for transcriptional activation of conserved ion transporters to support nitrogen fixation of *Sinorhizobium fredii* in soybean nodules. Mol Plant Microb Int 29:352–361

Kang BG, Kim WT, Yun HS, Chang SC (2010) Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnol Rep 4:179–183

Kasotia A, Choudhary DK (2014) Induced inorganic phosphate solubilization through N-methyl N´- nitro-N-Nitrosoguanidine treated mutants of *Pseudomonas koreensis* strain AK-1 (MTCC number 12058) under polyethylene glycol. Proc Natl Acad Sci India Sect B Biol Sci. https://doi.org/10.1007/s40011-014-0416-6

Knoche KL (2017) Role of nitrogenase and ferredoxin in the mechanism of bioelectrocatalytic nitrogen fixation by the cyanobacteria *Anabaena variabilis* SA-1 mutant immobilized on indium tin oxide (ITO) electrodes. Electrochim Acta 232:396–403

Kumari S, Vaishnav A, Jain S, Varma A, Choudhary DK (2016) Induced drought tolerance through wild and mutant bacterial strain *Pseudomonas simiae* in mung bean (*Vigna radiata* L.). World J Microbiol Biotechnol 32:4

Marroqui S, Zorreguieta A, Santamari CA, Temprano F, Sobero M, Megias ML, Downie JA (2001) Enhanced symbiotic performance by rhizobium tropici *g*lycogen synthase mutants. J Bacteriol 183:854–864

McCully ME (2001) Niches for bacterial endophytes in crop plants: a plant biologist's view. Aust J Plant Physiol 28:983–990

Mishra M, Goel R (1999) Development of a cold resistant mutant of plant growth promoting *Pseudomonas fluorescens* and its functional characterization. J Biotechnol 75:71–75

Mohamed HM, Ibrahim Ebaid MA (2011) Effect of inoculation with *Bacillus polymyxa* mutants on growth, phosphorus and iron uptake by tomato in calcareous soils. Int J Soil Sci 6:176–187

Montanez A, Rodriguez Blanco A, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects in vitro. Appl Soil Ecol 58:21–28

Moore D (1969) The mutagenic action of N-methyl-N nitrosoguanidine on *Coprinus lagopus*. J Gen Microbiol 55:121–125

Morita T, Yielding KL (1977) Induction of respiratory deficient mutants in *Saccharornyces cerevisiae* by mono and diazido analogs of ethidium. Mutation Res 56:21–30

Nilan RA, Sideris EG, Kleinhofs A, Sander C, Konzak CF (1973) Azide - a potent mutagen. Mutation Res 17:142–144

Oded E, Hanukoglu A, Hanukoglu I (2009) Simple and efficient site-directed mutagenesis using two single-primer reactions in parallel to generate mutants for protein structure-function studies. BMC Biotechnol 9(1):61

Owais WM, Kleinhofs A, Nilan RA (1979) In vivo conversion of sodium azide to a stable mutagenic metabolite in *Salmonella typhimurium*. Mutation Res Gen Toxicol 68:15–22

Paau AS (1989) Improvement of *Rhizobium* inoculants. Appl Environ Microbiol 55:862–865

Pankievicz V, Fernanda PA, Karina FDN, Beverly A, Youwen X, Schueller MJ, Ana CM (2015) Robust biological nitrogen fixation in a model grass–bacterial association. Plant J 81:907–919

Photini P, Jason MP, Claire GC (2004) Mechanism of 2-aminopurine-stimulated mutagenesis in *Escherichia coli*. Mutation Res Fund Mol Mech Mutagen 550(1):25–32

Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW (2001) Enhanced maize productivity with diazotrophic bacteria. Aust J Plant Physiol 28:829–836

Ryan PR, Dessaux Y, Thomashow LS, Weller DM (2009) Rhizo-sphere engineering and management for sustainable agriculture. Plant Soil 321:363–383

Shashi B, Sharma K, Sakadevan SS (1997) Mutations conferring azide resistance enhance symbiotic nitrogen fixation in *Rhizobium loti*. Plant Soil 189:221–229

Steenhoudt O, Vanderleyden J (2000) *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506

Steven PD, Chen H, Batley M, John WR, Barry GR (1987) Nitrogen fixation ability of exopolysaccharide synthesis mutants of Rhizobium sp. strain NGR234 and Rhizobium trifolii is restored by the addition of homologous exopolysaccharides

Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci U S A 108:20260–20264

Triplett EW (2009) Materials and methods for enhancing nitrogen fixation in plants. U.S. Patent 20:090:137:390

Trivedi P, Sa T (2008) *Pseudomonas corrugata* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. Curr Microbiol 56:140–144

Trivedi P, Pandey A, Palni LMS (2005) Carrier-based preparations of plant growth-promoting bacterial inoculants suitable for use in cooler regions. World J Microbiol Biotechnol 21:941–945

Van DA, Croonenborghs A, Spaepen S, Vanderleyden J (2009) Wheat growth promotion through inoculation with an ammonium-excreting mutant of *Azospirillum brasiliense*. Biol Fertil Soils 45:449–553

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Wagner SC (2012) Biological nitrogen fixation. Nat Edu Knowl 3:15–19

Westhoff P (2009) The economics of biological nitrogen fixation in the global economy. In: Emerich DW, Krishnan HB (eds.) Biological nitrogen fixation in non-legume plants agronomy monograph no. 52. American Society of Agronomy, Madison, pp 309–328

Yielding LW, White WE Jr, Yielding KL (1976) Production of frameshift mutations in *Salmonella* by a light sensitive aside analog of ethidium. Mutation Res 34:351–358

Chapter 18
Perspectives on Nitrogen-Fixing *Bacillus* Species

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Abstract Not all prokaryotes capable of biological nitrogen fixation require nodules to fix nitrogen (N_2). A wide range of *Bacillus* genus members have been reported for their N_2-fixing ability, as they can fix and provide N_2 to a wide range of host plants. Besides N_2 fixation, these bacteria possess several plant growth–promoting abilities such as growth hormone production, phosphate solubilization, and siderophore production for iron acquisition. They also have the capability to protect plants against phytopathogens through production of cell wall–degrading enzymes and antibiotic metabolites, and also through elicitation of plant defense systems. This chapter reviews and discusses the role of *Bacillus* spp. in N_2 fixation and perspectives on their agricultural development.

Keywords *Bacillus* · Nitrogen metabolism · Plant growth promoter

18.1 Introduction

Being a key component of nucleotides, proteins, and chlorophyll in plants, nitrogen is the most important element for life. It is found in abundance in the atmosphere, of which it constitutes about 78%, but it is actually inaccessible to plants because of its diatomic form (N_2), due to the presence of a double band (Galloway et al. 2003). The inert nature of nitrogen forces farmers to use chemical N_2 fertilizers to maintain optimum yields, but there are several drawbacks that necessitate implementation of alternative strategies to retain N_2 in soil in order to maintain plant growth. Besides being expensive, N_2 fertilizers contribute significantly to greenhouse gas emissions

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(Wang et al. 2007). Additionally, because of denitrification, volatilization, and leaching, soil becomes very vulnerable to loss of nitrogen, which is very reactive and mobile. Furthermore, its leached reactive form can cause several environmental problems and negative effects on human health (Williamson 2011; Robertson and Vitousek 2009). In view of the adverse effects on the ecosystem, along with the steadily increasing cost of N_2 fertilizer, cultivators have adapted crop rotation policies. Crop rotation benefits the agricultural system and adds fixed N_2 into soil through the process of biological N_2 fixation (Eskin et al. 2014; Peoples et al. 1995). Some prokaryotes have the capability to fix N_2 by reducing dinitrogen to ammonia in a process known as biological N_2 fixation (BNF). Symbiosis between leguminous plants and *Rhizobium* is the best example of N_2 fixation, but not all plants have achieved symbiosis with an N_2-fixing prokaryote (Vessey et al. 2005). Nodule formation is a primary requirement for rhizobia, and that is why they have a very selective host range (Mylona et al. 1995).

18.2 Role of *Bacillus* Species in Nitrogen Fixation

Bacillus, an endospore-forming genus, is a systematically diverse taxon, which includes aerobic and rod-shaped bacteria (Claus and Berkeley 1986). *Bacillus* spp. inhabit soil and contribute to crop productivity in direct and indirect ways. Some common survival traits that different *Bacillus* spp. possess are formation of stress-resistant endospores, production of a multilayered cell wall structure, and secretion of peptide signaling molecules, peptide antibiotics, and extracellular enzymes. Along with these traits, they have noteworthy variations in other important characteristics such as utilization of nutrients, ability to move, and physiochemical growth optima. Because of quantitative and qualitative variations in these characteristics, these bacteria show diversity and inhabit various niches in agroecosystems (Choudhary and Johri 2009; Gardener 2004). The potential to fix N_2 is broadly dispersed among phylogenetically different bacteria. Conventionally, N_2 fixation is catalyzed by an enzyme complex known as nitrogenase. There are two main components of the nitrogenase enzyme complex: the iron (Fe) protein, which is encoded by the *nifH* gene, and the molybdenum iron (MoFe) protein, which is encoded by *nifDK* genes. The sequence of the *nifH* gene is evolutionarily conserved and is used as marker for N_2 fixation. Primers designed on the basis of the *nifH* gene sequence are used extensively to detect the genetic potential of bacteria to fix N_2 in any ecological state (Auman et al. 2001; Rosch et al. 2002; Mehta et al. 2003).

Although very little research has been done regarding the role of *Bacillus* spp. in N_2 fixation, several researchers have isolated N_2-fixing isolates from different sources and checked them molecularly for N_2-fixing ability. In earlier studies, *Paenibacillus azotofixans*, *P. macerans*, and P. polymyxa were found to be N_2 fixers on the basis of their nitrogenase activity (Witz et al. 1967; Seldin et al. 1984). Additionally, other *Paenibacillus* spp.—namely, *P. odorifer*, P. graminis, *P. peoriae*, and *P. brasilensis*—were described as N_2 fixers in various research studies (Elo et al. 2001; Berge et al. 2002; Von der Weid et al. 2002). Besides

Paenibacillus spp., Xie et al. (1998) reported that many *Bacillus* members—namely, B. megaterium, B. cereus, B. pumilus, *B. circulans*, *B. licheniformis*, B. subtilis, B. brevis, and *B. firmus*—were N_2-fixing bacteria on the basis of their nitrogenase activity. However, it was only detection of the nifH gene that identified in *P. azotofixans*, *P. macerans*, *P. polymyxa*, *P. graminis*, and *P. odorifer* as nitrogen fixers (Achouak et al. 1999; Berge et al. 2002). Ding et al. (2005) confirmed the N_2 fixation potential of *B. marisflavi* and P. massiliensis by using degenerate primers for the *nifH* gene. Jain et al. (2016) also demonstrated the N_2 fixation ability of *Bacillus* sp. SJ-5 by growing it on Jensen's medium, which is devoid of N_2, and further confirmed it by amplification of the ferredoxin–nitrite reductase gene. Jiang et al. (2019) found a positive impact of using bio-organic fertilizer containing highly efficient nitrogen-fixing B. amyloliquefaciens on strawberry plants. In a recent study, the nitrogen fixation potential of B. megaterium and *B. mycoides* strains was confirmed by using the nitrogen balance and $^{15}N_2$ isotope dilution in different parts of sugarcane plants after inoculation (Singh et al. 2020).

Apart from use of an intact culture, coinoculation with other beneficial microbes may be a valuable addition for improved nutrient availability and plant growth. In an interesting study, Elkoca et al. (2007) observed better nodulation and a significant increase in the seed yield of chickpea plants when they were inoculated with *Rhizobium*, N_2-fixing B. subtilis (OSU-142), and P-solubilizing B. megaterium (M-3). In another study, significant increases in plant N_2 and phosphate accumulation were observed when phosphate-solubilizing B. subtilis strains were coinoculated with mycorrhizal *Glomus intraradices* (Toro et al. 1997). Recently, Karagoz and Dursun (2019) reported increased numbers of bulbs and improved quality of tulip cultivars after application of the nitrogen-fixing and phosphate-solubilizing bacteria B. megaterium TV-3D and P. polymyxa TV-12E, along with *Pantoea agglomerans* RK-79 and *Pantoea agglomerans* RK-92. In another study, it was reported that coinoculation of *B. velezensis* S141 with *Bradyrhizobium diazoefficiens* USDA110 resulted in enhanced nodulation and N_2-fixing efficiency in soybean plants, as they produced larger nodules (Sibponkrung et al. 2020).

18.2.1 Nitrogen Metabolism in Bacillus *Species*

Prior to the development of artificial methods for N_2 fixation to obtain fertilizers, roughly all N_2 present in living organisms was converted into ammonia by microbial reduction. Biological reduction of N_2 is a process of utmost significance and certainly has been vital for sustaining life on the earth (Ruiz-Herrera et al. 2015). Whereas enteric bacteria have a two-component Ntr regulatory system, rapidly metabolizable N_2 sources regulate N_2 metabolism genes in B. subtilis. In *Bacillus* spp., depending on nutrient availability, three regulatory proteins—namely, CodY, GlnR, and TnrA—independently control expression of gene products involved in N_2 metabolism. Under different nutritional conditions, each of these systems gets activated. CodY is suppressed in cells growing quickly with amino acids, GlnR is

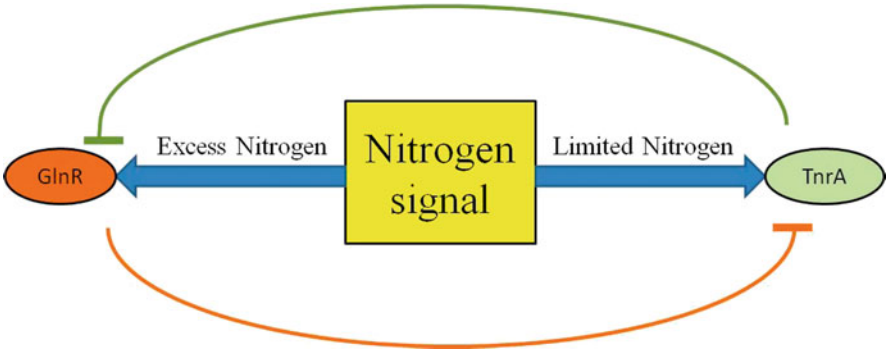

Fig. 18.1 Model for GlnR and TnrA transcriptional factor cross-regulation in *Bacillus subtilis*

suppressed only in cells growing with surplus nitrogen, and TnrA transcription is activated or suppressed only during nitrogen-deprived growth. All three proteins regulate *ureABC* (urease) expression, which gets elevated during N_2-limited conditions, along with asparaginase, γ-aminobutyric acid permease (*gabP*), a putative ammonium permease (*nrgA*), and nitrate assimilatory enzymes (*nasABCDEF*) in B. subtilis (Wray et al. 1997; Fisher 1999). GlnR and TnrA, which belongs to the MerR family of DNA-binding regulatory proteins, bind to similar DNA sequences (*glnRA*; dicistronic operons) under different nutritional conditions. The B. subtilis *glnRA* operon contains genes encoding glutamine synthetase and the negative regulatory protein GlnR. N_2-depriving conditions activate TnrA protein, while GlnR-dependent repression occurs in cells growing with surplus N_2 (Wray et al. 1998). In the case of excess N_2, GlnR not only represses the *glnRA* promoter but also represses expression of the *tnrA* and *ureABC* P3 promoters (Wray et al. 1997; Fisher 1999). Therefore, GlnR and TnrA cross-regulate each other's expression, along with regulating their own synthesis (Fig. 18.1).

The nitrogen signals regulating the activity of GlnR and TnrA have not been identified, and it is still not clear whether both of these respond to different signals or the same signals. However, it has been reported that glutamine synthetase is required for transduction of the signal for nitrogen availability to both proteins (Atkinson and Fisher 1991; Nakano et al. 1998; Ferson et al. 1996). Studies have suggested that regulated expression by both of these proteins permits cells to adapt to growth during N_2-limited conditions. Apart from these two proteins, CodY, a third regulatory protein, controls expression of several genes involved in N_2 metabolism, competence, and acetate metabolism in response to the growth rate. Cells growing rapidly in a medium rich in amino acids show the highest levels of CodY-dependent repression, while during a transition to nutrient-limited growth, this regulation is relieved (Ferson et al. 1996; Fisher et al. 1996; Wray et al. 1997). Expression of various enzymes in B. subtilis that are required for degradation of different amino acids is substrate inducible, but their expression is not regulated during nitrogen limitation by GlnR and TnrA. This model of regulation may reflect the fact that catabolism of amino acids produced by proteolysis during sporulation and

germination provides cells with substrates for energy production and macromolecular synthesis. As a result, expression of amino acid degradative enzymes may be regulated to ensure that high levels of these enzymes are present in sporulating cells and in dormant spores (Fisher 1999).

18.3 Applications of Nitrogen-Fixing *Bacillus* Species

Although N_2 fixation itself is very important for survival of plants and growth promotion, several members of the *Bacillus* genus perform different plant growth–promoting and plant–protecting activities, which can be exploited for sustainable agricultural development. Enhancements in plant health and yield are mediated by three different ecological mechanisms: (1) antagonism of pests and pathogens, (2) promotion of host nutrition and growth, and (3) elicitation of plant host defense systems.

18.4 *Bacillus* Species as Plant Growth Promoters

Plant growth–promoting bacteria (PGPBs) play a major role in sustainable agriculture. *Bacillus* spp. have been reported to be potent plant growth promoters and may promote plant health by influencing the plant host or mutualistic symbionts. Various isolates of B. amyloliquefaciens, B. cereus, *B. sphaericus*, B. subtilis, *B. mycoides*, and B. pumilus have been reported to induce the host's resistance pathways in a local or systemic manner. Different hormones produced by B. subtilis (e.g., auxins, gibberellins, and cytokinins, which are involved in plant cell enlargement, division, and enlargement of symbiotic and nonsymbiotic roots) may affect growth of host plants (Priest 1993). The root is the main part of a plant; it is responsible for nutrient acquisition from soil and forms a solid base from which to grow shoots. Several *Paenibacillu*s and *Bacillus* spp. have been reported to enhance root growth and hence nutrient uptake by secreting the plant growth hormone indole acetic acid (IAA) (Zerrouk et al. 2020; Weselowski et al. 2016; Zhou et al. 2016; Fan et al. 2016; Porcel et al. 2014). Levels of ethylene, the only gaseous hormone responsible for plant growth inhibition, increase in plants in stress conditions. Some *Bacillus* spp. reduce ethylene levels by degrading 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene, into 2-oxobutanoate and ammonia through production of ACC deaminase enzyme, thereby directly increasing plant growth (Gowtham et al. 2020; Wang et al. 2018; Barnawal et al. 2017, 2013). Bacterial polysaccharides can bind soil particles to form microaggregates and macroaggregates. Plants treated with exopolysaccharide (EPS)-producing bacteria display increased resistance to water stress due to improved soil structure. Jain et al. (2016) reported the EPS-producing activity of *Bacillus* sp. SJ-5. EPS can also bind to cations, including Na^+, thus making it unavailable to plants under saline conditions.

In addition, nutrient elements—such as phosphorus, potassium, iron, zinc, and copper—possess limited mobility in soil and are present in insoluble forms. Several *Bacillus* spp.—such as *B. circulans*, B. megaterium, *B. polymyxa*, B. subtilis, and *B. sircalmous*—reportedly have the potential to produce organic acids (gluconic acid, citric acid, etc.), which can convert insoluble inorganic phosphate compounds (tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate) into bioavailable phosphorus (Prakash and Arora 2019; Saeid et al. 2018; Satyaprakash et al. 2017). Like phosphorus, iron is present in the environment in a form (Fe^{3+}) that is inaccessible to plants. Some *Bacillus* spp. provide iron to plants by chelating it from soil through siderophore production (Wahyudi et al. 2011; Liu et al. 2017). *Bacillus* spp. can also catalyze total hydrolysis of proteins into peptides and amino acids by secretion of degradative enzyme proteases (López-Otín and Overall 2002). Apart from possessing these different plant growth–promoting properties, some *Bacillus* spp. can also help get rid of fungal pathogens by secreting cell wall–degrading enzymes.

18.5 *Bacillus* Species as Plant Protectors

Bacillus and *Paenibacillus* spp. work as plant protectors either directly by suppressing phytopathogens or producing various cell wall–degrading and antifungal metabolites, or indirectly by eliciting induced systemic resistance (ISR) against pathogens in plants. B. subtilis is one of the most prominent bacteria in this regard and been explored for its antagonistic properties against fungal phytopathogens for use as a biocontrol agent in agriculture (Xie et al. 2020; Asaka and Shoda 1996; Pinchuk et al. 2002). Various studies have documented direct antagonism by other species—including B. amyloliquefaciens, B. cereus, *B. licheniformis*, B. megaterium, *B. mycoides*, *and* B. pumilus—as well as some isolates of *P. macerans* and P. polymyxa (Govindasamy et al. 2010; Liu and Sinclair 1992; Timmusk and Wagner 1999). In different in vitro research studies, *Bacillus* sp. BPR7 has been found to strongly inhibit growth of several phytopathogens such as *Fusarium oxysporum*, *Macrophomina phaseolina*, *F. solani*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Colletotrichum* sp. (Kumar et al. 2012). B. subtilis CKT 1 has also been reported as a capable biocontrol agent against *F. oxysporum*, *S. sclerotiorum*, and R. solani, with multiple plant growth–promoting properties. Reported that B. cereus QQ308 possesses the capability to secrete different hydrolytic enzymes, such as chitinase, chitosanase, and protease. These enzymes inhibit growth of several important soilborne fungal plant pathogens, including *F. oxysporum*, *F. solani*, *and* P. ultimum. The culture itself or the culture supernatant can be used as an antifungal formulation. Chitinase produced by B. thuringiensis GS1 inhibits growth of R. solani (Seo et al. 2012). Basha and Ulaganathan (2002) observed antagonistic activity of the chitinolytic *Bacillus* sp. strain BC121 against *Curvularia lunata*. In another study, Heulin et al. (1994) reported the in vitro antifungal potential of P. polymyxa against the take-all wheat

disease–causing agent *Gaeumannomyces graminis* var. *tritici* and the *Fusarium* wilt disease pathogenic fungus *F. oxysporum*. P. polymyxa strains E681 and HKA-15 were also reported in separate studies to control damping-off disease in sesame plants and charcoal rot in soybean plants, respectively (Ryu et al. 2006; Senthilkumar et al. 2007). Besides the antifungal properties of *Bacillus* spp., the antibacterial property of lipopeptides produced by *Bacillus* spp. against the pathogen *Xanthomonas campestris* has been studied extensively (Monteiro et al. 2005; Salerno and Sagardoy 2003). Effective antagonism of *Bacillus* and *Paenibacillus* spp. against other bacterial plant pathogens that affect economically important crops has been also reported.

Apart from direct antagonistic activity, *Bacillus* spp. have been reported to colonize plant roots and elicit ISR via activation of different defense-related genes to provide plants with a heightened level of protection against future pathogen attacks. Kim et al. (2015) reported upregulation of the pathogenesis-related genes PR-2 (encoding β-1,3-glucanase) and acidic PR-3 (encoding chitinase) against the fungal pathogen R. solani and the oomycete *Phytophthora nicotianae* in tobacco plants treated with *Bacillus* sp. JS. In another study, Nie et al. (2017) found a reduced incidence of disease caused by *Botrytis cinerea* through activation of ISR by root drenching with B. cereus AR156. Increased enzymatic activities of defense-related enzymes—namely, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, lipoxygenase, and glucanase—against the fungal pathogens *F. oxysporum* and R. solani were observed in soybean plants upon ISR elicitation by *Bacillus* sp. SJ-5 (Jain et al. 2017).

18.6 Conclusion

Chemical fertilizers, which are now used frequently in a quest for high crop yields to fulfill the needs of the growing human population, are an ongoing matter of discussion because of environmental concerns and fears for consumer health. Therefore, there has recently been growing interest in environmentally friendly and sustainable agricultural practices. Although *Rhizobium* is a great nitrogen fixer, its selective host range and limiting factors, such as nodule formation, have prompted a search for other nitrogen-fixing microbes. Many other bacteria that exhibit such activities have now been reported. Nitrogen-fixing properties, along with different plant growth–promoting properties and a wide host range, make *Bacillus* spp. suitable for this purpose. Because of their spore-forming capability, they are readily adaptable to field applications and can resist a wide range of environmental stresses. Additionally, a lot of genomic research has already been done on *Bacillus* species, which can be further explored through application of biotechnology to develop improved transgenic strains that have manifold mechanisms of action and strains with precise formulation qualities. Endophytic colonization and biofilm formation are two of the most desirable characteristics for prospective biofertilizers and biocontrol agents. As studies have shown, *Bacillus* spp. possess these properties

and can be used as biofertilizers and biocontrol agents, which may be self-perpetuating within colonized host plants. Although many *Bacillus* spp. with potential for nitrogen fixation have been identified, the underlying mechanisms are still being investigated in ongoing research and should be confirmed in the future. To achieve sustainable crop yields in agriculture, application of *Bacillus* and *Paenibacillus* can notably decrease use of chemical fertilizers and pesticides, and maintain the fertility of soil.

References

Achouak W, Normand P, Heulin T (1999) Comparative phylogeny of *rrs* and *nifH* genes in the Bacillaceae. Int J Syst Bacteriol 49:961–967

Asaka O, Shoda M (1996) Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. Appl Environ Microbiol 62:4081–4085

Atkinson MR, Fisher SH (1991) Identification of genes and gene products whose expression is activated during nitrogen-limited growth in *Bacillus subtilis*. J Bacteriol 173:23–27

Auman AJ, Speake CC, Lidstrom ME (2001) *nifH* sequences and nitrogen fixation in type I and type II methanotrophs. Appl Environ Microbiol 67:4009–4016

Barnawal D, Maji D, Bharti N, Chanotiya CS, Kalra A (2013) ACC deaminase–containing *Bacillus subtilis* reduces stress ethylene–induced damage and improves mycorrhizal colonization and rhizobial nodulation in *Trigonella foenum-graecum* under drought stress. J Plant Growth Regul 32:809–822

Barnawal D, Pandey SS, Bharti N, Pandey A, Ray T, Singh S, Chanotiya CS, Kalra A (2017) ACC deaminase--containing plant growth--promoting rhizobacteria protect *Papaver somniferum* from downy mildew. J Appl Microbiol 122:1286–1298

Basha S, Ulaganathan K (2002) Antagonism of *Bacillus* species (strain BC121) towards *Curvularia lunata*. Curr Sci 82:1457–1463

Berge O, Guinebretiere MH, Achouak W, Normand P, Heulin T (2002) *Paenibacillus graminis* sp. nov. and *Paenibacillus odorifer* sp. nov., isolated from plant roots, soil and food. Int J Syst Evol Microbiol 52:607–616

Choudhary DK, Johri BN (2009) Interactions of *Bacillus* spp. and plants—with special reference to induced systemic resistance (ISR). Microbiolog Res 164:493–513

Claus D, Berkeley RCW (1986) Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, pp 1105–1139

Ding Y, Wang J, Liu Y, Chen S (2005) Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region. J Appl Microbiol 99:1271–1281

Elkoca E, Kantar F, Sahin F (2007) Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. J Plant Nutr 31:157–171

Elo S, Suominen I, Kampfer P, Juhanoja J, Salkinoja-Salonen M, Haahtela K (2001) *Paenibacillus borealis* sp. nov., a nitrogen-fixing species isolated from spruce forest humus in Finland. Int J Syst Evol Microbiol 51:535–545

Eskin N, Vessey K, Tian L (2014) Research progress and perspectives of nitrogen fixing bacterium, Gluconacetobacter diazotrophicus, in monocot plants. Int J Agron. https://doi.org/10.1155/2014/208383

Fan P, Chen D, He Y, Zhou Q, Tian Y, Gao L (2016) Alleviating salt stress in tomato seedlings using *Arthrobacter* and *Bacillus megaterium* isolated from the rhizosphere of wild plants grown on saline–alkaline lands. Inter J Phytoremed 18:1113–1121

Ferson AE, Wray LV, Fisher SH (1996) Expression of the *Bacillus subtilis gabP* gene is regulated independently in response to nitrogen and amino acid availability. Mol Microbiol 22:693–701

Fisher SH (1999) Regulation of nitrogen metabolism in *Bacillus subtilis*: vive la difference! Mole Microbiol 32:223–232

Fisher SH, Rohrer K, Ferson AE (1996) Role of CodY in regulation of the *Bacillus subtilis hut* operon. J Bacteriol 178:3779–3784

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The nitrogen cascade. AIBS Bull 53:341–356

Gardener BBMS (2004) Ecology of *Bacillus* and *Paenibacillus* spp. in agricultural systems. Phytopathol 94:1252–1258

Govindasamy V, Senthilkumar M, Magheshwaran V, Kumar U, Bose P, Sharma V, Annapurna K (2010) *Bacillus* and *Paenibacillus* spp.: potential PGPR for sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 333–364

Gowtham HG, Singh B, Murali M, Shilpa N, Prasad M, Aiyaz M, Amruthesh KN, Niranjana SR (2020) Induction of drought tolerance in tomato upon the application of ACC deaminase producing plant growth promoting rhizobacterium *Bacillus subtilis* Rhizo SF 48. Microbiol Res 234:126422

Heulin T, Berge O, Mavingui P, Gouzou L, Hebbar KP, Balandreau J (1994) *Bacillus polymyxa* and *Rahnella aquatilis*, the dominant N 2 -fixing bacteria associated with wheat rhizosphere in French soils. Eur J Soil Biol 30:35–42

Jain S, Kumari S, Vaishnav A, Choudhary DK, Sharma KP (2016) Isolation and characterization of plant growth promoting bacteria from soybean rhizosphere and their effect on soybean plant growth promotion. Int J Adv Sci Tech Res 5:398–410

Jain S, Vaishnav A, Kumari S, Varma A, Tuteja N, Choudhary DK (2017) Chitinolytic *Bacillus*-mediated induction of jasmonic acid and defense-related proteins in soybean (*Glycine max* L. Merrill) plant against *Rhizoctonia solani* and *Fusarium oxysporum*. J Plant Growth Regul 36:200–214

Jiang L, Kaiyun WA, Yuguo WU, Kaiyuan WA, Xinjun FA (2019) Application of bio-organic fertilizer containing high-efficient nitrogen-fixing *Bacillus amyloliquefaciens* on strawberry. Agri Biotechnol 8:2164–4993

Karagoz FP, Dursun A (2019) Effects of nitrogen fixing and phosphate solubilizing bacteria on growth and bulbs production of tulip cultivars. Ege Üniversitesi Ziraat Fakültesi Dergisi 56:241–248

Kim JS, Lee J, Lee CH, Woo SY, Kang H, Seo SG, Kim SH (2015) Activation of pathogenesis-related genes by the Rhizobacterium, *Bacillus* sp. JS, which induces systemic resistance in tobacco plants. Plant Pathol J 31:195

Kumar P, Dubey RC, Maheshwari DK (2012) *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res 167:493–499

Liu ZL, Sinclair JB (1992) Population dynamics of *Bacillus megaterium* strain B153–2-2 in the rhizosphere of soybean. Phytopathology 82:1297–1301

Liu D, Yang Q, Ge K, Hu X, Qi G, Du B, Liu K, Ding Y (2017) Promotion of iron nutrition and growth on peanut by Paenibacillus illinoisensis and Bacillus sp. strains in calcareous soil. Br J Microbiol. https://doi.org/10.1016/j.bjm.2017.02.006

López-Otín C, Overall CM (2002) Protease degradomics: a new challenge for proteomics. Nat Rev Mol Cell Biol 3:509–519

Mehta MP, Butterfield DA, Baross JA (2003) Phylogenetic diversity of nitrogenase (*nifH*) genes in deep-sea and hydrothermal vent environments of the Juan de Fuca Ridge. Appl Environ Microbiol 69:960–970

Monteiro L, Mariano R, de Lima R, Souto-Maior AM (2005) Antagonism of *Bacillus* spp. against *Xanthomonas campestris* pv. *campestris*. Braz Arch Biol Technol 48:23–29

Mylona P, Pawlowski K, Bisseling T (1995) Symbiotic nitrogen fixation. Plant Cell 7:869

Nakano MM, Hoffman T, Zhu Y, Jahn D (1998) Nitrogen and oxygen regulation of *Bacillus subtilis* nasDEF encoding NADH-dependent nitrite reductase by TnrA and ResDE. J Bacteriol 180:5344–5350

Nie P, Li X, Wang S, Guo J, Zhao H, Niu D (2017) Induced systemic resistance against *Botrytis cinerea* by *Bacillus cereus* AR156 through a JA/ET– and NPR1-dependent signaling pathway and activates PAMP-triggered immunity in *Arabidopsis*. Front Plant Sci 8:238

Peoples MB, Herridge DF, Ladha JK (1995) Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? In: Ladha JK, Peoples MB (eds) Management of biological nitrogen fixation for the development of more productive and sustainable agricultural systems. Springer, Dordrecht, pp 3–28

Pinchuk IV, Bressollier P, Sorokulova IB, Verneuil B, Urdaci MC (2002) Amicoumacin antibiotic production and genetic diversity of *Bacillus subtilis* strains isolated from different habitats. Res Microbiol 153:269–276

Porcel R, Zamarreño ÁM, García-Mina JM, Aroca R (2014) Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. BMC Plant Biol 14:36

Prakash J, Arora NK (2019) Phosphate-solubilizing Bacillus sp. enhances growth, phosphorus uptake and oil yield of Mentha arvensis L. 3 Biotech 9:126

Priest F (1993) Systematics and ecology of *Bacillus*: *Bacillus subtilis* and other Gram-positive bacteria, biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, pp 3–16

Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: balancing the cost of an essential resource. Annu Rev Environ Resour 34:97–125

Rosch C, Mergel A, Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68:3818–3829

Ruiz-Herrera J, León-Ramírez C, Vera-Nuñez A, Sánchez-Arreguín A, Ruiz-Medrano R, Salgado-Lugo H, Sánchez-Segura L, Peña-Cabriales JJ (2015) A novel intracellular nitrogen-fixing symbiosis made by *Ustilago maydis* and *Bacillus* spp. New Phytol 207:769–777

Ryu CM, Kima J, Choi O, Kima SH, Park CS (2006) Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. Biol Control 39:282–289

Saeid A, Prochownik E, Dobrowolska-Iwanek J (2018) Phosphorus solubilization by *Bacillus* species. Molecules 23:2897

Salerno CM, Sagardoy MA (2003) Antagonistic activity by *Bacillus subtilis* against *Xanthomonas campestris* pv. *glycines* under controlled conditions. Span J Agric Res 1:55–58

Satyaprakash M, Nikitha T, Reddi EU, Sadhana B, Vani SS (2017) Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. Int J Curr Microbiol App Sci 6:2133–2144

Seldin L, van Elsas JD, Penido EGC (1984) *Bacillus azotofixans* sp. nov., a nitrogen-fixing species from Brazilian soils and grass roots. Int J Syst Bacteriol 34:451–456

Senthilkumar M, Govindasamy V, Annapurna K (2007) Role of antibiosis in suppression of charcoal rot disease by soybean endophyte *Paenibacillus* sp. HKA-15. Curr Microbiol 55:25–29

Seo DJ, Nguyen DM, Song YS, Jung WJ (2012) Induction of defense response against *Rhizoctonia solani* in cucumber plants by endophytic bacterium *Bacillus thuringiensis* GS1. J Microbiol Biotechnol 22:407–415

Sibponkrung S, Kondo T, Tanaka K, Tittabutr P, Boonkerd N, Yoshida KI, Teaumroong N (2020) Co-inoculation of *Bacillus velezensis* strain S141 and *Bradyrhizobium* strains promotes nodule growth and nitrogen fixation. Microorganisms 8:678

Singh RK, Singh P, Li HB, Song QQ, Guo DJ, Solanki MK, Verma KK, Malviya MK, Song XP, Lakshmanan P, Yang LT (2020) Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: a comprehensive study of plant–microbe interactions for growth enhancement in *Saccharum* spp. BMC Plant Biol 20:1–21

Timmusk S, Wagner EGH (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant Microbe Interact 12:951–959

Toro M, Azcon R, Barea JM (1997) Improvement of arbuscular mycorrhizal development by inoculation with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (32P) and nutrient cycling. Appl Environ Microbiol 63:4408–4412

Vessey JK, Pawlowski K, Bergman B (2005) Root-based N_2-fixing symbioses: legumes, actinorhizal plants, *Parasponia* sp. and cycads. Plant Soil 274:51–78

Von der Weid I, Duarte GF, van Elsas JD (2002) *Paenibacillus brasilensis* sp. nov., a novel nitrogen-fixing species isolated from the maize rhizosphere in Brazil. Int J Syst Evol Microbiol 52:2147–2153

Wahyudi AT, Astuti RP, Widyawati A, Mery A, Nawangsih AA (2011) Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting rhizobacteria. J Microbiol Antimicrob 3:34–40

Wang S, Liu F, Chen C, Xu X (2007) Life cycle emissions of greenhouse gas for ammonia scrubbing technology. Korean J Chem Eng 24:495–498

Wang W, Wu Z, He Y, Huang Y, Li X, Ye BC (2018) Plant growth promotion and alleviation of salinity stress in *Capsicum annuum* L. by *Bacillus* isolated from saline soil in Xinjiang. Ecotox Environ Safe 164:520–529

Weselowski B, Nathoo N, Eastman AW, MacDonald J, Yuan ZC (2016) Isolation, identification and characterization of *Paenibacillus polymyxa* CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. BMC Microbiol 16:244

Williamson JM (2011) The role of information and prices in the nitrogen fertilizer management decision: new evidence from the Agricultural Resource Management Survey. J Agr Resour Econ 1:552–572

Witz DF, Detroy RW, Wilson PW (1967) Nitrogen fixation by growing cells and cell-free extracts of the Bacillaceae. Arch Microbiol 55:369–381

Wray LV, Ferson AE, Fisher SH (1997) Expression of the *Bacillus subtilis* ureABC operon is controlled by multiple regulatory factors including CodY, GlnR, TnrA, and Spo0H. J Bacteriol 179:5494–5501

Wray LV Jr, Zalieckas JM, Fisher SH (1998) Mutational analysis of the TnrA-binding sites in the *Bacillus subtilis nrgAB* and *gabP* promoter regions. J Bacteriol 180:2943–2949

Xie GH, Su BL, Cui ZJ (1998) Isolation and identification of N_2-fixing strains of *Bacillus* in rice rhizosphere of the Yangtze River Valley. Acta Microbiol Sin 38:480–483

Xie S, Liu J, Gu S, Chen X, Jiang H, Ding T (2020) Antifungal activity of volatile compounds produced by endophytic Bacillus subtilis DZSY21 against Curvularia lunata. Ann Microbiol. https://doi.org/10.1186/s13213-020-01553-0

Zerrouk IZ, Rahmoune B, Auer S, Rösler S, Lin T, Baluska F, Dobrev PI, Motyka V, Ludwig-Müller J (2020) Growth and aluminum tolerance of maize roots mediated by auxin- and cytokinin-producing *Bacillus toyonensis* requires polar auxin transport. Environ Exp Bot 18:104064

Zhou C, Guo J, Zhu L, Xiao X, Xie Y, Zhu J, Ma Z, Wang J (2016) *Paenibacillus polymyxa* BFKC01 enhances plant iron absorption via improved root systems and activated iron acquisition mechanisms. Plant Physiol Biochem 105:162–173

Chapter 19
Quorum Sensing Enhances Nitrogen Uptake in Plant

Meenu Saraf and Sheetal Sharma

Abstract Bacteria have cell communication in a population density dependent type which involves the production of pheromone like molecule called autoinducer. When a bacterial cell grows in population, the intracellular signals regulate differential gene expression which leads to specific behaviours. Primarily plants have the narrow recognizable range for low molecular weight nitrogen component, hence are dependent on products release through N-mineralization such as ammonia and nitrate for biomolecule production. Diazotrophic bacteria release signalling molecule like AHL (Acyl-homoserine lactone) which is involved in communication between plants and bacteria. This signalling channel promotes the production of exoenzymes like chitinase and protease to enhance mineralization of low molecular weight nitrogen source present in the soil. Symbiotic diazotrophic bacteria belonging to *Rhizobium spp* have role in quorum sensing through the production of signal molecules like AHL. The CinI gene present in *R. leguminosarum* expresses long-chain AHL. Nitrogen fixation was found to be decreased by 50% when mutant strain for CinI gene was inoculated in pea plant. Studies have been affirmed that the activity of glutamate synthetase is enhanced in response to AHL treatment. Moreover, glutamate in combination with glutamine plays a key role assimilation of ammonia into amino acids and further array to other nitrogen containing biomolecule. In rhizosphere soil of plant Glycine max and Vigna radiata nodule occurs due to correlation with diazotrophs and legumes. These symbiotic associations amalgamate AHL QS signals triggering the production of exopolysaccharides, increase nodulation efficiency and regulation of *nif*-genes. Many reports have suggested a significant role of QS in the regulation of nitrogen cycle. The review aims at recent developments at a molecular level, mechanism and methods for optimization of QS signal involve in the rhizosphere to aid nitrogen fixation in plants.

Keywords Quorum sensing · Nitrogen fixation · Acyl-homoserinelactone · Nodulation · Signals

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19.1 Introduction

The population density of soil microbiota present in the rhizosphere has a significant effect on enhancing the level of plant nutrition and regulating the strength of roots. Therefore the final above ground level growth of plants is controlled by the biodiversity of microbiota present in the rhizosphere (Bais et al. 2006). In the last few decades, it has been recognizing that in the rhizosphere there is a cross-talk and exchange of molecules between soil flora and micro-organisms surrounding the root surface. These complex signalling molecules consist of distinct types of cell signalling pathways which are forming a hidden network, that manipulate and control the physiological process, like the formation of biofilm, symbiosis defense between flora and microorganisms (Waters and Bassler 2005). Bacteria have cell communication in a population density dependent type which involves the production of pheromone-like molecule called autoinducer. When a bacterial cell grows in population, the intracellular signals regulate differential gene expression which leads to specific behaviours (Fuqua et al. 1994). The process of QS occurs in sequential order, firstly bacterial cell production and release biochemical pheromones in surrounding habitat and secondly recognition of autoinducer by the specific receptor which leads to change in gene regulation of receptor (Redfield 2002). The best investigated QS operating system is established in gram negative bacteria through the utilization of acetylated homoserine lactones (AHLs), quinolone, *p*-coumarate, and 3OH palmitic acid methyl ester as signalling molecules as well as in gram positive bacteria by utilizing oligopeptide as an autoinducer (Waters and Bassler 2005). Primarily plants have the narrow recognizable range for low molecular weight nitrogen component, hence are dependent on products release through N-mineralization such as ammonia and nitrate for biomolecule production. Diazotrophic bacteria release signalling molecule like AHL which promotes the production of exoenzymes like chitinase and protease to enhance mineralization of low molecular weight nitrogen source present in the soil (Schimel and Bennett 2004). Many symbiotic diazotrophic bacteria like *Rhizobium* and *Sinorhizobium* spp. can perform biological nitrogen fixation by forming nodulation in association with a plant. These both strains have complex QS system involving different chains for the synthesis of AHL (Braekenetal.2008). This review gives a glimpse about the molecular and mechanistic role of QS autoinducers involved in a symbiotic relationship with the plant for nitrogen fixation (Table 19.1).

19.2 How Bacteria Talk to each Other

Many reviews have described features like how quorum sensing is cell density dependent activity and involves autoinducers. Some information have reported on plant-associated bacteria showing quorum sensing mechanism. There are two classes of plant-associated bacteria linked with quorum sensing gene, one which is legume

Table 19.1 Quorum sensing system in diazotrophs

Rhizobacteria	Genes involve in QS	Autoinducer	References
Agrobacterium tumefaciens	*tarR/traI* (pTi)	3-oxo-C8-HSL	Piper et al. (1993), Fuqua et al. (1994)
Rhizobium leguminosarum	*cinR/cinI* (chromosome)	3-OH-C14:1-HSL	Lithgow et al. (2000)
	rhiR/rhiI (pRL1JI)	C6-HSL, *C7-HSL, C8-HSL*	Rodelas et al. (1999)
	traR/traI (pRL1JI)	3-oxo-C8-HSL, *C8-HSL*	Wilkinson et al. (2002), Danino et al. (2003)
Sinorhizobium meliloti RM1021	*sinR/sinI* (chromosome)	3-oxo-C14-HSL	Marketon et al. (2002)
Rhizobium etli CNF24	traR/traI (p42a)	3-oxo-C8-HSL, 3-OH-C14:1-HSL	Tun-Garrido et al. (2003)
Bradyrhizobium japonicum, CPAC15	*luxI/luxR*	3-OHC12-AHL, 3-OH-C14-AHL, 3-oxo-C14-AHL	dos Santos Lima Fagotti et al. (2019)
lebsiella pneumonia NG14	*luxI-luxR*	Unknown	Liu et al. (2011)
Azospirillum lipoferum B52	*luxI-luxR*	C6HSL 3O, C10HSL	

nodulating and other class of bacteria produces bioactive compounds to inhibit the growth of competitive microorganism present in the rhizosphere.

19.2.1 Synthesis and Regulator of AHL Compound

AHL synthases are mediated by two known protein family. The first group is LuxI type protein which is identical to *P. fischeri.* This group of the enzyme also catalyzes the ligation process of S-adenosylmethionine (SAM) with an acylated acyl carrier protein forming an HSL as a resulting component of AHL (Moré et al. 1996; Schaefer et al. 1996; Fuqua et al. 1994; Withers et al. 2001). The second group of protein is LuxM/AinS-type. The product of *ains* gene from *P. fischeri* and *luxM* gene from *V. harveyi* synthesizes AHL though they are similar to LuxI but there is no evidence for the presence of the similar gene in rhizobia family. This group of AHL synthases is directly involved in the synthesis of 3-OH-C4-HSL and C8HSL (Gilson et al. 1995; Milton 2006). The third set of AHL synthase is HdtS and has been identified in *Pseudomonas fluorescens*. The *hdts* maintains the production of three types of AHL (C10-HSL, 3-OH-C14:1, and C6-HSL). The *hdts* is identical to lysophosphatidic acid acyltransferase family. This regulates the transfer of acyl chain from acyl ACP to lysophosphatidic acid, producing an end product of phosphatidic acid (Laue et al. 2000). In rhizobia family, most of the AHL regulator discovered to date belongs to Lux-R regulator proteins having two distinct regions

with the conserved sequence for the binding domain of AHL and binding motif for DNA (Slock et al. 1990; Shadel et al. 1990).

19.2.2 AHL Reporters

The AHL based bioreporters had made feasible to understand the mechanism of QS in diverse bacteria. Though there is a wide range of reports exists, two of them have given focused for complete insights like *Agrobacterium tumefaciens* and *Chromobacterium violaceum.* With the help of thin layer chromatography both the model and the organism have been used for detection of AHL. The *A. tumefaciens* does not produce its bioreporters, instead of that, it initiates the traG:lacZ fusion which leads to TraR a homologue to LuxR. This type of AHL reporter is bit sensitive and can detect a low amount of AHL in the environment (Shaw et al. 1997). Whereas long chain reporters have been detected in *sinI* gene lacking strain of *S. meliloti* by integrating fusion of *sinI:lacZ* in the chromosome which leads to the end product of C12 to C18 detection AHL reporter (Llamas et al. 2004). In *C. violaceum* the production of purple pigment violacein is mediated by QS genes *cviI/cviR*. A *C. violaceum (cviI)* mutant is inadequate to produce any AHL but was able to produce pigment in the response of any external AHLs or a component that mimics AHL. It can produce any short chain AHLS up to the length of C4 to C8 (McClean et al. 1997).

19.2.3 AHL Degradation

In the rhizospheric environment when AHL producing microbiota in communities interact with microorganisms which are proficient of degrading AHL, produce signals and get involved in quorum sensing. Those enzymes which degrade AHL are categorized either based on bacterial spp. in which they are found or their mode of action (Zhang et al. 2007). These enzymes are significantly either belongs to lactonases or analyses group. The process of QS in *A. tumefaciens* begins when *attM* initiates degrading AHL molecule which hinders signal turnover. The gamma-butyrolactones act as a natural substrate for AttM and can hinder the expression of *attM* genes found in rhizobacteria (Carlier et al. 2004).

19.3 The Symbiotic Bacteria Interact through Nodulation

One of the best studied interactions is between legumes and diazotrophic bacteria which involves the signalling process back and forth from the host plant. The signal is initiated by the plants from the release of flavonoid molecule which stimulate

regulatory gene *nodD* and produce chitin molecule. On interaction with flavonoid, the NodD proteins make conformational changes to bind *nod* boxes in the promoters of *nod* genes which release end product pentameric lipochitooligosaccharide with nonreducing sugar N acylated fatty acid composed of sixteen or eighteen carbon atoms. Perhaps it has not been demonstrated that Nod factor binds to these receptors are having LysM domain (Jones et al. 2008; Madsen et al. 2010). When the alfalfa seedlings were treated with purified Nod factor with the lowest concentration it stimulates an ion flow across the plant plasma membrane and as a result of depolarization it causes calcium spiking. Because of which there is root hair deformation and production of empty nodules lead by exopolysaccharide mutant strain of *S. meliloti* (Ardourel et al. 1994). On the other hand, mutation in some significant Nod factor results in Nod^{-}, does not interfere with either calcium spiking or root hair deformation but is unable to form robust exopolysaccharide (Fujishige et al. 2008). The various biochemical and genetically conducted experiments had developed some approaches to studying high affinity binding sites for Nod factor receptor in legumes. One of them is located at the plasma membrane of alfalfa cells, namely NFBS2 which binds to *S. meliloti*. At high affinity binding region, NFBS2 binds to Nod factor but avoids selection of reducing sugar encoded with sulphate. Whereas when there is a requirement of optimal nodulation in alfalfa sulphated Nod factors of *S. meliloti* are recognized (Bono et al. 1995; Demont-Caulet et al. 1999; Niebel et al. 1997). The other best study mechanism for signalling pathway is "crack entry" where rhizobia from the infection thread trigger through intracellular space between roots. This is explained in *Aeschynomene indica* very well which was infected by *Bradyrhizobium* strain and it was evaluated that invasion of bacteria is independent of Nod genes (Giraud et al. 2007). However so far mutants in Nod altered the synthesis of purines and pyrimidines which gives rise to nodule development (Madsen et al. 2010). Considering in some cases signalling pathway of nodules require proteins for crack entry in *Aeschynomene*. Plants released phenolic compounds are induced to bind NodD and NodV proteins in certain rhizobia. The gene transcription for type III (T3SS) pathway is activated by adhering of Tts1 protein molecule to Nod factors of rhizobia strains (Deakin and Broughton 2009). The downstream gene that is mandatory for the expression of nodulation in plants is regulated by the presence of signal transducing proteins and receptors secreted by actinorhizal nodule and legume (Wang et al. 2010). The bacterial signals are necessary to initiate nodulation through Nod factors in plants. One of the Nod factors secreted by lotus japonicas which are (LysM) type encoded by NFR5 kinase genes well as a downstream component like SymRK encoded by leucine rich receptor kinase is required for the earliest detection to initiate nodulation (Madsen et al. 2010; Gherbi et al. 2008) (Fig. 19.1).

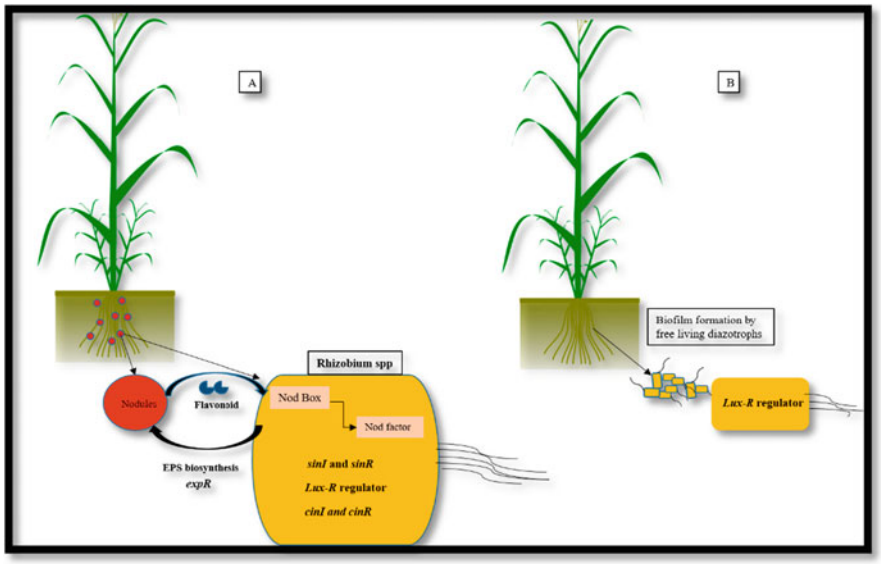

Fig. 19.1 Regulation of quorum sensing in (**a**) symbiotic (**b**) free living diazotrophs. In symbiotic association rhizobium spp. interact with root nodules where flavonoid activates Nod box (Nod transcriptional regulator) and expresses genes (*nod, noe, noI*) involved in nodulation. Expression of other quorum sensing genes *sinI & sinR, cinI & cinR* is also induced by rhizobium spp., when interacts with signal molecules like AHL. For EPS production expr gene is expressed by rhizobium which forms interaction with nodule. The Lux-R regulator expression is activated in both types of diazotrophs. However free living diazotrophs are clustered together by biofilm formation around the roots to propagate nitrogen fixation in plant

19.4 Role of the Plasmid in Signalling Symbiosis

The archetype to study quorum sensing based plasmid transfer was investigated in the bacteria *A. tumefaciens* which carries transfer of Ti plasmid in the plant which result in the production of opines (Zhu et al. 2000). When the bacteria are at late exponential phase and reach maximum population density, in the presence of opine, bacteria induce the plasmid transfer gene through Ti plasmid. This mode of action is possible due to the activity of the regulator TraR. This regulator is transcriptionally promoted by opines and generated by aggregation of 3-oxo-c8-HSL produced by TraI (Zhang et al. 1993; Piper et al. 1993). A crystallized form of TraR bound to AHL has been developed and it has proved that AHL is a necessary component that binds to dimers of TraR. This kind of bounding system is much more stable to proteolytic degradation rather than TraR is not bound to AHL (Zhu and Winans 1999). The product of *traM* gene governs the positive feedback of TraR and AHL and escapes them from any premature induction (Chen et al. 2004; Qin et al. 2004). In distinct *Rhizobium* spp., symbiotic Sym plasmid is involved with performing three different functions of nitrogen fixation, exopolysaccharides production and

nodulation (Banfalvi et al. 1985; Beynon et al. 1980; Huguet et al. 1983; Wijffelman et al. 1983). In contrast, spp. like *Bradyrhizobium* and *Azorhizobium* carry a cluster of information for plasmid on a specific chromosome. The successful horizontal transfer of plasmid containing multiple information from nitrogen-fixing enriched strain to nonsymbiotic strain has been demonstrated in *Mesorhizobium loti* (González and Marketon 2003). Apart from Sym plasmid, there is also another group of plasmid which is not identified yet functionally but is having similar homology for ti plasmid. There are two types of the plasmid in *S. meliloti.* One which carries nitrogen fixation and nodulation genes (pSymA plasmid) and other which carries information for symbiotic production of required exopolysaccharide, dicarboxylic acid transport and thiamine (pSymB plasmid) (Banfalvi et al. 1985; Huguet et al. 1983; Banfalvi et al. 1981). The similar plasmid based quorum sensing system has gained interest by many researchers, one of the best studied models is pRL1JI plasmid found in *R. leguminosarum*, pRL1JI belongs to *Rhizobium* spp. and nonsymbiotic plasmid such as p42a from *R. etli* strain CFN42 can be applied for systemic induction plasmid transfer. The *traI, traR,* and *traM* genes are shared by all these systems. Even though they utilize 3-oxo-C8 HSL to activate TraR, there can be other AHL like C8HSL and 3-OH-C8-HSL can be utilized to activate TraR (Wilkinson et al. 2002; Danino et al. 2003; Freiberg et al. 1997). The activity of induced transfer of plasmid is regulated by traR gene expression. The same mechanism is applied to *A. tumefaciens,* opine catabolism gene which is expressed from plant cells and combines with traR to induce plasmid transfer (Kim and Farrand 1998; Zhu et al. 2000). Unlike *A. tumefaciens* in *Rhizobium* spp. regulation of *traR* is different. The *bisR* gene present on the upstream of pRL1JI plasmid encodes for BisR a LuxR-type of a regulator that controls the expression of *traR* in response to AHL like 3-OH-C14:1-HSL (Danino et al. 2003). The CinR present in *R. leguminosarum* regulates gene cinI, which encodes for 3-OH-C14:1-HSL (Lithgow et al. 2000). The BisR gene has found to repress the expression of *cinI* as a result there is very low or no amount of 3-OH-C14:1-HSL is produced in *Rhizobium* strain which does have pRL1JI plasmid, and even though BisR is present in a cell there is little induction of *traR* is observed by BisR. The TraM of *R. leguminosarum* which is comparatively similar to TraM of *A. tumefaciens* inhibits low level induction of *traR* (Chen et al. 2004; Qin et al. 2004; Danino et al. 2003). There are some plasmids like pNGR234 from *Rhizobium sp.* NGR234 and pRme41a from *S. meliloti* strain Rm1021 which are having a low transfer rate, as both plasmid lack *bisR* gene for the regulation of plasmid transfer (Freiberg et al. 1997).

19.5 QS Signalling in Symbiotic Nitrogen Fixing Bacteria

The study conducted to differentiate phylogenetic bacteria based on *nif* gene involved in nitrogen fixation, *LuxA* and *LuxS* genes which are responsible for quorum sensing in bacteria. These analyses have given new insights to evolutionarily determine the relationship between diazotrophs and quorum sensing producing

bacteria. Among thirty different bacterial spp., only a few were able to attribute both characteristics. The major contribution has been given by *Rhizobium* spp. perhaps very few nonsymbiotic diazotrophic bacteria can show both characteristics (Chaphalkar and Salunkhe 2010).

19.5.1 Sinorhizobium Meliloti

Two strains of *S. meliloti* have been studied for the regulation of the quorum-sensing system. But one of them, Rm1021, is well characterized by the complete genome.

19.5.1.1 *sinI* and *sinR*

Moreover *sinI* gene produces a distinct type of long chain AHL in response to the different culture medium. Although there is variation in concentration of AHL in response to culture media, *sinI* has gained little interest to study the specific response of AHL (Marketon et al. 2002; Teplitski et al. 2003; Chen et al. 2004; Gao et al. 2005). One more example discussing *sinI* mutation effects on AHL synthesis was explained by Gao et al. More than 35 different proteins and 100 types of genes were genetically altered by a mutation in SinI gene. Apart from genetical alteration, a phenotypic difference like lacking swarming activity on a plate, devoid of producing mucoid colonies. However by the addition of 5 nm C16:1-HSL swarming was restored (Gao et al. 2005). The *sinR* is located adjacently to sinI on the chromosome; therefore, activation of *sinI* is effected by SinR. There was a huge difference in the production of AHL, when made by SinI (Marketon et al. 2002). When *sinI* dependent AHL was released there was enhanced production, also it was interfering with the expression of *sinR* mutant strain. Hence there is a possibility that *sinR* is regulated by *sinI* dependent AHL but when symbiotically nodule producing gene *fixSI* was expressed it dependent on *sinR* rather than *sinI*. There are chances that some another mechanism is also involved in regulation of *sinR* gene except SinI dependent pathway (Hoang et al. 2004).

19.5.1.2 *expR*

The best example of EPS II and quorum sensing correlation is explained in *S. meliloti*. The wild type of 1021 strain is normally deficient of producing EPS but there is an insertion sequence which interferes with *expR*. Other strain like Rm41 (*expR*$^{+}$) can produce an excellent amount of EPS which is controlled by ExpR regulator. ExpR is positively controlling the formation of EPS biosynthesis by expression of *exp* genes. However, by some mechanism expression of *exp* gene is accomplished by SinI. Therefore SinI mutant was deficient to produce EPS as well as

any AHL molecule. Moreover, sinI mutant was unable to produce nodule showing deficiency in nitrogen fixation (Marketon et al. 2003).

19.5.1.3 *Lux-R* Regulator

The genome sequence of strain 1021 also carries some genes which are homologous to Lux-R regulator of *P. aeruginosa*. Also six Lux-R regulators have been identified in 1021, all the regulators are involved in various metabolic activities. Some of the AHL receptor structure of homodimers and heterodimers were homologous to *P. aeruginosa* receptors (Ledgham et al. 2003). Even though strain 1021 lacks regulation of ExpR gene, there have been more than seventy five proteins are identified that were expressing AHL made by SinI regulatory system and AHL accumulation has been responsible for the expression of sixty other proteins. However strain 1021 lacks ExpR functions, the expression of 135 proteins is due to direct or indirect involvement of either SinI gene or one of the six other AHL receptor system is actively participating in for the production of AHL (Chen et al. 2003; Teplitski et al. 2003).

19.5.1.4 *traI*, *traR*, and *meII*

The mutation in *sinI* gene nullifies the synthesis of long chain AHL. However, there is no effect on the production of short chain AHL system like C8-HSL. Consequently, they are part of another quorum sensing system termed *mel* although there is no evidence for the presence of the gene in Rm1021 (Marketon et al. 2002). While another strain Rm41 has traI and traR system besides plasmid transfer gene (Teplitski et al. 2003). Recently published article (Acosta-Jurado et al. 2020) on the characterization of the quorum sensing system in *S. fredii* HH103 has revealed that the production of both long chain and short chain AHL is regulated by *traI* and AHL made by *SinI* proteins, at very low concentration. Perhaps the significant genes *expR* and *traR* are found to be spitted from each other and situated on two different ORF regions. This indicates the presence of carboxy terminal proteins that have a binding affinity for DNA motifs which might be regulating target genes by its own, rather than depending on AHL regulating system.

19.5.2 Rhizobium Leguminosarum

R. leguminosarum consists of three distinct types of biovars. 1) which nodulates vetch lentils and peas by *viciae,* 2) involves in nodulation of clover by trifolii, and 3) last one which nodulates phaseolus beans by *phaseoli*. Although among all the biovars, most focus has been given to *viciae.* Four (*rhi, rha, cin,* and *tra*) various

types of LuxI type of AHL synthase genes have been identified in *R. leguminosarum* strain.

19.5.2.1 *cinI* and *cinR*

CinR induces the expression of *cinR* gene in the presence of AHL to synthesize by CinI. When a complex cascade involved in quorum sensing was characterized it depicted that CinR is responsible for positive autoregulated loop hence it can master control the three other (*raiI/raiR, traI,traR, rhiI/rhiR*) quorum sensing system involved in AHL synthesis. Mutation in *cinI/cinR* abolishes the function of many AHL synthase which directly manipulate physiological features of rhizobial strain. Along with that it also manipulates the expression of *rhi* gene that generates nodulation and symbiotic plasmid transfer (Wisniewski-Dyé and Downie 2002). Lithgow et al. (2000) found that in artificial growth medium at a laboratory growth rate of mutants was relatively slow compared to wild type strains of *cinI/cinR*. However, these survival properties of culture may be correlated with the growth in soil or plants. The *cinI/cinR* quorums sensing system is completely cell density-dependent. During the starvation period of carbon and nitrogen source, when culture producing AHL (3-OH-C14:1-HSL) enters into stationary phase are still able to maintain viability at high cell density even after 20–60 days of incubation period.

Whereas when the culture was at low density appeared to show viability loss instantly compared to high cell density (Thorne and Williams 1999).

19.5.2.2 *raiI* and *raiR*

These both *raiI/raiR* genes are not found in the sequenced genome as well as by analytical method for. *R. leguminosarum* by *viciae.* But are indicated in *R. leguminosarum* by phaseoli on the large nonsymbiotic plasmid (Lithgow et al. 2000). Because of the uneven distribution of genes, chances are indicating, that mutation in *raiI/raiR* has no significant effect on phenotype of *R. leguminosarum* even though conditions are either symbiotic or free living (Wisniewski-Dyé and Downie 2002).

19.5.2.3 *rhiI* and *rhiR*

The *rhi* plays a vital role in nodule formation as they interact in the rhizosphere, like legumes of the pea plant. The rhiABC operon is controlled by RhiR but induced by 3OH, C14:1-HSL. Mutation in any region of *rhiI* is manipulated the expression level of *rhiA*. It was observed that mutation in *rhiI* increases the number of nodule formation in pea. The AHL produced by RhiI are C6-HSL and C8-HSL. However, when these both AHL are induced in *Agrobacterium* strain which is deficient of *rhiI*, it appears that rhiA-lacZ and rhiI-lacZ expression is auto-induced. However

mutation in rhiI decreases amount of AHL but does not show any significant effect on RhiA protein synthesis. These activities suggest that there may be additional loci which regulate the gene expression of *rhiI* gene or *rhiABC* gene (Rodelas et al. 1999). To induce expression of *raiI* a RaiR gene is required, whereas in other cases to induce expression of raiR, LuxR-type regulator and ExpR are required. Assuming that cinI and cinR mutant is related to the reduction of *raiI* expression, actively produced CinI made AHL was added to ExpR, hoping that this would induce raiI expression. But it was found that raiI expression is reduced and controlled by *cinS*.

19.5.2.4 *traI* and *traR*

The expression of *traI* gene is induced by TraR in presence of TraI made 3-oxo-C8-hSL. To activate plasmid transfer gene traI symbiosis with pRL1JI plasmid along with *bisR* gene which is inducing the Lux-R regulator.

Frederix et al. (2011) have studied coordination system for quorum sensing gene regulation in response to the accumulation of anti-repressor density. The *cinI* gene also transcribes gene *cinS*, which later activates *raiR* and *rhiR*. Once the genes are transcribed, all the genes are express in cooperate way with their AHL synthase and regulate the mechanism of quorum sensing. When cinS bounds to PraR transcriptional regulator it represses the expression of *rhiR* and *raiR*. However, during the anti-repressor function, there was no need for CinS made AHL synthase for inducing expression of *cinS*. The LuxR-type regulator ExpR represses the transcription of praR in order to induce normal expression of rhiR and raiR. Therefore when CinS assembles in a culture density dependent style, it activates the quorum sensing system.

19.5.3 Rhizobium Etli

Strains CNPAF512 and CFN42 have been characterized for studying quorum sensing mechanism in *R. etli*. Though both are having distinct features, still share an orthologous gene with similar other strains of *R. leguminosarum*.

19.5.3.1 *cinI* and *cinR*

Miao et al. (2018) have investigated the effect of *cinR* mutant and wild type strain CFN42 by co inoculating with *Rhizobium fabae* and characterized for nodule formation of *Phaseolus vulgaris* (common bean) under both sterile and unsterile condition of the soil. While wild type strain was deficient in showing any remarkable increase in the quantity of nodules in sterilized soil compared to *cinR* mutant. However when wildtype strain was cocultured with *Rhizobium fabae* in unsterilized soil there was a notable increase in biofilm formation, a number of nodules and root

attachment compared to *cinR* mutant. To confirm the effect of R fabae on quorum sensing system, the amount of AHL produced by both the strain was quantified. Interestingly it was concluded that *R. fabae* when cocultured with *cinR* mutant increases the amount of AHL rather than cocultured with wild type and this was also confirmed by thin layer chromatography. However, *cinR* mutant itself is unable to synthesize their own AHL individually, hence when comes in association with *R. fabae* increases the amount of AHL synthase. Furthermore it was also analysed that *R. fabae* interaction with *R. etli* further affects the AHL transcriptional expression or not. Interestingly AHL synthase genes like *cinI, raiI,* and *tarI* expression was induced by R. etli in presence of signal release by *R. fabae*. Whereas there was no sign of interaction with *cinR* mutant AHL synthase expression in presence of signals released by *R. fabae* (Zheng et al. 2015).

19.5.3.2 *raiI* and *raiR*

Mutation in *R. etli* of *raiI* gene has found to enhance the number of nodules as well as nitrogenase activity of plant. Interestingly when there was an increase in a number of nodule due to mutation in raiI, on the controversy there was no effect of the mutation on *raiR* gene in nodule development (Daniels et al. 2002).

19.5.3.3 *traI* and *traR*

The functional and structural premises of gene for plasmid pRL1JI from *R. leguminosarum* and p42a of *R. etli* CFN42 appeared to be identical and share same mechanism (Danino et al. 2003; Tun-Garrido et al. 2003). The conjugal transfer of plasmid was studied by in two patterns to evaluate the survival rate and nodulation frequency in plant roots. In the first method traM gene was used, which acts as an antiactivator for TraR. And it was noted that in the presence of expression of TraM gene the transfer of plasmid pRet42a was declined. Another strategy was applied by placing *traI* gene with the nifH promoter region. The huge plasmid was conserved and increase in nodulation was recorded when strains were containing TraM under the nifH promoter. However, on the infection thread, the transconjugation of genes like *traA* and *traI* was expressed. As it is quite complicated to revive bacteria from infection thread, details were confirmed by a technique like flow cytometry and confocal microscopy (Bañuelos-Vazquez et al. 2019).

19.6 Quorum Sensing Signalling in Free Living Diazotrophs

The quorum sensing in free living diazotrophic bacteria has not discussed much because of their low popularity but there are few experiments conducted showing evidence to increase the nitrogen content of plant in response to quorum sensing. In ecosystem, plants have limited absorption capacity of nutrition from soil because they lack the functional activity to convert nitrogen of high molecular weight to low molecular weight. When quorum sensing having bacteria inoculated in field they form biofilm and perform subsequential conversion of atmospheric nitrogen to the available form of amino acids and ammonia. There was an increase in the amount of dissolved nitrogen content of paddy field when treated with diazotrophic bacteria, producing extracellular enzymes and extent quorum sensing behaviour (Guo et al. 2012; Liu et al. 2013; Zhou et al. 2014). The experiment conducted by Santos Lima Fagotti et al. (2019) with two different strains of *Azospirillum brasilense* Ab-V6 and *Bradyrhizobium japonicum* CPAC15 to investigate outcome of cocultured inoculation in green house experiment with all these strains. A transconjugant strain of CPAC 15QS when cocultured with Ab-V6 and inoculated in soybean pot to evaluate the effect of plant growth promoting activity for soybean. It was observed that after the 35 days of plant study CPAC15QS + Ab-V6 was able to enhance nitrogen content of shoot and there was increase in nodulation (dos Santos Lima Fagotti et al. 2019). The whole genome analysis of endophytic nitrogen fixing bacteria *Gluconacetobacter diazotrophicus* Pal5 strain isolated from sugarcane field has emphasized the presence of three luxI-luxR type quorum sensing genes. According to which there are chances that QS genes may play significant role in nitrogen fixation and nodulation (Bertalan et al. 2009). *Klebsiella* has always been an organism of interest for studying nitrogen fixation. Recently published article has investigated that *Klebsiella* NG14 strain was efficiently showing a positive result for acetylene reduction assay and ^{15}N2-fixing activity. *Klebsiella* NG14 has significantly increased nitrogen content of rice by forming a biofilm which is always controlled by cell signalling like quorum sensing (Liu et al. 2011).

Vial et al. (2006) tested 40 strains of Azospirillum for their ability to produce AHLs. But only four-strains among forty were able to produce AHL, that strain was isolated from rice rhizosphere. Genes belong to AHL producing quorum sensing system were generated by the genomic library and *alpI* and *alpR* were directly related to the synthesis of AHL in *A. lipoferum* TVV3. Both the genes *alpI* and *alpR* were found to belong to LuxI and LuxR families and when cloned in suitable host, were able to synthesize a different type of AHL. However, it was noticed that these genes are not homologous to all the strains of *Azospirillum* spp. These genes show characteristics only in few strain of *Azospirillum*.

19.7 Conclusion

There are many experiments conducted on how rhizobacteria utilize these signalling behaviours to interact with plant, which leads to some major beneficial modification to enhance plant growth. Quorum sensing has gained attraction in many perspectives to study the biochemical reaction and gene resolution of rhizobacteria in response to plants. Since many years *Rhizobium* spp. has been consider a model organism to study molecular pinning involved in nitrogen fixation. Majority of *Rhizobium* spp. like *Mesorhizoboium, Sinorhizobium, Rhizobium etli, Bradyrhizobium* involves distinct mechanism and different types of gene regulation to induce quorum sensing. Many nonpathogenic bacteria have shown their involvement in the metabolic process like nitrogen fixation, exopolysaccharide production though production of autoinducer like AHL (Gao et al. 2005). Despite inducing genes like *cinI*, *sinI*, *rhiI,* other environmental factors may also play a role in regulating quorum sensing system. Nodulation study has been one of the easiest and convenient way to emphasize the role of quorum sensing in nitrogen fixation.

19.8 Future Prospective

Although many rhizobacteria have reported for manipulating symbiotic signalling with plants, only a few of them are practically applied. Many reports have explained the nodulation process through quorum sensing but a detailed correlation between nifH cluster and quorums sensing has not been focused yet. Apparently, some free living diazotrophs are also capable of inducing gene expression through quorum sensing system. However very few strains like *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, *and Klebsiella* NG14 have been efficiently involved in nitrogen fixation through quorum sensing mechanism. Quorum sensing is depended on another mechanism like EPS production and biofilm formation. In future, there should be more focus on investigating quorum sensing signalling in free living diazotrophs and plants in the rhizosphere. AHL produced by the *S. meliloti* has significantly affected the senescence of nodulation.

References

Acosta-Jurado S, Alías-Villegas C, Almozara A, Espuny MR, Vinardell J-M, Pérez-Montaño F (2020) Deciphering the symbiotic significance of quorum sensing systems of sinorhizobium fredii HH103. Microorganisms 8:68

Ardourel M, Demont N, Debellé F, Maillet F, de Billy F, Promé J-C, Dénarié J, Truchet G (1994) Rhizobium meliloti lipooligosaccharide nodulation factors: different structural requirements for bacterial entry into target root hair cells and induction of plant symbiotic developmental responses. Plant Cell 6:1357–1374

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Banfalvi Z, Sakanyan V, Koncz C, Kiss A, Dusha I, Kondorosi A (1981) Location of nodulation and nitrogen fixation genes on a high molecular weight plasmid of R. meliloti. Mol Gen Genet MGG 184:318–325

Banfalvi Z, Kondorosi E, Kondorosi A (1985) Rhizobium meliloti carries two megaplasmids. Plasmid 13:129–138

Bañuelos-Vazquez LA, Torres Tejerizo G, Cervantes-De La Luz L, Girard L, Romero D, Brom S (2019) Conjugative transfer between rhizobium etli endosymbionts inside the root nodule. Environ Microbiol 21:3430–3441

Bertalan M, Albano R, de Pádua V, Rouws L, Rojas C, Hemerly A, Teixeira K, Schwab S, Araujo J, Oliveira A (2009) Complete genome sequence of the sugarcane nitrogen-fixing endophyte Gluconacetobacter diazotrophicus Pal5. BMC Genomics 10:450

Beynon J, Beringer J, Johnston A (1980) Plasmids and host-range in rhizobium leguminosarum and rhizobium phaseoli. Microbiology 120:421–429

Bono J-J, Riond J, Nicolaou KC, Bockovich NJ, Estevez VA, Cullimore JV, Ranjeva R (1995) Characterization of a binding site for chemically synthesized lipo-oligosaccharidic NodRm factors in particulate fractions prepared from roots. Plant J 7:253–260

Carlier A, Chevrot R, Dessaux Y, Faure D (2004) The assimilation of γ-butyrolactone in agrobacterium tumefaciens C58 interferes with the accumulation of the N-acyl-homoserine lactone signal. Mol Plant-Microbe Interact 17:951–957

Chaphalkar A, Salunkhe N (2010) Phylogenetic analysis of nitrogen-fixing and quorum sensing bacteria. Int J Bioinf Res 2:17–32

Chen H, Teplitski M, Robinson JB, Rolfe BG, Bauer WD (2003) Proteomic analysis of wild-type Sinorhizobium meliloti responses to N-acyl homoserine lactone quorum-sensing signals and the transition to stationary phase. J Bacteriol 185:5029–5036

Chen G, Malenkos JW, Cha M, Fuqua C, Chen L (2004) Quorum-sensing antiactivator TraM forms a dimer that dissociates to inhibit TraR. Mol Microbiol 52:1641–1651

Daniels R, De Vos DE, Desair J, Raedschelders G, Luyten E, Rosemeyer V, Verreth C, Schoeters E, Vanderleyden J, Michiels J (2002) The cin quorum sensing locus of rhizobium etli CNPAF512 affects growth and symbiotic nitrogen fixation. J Biol Chem 277:462–468

Danino VE, Wilkinson A, Edwards A, Downie JA (2003) Recipient induced transfer of the symbiotic plasmid pRL1JI in rhizobium leguminosarum bv. Viciae is regulated by a quorum sensing relay. Mol Microbiol 50:511–525

Deakin WJ, Broughton WJ (2009) Symbiotic use of pathogenic strategies: rhizobial protein secretion systems. Nat Rev Microbiol 7:312–320

Demont-Caulet N, Maillet F, Tailler D, Jacquinet J-C, Promé J-C, Nicolaou KC, Truchet G, Beau J-M, Dénarié J (1999) Nodule-inducing activity of synthetic Sinorhizobium meliloti nodulation factors and related lipo-chitooligosaccharides on alfalfa. Importance of the acyl chain structure. Plant Physiol 120:83–92

dos Santos Lima Fagotti D, Abrantes JLF, Cerezini P, Fukami J, Nogueira MA, del Cerro P, Valderrama Fernández R, Ollero FJ, Megías M, Hungria M (2019) Quorum sensing communication: Bradyrhizobium-Azospirillum interaction via N- acyl-homoserine lactones in the promotion of soybean symbiosis. J Basic Microbiol 59:38–53

Frederix M, Edwards A, McAnulla C, Downie JA (2011) Coordination of quorum-sensing regulation in rhizobium leguminosarum by induction of an anti-repressor. Mol Microbiol 81:994–1007

Freiberg C, Fellay R, Bairoch A, Broughton WJ, Rosenthal A, Perret X (1997) Molecular basis of symbiosis between rhizobium and legumes. Nature 387:394–401

Fujishige NA, Lum MR, De Hoff PL, Whitelegge JP, Faull KF, Hirsch AM (2008) Rhizobium common nod genes are required for biofilm formation. Mol Microbiol 67:504–515

Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176:269

Gao M, Chen H, Eberhard A, Gronquist MR, Robinson JB, Rolfe BG, Bauer WD (2005) sinI-and expR-dependent quorum sensing in Sinorhizobium meliloti. J Bacteriol 187:7931–7944

Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Giczey G, Auguy F, Péret B, Laplaze L, Franche C (2008) SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankiabacteria. Proc Natl Acad Sci 105:4928–4932

Gilson L, Kuo A, Dunlap PV (1995) AinS and a new family of autoinducer synthesis proteins. J Bacteriol 177:6946–6951

Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, Avarre J-C, Jaubert M, Simon D, Cartieaux F, Prin Y (2007) Legumes symbioses: absence of nod genes in photosynthetic bradyrhizobia. Science 316:1307–1312

González JE, Marketon MM (2003) Quorum sensing in nitro- gen-fixing rhizobia. Microbiol Mol Biol Rev 67:574–592

Guo Y, Zhang Y, Li J-L, Wang N (2012) Diffusible signal factor-mediated quorum sensing plays a central role in coordinating gene expression of Xanthomonas citri subsp. citri. Mol Plant-Microbe Interact 25:165–179

Hoang HH, Becker A, González JE (2004) The LuxR homo- log ExpR, in combination with the sin quorum sensing system, plays a central role in Sinorhizobium meliloti gene expression. J Bacteriol 186:5460–5472

Huguet T, Rosenberg C, Casse-Delbart F, De Lajudie P, Joua L, Boistard JBP, Dénarié J (1983) Studies on rhizobium meliloti plasmids and on their role in the control of nodule formation and nitrogen fixation: the pSym megaplasmids and the other large plasmids. Oceania 54:2

Jones KM, Sharopova N, Lohar DP, Zhang JQ, Vanden-Bosch KA, Walker GC (2008) Differential response of the plant Medicago truncatula to its symbiont Sinorhizobium meliloti or an exopolysaccharide-deficient mutant. Proc Natl Acad Sci 105:704–709

Kim H, Farrand SK (1998) Opine catabolic loci from agrobacterium plasmids confer chemotaxis to their cognate substrates. Mol Plant-Microbe Interact 11:131–143

Laue BE, Jiang Y, Chhabra SR, Jacob S, Stewart GS, Hardman A, Downie JA, O'Gara F, Williams P (2000) The biocontrol strain Pseudomonas fluorescens F113 produces the rhizobium small bacteriocin, N-(3-hydroxy-7-cis-tetradecenoyl) homoserine lactone, via HdtS, a putative novel N-acylhomoserine lactone synthase. Microbiology 146:2469–2480

Ledgham F, Ventre I, Soscia C, Foglino M, Sturgis JN, Lazdunski A (2003) Interactions of the quorum sensing regulator QscR: interaction with itself and the other regulators of Pseudomonas aeruginosa LasR and RhlR. Mol Microbiol 48:199–210

Lithgow JK, Wilkinson A, Hardman A, Rodelas B, Wisniew-ski-Dyé F, Williams P, Downie JA (2000) The regulatory locus cinRI in rhizobium leguminosarum controls a network of quorum-sensing loci. Mol Microbiol 37:81–97

Liu Y, Wang H, Sun X, Yang H, Wang Y, Song W (2011) Study on mechanisms of colonization of nitrogen-fixing PGPB, Klebsiella pneumoniae NG14 on the root surface of rice and the formation of biofilm. Curr Microbiol 62:1113–1122

Liu H, Srinivas S, He H, Gong G, Dai C, Feng Y, Chen X, Wang S (2013) Quorum sensing in Vibrio and its relevance to bacterial virulence. J Bacteriol Parasitol 4:3

Llamas I, Keshavan N, González JE (2004) Use of Sinorhizobium meliloti as an indicator for specific detection of long-chain N-acyl homoserine lactones. Appl Environ Microbiol 70:3715–3723

Madsen LH, Tirichine L, Jurkiewicz A, Sullivan JT, Heck- mann AB, Bek AS, Ronson CW, James EK, Stougaard J (2010) The molecular network governing nodule organogenesis and infection in the model legume Lotus japonicus. Nat Commun 1:1–12

Marketon MM, Gronquist MR, Eberhard A, González JE (2002) Characterization of the Sinorhizobium meliloti sinR/sinI lo- cus and the production of novel N-acyl homoserine lactones. J Bacteriol 184:5686–5695

Marketon MM, Glenn SA, Eberhard A, González JE (2003) Quorum sensing controls exopolysaccharide production in Sino- rhizobium meliloti. J Bacteriol 185:325–331

McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, Daykin M, Lamb JH, Swift S, Bycroft BW (1997) Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiology 143:3703–3711

Miao J, Zhang N, Liu H, Wang H, Zhong Z, Zhu J (2018) Soil commensal rhizobia promote rhizobium etli nodulation efficiency through CinR-mediated quorum sensing. Arch Microbiol 200:685–694

Milton DL (2006) Quorum sensing in vibrios: complexity for di- versification. Int J Med Microbiol 296:61–71

Moré MI, Finger LD, Stryker JL, Fuqua C, Eberhard A, Winans SC (1996) Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. Science 272:1655–1658

Niebel A, Bono J-J, Ranjeva R, Cullimore JV (1997) Identification of a high affinity binding site for lipo-oligosaccharidic NodRm factors in the microsomal fraction of Medicago cell suspension cultures. Mol Plant-Microbe Interact 10:132–134

Piper KR, von Bodman SB, Farrand SK (1993) Conjugation factor of agrobacterium tumefaciens regulates Ti plasmid transfer by autoinduction. Nature 362:448–450

Qin Y, Smyth AJ, Su S, Farrand SK (2004) Dimerization properties of TraM, the antiactivator that modulates TraR-mediated quorum-dependent expression of the Ti plasmid tra genes. Mol Microbiol 53:1471–1485

Redfield RJ (2002) Is quorum sensing a side effect of diffusion sensing? Trends Microbiol 10:365–370

Rodelas B, Lithgow JK, Wisniewski-Dye F, Hardman A, Wilkinson A, Economou A, Williams P, Downie JA (1999) Analysis of quorum-sensing-dependent control of rhizosphere-ex- pressed (rhi) genes in rhizobium leguminosarum bv. Viciae. J Bacteriol 181:3816–3823

Schaefer AL, Hanzelka BL, Eberhard A, Greenberg EP (1996) Quorum sensing in Vibrio fischeri: probing autoinducer- LuxR interactions with autoinducer analogs. J Bacteriol 178:2897–2901

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Shadel GS, Young R, Baldwin TO (1990) Use of regulated cell lysis in a lethal genetic selection in Escherichia coli: identification of the autoinducer-binding region of the LuxR protein from Vibrio fischeri ATCC 7744. J Bacteriol 172:3980–3987

Shaw PD, Ping G, Daly SL, Cha C, Cronan JE, Rinehart KL, Farrand SK (1997) Detecting and characterizing N-acyl-ho-moserine lactone signal molecules by thin-layer chromatography. Proc Natl Acad Sci 94:6036–6041

Slock J, VanRiet D, Kolibachuk D, Greenberg EP (1990) Critical regions of the Vibrio fischeri luxR protein defined by mutational analysis. J Bacteriol 172:3974–3979

Teplitski M, Eberhard A, Gronquist MR, Gao M, Robinson JB, Bauer WD (2003) Chemical identification of N-acyl ho- moserine lactone quorum-sensing signals produced by Sinorhizobium meliloti strains in defined medium. Arch Microbiol 180:494–497

Thorne SH, Williams HD (1999) Cell density-dependent starvation survival of rhizobium leguminosarum bv. Phaseoli: identification of the role of an N-acyl Homoserine lactone in adaptation to stationary-phase survival. J Bacteriol 181:981–990

Tun-Garrido C, Bustos P, González V, Brom S (2003) Conjugative transfer of p42a from rhizobium etli CFN42, which is required for mobilization of the symbiotic plasmid, is regulated by quorum sensing. J Bacteriol 185:1681–1692

Vial L, Cuny C, Gluchoff-Fiasson K, Comte G, Oger PM, Faure D, Dessaux Y, Bally R, Wisniewski-Dyé F (2006) N-acyl-homoserine lactone-mediated quorum-sensing in Azospirillum: an exception rather than a rule. FEMS Microbiol Ecol 58:155–168

Wang B, Yeun LH, Xue J-Y, Liu Y, Ané J-M, Qiu Y-L (2010) Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. New Phytol 186:514–525

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Wijffelman C, Pees E, Van Brussel A, Hooykaas P (1983) Repression of small bacteriocin excretion in rhizobium leguminosarum and rhizobium trifolii by transmissible plasmids. Mol Gen Genet MGG 192:171–176

Wilkinson A, Danino V, Wisniewski-Dyé F, Lithgow J, Downie J (2002) N-acyl-homoserine lactone inhibition of rhizobial growth is mediated by two quorum-sensing genes that regulate plasmid transfer. J Bacteriol 184:4510–4519

Wisniewski-Dyé F, Downie JA (2002) Quorum-sensing in Rhizobium. Antonie Van Leeuwenhoek 81:397–407

Withers H, Swift S, Williams P (2001) Quorum sensing as an integral component of gene regulatory networks in gram-negative bacteria. Curr Opin Microbiol 4:186–193

Zhang L, Murphy PJ, Kerr A, Tate ME (1993) Agrobacterium conjugation and gene regulation by N-acyl-L-homoserine lactones. Nature 362:446–448

Zhang H-B, Wang L-H, Zhang L-H (2007) Detection and analysis of quorum-quenching enzymes against acyl homoserine lactone quorum-sensing signals. Curr Protoc Microbiol 5:1C–3C

Zheng H, Mao Y, Zhu Q, Ling J, Zhang N, Naseer N, Zhong Z, Zhu J (2015) The quorum sensing regulator CinR hierarchically regulates two other quorum sensing pathways in ligand-dependent and-independent fashions in rhizobium etli. J Bacteriol 197:1573–1581

Zhou M, Guo Z, Yang Y, Duan Q, Zhang Q, Yao F, Zhu J, Zhang X, Hardwidge PR, Zhu G (2014) Flagellin and F4 fimbriae have opposite effects on biofilm formation and quorum sensing in F4ac+ enterotoxigenic Escherichia coli. Vet. Microbiol 168:148–153

Zhu J, Winans SC (1999) Autoinducer binding by the quorum- sensing regulator TraR increases affinity for target promoters in vitro and decreases TraR turnover rates in whole cells. Proc Natl Acad Sci 96:4832–4837

Zhu J, Oger PM, Schrammeijer B, Hooykaas PJ, Farrand SK, Winans SC (2000) The bases of crown gall tumorigenesis. J Bacteriol 182:3885–3895

Part VI
Fungi in Nitrogen Fixation

Chapter 20
Fungi and Nitrogen Cycle: Symbiotic Relationship, Mechanism and Significance

Mani Singh

Abstract Fungi are integral components of any natural ecosystem and play a significant role in maintaining nitrogen cycle. Nitrogen is the essential constituent of any organisms as several biomolecules like proteins and nucleic acids are made up of nitrogen. As such nitrogen caters to the requirements of different species at different trophic levels of the ecosystem through its various compounds formed in a nitrogen cycle. Like other microbes, fungi form different symbiotic relationship with plants in which they get carbon inputs from plants in return of nitrogenous and other nutrients. This chapter explains the role of fungi in nitrogen cycle and explores their molecular mechanism. While the first half of nitrogen cycle is led by bacteria, the second half is undoubtedly led by fungi. The major fungal genes involved are *P450nor*, *Nirk*, *dNar/aNar*. The emerging knowledge on the plant–fungi relationship in general and nitrogen cycle on particular will find wider applications in plant productivity and sustainability of the ecosystem.

Keywords Fungi · Denitrification · Co-denitrification · *P450nor* · *Nirk* · *dNar/aNar*

20.1 Introduction

Nitrogen is an essential element for all living organisms including plants, animals and microbes. It is essentially required by the organism in the formation of proteins, nucleic acids and other nitrogenous compounds. Atmospheric nitrogen is the ultimate source of nitrogen; however, it is of rather inert nature and not bioavailable. Hence most of the organisms are not able to utilize it as such. Nitrogen is used as ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) ions by the plants. Plants absorb NO_3^- from the soil as mineral metabolites which are converted into amino (R-NH3) group and other nitrogenous compounds that create living matters. Soil

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fertilizers (added by man) and rocks dissolute by water act as other sources of nitrates for the plants. The lightening energy helps combine the nitrogen and oxygen to form atmospheric nitrates, which settle down to the earth through the rain. In factory setup it requires high energy and pressure to form nitrogenous fertilizers. Alternatively, nitrogen fixation is done by the microbes which facilitate almost 90% of the nitrogen fixation.

20.2 Nitrogen Cycle and Microbes

Nitrogen is found in inorganic (ammonia, nitrate) and organic (amino and nucleic acids) forms besides the elemental form. The nitrogen undergoes different transformations in nitrogen cycle. These transformational changes as different compounds of nitrogen are very important phenomenon in the biosphere that is responsible for high productivity of the ecosystem. Nitrogen fixation, nitrification, denitrification, anammox and ammonification are major steps of nitrogen cycle (Fig. 20.1 and 20.2).

The NO_3 and NO_2 utilized by the organisms and released into the environment in the form of ammonia (NH_3). The ammonia is used for other atmospheric/soil bacteria. NH_3 is oxidized to the NO_2^- and then to the NO_3^- by nitrifying bacteria and becomes bioavailable to the plants. Some part of this nitrate gets converted to the atmospheric nitrogen by the denitrifying bacteria and fungi. The whole process constitutes Nitrogen Cycle, where microbes play significant job in maintaining the cycle (Fig. 20.1).

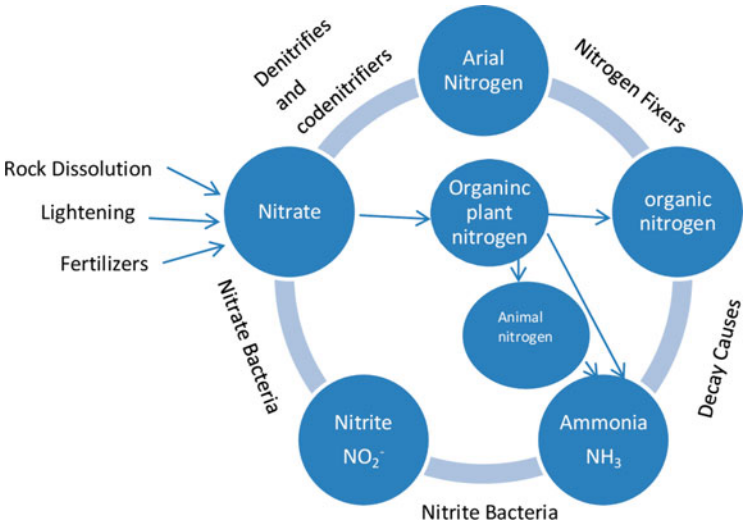

Fig. 20.1 Nitrogen Cycle

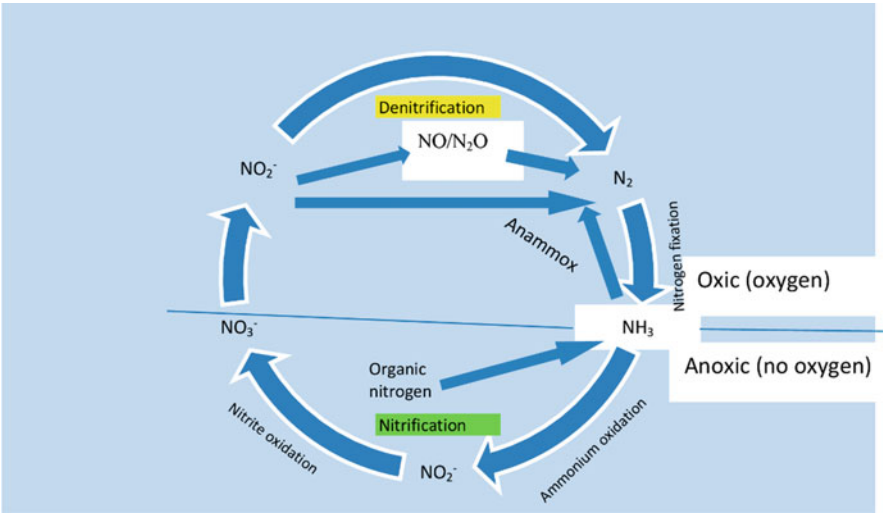

Fig. 20.2 Major transformations in the nitrogen cycle

20.2.1 Nitrogen Fixation

Nitrogen fixation is the conversion of molecular or atmospheric nitrogen into form utilizable to plant by nitrogen fixing microorganisms using an enzyme system called nitrogenase. This is also known as Biological Nitrogen Fixation (BNF). BNF includes both symbiotic nitrogen fixation and the free living nitrogen fixing system. Presence of triple bond in nitrogen gas makes it a stable compound that requires large amount of energy to break as provided below:

$$N_2 + 16ATP + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

20.2.2 Nitrification

Nitrification is the biological oxidation of ammonia to nitrite followed by the oxidation of the nitrite to nitrate. It completes in two steps. *Ammonium oxidation* is the first step of nitrification which is not common among the prokaryotes. Recent discoveries support the role of an archaeon in ammonia oxidation (Koenneke 2005). Many other ammonia oxidizing archaea have been found to be abundant in ocean, soils and salt marshes. These archaea act as ammonia oxidizing organism. Oxidation of nitrite into nitrate is the second step in nitrification and is mostly carried out by nitrogen oxidizing bacteria (prokaryotes). Example of these bacteria is *Nitrospira, nitrobacter, nitrococcus* and *Nitrospina.* It involves the following steps:

$$NH_3 + O_2 + 2e^- \rightarrow NH2OH + H_2O$$

$$NH2OH + H_2O \rightarrow NO_2{}^- + 5H^+ + e^-$$

$$NO_2{}^- + 1/2\,O_2 \rightarrow NO_3{}^-$$

Besides aerobic nitrification, anoxic or anaerobic nitrification of ammonia also occurs (Strous et al. 1999) that is called Anammox (anaerobic ammonia oxidation). Members of planctomycetes phylum of bacteria carry out anammox and use nitrite as the electron acceptor to produce gaseous nitrogen.

In *Ammonification,* decomposition of excretory products or bodies of animals and human takes place by various fungi and prokaryotes that release inorganic nitrogen back into the ecosystem as ammonia. This makes ammonia available for uptake by plants and other microorganisms. Ammonification is followed by denitrification process.

20.2.3 Denitrification

Denitrification is carried out mostly by different genera of prokaryotes (bacteria and archaea) (Risgaard-Petersen 2006). Some examples of denitrifying bacteria are *Bacillus* and *Pseudomonas*, whereas prominent denitrifying fungi are *Fusarium* and *Cylindrocarpon*. However, recent evidences establish that some of the eukaryotes (fungi) are also capable of denitrification. The denitrifiers require mostly organic carbon and are called chemo-organotrophs. Denitrification releases nascent nitrogen back into atmosphere that completes the nitrogen cycle. Many other studies have also mentioned that fungal denitrification is a major process of the nitrogen cycle (Laughlin and Stevens 2002, Ma et al. 2008, Sutka et al. 2008, Cathrine and Raghukumar 2009). Denitrification involves the following steps:

$$NO_3 \rightarrow NO_2$$

$$NO_2 \rightarrow NO + N2O$$

$$2NO_3{}^- + 10e^- + 12\,H^+ \rightarrow N2 + 6H_2O$$

20.2.4 Co-Denitrification

Recent findings have revealed a parallel process of dentrification, known as co-denitrification. Co-denitrification is the process in which hybrid N_2O and/or N_2 formation occurs in the presence of inorganic and organic nitrogen (Su et al. 2004;

Spott et al. 2011; Long et al. 2013). It is the process which is responsible for high soil emission of N_2O and N_2 through fungal interventions.

$$NO_2^- + R\,NH_2 \rightarrow N_2$$

$$NO_2^- + R\,NHOH \rightarrow N_2O$$

Anthropogenic activities since mid-1990s have spiked the release of N_2 in the environment. According to Vitousek (2002) by 2030 anthropogenic introduction of nitrogen into the ecosystem will be high enough to be consumed by the microbial nitrogen fixation. The increased environmental nitrogen may have an adverse impact on primary productivity of the ecosystem (Galloway et al. 1994).

20.2.5 Microbes Involved in Nitrogen Cycle

Microbes are the key components of any ecosystem. Apart from nitrogen fixing bacteria, many other microbes (bacteria, archaea bacteria and various fungi) are associated with the nitrogen cycle. A brief description is given in Table 20.1.

Table 20.1 List of various microbes and their roles in Nitrogen cycle

Microorganism	Relationship	Role in nitrogen cycle
Bacteria		
Rhizobium spp	Symbiotic	Nitrogen fixation
Achromobacter	Symbiotic	Nitrogen fixation
Azorhizobium sp.	Symbiotic	Nitrogen fixation
Frankia sp.	Symbiotic	Nitrogen fixation
Azotobacter	Non-symbiotic	Nitrogen fixation
Pseudomonas sp.	Symbiotic/commensals/ pathogen	Ammonification
Bacillus sp.	Symbiotic	Ammonification
Nitrobacter sp.	Symbiotic/non-symbiotic	Nitrification
Nitrosomonas sp.	Non-symbiotic	Nitrification
Fungi		
Fusarium oxysporum	Commensals/symbiotic/ pathogenic	Denitrification
Cylindrocarpon tonkinense		Denitrification
Bipolaris sorokiniana	Non-symbiotic/pathogenic	Denitrification and codentrification
Fusarium solani	Non-symbiotic/pathogenic	Co-denitrification

20.3 Bacteria Vs Fungi: Role in Nitrogen Cycle

Similar to bacteria, fungi also contribute significantly in nitrogen cycle from decomposition to denitrification. Earlier, nitrogen fixation was suggested in most kind of mycorrhizal systems, however now it is accepted that only prokaryotic organisms can fix atmospheric nitrogen and that ecto- and endo-mycorrhizal fungi lack this capacity. Though, arbuscular mycorrhizal fungi (AMF) are known to help enhance the nitrogen fixing capacity of bacteria like *Rhizobia* and *Azospirillum* in rhizosphere (Biró et al. 1999, Michael et al. 2000). At least 21 AMF spp. have been found in symbiotic interactions with nitrogen fixing bacteria (MiguelBarea and Azcon et al. 1992). The fungal species involved in decomposition of dead organic materials free the organic nitrogen for nitrification and ammonification.

However, various fungal species play important role in the denitrification and co-denitrification (Maeda et al. 2015, Phillips et al. 2016). *Bipolaris sorokiniana* produces N_2O and CO_2 in the presence of organic and inorganic nitrogenous substrate under microaerobic and anaerobic conditions (Phillips et al. 2016). The N_2O production depends on the source of nitrogen, soil pH, soil microbial community.

The process of denitrification of nitrogen cycle was previously thought to be restricted to bacteria only but in later years the dominant role of fungi and yeasts (eukaryotes) was established in distinct denitrifying (Shoun and Tanimoto 1991, Shoun et al. 1992, Tsuruta et al. 1998, Averill 1996, Phillips et al. 2016) and co-denitrifying activities (Shoun et al. 2012, Rex et al. 2019). Cytochrome P450 as nitric oxide reductase (P450nor) is the most characteristic component of fungal denitrification system (Nakahara et al. 1993; Takaya and Shoun 2000). According to Shoun et al. (2012) fungal denitrification system comprises NirK (copper containing nitrite reductase) and P450nor (cytochrome P450 nitric oxide reductase) to reduce nitrite to nitrous oxide.

20.4 Fungi as Symbionts

Fungi undertake denitrification in symbiotic and nonsymbiotic manners. Mycorrhizae are structures formed by the association of fungi and plant roots. Mycorrhiza may be categorized as ectotrophic, endotrophic and extendotrophic mycorrhizae. Mycorrhiza increases nutrient absorption from soil which is supplied to the plants in instalments. The movement of minerals from the fungi to the plants is a metabolically controlled process.

Widely studied arbuscular mycorrhizal fungi (AMF) are endotrophic mycorrhizae and are ubiquitous symbionts of all groups of plants. AMF can improve plant nitrogen acquisition, though have a limited ability to access organic nitrogen. Nonetheless, the synergistic relationship among AMF, *Rhizophagus irregularis* and soil microbial communities doubles the nitrogen acquisition that mycorrhizal

plants acquire from organic matter. This increase in nitrogen acquisition is tenfolds when compared to non-mycorrhizal plants grown in the absence of soil microbial communities (Hestrin et al. 2019).

AMF–plant symbiotic association which is found in majority of land plants can reduce the N_2O emission from the soil by decline in *nirk* gene functions. This association increases *nosZ* activities which consume N_2O, thus the AMF–plant association alters N_2O emission from the soil (Bender et al. 2014). Intense agricultural practices destroy AMF associated with plants. This may lead to the increased soil N_2O emission due to fungal denitrification and co-denitrification in the agricultural lands, pastures and forest soil (Phillips et al. 2016, Rex et al. 2019). Acid rain declines soil nutrients, amoA gene abundance and gene functions of bacteria and these effects are ameliorated by the introduction of ecto-AMF and soil substrate nutrients due to the AMf–bacteria–plant symbiotic association (Li et al. 2019).

AMF–plant symbiotic association can find commercial application as biofertilizer as an alternative to the chemical fertilizers. The application of biofertilizer containing mycorrhizal fungi and bacterial species promotes the plant growth. The results of this biofertilizer application were matching to the organic as well as the chemical fertilizers. Such biofertilizers improve organic and nitrogen content in soil and enhance the nutritional assimilation of plants (total N, P and K) also (Wu et al. 2005).

In a new case, animal–fungus association has shown N_2 fixation activities. N_2 fixation in the leaf cutter ant-microbe symbiosis is an example of a completely new nitrogen source in neotropical ecosystems (Pinto-Tomás et al. 2009). The symbiotic association of termites and fungi is another example of nitrogen fixation (Sapountzis et al. 2016). Termites fulfil their nitrogen requirements by using atmospheric N_2 fixing capacity of diazotrophic bacteria. Fungus-growing termites (subfamily Macrotermitinae) host a fungal exosymbiont (*Termitomyces*) that provides digestive services and the food source for the termites. This has been thought to obviate the need for N_2-fixation by bacterial symbionts. Thus fungi have a distinct and varied role in nitrogen cycle.

20.5 Denitrification and Co-Denitrification Mechanism

Microbial pathways of nitrification, denitrification and co-denitrification produce N_2O. Fungal denitrification has two pathways. One is the classical denitrification process which involves conversion of nitrate or nitrite into N_2O in low concentration of oxygen insufficient to support aerobic respiration. The other one is co-denitrification in which a hybrid N2O and N2 formation occurs with the association of two different nitrogen atoms coming from two different nitrogen sources.

Co-denitrification gives hybrid N_2O through the reaction of N-donor like NH4+, NH_2OH, phenylalanine and glycine with a nitrosyl compound (Rex et al. 2019). A pasture soil having high concentration of urea witnesses co-denitrification via multiple pathways like formation of N_2O from anyone of the nitrogen sources

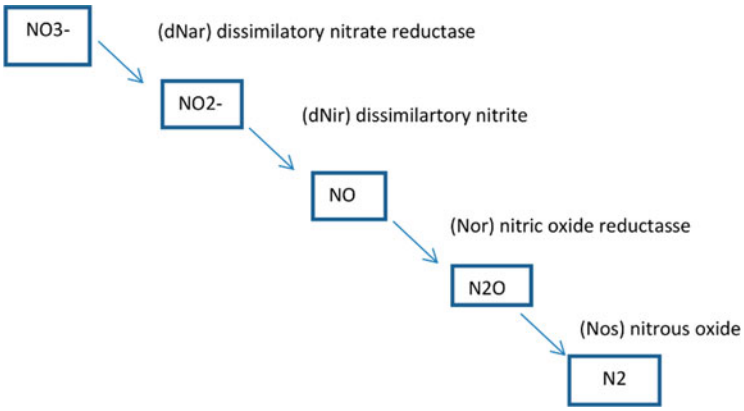

Fig. 20.3 Bacterial denitrifying system

such as ammonium ion, glycine, phenylalanine and NH_2OH. Among these, co-denitrification in the presence of NH_2OH is the dominant process when there is a high concentration of urea-N in pasture soil due to lots of urine patches.

Bacterial denitrification is a function of anaerobic respiration. The molecular mechanism of bacterial denitrifying system is well characterized (Berks et al. 1995, Zumft 1997 and Ferguson 1998). The denitrifying system of bacteria consists of four reducing steps catalysed by specific assimilatory genes (Fig. 20.3).

Characterization of the fungal denitrification system reveals that many fungi (Eukaryotes) perform specific denitrifying activities due to the presence of denitrification system possessing nirk and p 450nor genes. NirK is a copper containing nitrite reductase and P 450nor a member of CYP family, a cytochrome P450 nitric oxide (NO) reductase (Nor). Both enzymes reduce nitrite to nitrous oxide (N_2O) (Shoun et al. 2012). Molecular characterization of denitrification system for both proteins and genes in two fungal species *Fusarium oxysporium* MT811(JCM11502) and *Cylindrocarpon tonkinense* IFO (NITE Biological Resource Center; NBRC) discloses that the fungal denitrification system is restricted to mitochondrial functioning during anaerobic respiration (Fig. 20.4).

Dissimilatory and assimilatory nitrate reductase has also been used by some of the fungal system. Phylogenetic analysis establishes the ancestral relationship between bacteria and fungi in regard to NirK gene (Shoun et al. 2012). The horizontal transfer of the gene P450 from bacteria to fungi is responsible for modulated function of p450nor activity which replaced the original p450 (Kizawa et al. 1991). According to Kizawa et al. (1991) amino acid sequence of CYP55 of superfamily P450 possesses 40% similar sequence to the bacterial CYP105 of actinomycetes. Hence fungi are supposed to have acquired the P450nor from actinomycetes through horizontal transfer (Kizawa et al. 1991). P450nor is a haem protein and possesses lypooxygenase activity along with properties of P450 (Shoun et al. 1983). Cytochrome P450nor gets electrons from nicotinamide adenine dinucleotide (NAD) to reduce NO to N_2O. The mechanism of the electron transfer from nicotinamide

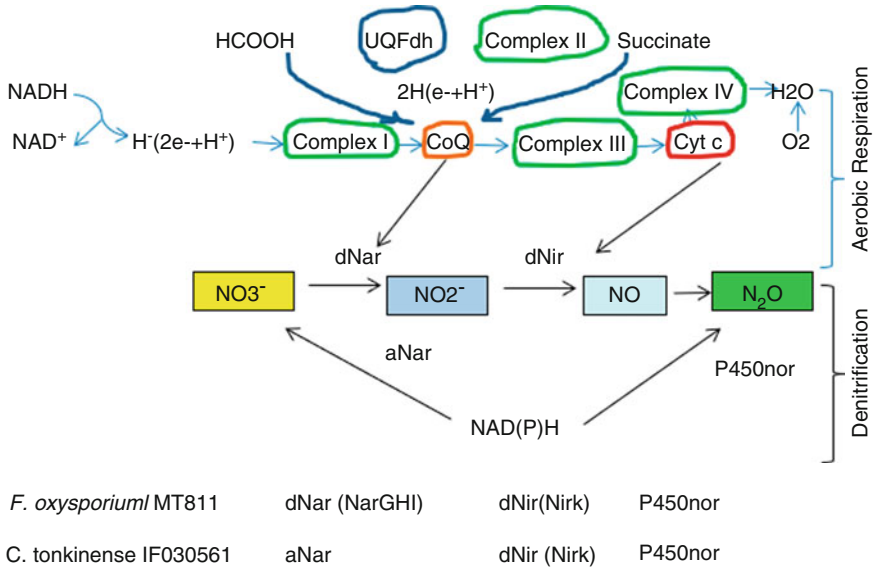

Fig. 20.4 Mitichondiral –denitrifying system of *Fusarium oxysporium* MT811 and *Cylindrocarpon tonkinense* sharing the respiratory chain with oxygen respiration. Source: Shoun et al. (2012)

adenine dinucleotide to P450nor is explored and elucidated thoroughly by many researchers (Shoun et al. 2012, Higgins et al. 2016, Phillips et al. 2016, Rex et al. 2019). The ability of P450nor to receive two energy electrons of NADH is against the central dogma of physiological electron transfer because it does not require a redox partner like usual P450 reductase (Fig. 20.5). P450nor is found both in mitochondria and cytoplasm of the fungal cells. P450nor A1 gives two isoforms P450norA and P450norB. Formation of P450norA from first initiation codon of gene 450nor localizes it to mitochondria and second initiation codons restrict norB to cytoplasm (Shoun et al. 2012).

Continuous and extensive studies have given a clear picture of the reaction mechanism of P450nor. The overall reaction has a very rapid turnover, i.e. 1000 s^{-1} at 10 degree Celsius (Shiro et al. 1995, Daiber et al. 2002). The overall reaction is as follows:

$$2NO + NADH + H^+ \rightarrow N2O + H_2O + NAD^+$$

This reaction has three steps, which are follows :

$$\text{a)} \underset{(\text{ferricP450nor})}{Fe^{3+}} + NO \rightarrow \underset{(\text{ferric-NO complex})}{Fe^{3+}\text{-}NO}$$

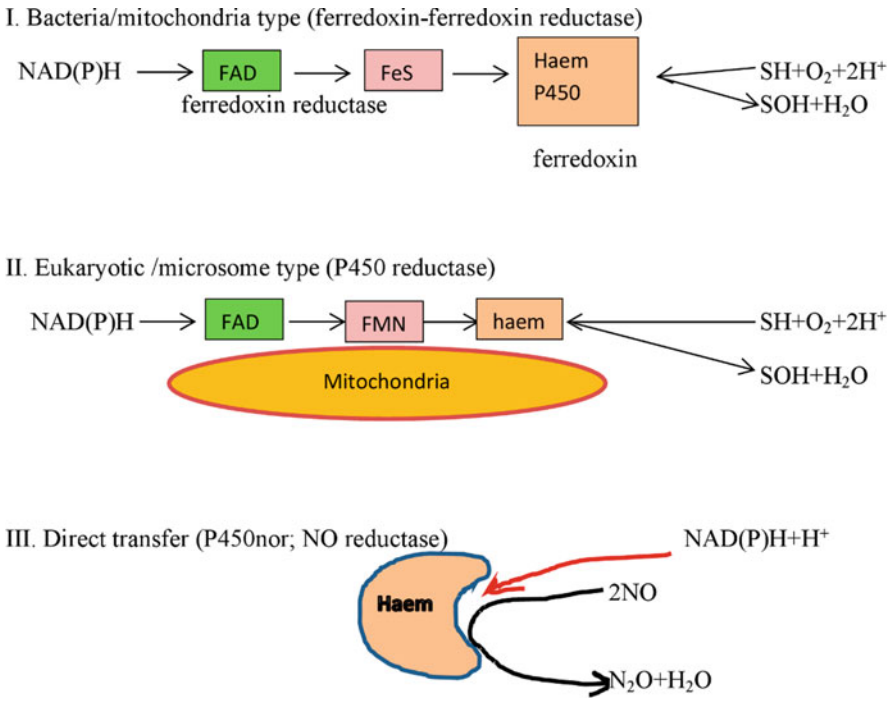

Fig. 20.5 Classification of P450 based on the types of redox partner dependency. Figure clearly depict the electron transfer from NAD(P)H to P450 in the forms of arrows. In I and II type of classification pathways show uses of redox partners by the typical bacterial and Eukaryotic system but in type III P450nor directly receive the electrons from NAD(P)H and does not require any redox partner. Bacterial P450 possess water soluble components and it is bound to inner mitochondrial membrane, whereas ferredoxin reductase and ferredoxin are matrix soluble. In type II microsomal type all components are bound to membrane. P450nor is restricted to both mitochondria and cytosol of fungal cell. SH is the symbol of organic substrate which gets hydroxylated by P450 reaction. Source: Shoun et al. (2012)

$$\text{b)}\ \underset{\text{Reduction}}{Fe^{3+}\text{-NO} + NADH + H^{+}} \rightarrow \text{Intermediate(I)} + NAD^{+}$$

$$\text{c)}\ I + NO \rightarrow N2O + H_2O$$

The intermediate (I) may be ferric-hydroxylamine radical complex (Daiber et al. 2002). A quantum chemical calculation supports this reaction mechanism (Lehnert et al. 2006). Most of the fungal denitrification system consists of *Nirk* and *P450nor*. These two genes are essential to ensure fungal denitrification. Some exceptional fungi like *F. oxysporium* and *C. tonkinense* use dNar/aNar for denitrification of nitrate. Substantially the Nirk and dNar are connected to mitochondrial respiratory chain which synthesizes ATP. This phenomenon is supposed to be the first example of anaerobic respiration occurred in mitochondria. Whereas P450 and aNar work as a sink for electrons under anaerobic conditions because both of them gain electrons

directly from NAD(P)H and are not integrated with respiratory chain (Shoun et al. 2012). Possible ancestors of Eukaryotic *nirK* are supposed to be proto mitochondrion, which possess Nirk-type dNir (Kim et al. 2009). The horizontal transfer of P450nor from bacteria to fungi and substitution of Serene (S73 and S75) with Glycine (G73 and G75) in the gene is the possible reason for the modified properties of usual monooxygenase nature of P450 gene to give Nor activity because P450nor is restricted to fungi only (Shoun et al. 2012). The stereospecific transfer of electrons from NADH to haem of P450nor makes the gene unique to fungi. This gene may also catalyse codenitrification reactions in fungus. It also exhibits NADH-peroxidase activity. Thus P450nor is a multifunctional enzyme.

Co-denitrification usually goes along with fungal denitrification. It is a process of formation of hybrid N2 or N2O species (Shoun et al. 1992, Nakahara et al. 1992, Shoun et al. 2012, Phillips et al. 2016, Rex et al. 2019). In this process one nitrogen atom comes from nitrite and another one comes from different nitrogen donors (organic nitrogen compound, amine, imines, etc.). The denitrification and co-denitrification ratio vary with fungal strains. Nitrogen donors Amines give N_2 and N2O is provided by imines or azides. Shoun et al. (2012) cultured the *Fusarium solani* IFO 9425 using labelled nitrogen in potassium nitrite and aniline and found that the product of the reactions of denitrification was consisting of one labelled nitrogen and the other was normal nitrogen in case of both N_2 and N $_2$O. Thus they confirmed the process of co-denitrification. The reactions are as follows:

$$^{15}NO^{-}{}_{2} + R^{14}NH_2 \rightarrow {}^{15}N^{14}N$$

$$^{15}NO^{-}{}_{2} + R^{14}NHOH \rightarrow {}^{15}N^{14}NO$$

P450 catalyses the co-denitrification reaction in which the reaction between NO and azide gives N_2O and N_2. For co-denitrification NADH is not obligatory as electron donor because nitrogen donor also acts as a reducing agent for NO reduction. This suggests that NO is a direct reactant rather than nitrite in co-denitrification. Co-denitrification starts with reduction of nitrite into NO by *dNir (Nirk)* followed by denitrification of NO to N_2O catalysed by P450nor.

Soil type and characters also play an important role in determination of occurrence of co-denitrification or denitrification or both. A typical denitrification pathway along with the co-denitrification pathway is a characteristic feature of soil of pastureland and agricultural land (Kumar et al. 2002). Rex et al. (2019) explained how denitrification and co-denitrification occur in pasture soil. They also noticed the higher contribution of fungi in comparison to bacteria in all type of ecosystem whether it is pasture land or agricultural land or forest. Rex et al. (2019) described two pathways of codentrification. In one pathway acidic soil in presence of O_2 leads to abiotic nitrosation giving nitrous acid which reacts with amino compounds, NH_2OH, $NH_4{}^{+}$or other organic N compounds to give hybrid N_2O finally. By contrast in the other pathway, acidic soil converts to alkali due to absence of O_2. Actually alkali soil leads biologically mediated nitrosation (Selbie 2015) that causes

replacement of H of organic compound with nitroso group (RN=O) and reacting with nucleophile compounds followed by formation of hybrid N_2O.

20.6 Significance of Fungus in Nitrogen Cycle

Fungi not only have beneficial symbiotic relationship with the plants forming mycorrhiza with the roots, but it also plays direct role in main steps of nitrogen cycle through denitrification and nitrogen fixation. Co-denitrification is the parallel process to denitrification in the nitrogen cycle in which two atoms of nitrogen come from different sources of nitrogen forming a hybrid N_2O and N_2.

Fungi have major contribution in denitrification than bacteria and play dominant role in the soil emission of greenhouse gas N_2O (Laughlin and Stevens 2002). Denitrifying soil bacteria contain nitrogen dioxide reductase gene (nosZ) which converts N_2O to N2. Though, fungi lack this gene, they are able to perform denitrification and co-denitrification in various types of soils using nitric oxide reductase cytochrome *p450nor* and *Nirk* genes. Some of the fungi, specially belonging to the order Hypocreales (e.g. Fusarium) are capable of producing N_2O in absence of oxygen (Shoun et al. 2012, Maeda et al. 2015) and in the presence of little amount of oxygen (Takaya 2009, Phillips et al. 2016).

Bipolaris sorokiniana an exclusive denitrifier is a fungal species of order Pleosporales (Phillips et al. 2016). It is widely distributed in mid-latitudes in grasslands and agricultural fields (Kumar et al. 2002). It is known to contribute in denitrification process taking place in agricultural fields. According to Erisman et al. (2013) the process of denitrification in the soil and sediments is a very important ecosystem service which has positive impact on the water quality, however at the same time it is harmful to the stratospheric ozone. N_2O is a potent greenhouse gas and acts as a precursor of two different reactions which lead to depletion of stratospheric Ozone (Ravishankara et al. 2009).

Fungi do not possess nitrous oxide reductase (nosZ) gene as found in bacteria (Shoun et al. 2012). This gene reduces N_2O to N_2. Therefore fungi give N_2O as an end product of denitrification in place of N_2 in the case of bacteria. P450 catalyses the assimilation and polycyclic aromatic hydrocarbons in Hypocreale and Eurotiale orders of fungi. These substrates are biogenic byproducts of industrial processes (Huarte-Bonnet et al. 2018). According to Huarte-Bonnet et al. the family CYP52 does the first step that is hydroxylation in assimilation process of alkyl hydrocarbons whereas the family CYP53 is associated with oxidation process of poly aromatic hydrocarbons. Thus these fungal species have a great potential of bioremediation of oilspills and other types of pollutions.

Co-denitrification also contributes in the introduction of N_2O and N2 in the atmosphere. Thus denitrification and co-denitrification both dominantly accomplished by the soil fungi lead to the release of organic and inorganic nitrogen to the atmosphere in the form of nitrous oxide and nitrogen gas. This maintains the nitrogen cycle which is very important for a balanced and sustainable ecosystem.

Laughlin and Stevens (2002) observed the domination of fungal co-denitrification in hybrid N_2 formation due to depletion of NO_3^- where 92% of N_2O evolved was co-denitrification driven.

Therefore it can be concluded that apart from the synergistic relationship of mycorrhiza which supplies phosphorus to the soil microbiome, many of the fungal genera play a crucial role in the release of biological nitrogen in the forms of N_2 and N_2O gases into the atmosphere. The fungi act as a regulatory unit for the nitrogen cycle.

20.7 Conclusion

Fungi play a crucial role in nitrogen cycle. Though some of the fungal species also act as a nitrogen fixers and nitrifiers but mainly they play a major role in denitrification and co-denitrification. *dNar, aNar*, *Nirk* and *P450nor* are the main genes responsible for denitrification and co-denitrification functions of the fungal species. *P450nor* directly receives the electrons from NADH. The fungal species undertake denitrification or co-denitrification and get energy for their vital activities at the same time help return back the N_2O and N_2 into the atmosphere. These processes are important for maintaining nitrogen cycle and ecological balance. The process contribute in release of N_2O in atmosphere causing global warming due to its greenhouse effect.

Fungal species use aliphatic and aromatic hydrocarbons as substrate for nitrogen uptake. Therefore they act as bioremediators for the removal of poly aromatic hydrocarbons and aliphatic pollutants. Better understanding and commercial applications of the knowledge will help reduce the dependence on chemical inputs in the form of fertilizer and pesticides which will be a step towards a sustainable ecosystem. New study techniques and genomic research are required to unravel the mystery and unlock the potential of useful fungal application in agriculture and ecosystem sustainability.

20.8 Future Scope of Research

Higgins et al. (2016) used a new PCR primer as a tool to assess the diversity of P450nor in 15 different groups of 214 fungal isolates from agricultural lands. These new PCR primers may have application in various kinds of biomes for the assessment of emission of N_2O and contribution of fungal isolates for N_2 loss from agricultural soil. It was found that all fungal isolates were possessing *P450nor* that produces N_2O from NO_2^- though *nirk* gene . . .was found in only 13–74% of N_2O producing isolates. Thus P450nor targeted PCR primers are highly useful in the assessment studies regarding fungal contributions to N_2O formation and denitrification.

Thus there is ample scope of development of such targeting PCR primers for the accurate assessment of fungal contributions to co-denitrification too. The molecular mechanism of denitrification is almost explored and explained. There is a need of elaborated information on the molecular mechanism of co-denitrification.

References

Averill BA (1996) Dissimilatory nitrite and nitric oxide reductases. Chem Rev 96:2951–2964. https://doi.org/10.1021/cr950056p

Bender S, Plantenga F, Neftel A et al (2014) Symbiotic relationships between soil fungi and plants reduce N2O emissions from soil. ISME J 8:1336–1345. https://doi.org/10.1038/ismej.2013.224

Berks BC, Ferguson SJ, Moir JW, Richardson DJ (1995) Enzymes and associated electron transport system that catalyse the respiratory reduction of nitrogen oxides and oxyanions. Biochim Biophys Acta 1232:97–173. https://doi.org/10.1016/0005-27289500092-5

Biró B, Köves-Péchy K, Vörös I (1999) Interrelations between Azospirillum and rhizobium nitrogen-fixer sand arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions. Appl Soil Ecol 15:159–168

Cathrine SJ, Raghukumar C (2009) Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India. Mycol Res 113:100–109. https://doi.org/10.1016/j.mycres.2008.08.009

Daiber A, Nauser T, Takaya N (2002) Isotope effects and intermediates in the reduction of NO by P450. J Inorg Biochem 88:343–352. https://doi.org/10.1016/S0162-0134(01)00386-5

Erisman JW, Galloway JN, Seitzinger S (2013) Consequences of human modification of the global nitrogen cycle. Philos T R Soc B:368. https://doi.org/10.1098/rstb.2013.0116

Ferguson SJ (1998) Nitrogen cycle enzymology. Curr Opin Chem Biol 2:182–193. https://doi.org/10.1016/S1367-5931(98)80059-8

Galloway JN et al (1994) Year 2020: consequences of population growth and development on deposition of oxidized nitrogen. Ambio 23:120–123

Hestrin R, Hammer EC, Mueller CW, Lehmann J (2019) Synergies between mycorrhizal fungi and soil microbial communities increase plant nitrogen

Higgins SA, Welsh A, Orellana H et al (2016) Detection and diversity of fungal nitric oxide reductase genes (P450nor) in agricultural soil. Appl Environ Microbiol 82(10):2919–2928. https://doi.org/10.1128/AEM.00243-16

Huarte-Bonnet C, Kumar S, Saparrat MCN et al (2018) Insights into hydrocarbon assimilation by Eurotialean and Hypocrealean fungi: roles for CYP52 and CYP53 clans of cytochrome P450 genes. Appl Biochem Biotechnol 184:1047–1060. https://doi.org/10.1007/s12010-017-2608-z

Kim SW, Fushinobu S, Zhou S (2009) Eukaryotic nirK genes encoding copper-containing nitrite reductase: originating from the protomitochondrion? Appl Environ Microbiol 75:2652–2658. https://doi.org/10.1128/AEM.02536-08

MiguelBarea J, Azcon R (1992) ConcepcionAzcón-Aguilar 21 vesicular-arbuscular mycorrhizal fungi in nitrogen-fixing systems. Methods Microbiol 24:391–416

Kizawa H, Tomura D, Oda M, Fukamizu A, Hoshino T, Gotoh O, Yasui T, Shoun H (1991) Nucleotide sequence of the unique nitrate/nitrite-inducible cytochrome P-450 cDNA from Fusarium oxysporum. J Biol Chem 266:10632–10637

Koenneke M (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546

Kumar J, Schafer P, Huckelhoven R et al (2002) Bipolaris sorokoniana, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Mol Plant Pathal 3:185–195

Laughlin RJ, Stevens RJ (2002) Evidence for fungal dominance of denitrification and co-denitrification in a grassland soil. Soil Sci Soc Am J 66:1540–1548. https://doi.org/10.2136/sssaj2002.1540

Lehnert N, Praneeth VK, Paulat F (2006) Electronic structure of iron(II)-porphyrin nitroxyl complexes: molecular mechanism of fungal nitric oxide reductase (P450nor). J Comput Chem 27:1338–1351. https://doi.org/10.1002/jcc.20400

Li Y, Chen Z, He J-Z (2019) Ectomycorrhizal fungi inoculation alleviates simulated acid rain effects on soil ammonia oxidizers and denitrifiers in Masson pine forest. Environ Microb 21:299–313. https://doi.org/10.1111/1462-2920.14457

Long A, Heitman J, Tobias G (2013) Co-occurring anammox, denitrification and co-denitrification in agricultural soils. Appl Environ Microb 79:168–176

Ma WK, Farrell RE, Siciliano SD (2008) Soil formate regulates the fungal nitrous oxide emission pathway. Appl Environ Microbiol 74:6690–6696. https://doi.org/10.1128/AEM.00797-08

Maeda K, Spor A, Edel-Herman V (2015) N2O production a widespread trait in fungi. Sci Rep 5:9697

Michael MT, Eggenberg P, Biro B, Köves-Pechy K, Vörös I, Strasser RJ (2000) Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and Azospirillum and rhizobium nitrogen-fixers on the photosynthetic activity of alfalfa, probed by the polyphasic chlorophyll a fluorescence transient O-J-I-P. Appl Soil Ecol 15(2, 1):169–182. https://doi.org/10.1016/S0929-1393(00)00093-7

Nakahara K, Tanimoto T, Hatano K, Usuda K, Shoun H (1993) Cytochrome-P-450-55A1 (P-450dNIR) acts as nitric oxide reductase employing NADH as the direct electron-donor. J Biol Chem 268:8350–8355

Phillips R, Grelet G, McMillan A (2016) Fungal denitrification: Bipolaris sorokiniana exclusively denitrifies inorganic nitrogen in the presence and absence of oxygen. FEEMS Microb Lett 363:1–5. https://doi.org/10.1095/femsle/fnw007

Pinto-Tomás AA, Anderson MA, Suen G (2009) Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. Science 326:1120–1123. https://doi.org/10.1126/science.1173036

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N2O): 2009. The dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125

Rex D, Clough TJ, Richards KG, Condron LM, de Klein CAM, Morales SE, Lanigan GJ (2019) Impact of nitrogen compounds on fungal and bacterial contributions to co-denitrification in a pasture soil

Risgaard-Petersen N (2006) Evidence for complete denitrification in a benthic foraminifer. Nature 443:93–96

Sapountzis P, Verges JD, Rousk K (2016) Potential for nitrogen fixation in the fungus-growing termite symbiosis. Front Microbiol. https://doi.org/10.3389/fmicb.2016.01993

Selbie DR (2015) Confirmation of co-denitrification in grazed grasslands. Sci Rep 5:17361

Shiro Y, Fujii M, Iizuka T, Adachi S, Tsukamoto K, Nakahara K, Shoun H (1995) Spectroscopic and kinetic studies on reaction of cytochrome P450nor with nitric oxide. Implication for its nitric oxide reduction mechanism. J Biol Chem 270:1617–1623. https://doi.org/10.1074/jbc.270.4.1617

Shoun H, Tanimoto T (1991) Denitrification by the fungus Fusarium oxysporum and involvement of cytochrome P-450 in the respiratory nitrite reduction. F Biol Chem 266:11078–11082

Shoun H, FushinobuS JL et al (2012) Fungal denitrification and nitric oxide reductase cytochrome P45nor. Philos T R Soc B 367:1186–1194

Shoun H, Kim DH, Uchiyama H, J (1992) Denitrification by fungi. FEMS Microb Lett 73:277–281. Doi:https://doi.org/10.1111/j.1574-6968.1992.tb05331.x

Shoun H, Sudo Y, Seto Y et al (1983) Purification and properties of a cytochrome P-450 of a fungus, Fusarium oxysporum. J Biochem 94:1219–1229

Spott O, Russow R, Stang GF (2011) Formation N2O and N_2 due to co-denitrification: first review of a barley considered process of microbially mediated N-nitrosation. Soil Biol Chem 43:1995–2011

Strous M, Fuerst JA, Kramer EH (1999) Missing lithotroph identified as new planctomycete. Nature 400:446–449. https://doi.org/10.1038/22749

Su F, Takaya N, Shoun H (2004) Nitrous oxide-forming co-denitrification catalyzed by cytochrome P450nor. Biosci Biotechnol Biochem 68:473–475. https://doi.org/10.1271/bbb.68.473

Sutka RL, Adams GC, Ostrom NE (2008) Isotopologue fractionation during N2O production by fungal denitrification. Rapid Commun Mass Spectrom 22:3989–3996. https://doi.org/10.1002/rcm.3820

Takaya N, Shoun H (2000) Nitric oxide reduction, the last step in denitrification by Fusarium oxysporum, is obligatorily mediated by cytochrome P450nor. Mol Gen Genet 263:342–348. https://doi.org/10.1007/s004380051177

Tsuruta S, Takaya N, Zhang L, Shoun H, Kimura K, Hamamoto M, Nakase T (1998) Denitrification by yeasts and occurrence of cytochrome P450nor in Trichon cutaneum. FEMS Microbiol Lett 168:105–110. https://doi.org/10.1111/j.1574-6968.1998tb1362.x

Vitousek PM (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57:1–45

Wu SC, Cao ZH, Li ZG (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61:533–616

Chapter 21
Arbuscular Mycorrhiza in Sustainable Plant Nitrogen Nutrition: Mechanisms and Impact

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Abstract Nitrogen (N) is an important macronutrient that has a significant role in plant growth and development. Therefore, optimum levels of N should be supplied to the plants for sustainable crop production. Besides the chemical fertilization of plants, beneficial soil microorganisms like the arbuscular mycorrhizal (AM) fungi form a mutualistic association with the majority of plant roots and enhance plant growth and nutrient uptake. The importance of AM fungi is mostly attributed to phosphorus (P) acquisition in plants. Nevertheless, the role of AM fungi in the N nutrition of plants is also well known. Plants N acquisition is mediated by direct and indirect pathways through plant roots or via a common mycelium network of AM fungi. Organic and inorganic N is the major source of N in the soils. Influence of AM fungi on other symbiotic and asymbiotic microorganisms is also known to contribute to dinitrogen (N_2) fixation in plants. This chapter highlights the role of AM fungi in N nutrition, N_2 fixation, and uptake of organic and inorganic N from the soils. Also, the importance of AM fungi in the N cycle and the impact of different AM fungal species on plant N uptake are discussed.

Keywords Agroecosystems · Ammonia oxidizers · Arbuscule · Common mycelial network · Extraradical hyphae · Nitrogen cycle

21.1 Introduction

Globally, it is important to improve and increase the crop yield to meet the ever-growing human population. Therefore, the agricultural soils are intensely fertilized to increase crop yields and to prevent the depletion of nutrients in the soils (Kobae 2019). Nevertheless, the application of chemical fertilizers stimulates environmental

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pollution and also leads to the degradation of natural resources (Fan et al. 2011). The growth and development of crop plants basically rely on the combination and concentration of nutrients existing in the soil solution. The significant challenge for plants is to acquire adequate quantities of these mineral nutrients essential for their basic cellular process and development from the soil. This is caused mainly due to the immobile nature of plants as well as the large number of essential mineral nutrients including both macro- and micronutrients required for their growth and sustenance (Morgan and Connolly 2013).

Among the various macronutrients, nitrogen (N) forms an integral part of the plant body as it constitutes around 3–4% of plant dry weight (Tang et al. 2018). Also, N becomes a limiting factor for plant growth and yield in certain conditions and it influences the development of leaves, formation, and functioning of chlorophyll and is a major constituent of amino acids (Ueda et al. 2017). Therefore, N is a vital component in agricultural fertilization for any crop species (Torres-Olivar et al. 2014). In fact, Gojon (2017) suggested that the quantity of N fertilizers used in agriculture is enormous and nearly equals the natural global dinitrogen (N_2) fixation into the bio-lithosphere from the atmosphere. The source of N for plants in the soil is available as a complex mixture of organic and inorganic compounds (Christou et al. 2006). Plants generally take up inorganic forms of N mostly nitrate (NO_3^-) and ammonium (NH_4^+) from the soil (Hawkins and Robbins 2010). Nevertheless, some studies have shown that amino acids are used as a primary source of N in certain plant species (Inselsbacher and Näsholm 2012; Dion et al. 2018). Moreover, plants incorporating organic N into their protein tend to have reduced carbon (C) cost for protein synthesis (Franklin et al. 2017).

The nutrient transport in plants not only involves the unique transport system present on the root epidermis but also the nutrient transport system imparted by particular soil microbial communities (Kobae 2019). Arbuscular mycorrhizal (AM) fungi are ubiquitous soil-borne microorganisms associating with nearly 60–70% of land plants (Smith and Read 2008). These fungi have a pivotal role in natural and agricultural ecosystems through their influence on plant nutritional status by altering the soil's biological activity and availability of nutrients to plants (Smith and Read 2008; Ingraffia et al. 2019). It has been estimated that plants assimilate more than 50% of their total N through mycorrhizal symbiosis regardless of the host type (Veresoglou et al. 2012). The extraradical mycelia (ERM) of AM fungi in the soil forms a common mycelial network interconnecting the roots of almost all the plants existing in a plant community (He et al. 2019). This facilitates the transport of nutrients such as phosphorus (P), C, N, and other nutrients between multiple plant hosts and also permits the exchange of signals among the AM fungal network in interconnected plants (Bücking et al. 2016).

The efficient role of AM fungi in the uptake and transport of P has been well evidenced in different plant species (Seguel et al. 2015; Mackay et al. 2017; Ortas et al. 2019). Nevertheless, Wang et al. (2018) pointed out that the importance of AM fungi in enhancing the N nutrition is not adequately studied. Moreover, in a review, Bücking and Kafle (2015) also claimed that the balance between N immobilization and N transfer to plants by AM mycelium as a function of N availability in soil is

Table 21.1 Example of studies showing different types of arbuscular mycorrhizal (AM) fungi mediated nitrogen (N) nutrition in plants

Mechanisms	Studies
Direct uptake and translocation	Liese et al. (2018); Wang et al. (2018); Zhu et al. (2016); Scandellari (2017); Liu et al. (2020); Wu et al. (2020); Yang et al. (2020)
Indirect role	
Solubilization of organic N	Hodge et al. (2001); Leigh et al. (2009); Hodge and Fitter (2010); Whiteside et al. (2012a, 2012b); Hodge (2014); Paterson et al. (2016)
Stimulation of soil microbial activity	Herman et al. (2012); Arriagada et al. (2014); Almonacid et al. (2015); Taylor et al. (2016); Gui et al. (2017)
Increased dinitrogen (N_2) fixation by asymbiotic microorganisms	Montenegro-Gómez et al. (2017); Dutta and Neog (2017); Mohammadi et al. (2019); Dal Cortivo et al. (2020); Varinderpal-Singh et al. (2020)
Improved symbiotic N_2 fixation	Bulgarelli et al. (2017); Püschel et al. (2017); Hack et al. (2019); Hao et al. (2019); Ingraffia et al. (2019); Lindsay et al. (2019); de Novais et al. (2020); Musyoka et al. (2020)
Interplant transfer of N	Montesinos-Navarro et al. (2016); Fernandez et al. (2020); He et al. (2019); Lu et al. (2020); Yu et al. (2020)

poorly understood. Though in recent years, researchers have carried out experiments demonstrating the impact of AM fungi on the N uptake in different host plants, their mechanisms (Table 21.1) are not well deciphered as for plants P acquisition (Johnson et al. 2015). Therefore, in this chapter, the significant role of AM fungi in N uptake in plants through various pathways and mechanisms is discussed. Additionally, the role of AM fungi in N_2 fixation and the impact of different AM fungal species on N uptake by different plant species are also described.

21.2 Arbuscular Mycorrhizal Fungi

The AM fungi are obligate biotrophs belonging to the subphylum Glomeromycotina and Mucoromycotina (Schüβler et al. 2001; Spatafora et al. 2016). The roots of most terrestrial plants associate symbiotically with AM fungi that contribute to superior plant growth by increasing the acquisition of immobile mineral nutrients (Brundrett 2009; Wang et al. 2018). The vast body of available literature has proved the beneficial aspects of AM fungi in terms of enhanced plant growth, nutrient uptake, and improved soil fertility, tolerance against several biotic and abiotic stresses (Chen et al. 2018; Sun et al. 2018). Moreover, AM fungal species influence the plant N nutrition in different plant species (Table 21.2). The AM fungi also facilitate a sequence of complex communication events among the fungus and the plant that leads to increased photosynthetic rate and other characters related to gas-exchange (Birhane et al. 2012). An AM fungus depends exclusively on the host plant for the photosynthetically fixed C and completion of its life cycle in exchange for nutrients

Table 21.2 Impact of arbuscular mycorrhizal (AM) fungi on plant nitrogen (N) nutrition under different experimental conditions

Host plant	AM fungal species	Observations	Conditions	Reference
Andropogon gerardii Vitman	*Rhizophagus irregularis*	Decreased shoot N (5.3 mg plant^{-1}, 8.2 mg plant^{-1}) and root N (3.9 mg plant^{-1}, 8.4 mg plant^{-1}) under non-fertilized and basic fertilization than uninoculated plants	Greenhouse	Püschel et al. (2016)
Bidens pilosa L.	*Claroideoglomus etunicatum*	Increased N accumulation (47.49 mg plant^{-1}), ^{15}N content (3.50 μg plant^{-1}), N transfer (0.22%), and N transfer amount (0.05%) compared to uninoculated plants	Greenhouse	He et al. (2019)
Broussonetia papyrifera (L.) L'Hér. Ex vent.	*C. etunicatum*	More N accumulation (49.79 mg plant^{-1}), ^{15}N content (3.00 μg plant^{-1}), N transfer (0.19%), and N transfer amount (0.04%) than uninoculated plants	Greenhouse	He et al. (2019)
Calamagrostis epigejos (L.) Roth	*R. irregularis, Claroideoglomus claroideum, Funneliformis caledonium*	Increased shoot N concentration (6.3 mg plant^{-1}) at younger succession stage than uninoculated plants	Greenhouse	Rydlová et al. (2016)
Chrysanthemum morifolium Ramat	*Funneliformis mosseae, Diversispora versiformis*	Increased shoot N concentration by 29%, 6%, and 13% under 200 mM NaCl when AM fungi were inoculated individually or in combination	Greenhouse	Wang et al. (2018)
Cicer arietinum L. (var Pusa 329 and Pusa 240)	*F. mosseae*	Both leaf (24.2% & 18.89%) and root N (24.68% & 15.65%) content was higher in AM inoculated Pusa 329 and Pusa 240 chick pea genotype than uninoculated plants	Greenhouse	Garg and Chandel (2011)
Cinnamomum camphora (L.) J.Presl	*C. etunicatum*	N accumulation (11.73 mg plant^{-1}), ^{15}N content (1.40 μg plant^{-1}), N transfer (0.09%), and N transfer amount (0.02%) was greater in AM inoculated plants than uninoculated plants	Greenhouse	He et al. (2019)
Cucumis sativus L.	*Claroideoglomus* sp., *Funneliformis* sp., *Diversispora* sp., *Glomus* sp., *Rhizophagus* sp.	Combined AM fungal inoculation increased the plant N uptake by 91.16% than uninoculated plants	Greenhouse	Chen et al. (2017)
Eleusine coracana (L.) Gaertn	*Rhizophagus aggregatus, Acaulospora scrobiculata, Rhizophagus intraradices*	Shoot (77.30%) and root N contents (48.22%) were higher in AM inoculated plants than uninoculated plants in Alfisol soil	Greenhouse	Kandhasamy et al. (2020)

Fragaria × *ananassa* var. Elsanta	*F. mosseae, R. irregularis*	Enhanced translocation of ^{15}N in leaves (63.3%), crowns (5.6%), fruits (3.4%), and root (5.1%); increased inorganic N uptake with AM fungal inoculation	Greenhouse	Tomè et al. (2015)
Glycine max L.	*Rhizophagus clarus*	Increased N uptake (0.28 g plant^{-1})	Field	Cely et al. (2016)
Gossypium hirsutum L.	*R. clarus*	Enhanced shoot N concentration (0.35 g plant^{-1})	Field	Cely et al. (2016)
Leymus chinensis (Trin.) Tzvelev	*F. mosseae*	Increase in N level decreased the AM fungal colonization	Greenhouse	Lin et al. (2017)
Medicago sativa L.	*R. irregularis*	AM fungal inoculation increased leaf N content (28 g kg^{-1}) under well-watered condition and root N content (16 g kg^{-1}) under drought conditions	Greenhouse	He et al. (2017)
Plantago lanceolata L.	*Simiglomus hoi*	Increased shoot (3.18%) and root (2.08%) N content derived from plant root litter. Enhanced N concentration in roots (15.2 mg plant^{-1}) and shoots (24.7 mg plant^{-1}) under AM inoculation	Greenhouse	Herman et al. (2012)
Populus × *canadensis* Neva	*R. irregularis*	Enhanced leaf N (17.6 g kg^{-1}) content of plants under 15 mmol L^{-1} NH_4NO_3 fertilization than other treatments	Nursery	Wu et al. (2017)
Solanum lycopersicum L.	*R. irregularis*	Increased shoot (1.73%) and root (1.74%) N content under regular pattern and low amount of water; in medium level of water, shoot and root N concentration was 1.90% and 1.86%, respectively; and at high level of water, shoot N was 1.77% and root N content was 1.84%	Glasshouse	Bowles et al. (2018)
Sorghum bicolor (L.) Moench	*R. irregularis*, *Rhizophagus arabicus*	*R. arabicus* increased total N concentration under drought (27 mg plant^{-1}) and well-watered condition (29 mg plant^{-1})	Microcosm/ growth chamber	Symanczik et al. (2018)

(continued)

Table 21.2 (continued)

Host plant	AM fungal species	Observations	Conditions	Reference
Stevia rebaudiana Bertoni	*R. irregularis*	Higher leaf (0.87 g plant^{-1}), stem (0.33 g plant^{-1}), and root (0.39 g plant^{-1}) N concentration and nutrient use efficiency than uninoculated plants treated with higher P (^{50}P) dosage	Greenhouse	Tavarini et al. (2018)
Triticum aestivum L.	*Glomus* spp.	Increased shoot N content by 31.3%, 46.5%, and 53.9% and leaf nitrate content by 27.3%, 33.3%, and 44.0% under different salinity levels than non-AM plants	Greenhouse	Talaat and Shawky (2014)
Triticum durum Desf. -*Vicia faba* L. (intercropping)	*Gigaspora margarita*, *F. mosseae*, *R. irregularis*, *R. clarum*, *Septoglomus deserticola*, *Funneliformis monosporus*, *Paraglomus brasilianum*, *R. aggregatus*	Increased N transfer from fababean to cereal (2.03% to 2.67%). Inoculation of AM fungal mixture enhanced N transfer by 20%	Wire-house protected from rain in farm	Ingraffia et al. (2019)
T. aestivum	*R. irregularis*	Enhanced N uptake (0.24 g plant^{-1}), ^{15}N recovery (90.9%) and N use efficiency (NUE) (29.5%) at elevated CO_2 treatments	Climate-controlled glasshouse	Zhu et al. (2016)
T. durum	*F. mosseae*	Increased N uptake in AM inoculated plants by 20% than uninoculated plants and enhanced the soil N mineralization	Climate-controlled glasshouse	Saia et al. (2014)
Triticum turgidum L.	*R. irregularis*	AM inoculation enhanced the N concentration in grains by 36%	Field	Ercoli et al. (2017)
Zea mays L. -*Medicago sativa* L. (intercropping)	Indigenous AM fungal species	Intercropping increased total N content by 114% and 32% than monoculture of maize and alfalfa plants; N transfer ranged from 7 to 10 mg N plant^{-1} from alfalfa to maize	Field	Zhang et al. (2020)

acquired and transported to plants (Fellbaum et al. 2012a). The AM fungi are also regarded as natural plant growth regulators and are often used as bio-inoculants or biofertilizers in sustainable crop production systems (Barrow 2012).

The unique structures like arbuscules, and vesicles that are formed by AM fungi in the plant roots and also spores and hyphae that develop in the soil surrounding roots have different functions (Smith and Read 2008; Begum et al. 2019). Initially, the fungal hyphae penetrate the root through the epidermis and extend within plant roots inter- or intracellularly until it reaches the root cortex where it forms arbuscules. The arbuscules are short-lived and are the transit points as they regulate the nutrient exchange between the fungus and the plant (Bonfante and Genre 2010). The vesicles are presumed to be storage reserves of minerals and lipids of the fungi within the plant roots and are utilized by the fungi when their supply from the host plant is limited (Smith and Read 2008). The mycelium is a key component in AM fungal symbiosis consisting of ERM and intraradical mycelium (IRM). The ERM network in the soil not only helps in accessing the nutrients but also enlarges the surface area of the roots in the soil thereby promoting plant growth (Bowles et al. 2016). The ERM takes up nutrients from the soil and transport to the host plant roots, whereas the IRM liberates the nutrients to apoplast and exchanges C from the host plant. These C resources are utilized by the fungus to enlarge and maintain ERM, for thallus metabolisms, and also for spore development (van Aarle and Olsson 2008; Smith and Smith 2011).

21.3 Uptake of N by AM Fungi

The roots of AM plants acquire nutrients via two pathways, either through root epidermis and root hairs directly or by symbiotic interfaces that are provided by arbuscules or hyphal/arbusculate coils formed inside the root cortical cells (Fig. 21.1; Smith and Smith 2011). It has been suggested that the N assimilation by plants is carried out by mycorrhizal symbiosis regardless of the host type they are associated with (Veresoglou et al. 2012). Nitrogen uptake by AM fungi could be divided into two major divisions: (1) organic N uptake and (2) inorganic N uptake.

21.3.1 Organic N Uptake

The organic matter present in the soil is the fundamental source of N for plants in terrestrial ecosystems and maintaining this organic matter is crucial for the sustainability of both natural and agricultural ecosystems (Frey 2019). As mentioned previously, AM fungi capture low mobile mineral nutrients that are not available to the plant roots through their widespread hyphal network that extends beyond the nutrient depletion zone surrounding the roots (Smith et al. 2011; Kobae 2019). However, it was believed that AM fungi might play only a minor role in N nutrition

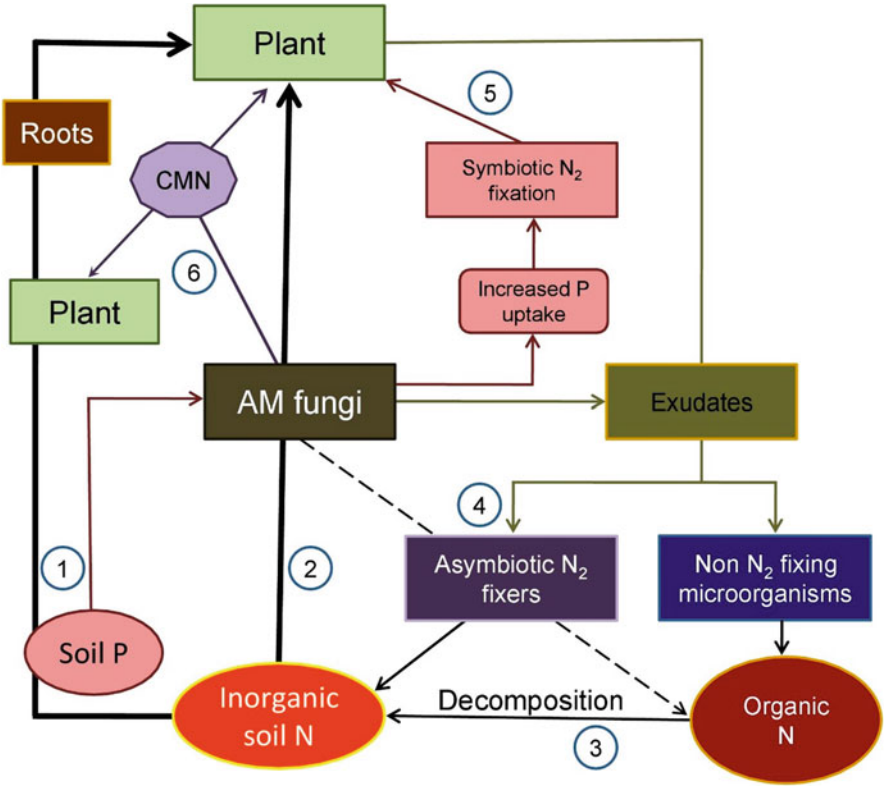

Fig. 21.1 Role of arbuscular mycorrhizal (AM) fungi in direct (**1–2**) and indirect (**3–6**) nitrogen (N) nutrition of plants. **1–2.** Direct uptake and transfer of soil N by plant roots (**1**) or the extraradical hyphae of AM fungi (**2**); **3–4.** Increased availability of inorganic N in the soil due to the enhanced decomposition of organic N and/or dinitrogen (N_2) fixation by asymbiotic microorganisms; **5.** Improved symbiotic N_2 fixation in leguminous and actinorhizal plants through improved plant phosphorus (P) nutrition; **6.** Interplant transfer of N between plants by common mycelial network (CMN). As the direct decomposition of organic matter by AM fungi is not well resolved it is shown by broken line arrow

of plants due to their obligate biotrophic nature and the lack of saprotrophic capability that is required for obtaining N from organic sources (Smith and Read 2008). Also, AM fungi do not possess or have the minimum capability to produce soil hydrolytic enzymes that have the capacity to decompose organic compounds with high molecular weight (Smith and Smith 2011; Phillips et al. 2013).

Nevertheless, numerous studies have evidenced the importance of AM fungi in the uptake of organic N from the soil (Whiteside et al. 2012a; Bukovská et al. 2016). For example, Hodge and Fitter (2010) in a microcosm experiment raised a pair of *Plantago lanceolata* plants both in the presence and absence of AM fungus *Simiglomus hoi* (=*Glomus hoi*) that permitted the fungus alone to access organic patch labelled with $^{15}N/^{13}C$. The results of the study showed that only the fungal

ERM was enriched with ^{15}N, and not with ^{13}C confirming that AM fungi do not possess any saprophytic capabilities and the fungus obtained ^{15}N as a product of decomposition from the organic patches. In the same experiment, the fungal hyphae of the AM fungi *S. hoi* and *Funneliformis mosseae* (=*Glomus mosseae*) exploited the organic material and were able to colonize new host plant, thus indicating the fungal growth. Therefore they proposed that AM fungi could proliferate especially in organic patches, where they can obtain one-third of N from the decomposing organic material (Hodge and Fitter 2010). The AM fungi could indirectly increase the mineralization of N from organic matter (Atul-Nayyar et al. 2009) through their effect on the C flow during the decomposition process via soil microbial communities (Herman et al. 2012; Nuccio et al. 2013).

The AM fungal spore formation, as well as the establishment of the ERM in the soil, is mainly facilitated by the organic amendments which are rich in N content such as plant remains, animal manure, and chitin present in the soil (Quilliam et al. 2012; Bukovská et al. 2016). The microorganisms in the soil that solely depend upon the organic matter for their nutrition, liberate nutrients such as N or P from them and generate other secondary metabolites. Thus, these secondary metabolites and nutrients may also be involved in the AM fungal hyphal response (Bukovská et al. 2016). In addition, microorganisms may directly associate with the surface or the hyphosphere of AM fungi either partially or completely to receive C from the hyphal exudates (Jansa and Gryndler 2010) and these microorganisms degrade the soil organic matter to release nutrients for themselves or for their fungal hosts (Jansa et al. 2013). The AM fungi are capable to perceive and respond to organic compounds that are soluble, which stimulate specific transcriptional responses in the ERM. For instance, the ERM of *Rhizophagus irregularis* (=*Glomus intraradices*) originating from the hairy roots of carrot was able to recognize and respond at the transcriptional level to the shortfall of N in the culture medium. However, this ability was not evident in the IRM or in the asymbiotic stage suggesting that N could act as a signalling molecule for the AM fungus (Cappellazzo et al. 2007). This clearly shows that the hyphae of AM fungi could detect the presence of organic matter containing decomposing patches, proliferate inside them and obtain inorganic N. In a study, Tanaka and Yano (2005) showed that around 75% of N accumulated in the shoots and roots of maize plants was transported through the hyphae of the AM fungus *Rhizophagus aggregatus* (=*Glomus aggregatum*).

Apart from primary decomposers that catalyse the release of N molecules which are captured by the microbial cells, some of the microbial grazers like protists or nematodes in the soil also have a vital role in the acquisition of N by AM fungal hyphae from the organic sources (Bukovská et al. 2018). These microbial grazers excrete large quantities of N that are taken up from their prey in the form of free NH_4^+ ions into the soil solution (Trap et al. 2016). Therefore, AM fungal hyphae can uptake these N from the soil solution to meet their N demand or transfer it to the associated host plant (Thirkell et al. 2016; Bukovská et al. 2018). Several studies have documented that free NH_4^+ ions in the soil to be the major source (Bukovská et al. 2018; Storer et al. 2018) and also the preferred form of N that is taken up by the AM fungal hyphae (Tanaka and Yano 2005).

Even though soil microbial biota mineralizes organic N into an accessible form for the plants, they could also concurrently compete for the liberated N. In a recent study, Hestrin et al. (2019) performed an experiment using a grass species *Brachypodium distachyon* and revealed that the synergistic effect between the AM fungus, *R. irregularis* and soil microbial communities possesses a non-additive impact on N uptake. These microbial synergies consequently lead to doubling-up of N that were derived from organic matter in mycorrhizal plants and also a tenfold enhancement in N uptake than the non-mycorrhizal plants raised in soils free of microbial communities. In addition, they also showed that more than 50% of the N acquired by AM inoculated plants from organic material may be ascertained to the synergistic effect between AM plants and their associated microbial communities in the soil (Hestrin et al. 2019).

The AM fungi can also capture various types of free amino acids such as glutamic acid, glycine, or glutamine from the soil and these amino acids represent the major proportion of N resources present in the soil (Breuninger et al. 2004; Näsholm et al. 2009; Whiteside et al. 2012b). Some of the transcriptome studies have revealed the expression and upregulation of amino acid transporter genes in the ERM of AM fungi (Tisserant et al. 2012). For example, the amino acid permease found in the fungal membrane of an AM fungus, *F. mosseae* was found to play an important role in the initial stages of amino acid acquisition thereby allowing the amino acid uptake directly from the soil (Cappellazzo et al. 2007). The uptake of different types of amino acids was determined using quantum dots in *Sorghum bicolor* plants inoculated with AM fungal consortia including *R. irregularis, Claroideoglomus etunicatum, F. mosseae,* and *R. aggregatus* under greenhouse conditions (Whiteside et al. 2012b). These authors documented that the AM colonized plants had enhanced uptake of most of the amino acids such as arginine, methionine, histidine, phenylalanine, and tryptophan than non-AM plants. They also found that AM fungi stimulated the uptake of positively and neutrally charged amino acids more than the amino acids with a negative charge. This clearly shows that AM fungal colonization increases the uptake of organic N through their mycelium and transfers it to their host plants (Whiteside et al. 2012b).

The examination of distinct soil patches in the root free zone by AM fungal species (*R. irregularis* and *Claroideoglomus claroideum*) colonizing the roots of a legume plant, *Medicago truncatula* was examined by Bukovská et al. (2016). The amplicon sequencing was used to determine fungal and prokaryotic communities and real-time PCR through AM taxon-specific markers to access hyphal colonization. The results of the experiments revealed that the hyphal density of both the AM fungi was enhanced in the presence of organic materials containing N compared to those possessing inorganic N. In addition, the abundance of numerous prokaryotes such as ammonia oxidizers and *Acanthamoeba* were strongly and positively correlated with the hyphal response of *R. irregularis* to the soil amendments. This study clearly documents the proliferation of AM fungi in organic N sources, in spite of host plant bearing N_2 fixing root nodules. Therefore, the proliferation of AM fungal hyphae in the soil tends to be caused basically by the demand for N in the fungus than the host plant (Bukovská et al. 2016).

21.3.2 Inorganic N Uptake

Inorganic N sources like NH_4^+ and NO_3^- are comparatively available in the soil as mobile forms, consequently decreasing the efficient benefit to the mycorrhizal plants for N uptake (Smith and Read 2008). Moreover, AM plants often possess higher nitrification rates in the ecosystem (Veresoglou et al. 2011). However, the limitation of N can also take place in these ecosystems, and in such a situation, AM fungi could contribute to NO_3^- uptake in a competitive environment and transfer them to the host plants. Nitrate plays a central role as a N source for crop plants and is more mobile when compared to ammonium (Gregory 2006; Marschner 2011). Both NH_4^+ and NO_3^- are captured by the ERM of AM fungi (Hawkins et al. 2000). Nevertheless, owing to its lower energy cost, NH_4^+ is commonly preferred than NO_3^- (Hodge et al. 2010). The uptake of NH_4^+ through AM symbiosis by plants is five times greater than NH_4^+ uptake by the plants themselves (Tian et al. 2010). In addition, the environmental conditions also affect the form of inorganic N taken up by the plants. For instance, it is familiar that uptake of NO_3^- is more sensitive than NH_4^+ at low temperature. On contrary, AM fungal hyphae may have enhanced access to N in the less mobile form NH_4^+ and uptake this form of N through its extensive ERM network before its conversion into NO_3^- (Hodge and Storer 2015).

According to Fellbaum et al. (2012b), the inorganic N forms NH_4^+ and NO_3^- taken up by the ERM are converted into arginine and then to polyphosphates. The polyphosphates are then transported into the IRM which are again converted to arginine (Cruz et al. 2007). The arginine in the IRM is then converted to urea and then to NH_4^+ with an intermediate product Glutamate (Tian et al. 2010). Thus, NH_4^+ in the IRM is translocated into the plant root cortex. In root organ cultures, carrot roots colonized with AM fungi had significantly higher glutamine synthetase and glutamate dehydrogenase activities than non-mycorrhizal plantlets (Toussaint et al. 2004). The plant biomass, densities of AM fungal hyphae, and transport of N to the plants through the fungal hyphae were lower when NH_4^+ was the only N source for mycorrhizal plants when compared to those supplied with NO_3^- along with NH_4^+ (Hawkins and George 2001). Also, a reduction in vesicle and arbuscule abundance has been reported in mycorrhizal plants supplied with NH_4^+ than NO_3^- (Valentine et al. 2002).

In a study, Tomè et al. (2015) conducted a dual labelling experiment to examine the allocation of C to AM fungal hyphae and also the transport of N assimilated by the fungal hyphae to strawberry plants. In this experiment, a mixture of AM fungal species comprising of *F. mosseae* and *R. irregularis* was inoculated on to the strawberry root stolons and ^{15}N as an inorganic N source was added to the hyphal compartment simultaneously with $^{13}CO_2$ pulse labelling. This study showed that 10% of fixed C was allocated to the plant roots and 4.3% to the mycorrhizal hyphae. Besides, 23% of inorganic N absorbed by the mycorrhizal fungi was reserved in the fungal mycelium. A strong positive correlation was observed between plant fixed C and N absorption capacity of fungal hyphae (Tomè et al. 2015).

21.4 Translocation of N via AM Fungi

The ERM of AM fungi reaches the soil, uptakes N, and transfers it into the IRM of the fungus and then to plant root cortical cells (Vangelisti et al. 2018; Xu et al. 2019). The extraradical fungal hyphae can also directly assimilate NH_4^+, NO_3^-, and amino acids from the external medium (Basu et al. 2018). In the N cycle, mycorrhizal fungi transfer the soil N into the plant cells via the arbuscules (Vergara et al. 2019). Thus, in addition to N translocation (Klironomos et al. 2005; Leifheit et al. 2014) AM fungi also enhance N use efficiency (NUE) and equalize extra N fertilization (Rosolem et al. 2017). The establishment of AM fungi–plant symbiosis takes place by the release of fungal 'Myc factors' and plant root exudates (Basu et al. 2018).

Productive crop genotypes have the ability to expand their symbiotic association with AM fungi to the non-symbiotic N_2 fixing microorganisms under the low N situation, which is the most effective measure for enhancing N translocation and NUE (Han et al. 2015; Cormier et al. 2016). The inorganic N taken up by AM fungal ERM from the soil is incorporated into the ERM of *R. irregularis* through the GS-GOGAT pathway as revealed through mRNA determination and quantitative real-time PCR techniques (Govindarajulu et al. 2005). The bidirectional transport and breakdown of arginine by the AM fungus *R. irregularis* mycelium associated with transformed carrot roots was reported by Jin (2009). The N released from arginine may be transmitted to the partner plant or used to biosynthesize more amino acids and nucleotides essential for fungal growth. The arginine is degraded, and transported as NH_3/NH_4^+ before it is released into arbuscules of AM fungi for their subsequent transfer to the plant host through NH_4^+ transporter. Kobae et al. (2010) showed that the liberated NH_4^+ was transferred transversely in the periarbuscular membrane to the root cortical cells and throughout this process; the NH_4^+ transporter gene (GmAMT) was predominantly transcribed and particularly expressed in the arbusculated cortical cells. Furthermore, Govindarajulu et al. (2005) showed that *R. irregularis* increases NH_4^+ accessibility in the fungal region and produced NH_4^+ during urea degradation in the IRM. These conclusions specified that NH_4^+ is transferred from IRM to the plant cell. The urea in the IRM is converted to NH_4^+ with the help of the urease enzyme. Finally, amino acids are produced from NH_4^+ and ornithine either within the IRM or within the plant host cells. This is demonstrated by the experiment which recorded that the genes of N incorporated compounds are expressed in the extraradical tissues, and genes linked with arginine breakdown are mostly expressed in the IRM (Govindarajulu et al. 2005).

Numerous studies were conducted on N uptake, translocation, and transfer via AM fungi through ^{15}N isotopic, estimation of enzymes, and investigation of expressions of related genes (Tian et al. 2010). The expression of a putative nicotinamide adenine dinucleotide (NAD)-dependent glutamate dehydrogenase (GDH) gene localized in the ERM tissue was down-regulated when provided with either NO_3^- or NH_4^+ confirming the catabolic role of GDH (Vallorani et al. 2002). This suggests that during C scarcity, NAD-dependent GDH catabolize glutamate to generate C skeletons to restore the tricarboxylic acid cycle (Jin et al. 2012). Jin (2009) reported

that isotopically marked substrates under in vitro AM fungus cultures of *R. irregularis* grown in medium enclosed $^{15}NH_4Cl$ produced the maximum free amino acids in the ERM in which arginine was predominant with more than 90% of the total ^{15}N in the free amino acids.

21.5 Role of AM Fungi in Symbiotic N_2 Fixation

Symbiotic N_2 fixation is a mutualistic association where plants offer photosynthetic C and a niche to the bacteria in exchange for fixed N (Sulieman and Tran 2004). Symbiotic N_2 fixation is mostly restricted to legumes in agricultural systems and around 200 species of woody perennials from 20 unrelated genera of non-leguminous angiosperms. In the nodular symbiosis, legumes and actinorhizal plants associate with bacteria that stimulate the nodule formation in the roots. Rhizobia belonging to different groups of α- and β-proteobacteria fix atmospheric N_2 and supply in the form of ammonia to the plants which can be absorbed easily by the leguminous plants (Chen and Hicks 2003; Masson-Boivin and Sachs 2018). However, the strains of *Frankia* that associate with the non-leguminous plants in actinorhizal symbiosis are specific to a plant species. The symbiotic bacteria in the nodules promote the provision for nutrients in legumes and actinorhizal plants and significantly contribute to their N acquisition (Larimer et al. 2014). The legumes (except in the genus *Lupinus*) and actinorhizal plants establish simultaneous root symbiosis with both *Rhizobium/Frankia* and AM fungi. The synergetic effect among the root symbionts (AM fungi and *Rhizobium*/*Frankia*) could enhance the nutrient acquisition and growth in nodulating N_2 fixing plants (Hack et al. 2019).

As mentioned earlier, in AM colonized plant roots, the fungal hyphae move into the surrounding rhizosphere soil by forming an extensive hyphal network reaching soil up to greater volume to obtain the mobile mineral nutrients (Smith and Read 2008; Molla and Solaiman 2009). The influence of mixed AM fungal inoculation (*F. mosseae*, *R. irregularis, S. hoi*) on plant growth, biological N_2 fixation (BNF) of a forage legume, *Melilotus alba* treated with *Rhizobium meliloti* suspension was analysed (Hack et al. 2019). The findings of the study revealed increased mycorrhizal colonization, plant biomass, nutrient uptake (N and P), and BNF under AM fungal inoculation. Further, the results of the study suggested that AM fungi did not make any significant impact on plant nutrition and biomass under low soil P availability, regardless of the degree of AM fungal colonization. However, AM symbiosis positively influenced the nutrient accumulation, N uptake, and plant biomass under high P availability in the soil, which is related to the improved BNF in *M. alba*.

Seedlings of *Discaria trinervis* spot inoculated with *Frankia* strain BCU110501 and co-inoculated with the AM fungus *Gigaspora rosea* (BEG 9) had improved growth and nodulation compared to those inoculated with *Frankia* alone (Obertello and Wall 2015). Likewise inoculation of *Casuarina equisetifolia* subsp. *equisetifolia* seedlings with AM fungal inoculum originating from Australian and Senegalese

soils increased the growth and N content of the seedlings (Diagne et al. 2018). These studies clearly shows that AM fungi improve nodulation and N_2 fixation in actinorhizal plants apart from improving plant growth.

Phosphorus is regarded pivotal for nodulation and N_2 fixation. This is evident from studies where P supply was directly related to nodulation and N_2 fixation (Pérez-Fernández et al. 2017; Míguez-Montero et al. 2020). Subsequently, the requirement of P for N_2 fixing plants is always higher than those of other plants. Biological N_2 fixation is an expensive process as Chen et al. (2014) showed that nodules in soybean accounted for around 11.5–28.0% of the photosynthetic C allocated to the belowground organs. Therefore plants tend to avoid this process in soils that are sufficient in N or P. For instance, shrubby legumes like *Cytisus multiflorus*, *Cytisus balansae*, *Cytisus scoparius*, and *Cytisus striatus* acquired their N from inorganic sources when the soils were high in P (Míguez-Montero et al. 2020). Contrarily, in soils where P is deficient and limiting, the plant growth in these legumes were forced to rely on BNF and accumulated greater N in the whole plant (Míguez-Montero et al. 2020).

Most of the studies have shown that AM fungi could enhance the symbiotic N_2 fixation in plants under stressful conditions. For instance, Bulgarelli et al. (2017) reported improved N_2 fixation and higher growth performance in nodulated (*Bradyrhizobium elkanii*) soybean plants and inoculated with AM fungus (*Glomus macrocarpum*) under P starvation. The AM symbiosis promoted the symbiotic N_2 fixation by increasing the nitrogenase activity, P concentration in nodules, and N content in the leaf and decreased the metabolic constraint of photosynthesis under P deficiency. Therefore, AM fungi have a key role in stimulating N_2 fixation in nodulated plants like soybean. Likewise, Püschel et al. (2017) also evidenced enhancement of BNF by AM fungi in a pot experiment including two species of *Medicago* (*Medicago truncatula* and *Medicago sativa*) treated with rhizobia and inoculated with or without AM fungus *R. irregularis* 'PH5' under three different levels of P. The findings suggested that inoculation of AM fungi under low P level increased the P uptake in both the plants and also enhanced the BNF efficiency and also observed a positive correlation between N derived from BNF and total P content of the plants.

21.6 Asymbiotic Soil Microorganisms in N Nutrition

The application of microbial inoculants in agriculture is one of the promising strategies to enhance environmental sustainability. Besides AM fungi, other microbes like diazotrophs the non-symbiotic N_2 fixers inhabiting the rhizosphere have also been reported to help in the solubilization of low mobility mineral nutrients and N_2 fixation in plants (Hsu and Buckley 2009; Mus et al. 2016). The diazotrophs could access N through N_2 fixation or by taking up N available from external sources that include organic N sources of both low and high molecular weight (Norman and Friesen 2017). The *nifH* gene that encodes the protein subunit of nitrogenase is

largely conserved among free-living asymbiotic N_2 fixers and provides substantial support for efficient N_2 fixation in the diazotrophs (Coelho et al. 2009). *Azotobacter chroococcum*, a free-living bacteria have a key role in the uptake of N by binding atmospheric N_2 and liberating N as NH_4^+ ions, thus making N available to the plants (Mrkovacki and Milic 2001). They may also act as mycorrhiza helper bacteria by improving the environmental conditions for the establishment and occurrence of AM fungi by hindering antagonists or through the production of growth factors (Frey-Klett et al. 2007). Moreover, a concurrent association of AM fungi and non-symbiotic N_2 fixers could have a synergistic effect on plant nutrient uptake (Sabannavar and Lakshman 2011).

Most of the studies have demonstrated that combined inoculation of AM fungi and asymbiotic N_2 fixers stimulates N_2 fixation and improves the plant yield. Nevertheless, the efficiency of combined inoculation of non-symbiotic N_2 fixers and AM fungi depends mostly on the compatibility between the interacting microbial partners in the rhizosphere and may differ with soil types and host plants. Rabie and Almadini (2005) examined the effect of single or dual inoculation of *Vicia faba* with asymbiotic N_2 fixing bacteria (*Azospirillum brasilense*) and AM fungi *Rhizophagus clarus* (=*Glomus clarum*) in pot culture under different NaCl levels and observed a significant increase in nodule number, N content, nitrogenase enzymes and also tolerance to salinity in AM inoculated plants when compared to non-AM plants either with or without *A. brasilense* inoculation. In another study, Mohammadi et al. (2019) compared the plant growth of *Oenothera biennis* inoculated with AM fungus (*F. mosseae*), asymbiotic N_2 fixing bacteria (*Azospirillum lipoferum*), and chemical fertilizers under water deficit conditions. The findings of the study suggested that AM fungal inoculation improved the response of physiological stresses by enhancing antioxidant pigments, total plant biomass of *O. biennis* under water stress when compared to chemical fertilizers and N_2 fixing bacteria.

For instance, in a recent field experiment, Dal Cortivo et al. (2020) compared the inoculation of bacterial consortia (*Azospirillum* sp. + *Azoarcus* sp. + *Azorhizobium* sp.) and AM fungal–bacterial consortia (*R. irregularis* + *Azotobacter vinelandii*, *R. irregularis* + *Bacillus megaterium* + *Frateuria aurantia*) on wheat seeds and reported enhanced plant growth, crop yield as well as the N accumulation in wheat plants on inoculation with microbial consortia than uninoculated plants. The enhancement in plant growth was ascribed to nutrient solubilizing and N_2 fixing capability of the applied microorganisms and due to the production of plant growth-promoting compounds (Dal Cortivo et al. 2020). Therefore, dual inoculation of AM fungi with asymbiotic N_2 fixer (*R. irregularis* + *A. vinelandii*) also contributes to the enhancement of the N_2 fixation in wheat plants.

21.7 Nitrogen Uptake under Intercropping System

Introduction of N_2 fixing legume plant is one of the strategies to enhance the N availability in cropping systems (Chapagain and Riseman 2014; Nygren and Leblanc 2015). The N transfer to cereals from legumes involves both direct and indirect

pathways in the intercropping system (He et al. 2009). In the indirect pathway, the N released from decomposed tissues and also the root exudates released into the rhizosphere by legumes is taken up by the roots of cereal plants through soil solution, whereas, in the direct method, N is imparted between coexisting legumes and cereals through common mycelium network of the AM fungi (Bahadur et al. 2019). However, the N demand by the receiver (cereal) plant may differ widely. Moreover, decomposition of roots, root exudates that are rich in soluble nitrogenous elements, and root nodules in legume plants could also increase the N content in the rhizosphere soil in an intercropping system (Thilakarathna et al. 2016). In fababean/maize intercropped system, the root exudates released by fababean increased the acidification of the rhizosphere and P acquisition in maize plants (Li et al. 2007). Further, the root exudates released by maize improved N_2 fixation as well as the synthesis of flavonoids in fababean (Li et al. 2016).

In the intercropping system, the AM fungal mycelial network linking the root system of nearby plants results in the exchange of carbohydrates and essential mineral nutrients between the plants (Meng et al. 2015; Ingraffia et al. 2019). For example, Li et al. (2009) showed that inoculation of AM fungal species, *Funneliformis caledonium* (=*Glomus caledonium*) in rice/mung bean increased the N uptake by 64% and nodulation by 54% in mung bean under the intercropping system. Also, 5.4% to 15.7% of total N was transferred from the leaves of mung bean to the leaves of rice plants in response to AM fungal inoculation (Li et al. 2009). In another study, Meng et al. (2015) showed that inoculation of both AM fungi and *Rhizobium* in soybean/maize intercropping system enhanced the efficiency of N_2 fixation in soybean and also stimulated N transfer from soybean to maize, in turn leading to improvement of growth and yield of both the crops under intercropping system. Similarly, the application of AM fungi (*R. irregularis*) and *Rhizobium* (*Bradyrhizobium elkanii* strain BXYD3) in soybean–maize intercropping system on N transfer and C allocation revealed that higher growth performance of maize than soybean was attributed to increased N uptake by maize through common mycelium network and contributed less C into common mycelium networks when compared to soybean under co-inoculation conditions (Wang et al. 2016).

The AM fungal community gets altered in the rhizosphere due to interspecific competition, thus facilitating the availability of nutrients for both competitive plant species (Li et al. 2016). In the legume–cereal intercropping system, the BNF facilitated by AM fungi increases the N availability than in unfertilized crops. Therefore, N uptake in cereal leads to the stimulation of N_2 fixation in legumes (Rodriguez et al. 2020). In a study, competitive interaction between the mycorrhizal individuals (with and without AM fungi) of legume (*Vicia faba*) and grass (*Hordeum vulgare)* under the intercropping system under N fertilization was evaluated by Bahadur et al. (2019). The study reported that AM fungi favoured the growth and uptake of N in *V. faba* than *H. vulgare*. Also, AM fungi modulated the degree of colonization in both the plants thus strongly affecting the interspecific competitive interactions. Thus, AM symbiosis has a significant role in nutrient acquisition in mixed legume/cereal cropping systems (Saharan et al. 2018; Ingraffia et al. 2019).

The N transfer between heterospecific and conspecific seedlings of *Broussonetia papyrifera*, *Cinnamomum camphora,* and *Bidens pilosa* raised concurrently in the microcosm in the presence and absence of AM fungal species (*C. etunicatum*) was examined using ^{15}N, a stable isotope to track the N transport between common mycelium network in greenhouse conditions (He et al. 2019). The findings of the study showed significantly higher N accumulations, plant biomass, ^{15}N content, and N transfer among receiver plant species in the order *B. papyrifera*≈*B. pilosa* > *C. camphora* both in the presence and absence of AM fungi. Hence, the study suggests that a common mycelium network could effectively mediate the N transfer from a donor species to heterospecific receiver plant species, wherein the ratio of N acquired from transfer relies upon the biomass production of the individual species (He et al. 2019). In a recent study, Lu et al. (2020) showed that mixed plantation of *Santalum album/Acacia confusa/Dalbergia odorifera* possesses a greater amount of interplant nutrient transfer and also suggested that herbaceous plants may acquire a certain amount of C and N through both direct and indirect transfers from these plants, suggesting translocation of nutrient among plant species within the community. This kind of study provides a new insight into the mixed plantation system for nutrient resource acquisition (Lu et al. 2020). Therefore, the role of mycorrhizal symbiosis in the nutrient transfer between plants in the intercropping system could help in sustainable crop management.

21.8 Stimulation of Soil Microbial Activity by AM Fungi

Several studies have shown that soil microbial activity relies upon the plant species and the occurrence of AM fungi (López-Gutiérrez et al. 2004; Marschner and Timonen 2006). The alterations caused by AM fungi influence the soil microbial population both qualitatively and quantitatively in the rhizosphere region (Zarea et al. 2011). The AM symbiosis alters the root exudate composition and the supply of C to the microbial communities by the hyphae of the AM fungi leads to physical alterations in the root zones (the hyposphere effect) (Barea et al. 2005). In a study, Zarea et al. (2011) showed that combined effects of mixed cropping system (Berseem clover/Persian clover) and AM fungal inoculation (*F. mosseae*) increased the plant growth, nitrogenase activity of root nodules, N content in shoots, and soil microbial activity. The increase in soil microbial activity was due to the higher production of organic matter by root and decomposition of external AM fungal mycelium (Zarea et al. 2011).

In addition, AM fungi can increase bacterial activity associated with the availability of N in the soil, by affecting the plant N cycle or by utilizing raw organic matter as a substrate (Saia et al. 2014). The AM fungi could stimulate the decomposition of complex organic material and thus consequently affecting the decomposition of litter by altering the activity of bacterial in the hyphosphere region (Hodge et al. 2001). Furthermore, Herman et al. (2012) also revealed that the inoculation of AM fungi alters the C flow resulting from the alteration of microbial communities in

soil. The AM fungus, *F. mosseae* significantly influenced the several bacteria of various genera that were involved in the litter degradation in forest soils (Gui et al. 2017).

The litter decomposition has a key role in mineral nutrient cycling including N that is driven by the soil microbial communities (Cheng et al. 2012). Taylor et al. (2016) examined the effect of the mycorrhizal association on organic matter decomposition in both natural and laboratory conditions using litters and soils collected from four different AM and ectomycorrhizal tree species and found that AM tree litters had lower C:N microbial biomass and decompose rapidly than the litter of ectomycorrhizal tree species. Furthermore, under natural conditions, the soils of AM tree consist of lesser total C and microbial biomass C:N when compared to ectomycorrhizal tree soils. This shows that mycorrhizal symbiosis differentially and indirectly affects the soil decomposer activity and the quality of organic matter.

21.9 Modulation of Plant N Use Efficiency

The N use efficiency is defined as the yield in biomass or grain obtained per unit of available N provided by the soil and the added N fertilizer. NUE depends upon the ability of the plants to take N from the soil as well as its efficient utilization in the production of biomass and grains. However, in spite of a 10-fold increase in N fertilizer application to cultivated soils worldwide, the overall increase in grain yield is less than 3-fold (Tilman et al. 2002). Therefore an increase in NUE of plants could reduce the application of N fertilizers and subsequently decrease the detrimental effect on the ecosystems in addition to the economical benefits. One of the mechanisms adopted by productive crop genotypes to soil low in available N is to associate with AM fungi and non-symbiotic N_2-fixing microorganisms. Studies have shown that plants associated with AM fungi could modulate their NUE under low N levels and other stress conditions (Verzeaux et al. 2017).

Nitrogen has an important role in the C metabolism that is mediated through AM fungi as C flux is accompanied by assimilation and transfer of N (Liu et al. 2019). For instance, inoculation of *R. irregularis* in wheat plants increased the C and N accumulation in the roots and NUE under elevated CO_2 levels (Zhu et al. 2016). The increase in NUE was attributed to AM symbiosis that favoured the accumulation of C and N uptake in wheat plants. Likewise, soybean plants inoculated with *R. irregularis* under water deficit condition had improved concentrations of N in their roots and a higher NUE when compared to uninoculated seedlings (Liu et al. 2019). The impact of dual inoculation of AM fungi (*F. mosseae*), *Rhizobium* (*Rhizobium leguminosarum*) and inorganic fertilizer (P and N) application on plant productivity and nutrient use efficiencies under field conditions in *Pisum sativum* was investigated (Bai et al. 2016). The results of the study suggested that dual inoculation of AM fungi and *Rhizobium* increased the nutrient use efficiency, but at a higher level of P and N, the dual inoculations decreased both the NUE and P use efficiency of *P. sativum* plants. In a recent study, inoculation of *R. irregularis*

increased the NUE of the grafted eggplants by 13.3% than those uninoculated with the AM fungus (Sabatino et al. 2020). Application of AM fungi along with other microbial consortia like the plant growth-promoting rhizobacteria improved plant growth and the NUE of wheat plants and reduced the N fertilizer application by 16.7% (Varinderpal-Singh et al. 2020). The presented evidence indicates that AM symbiosis could improve the NUE in plants and thereby minimizing the use of synthetic N fertilizers. This could have a substantial role in sustainable crop production.

21.10 AM Fungi in the N Cycle

The contribution of AM fungi in the N cycle is mainly attributed to the efficient nutrient transportation in soils. Veresoglou et al. (2012) described some common pathways through which AM fungi influence the N cycle includes: i) availability of substrate; ii) alteration in soil abiotic factors; iii) shift in microbial and plant communities; and iv) effect of individual host plants. One of the crucial steps in the N cycle is N_2 fixation that occurs in the plant root zones (Hamel 2004). As already discussed in the above sections, AM fungi affect both asymbiotic and symbiotic N_2 fixation. Apart from N_2 fixation, other transformation rates involved in N cycling could be mediated through substrate availability. The available substrate for free-living microbes occurring in N cycling may be reduced by AM fungi either through immobilization of inorganic N to their mycelium or transport nutrients to their host plant (Veresoglou et al. 2012). Also, the exudates of AM fungal hyphae could enhance soil across the root zone directly influencing the C limitation in some heterotrophic microorganisms in the N cycle (Toljander et al. 2007).

Abiotic factors like soil moisture, pH, temperature, and aeration affect the availability of N compounds (Avrahami and Bohannan 2007). Nitrification and denitrification are the two important processes involved in N cycling. Both these processes are often limited by the availability of their precursors (NH_4^+ and NO_3^-), and both have the potential to produce nitrous oxide (N_2O), a potent greenhouse gas (Hino et al. 2010). However, AM fungi have been reported to affect the soil aggregation and pH, thus altering the nitrogenous compounds in the soil. Cheng et al. (2012) demonstrated the role of AM fungi in obtaining N from organic matter under elevated CO_2 conditions through a sophisticated microcosm experiment. The authors showed that under elevated CO_2, the decomposition rate of fresh aboveground dead organic material was faster in the presence of AM fungi thereby increasing the N content of the host plant. Nevertheless, Verbruggen et al. (2013) pointed out that microcosm experiment like these that are conducted for the short term; therefore, such results should not be directly correlated with long-term studies. It is well known that AM fungi possess the molecular mechanisms allowing the acquisition of both inorganic and organic forms of N from the soil solution (Garcia et al. 2016).

Some of the studies have documented that AM fungal hyphae are extremely efficient in the uptake of N released by bacterial grazers, like the specific soil protists (Herdler et al. 2008; Koller et al. 2013). Around one-third of the consumed N by the protists is released to the soil solution in the form of free NH_4^+ ions (Trap et al. 2016). Other microbial groups like AM fungi or ammonia oxidizers take up these NH_4^+ ions released by the protists from the soil solution (Nannipieri and Eldor 2009; Rosenberg et al. 2009). Therefore, AM fungi have an essential role in the recycling of soil N by modifying the composition of the soil microbial community in the rhizosphere and thereby altering the denitrifying, nitrifying, and diazotrophic symbiotic or non-symbiotic bacteria (Veresoglou et al. 2011).

21.11 Reduction of Nitrous Oxide (N_2O) Emission

Nitrous oxide (N_2O) is one of the intermediate products of denitrification or nitrification process in the N cycle. It is an important greenhouse gas and is mostly emitted from agricultural soils and pastures where N fertilizers or manures rich in N are used (An et al. 2020). Agricultural soils are the major source of N_2O emission as only less than half of the inorganic N applied as fertilizers to the agricultural soils are utilized by plants and the residual N becomes the substrate for N transformation in the soil (Tonhauzer et al. 2020). In aerobic soils, nitrification, mediated by microorganisms is the prime cause of N_2O emission and the leftover N from fertilizers further accelerates this process in agricultural ecosystems. Studies have shown that AM symbiosis could reduce the emission of N_2O from agricultural soils. It has been hypothesized that the outcompetition of the weak competing nitrifiers by the fungal hyphae for NH_4^+ acquisition was the reason for the reduced N_2O emission in AM fungal soils (Storer et al. 2018). However, the results on the competition between microorganisms involved in N cycling and AM fungi are often inconsistent and unclear (Cavagnaro et al. 2012; Teutscherova et al. 2019). For instance, Teutscherova et al. (2019) showed that the mycorrhization of tropical grass *Brachiaria decumbens* reduced the N_2O emission after urea application by 46% despite an increased abundance of ammonia oxidizers. This reduced N_2O emission despite the increased populations of ammonia oxidizers bacteria was attributed to the AM fungi induced changes in the bacterial activity (Teutscherova et al. 2019). Contrarily in a recent mesocosm experiment involving a mixture of European grassland plant species, inoculation of *R. irregularis* to an agricultural soil containing indigenous AM fungal community failed to reduce the potential N_2O emission (Okiobe et al. 2020). Based on these results Okiobe et al. (2020) concluded that plant diversity has a strong influence on N_2O emission than AM fungal diversity or abundance. In a compartmentalized mesocosm study Okiobe et al. (2019) showed that AM fungi reduced N_2O emission through enhanced soil aggregation. Therefore the influence of AM fungi on N_2O emission from soils could be due to the direct or indirect effects of AM fungi on the various processes of the N cycle.

21.12 Conclusion

The AM symbiosis contributes to plant N uptake in addition to P acquisition. The recent advancements in direct and indirect uptake of N by AM fungi have been documented. The root colonization by AM fungi provides P supply to plants that in turn increase the N uptake and stimulates N_2 fixation in plants. The AM fungi also interact with other soil microbes synergistically leading to improved N uptake, root nodulation, and plant growth. The N transfer in plants is investigated through the isotope labelling method in AM symbiosis. Recent studies have carried out transcriptome and genomic techniques for determining the actual and precise method to study the plant N regulations mediated by AM fungi. Nevertheless, only some of the AM fungal species have been used to investigate N transfer and its regulations. Hence, further studies on the effect of other indigenous AM fungal species in different host plants could improve the understanding of plant N nutrition. The application of AM fungi bioinoculum could decrease the reliance on chemical fertilizers. Therefore, the AM symbiosis is of great importance for the sustainable agricultural system.

References

Almonacid L, Fuentes A, Ortiz J, Salas C, Garcia-Romera I, Ocampo J, Arriagada C (2015) Effect of mixing soil saprophytic fungi with organic residues on the response of *Solanum lycopersicum* to arbuscular mycorrhizal fungi. Soil Use Manag 31:155–164

An H, Owens J, Stoeckli J, Hao X, Beres B, Li Y (2020) Nitrous oxide emissions following split fertilizer application on winter wheat grown on Mollisols of southern Alberta, Canada. Geoderma Reg 21:e00272. https://doi.org/10.1016/j.geodrs.2020.e00272

Arriagada C, Almonacid L, Cornejo P, Garcia-Romera I, Ocampo J (2014) Influence of an organic amendment comprising saprophytic and mycorrhizal fungi on soil quality and growth of *Eucalyptus globulus* in the presence of sewage sludge contaminated with aluminium. Arch Agron Soil Sci 60:1229–1248

Atul-Nayyar A, Hamel C, Hanson K, Germida J (2009) The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. Mycorrhiza 19:239–246

Avrahami S, Bohannan BJ (2007) Response of *Nitrosospira* sp. strain AF-like ammonia oxidizers to changes in temperature, soil moisture content, and fertilizer concentration. App Environ Microbiol 73:1166–1173

Bahadur A, Jin Z, Long X, Jiang S, Zhang Q, Pan J, Liu Y, Feng H (2019) Arbuscular mycorrhizal fungi alter plant interspecific interaction under nitrogen fertilization. Eur J Soil Biol 93:103094. https://doi.org/10.1016/j.ejsobi.2019.103094

Bai B, Suri VK, Kumar A, Choudhary AK (2016) Influence of dual inoculation of AM fungi and *Rhizobium* on growth indices, production economics, and nutrient use efficiencies in garden pea (*Pisum sativum* L.). Commun Soil Sci Plant Anal 47:941–954

Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Barrow CJ (2012) Biochar potential for countering land degradation and for improving agriculture. Appl Geogr 34:21–28

Basu S, Rabara RC, Negi S (2018) AMF: the future prospect for sustainable agriculture. Physiol Mol Plant Pathol 102:36–45

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ahmed N, Ashraf M, Zhang L (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci 10:1068. https://doi.org/10.3389/fpls.2019.01068

Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW (2012) Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169:895–904

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:1–11

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE (2016) Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. Sci Total Environ 566:1223–1234

Bowles TM, Jackson LE, Cavagnaro TR (2018) Mycorrhizal fungi enhance plant nutrient acquisition and modulate nitrogen loss with variable water regimes. Glob Chang Biol 24:e171–e182. https://doi.org/10.1111/gcb.13884

Breuninger M, Trujillo CG, Serrano E, Fischer R, Requena N (2004) Different nitrogen sources modulate activity but not expression of glutamine synthetase in arbuscular mycorrhizal fungi. Fungal Genet Biol 41:542–552

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77

Bücking H, Kafle A (2015) Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. Agronomy 5:587–612

Bücking H, Mensah JA, Fellbaum CR (2016) Common mycorrhizal networks and their effect on the bargaining power of the fungal partner in the arbuscular mycorrhizal symbiosis. Commun Integr Biol 9:e1107684. https://doi.org/10.1080/19420889.2015.1107684

Bukovská P, Gryndler M, Gryndlerová H, Püschel D, Jansa J (2016) Organic nitrogen-driven stimulation of arbuscular mycorrhizal fungal hyphae correlates with abundance of ammonia oxidizers. Front Microbiol 7:711. https://doi.org/10.3389/fmicb.2016.00711

Bukovská P, Bonkowski M, Konvalinková T, Beskid O, Hujslová M, Püschel D, Řezáčová V, Gutiérrez-Núñez MS, Gryndler M, Jansa J (2018) Utilization of organic nitrogen by arbuscular mycorrhizal fungi—is there a specific role for protists and ammonia oxidizers? Mycorrhiza 28:269–283

Bulgarelli RG, Marcos FC, Ribeiro RV, de Andrade SA (2017) Mycorrhizae enhance nitrogen fixation and photosynthesis in phosphorus-starved soybean (*Glycine max* L. Merrill). Environ Exp Bot 140:26–33

Cappellazzo G, Lanfranco L, Bonfante P (2007) A limiting source of organic nitrogen induces specific transcriptional responses in the extraradical structures of the endomycorrhizal fungus *Glomus intraradices*. Curr Genet 51:59. https://doi.org/10.1007/s00294-006-0101-2

Cavagnaro TR, Barrios-Masias FH, Jackson LE (2012) Arbuscular mycorrhizas and their role in plant growth, nitrogen interception and soil gas efflux in an organic production system. Plant Soil 353:181–194

Cely MV, De Oliveira AG, De Freitas VF, de Luca MB, Barazetti AR, Dos Santos IM, Gionco B, Garcia GV, Prete CE, Andrade G (2016) Inoculant of arbuscular mycorrhizal fungi (*Rhizophagus clarus*) increase yield of soybean and cotton under field conditions. Front Microbiol 7:720. https://doi.org/10.3389/fmicb.2016.00720

Chapagain T, Riseman A (2014) Barley–pea intercropping: effects on land productivity, carbon and nitrogen transformations. Field Crops Res 166:18–25

Chen H, Hicks W (2003) High asymbiotic N_2 fixation rates in woody roots after six years of decomposition: controls and implications. Basic Appl Ecol 4:479–486

Chen Y, Yu Z, Wang J, Zhang X (2014) Allocation of photosynthetic carbon to nodules of soybean in three geographically different Mollisols. Euro J Soil Biol 62:60–65

Chen S, Zhao H, Zou C, Li Y, Chen Y, Wang Z, Jiang Y, Liu A, Zhao P, Wang M, Ahammed GJ (2017) Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. Front Microbiol 8:2516. https://doi.org/10.3389/fmicb.2017.02516

Chen M, Arato M, Borghi L, Nouri E, Reinhardt D (2018) Beneficial services of arbuscular mycorrhizal fungi–from ecology to application. Front Plant Sci 9:1270. https://doi.org/10.3389/fpls.2018.01270

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337:1084–1087

Christou M, Avramides EJ, Jones DL (2006) Dissolved organic nitrogen dynamics in a Mediterranean vineyard soil. Soil Biol Biochem 38:2265–2277

Coelho MR, Marriel IE, Jenkins SN, Lanyon CV, Seldin L, O'Donnell AG (2009) Molecular detection and quantification of nifH gene sequences in the rhizosphere of sorghum (*Sorghum bicolor*) sown with two levels of nitrogen fertilizer. Appl Soil Ecol 42:48–53

Cormier F, Foulkes J, Hirel B, Gouache D, Moënne-Loccoz Y, Le Gouis J (2016) Breeding for increased nitrogen-use efficiency: a review for wheat (*T. aestivum* L.). Plant Breed 135:255–278

Cruz C, Egsgaard H, Trujillo C, Ambus P, Requena N, Martins-Loução MA, Jakobsen I (2007) Enzymatic evidence for the key role of arginine in nitrogen translocation by arbuscular mycorrhizal fungi. Plant Physiol 144:782–792

Dal Cortivo C, Ferrari M, Visioli G, Lauro M, Fornasier F, Barion G, Panozzo A, Vamerali T (2020) Effects of seed-applied biofertilizers on rhizosphere biodiversity and growth of common wheat (*Triticum aestivum* L.) in the field. Front Plant Sci 11:72. https://doi.org/10.3389/fpls.2020.00072

de Novais CB, Sbrana C, da Conceição Jesus E, Rouws LF, Giovannetti M, Avio L, Siqueira JO, Júnior OJ, da Silva EM, de Faria SM (2020) Mycorrhizal networks facilitate the colonization of legume roots by a symbiotic nitrogen-fixing bacterium. Mycorrhiza 25:1–8

Diagne N, Baudoin E, Svistoonoff S, Ouattara C, Diouf D, Kane A, Ndiaye C, Noba K, Bogusz D Franche C, Duponnois R (2018) Effect of native and allochthonous arbuscular mycorrhizal fungi on *Casuarina equisetifolia* growth and its root bacterial community. Arid Land Res Manag 32:212–228

Dion PP, Jämtgård S, Bertrand A, Pepin S, Dorais M (2018) Organic nitrogen uptake and assimilation in *Cucumis sativus* using position-specific labeling and compound-specific isotope analysis. Front Plant Sci 9:1596. https://doi.org/10.3389/fpls.2018.01596

Dutta SC, Neog B (2017) Inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in modulating phosphorus dynamics in turmeric rhizosphere. Natl Acad Sci Lett 40:445–449

Ercoli L, Schüßler A, Arduini I, Pellegrino E (2017) Strong increase of durum wheat iron and zinc content by field-inoculation with arbuscular mycorrhizal fungi at different soil nitrogen availabilities. Plant Soil 419:153–167

Fan F, Yang Q, Li Z, Wei D, Cui XA, Liang Y (2011) Impacts of organic and inorganic fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer community. Microb Ecol 62:982–990

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bücking H (2012a) Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci U S A 109:2666–2671

Fellbaum CR, Mensah JA, Pfeffer PE, Kiers ET, Bücking H (2012b) The role of carbon in fungal nutrient uptake and transport: implications for resource exchange in the arbuscular mycorrhizal symbiosis. Plant Signal Behav 7:1509–1512

Fernandez M, Malagoli P, Vernay A, Améglio T, Balandier P (2020) Below-ground nitrogen transfer from oak seedlings facilitates Molinia growth: ^{15}N pulse-chase labelling. Plant Soil 20:1–4

Franklin O, Cambui CA, Gruffman L, Palmroth S, Oren R, Näsholm T (2017) The carbon bonus of organic nitrogen enhances nitrogen use efficiency of plants. Plant Cell Environ 40:25–35

Frey SD (2019) Mycorrhizal fungi as mediators of soil organic matter dynamics. Ann Rev Ecol Evol Syst 50:237–259

Frey-Klett P, Garbaye JA, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Garcia K, Doidy J, Zimmermann SD, Wipf D, Courty PE (2016) Take a trip through the plant and fungal transportome of mycorrhiza. Trends Plant Sci 21:937–950

Garg N, Chandel S (2011) Effect of mycorrhizal inoculation on growth, nitrogen fixation, and nutrient uptake in *Cicer arietinum* (L.) under salt stress. Turk J Agric For 35:205–214

Gojon A (2017) Nitrogen nutrition in plants: rapid progress and new challenges. J Exp Bot 68:2457–2462

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Gregory PJ (2006) Roots, rhizosphere and soil: the route to a better understanding of soil science? Eur J Soil Sci 57:2–12

Gui H, Purahong W, Hyde KD, Xu J, Mortimer PE (2017) The arbuscular mycorrhizal fungus *Funneliformis mosseae* alters bacterial communities in subtropical forest soils during litter decomposition. Front Microbiol 8:1120. https://doi.org/10.3389/fmicb.2017.01120

Hack CM, Porta M, Schäufele R, Grimoldi AA (2019) Arbuscular mycorrhiza mediated effects on growth, mineral nutrition and biological nitrogen fixation of *Melilotus alba* Med. in a subtropical grassland soil. Appl Soil Ecol 134:38–44

Hamel C (2004) Impact of arbuscular mycorrhizal fungi on N and P cycling in the root zone. Can J Soil Sci 84:383–395

Han J, Shi J, Zeng L, Xu J, Wu L (2015) Effects of nitrogen fertilization on the acidity and salinity of greenhouse soils. Environ Sci Pollut Res 22:2976–2986

Hao Z, Xie W, Jiang X, Wu Z, Zhang X, Chen B (2019) Arbuscular mycorrhizal fungus improves *Rhizobium–Glycyrrhiza* seedling symbiosis under drought stress. Agronomy 9:572. https://doi.org/10.3390/agronomy9100572

Hawkins HJ, George E (2001) Reduced ^{15}N nitrogen transport through arbuscular mycorrhizal hyphae to *Triticum aestivum* L. supplied with ammonium vs. nitrate nutrition. Ann Bot 87:303–311

Hawkins BJ, Robbins S (2010) pH affects ammonium, nitrate and proton fluxes in the apical region of conifer and soybean roots. Physiol Plant 138:238–247

Hawkins HJ, Johansen A, George E (2000) Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant Soil 226:275–285

He X, Xu M, Qiu GY, Zhou J (2009) Use of ^{15}N stable isotope to quantify nitrogen transfer between mycorrhizal plants. J Plant Ecol 2:107–118

He S, Long M, He X, Guo L, Yang J, Yang P, Hu T (2017) Arbuscular mycorrhizal fungi and water availability affect biomass and C: N: P ecological stoichiometry in alfalfa (*Medicago sativa* L.) during regrowth. Acta Physiol Plant 39:199. https://doi.org/10.1007/s11738-017-2493-7

He Y, Cornelissen JH, Wang P, Dong M, Ou J (2019) Nitrogen transfer from one plant to another depends on plant biomass production between conspecific and heterospecific species via a common arbuscular mycorrhizal network. Environ Sci Pollut Res 26:8828–8837

Herdler S, Kreuzer K, Scheu S, Bonkowski M (2008) Interactions between arbuscular mycorrhizal fungi (*Glomus intraradices*, Glomeromycota) and amoebae (*Acanthamoeba castellanii*, Protozoa) in the rhizosphere of rice (*Oryza sativa*). Soil Biol Biochem 40:660–668

Herman DJ, Firestone MK, Nuccio E, Hodge A (2012) Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiol Ecol 80:236–247

Hestrin R, Hammer EC, Mueller CW, Lehmann J (2019) Synergies between mycorrhizal fungi and soil microbial communities increase plant nitrogen acquisition. Commun Biol 2:1–9

Hino T, Matsumoto Y, Nagano S, Sugimoto H, Fukumori Y, Murata T, Iwata S, Shiro Y (2010) Structural basis of biological N_2O generation by bacterial nitric oxide reductase. Science 330:1666–1670

Hodge A (2014) Interactions between arbuscular mycorrhizal fungi and organic material substrates. Adv Appl Microbiol 89:47–99
Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci USA 107:13754–13759
Hodge A, Storer K (2015) Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant Soil 386:1–19
Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299
Hodge A, Helgason T, Fitter AH (2010) Nutritional ecology of arbuscular mycorrhizal fungi. Fungal Ecol 3:267–273
Hsu SF, Buckley DH (2009) Evidence for the functional significance of diazotroph community structure in soil. ISME J 3:124–136
Ingraffia R, Amato G, Frenda AS, Giambalvo D (2019) Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N_2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. PLoS One 14:e0213672. https://doi.org/10.1371/journal.pone.0213672
Inselsbacher E, Näsholm T (2012) The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. New Phytol 195:329–334
Jansa J, Gryndler M (2010) Biotic environment of the arbuscular mycorrhizal fungi in soil. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function, 2nd edn. Springer, Heidelberg, pp 209–236
Jansa J, Bukovská P, Gryndler M (2013) Mycorrhizal hyphae as ecological niche for highly specialized hypersymbionts–or just soil free-riders? Front Plant Sci 4:134. https://doi.org/10.3389/fpls.2013.00134
Jin H (2009) Arginine bi-directional translocation and breakdown into ornithine along the arbuscular mycorrhizal mycelium. Sci China Ser C 52:381–389
Jin H, Liu J, Liu J, Huang X (2012) Forms of nitrogen uptake, translocation, and transfer via arbuscular mycorrhizal fungi: a review. Sci China Life Sci 55:474–482
Johnson NC, Wilson GW, Wilson JA, Miller RM, Bowker MA (2015) Mycorrhizal phenotypes and the law of the minimum. New Phytol 205:1473–1484
Kandhasamy N, Ravichandran KR, Thangavelu M (2020) Interactive influence of soil and plant genotypes on mycorrhizal dependency in finger millet. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-020-00212-2
Klironomos JN, Allen MF, Rillig MC, Piotrowski J, Makvandi-Nejad S, Wolfe BE, Powell JR (2005) Abrupt rise in atmospheric CO_2 overestimates community response in a model plant–soil system. Nature 433:621–624
Kobae Y (2019) Dynamic phosphate uptake in arbuscular mycorrhizal roots under field conditions. Front Environ Sci 6:159. https://doi.org/10.3389/fenvs.2018.00159
Kobae Y, Tamura Y, Takai S, Banba M, Hata S (2010) Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean. Plant Cell Physiol 51:1411–1415
Koller R, Rodriguez A, Robin C, Scheu S, Bonkowski M (2013) Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. New Phytol 199:203–211
Larimer AL, Clay K, Bever JD (2014) Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. Ecology 95:1045–1054
Leifheit EF, Verbruggen E, Rillig MC (2014) Rotation of hyphal in-growth cores has no confounding effects on soil abiotic properties. Soil Biol Biochem 79:78–80
Leigh J, Hodge A, Fitter AH (2009) Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. New Phytol 181:199–207
Li L, Li SM, Sun JH, Zhou LL, Bao XG, Zhang HG, Zhang FS (2007) Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc Natl Acad Sci U S A 104:11192–11196
Li Y, Ran W, Zhang R, Sun S, Xu G (2009) Facilitated legume nodulation, phosphate uptake and nitrogen transfer by arbuscular inoculation in an upland rice and mung bean intercropping system. Plant Soil 315:285–296

Li B, Li YY, Wu HM, Zhang FF, Li CJ, Li XX, Lambers H, Li L (2016) Root exudates drive interspecific facilitation by enhancing nodulation and N_2 fixation. Proc Natl Acad Sci U S A 113:6496–6501

Liese R, Lübbe T, Albers NW, Meier IC (2018) The mycorrhizal type governs root exudation and nitrogen uptake of temperate tree species. Tree Physiol 38:83–95

Lin J, Wang Y, Sun S, Mu C, Yan X (2017) Effects of arbuscular mycorrhizal fungi on the growth, photosynthesis and photosynthetic pigments of *Leymus chinensis* seedlings under salt-alkali stress and nitrogen deposition. Sci Total Environ 576:234–241

Lindsay PL, Williams BN, MacLean A, Harrison MJ (2019) A phosphate-dependent requirement for transcription factors IPD3 and IPD3L during arbuscular mycorrhizal symbiosis in *Medicago truncatula*. Mol Plant-Microbe Interact 32:1277–1290

Liu H, Song F, Liu S, Li X, Liu F, Zhu X (2019) Arbuscular mycorrhiza improves nitrogen use efficiency in soybean grown under partial root-zone drying irrigation. Arch Agron Soil Sci 65:269–279

Liu J, Chen J, Xie K, Tian Y, Yan A, Liu J, Huang Y, Wang S, Zhu Y, Chen A, Xu G (2020) A mycorrhiza-specific H^+-ATPase is essential for arbuscule development and symbiotic phosphate and nitrogen uptake. Plant Cell Environ 43:1069–1108

López-Gutiérrez JC, Henry S, Hallet S, Martin-Laurent F, Catroux G, Philippot L (2004) Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR. J Microbiol Meth 57:399–407

Lu JK, Li ZS, Yang FC, Wang SK, Liang JF, He XH (2020) Concurrent carbon and nitrogen transfer between hemiparasite *Santalum album* and two N_2-fixing hosts in a sandalwood plantation. Forest Ecol Manag 464:118060. https://doi.org/10.1016/j.foreco.2020.118060

Mackay JE, Macdonald LM, Smernik RJ, Cavagnaro TR (2017) Organic amendments as phosphorus fertilisers: chemical analyses, biological processes and plant P uptake. Soil Biol Biochem 107:50–59

Marschner H (2011) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, London

Marschner P, Timonen S (2006) Bacterial community composition and activity in rhizosphere of roots colonized by arbuscular mycorrhizal fungi. In: Mukerji KG, Manoharachary C, Singh J (eds) Microbial activity in the rhizosphere. Soil biology, vol 7. Springer, Berlin, pp 139–154

Masson-Boivin C, Sachs JL (2018) Symbiotic nitrogen fixation by rhizobia—the roots of a success story. Curr Opin Plant Biol 44:7–15

Meng L, Zhang A, Wang F, Han X, Wang D, Li S (2015) Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Front Plant Sci 6:339. https://doi.org/10.3389/fpls.2015.00339

Míguez-Montero MA, Valentine A, Pérez-Fernández MA (2020) Regulatory effect of phosphorus and nitrogen on nodulation and plant performance of leguminous shrubs. AoB Plants 12:plz047. https://doi.org/10.1093/aobpla/plz047

Mohammadi M, Modarres-Sanavy SA, Pirdashti H, Zand B, Tahmasebi-Sarvestani Z (2019) Arbuscular mycorrhizae alleviate water deficit stress and improve antioxidant response, more than nitrogen fixing bacteria or chemical fertilizer in the evening primrose. Rhizosphere 9:76–89

Molla MN, Solaiman AR (2009) Association of arbuscular mycorrhizal fungi with leguminous crops grown in different agro-ecological zones of Bangladesh. Arch Agron Soil Sci 55:233–245

Montenegro-Gómez SP, Gómez-Posada S, Barrera-Berdugo SE (2017) Effect of poultry manure on *Azotobacter* sp., *Azospirillum* sp. and arbuscular mycorrhizal fungi in anion culture (*A. fistulosum*). Entramado 13:250–257

Montesinos-Navarro A, Verdú M, Querejeta JI, Sortibrán L, Valiente-Banuet A (2016) Soil fungi promote nitrogen transfer among plants involved in long-lasting facilitative interactions. Perspect Plant Ecol Evol Syst 18:45–51

Morgan JB, Connolly EL (2013) Plant-soil interactions: nutrient uptake. Nat Educ Knowl 4:2

Mrkovacki N, Milic V (2001) Use of *Azotobacter chroococcum* as potentially useful in agricultural application. Ann Microbiol 51:145–158

Mus F, Crook MB, Garcia K, Costas AG, Geddes BA, Kouri ED, Paramasivan P, Ryu MH, Oldroyd GE, Poole PS, Udvardi MK (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Appl Environ Microbiol 82:3698–3710

Musyoka DM, Njeru EM, Nyamwange MM, Maingi JM (2020) Arbuscular mycorrhizal fungi and *Bradyrhizobium* co-inoculation enhances nitrogen fixation and growth of green grams (*Vigna radiata* L.) under water stress. J Plant Nutr 43:1036–1047

Nannipieri P, Eldor P (2009) The chemical and functional characterization of soil N and its biotic components. Soil Biol Biochem 41:2357–2369

Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. New Phytol 182:31–48

Norman JS, Friesen ML (2017) Complex N acquisition by soil diazotrophs: how the ability to release exoenzymes affects N fixation by terrestrial free-living diazotrophs. ISME J 11:315–326

Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK (2013) An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environ Microbiol 15:1870–1881

Nygren P, Leblanc HA (2015) Dinitrogen fixation by legume shade trees and direct transfer of fixed N to associated cacao in a tropical agroforestry system. Tree Physiol 35:134–147

Obertello M, Wall LG (2015) Interactions between *Frankia* BCU110501 (actinorhiza) and *Gigaspora rosea* (arbuscular mycorrhiza) with *Discaria trinervis* studied by spot inoculation. Symbiosis 66:13–20

Okiobe ST, Augustin J, Veresoglou SD (2019) Disentangling direct and indirect effects of mycorrhiza on nitrous oxide activity and denitrification. Soil Biol Biochem 134:142–151

Okiobe ST, Rillig MC, Mola M, Augustin J, Parolly G, Veresoglou SD (2020) Arbuscular mycorrhiza has little influence on N_2O potential emissions compared to plant diversity in experimental plant communities. FEMS Microbiol Ecol 96:fiz208. https://doi.org/10.1093/femsec/fiz208

Ortas I, Iqbal T, Yücel YC (2019) Mycorrhizae enhances horticultural plant yield and nutrient uptake under phosphorus deficient field soil condition. J Plant Nutr 42:1152–1164

Paterson E, Sim A, Davidson J, Daniell TJ (2016) Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. Plant Soil 408:243–254

Pérez-Fernández MA, Calvo-Magro E, Rodríguez-Sánchez J, Valentine A (2017) Differential growth costs and nitrogen fixation in *Cytisus multiflorus* (L'Hér.) Sweet and C*ytisus scoparius* (L.) Link are mediated by sources of inorganic. N Plant Biol 19:742–748

Phillips RP, Brzostek E, Midgley MG (2013) The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. New Phytol 199:41–51

Püschel D, Janoušková M, Hujslová M, Slavíková R, Gryndlerová H, Jansa J (2016) Plant–fungus competition for nitrogen erases mycorrhizal growth benefits of *Andropogon gerardii* under limited nitrogen supply. Ecol Evol 6:4332–4346

Püschel D, Janoušková M, Voříšková A, Gryndlerová H, Vosátka M, Jansa J (2017) Arbuscular mycorrhiza stimulates biological nitrogen fixation in two *Medicago* spp. through improved phosphorus acquisition. Front Plant Sci 8:390. https://doi.org/10.3389/fpls.2017.00390

Quilliam RS, Marsden KA, Gertler C, Rousk J, DeLuca TH, Jones DL (2012) Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. Agric Ecosyst Environ 158:192–199

Rabie GH, Almadini AM (2005) Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. Afr J Biotechnol 4:210–222

Rodriguez C, Carlsson G, Englund JE, Flöhr A, Pelzer E, Jeuffroy MH, Makowski D, Jensen ES (2020) Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. Eur J Agron 118:126077. https://doi.org/10.1016/j.eja.2020.126077

Rosenberg K, Bertaux J, Krome K, Hartmann A, Scheu S, Bonkowski M (2009) Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. ISME J 3:675–684

Rosolem CA, Ritz K, Cantarella H, Galdos MV, Hawkesford MJ, Whalley WR, Mooney SJ (2017) Enhanced plant rooting and crop system management for improved N use efficiency. In: Sparks DL (ed) Advances in agronomy, vol 146. Academic Press, Cambridge, pp 205–239

Rydlová J, Püschel D, Dostálová M, Janoušková M, Frouz J (2016) Nutrient limitation drives response of *Calamagrostis epigejos* to arbuscular mycorrhiza in primary succession. Mycorrhiza 26:757–767

Sabannavar SJ, Lakshman HC (2011) Synergistic interactions among *Azotobacter*, *Pseudomonas*, and arbuscular mycorrhizal fungi on two varieties of *Sesamum indicum* L. Commun Soil Sci Plant Anal 42:2122–2133

Sabatino L, Iapichino G, Consentino BB, D'Anna F, Rouphael Y (2020) Root stock and arbuscular mycorrhiza combinatorial effects on eggplant crop performance and fruit quality under greenhouse conditions. Agronomy 1:693. https://doi.org/10.3390/agronomy10050693

Saharan K, Schütz L, Kahmen A, Wiemken A, Boller T, Mathimaran N (2018) Finger millet growth and nutrient uptake is improved in intercropping with pigeon pea through biofertilization and bioirrigation mediated by arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria. Front Environ Sci 6:46. https://doi.org/10.3389/fenvs.2018.00046

Saia S, Benítez E, García-Garrido JM, Settanni L, Amato G, Giambalvo D (2014) The effect of arbuscular mycorrhizal fungi on total plant nitrogen uptake and nitrogen recovery from soil organic material. J Agric Sci 152:370–378

Scandellari F (2017) Arbuscular mycorrhizal contribution to nitrogen uptake of grapevines. Vitis 56:147–154

Schüβler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Seguel A, Barea JM, Cornejo P, Borie F (2015) Role of arbuscular mycorrhizal symbiosis in phosphorus-uptake efficiency and aluminium tolerance in barley growing in acid soils. Crop Pasture Sci 66:696–705

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London

Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu Rev Plant Biol 62:227–250

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–1057

Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A, James TY (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108:1028–1046

Storer K, Coggan A, Ineson P, Hodge A (2018) Arbuscular mycorrhizal fungi reduce nitrous oxide emissions from N_2O hotspots. New Phytol 220:1285–1295

Sulieman S, Tran LS (2004) Symbiotic nitrogen fixation in legume nodules: metabolism and regulatory mechanisms. Int J Mol Sci 15:19389–19393

Sun Z, Song J, Xin XA, Xie X, Zhao B (2018) Arbuscular mycorrhizal fungal 14-3-3 proteins are involved in arbuscule formation and responses to abiotic stresses during AM symbiosis. Front Microbiol 9:91. https://doi.org/10.3389/fmicb.2018.00091

Symanczik S, Lehmann MF, Wiemken A, Boller T, Courty PE (2018) Effects of two contrasted arbuscular mycorrhizal fungal isolates on nutrient uptake by *Sorghum bicolor* under drought. Mycorrhiza 28:779–785

Talaat NB, Shawky BT (2014) Protective effects of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) plants exposed to salinity. Environ Exp Bot 98:20–31

Tanaka Y, Yano K (2005) Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. Plant Cell Environ 28:1247–1254

Tang Z, Xu W, Zhou G, Bai Y, Li J, Tang X, Chen D, Liu Q, Ma W, Xiong G, He H (2018) Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity in China's terrestrial ecosystems. Proc Natl Acad Sci U S A 115:4033–4038

Tavarini S, Passera B, Martini A, Avio L, Sbrana C, Giovannetti M, Angelini LG (2018) Plant growth, steviol glycosides and nutrient uptake as affected by arbuscular mycorrhizal fungi and phosphorous fertilization in *Stevia rebaudiana* Bert. Ind Crop Prod 111:899–907

Taylor MK, Lankau RA, Wurzburger N (2016) Mycorrhizal associations of trees have different indirect effects on organic matter decomposition. J Ecol 104:1576–1584

Teutscherova N, Vazquez E, Arango J, Arevalo A, Benito M, Pulleman M (2019) Native arbuscular mycorrhizal fungi increase the abundance of ammonia-oxidizing bacteria, but suppress nitrous oxide emissions shortly after urea application. Geoderma 338:493–501

Thilakarathna MS, McElroy MS, Chapagain T, Papadopoulos YA, Raizada MN (2016) Below-ground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. Agron Sustain Dev 36:58. https://doi.org/10.1007/s13593-016-0396-4

Thirkell TJ, Cameron DD, Hodge A (2016) Resolving the 'nitrogen paradox' of arbuscular mycorrhizas: fertilization with organic matter brings considerable benefits for plant nutrition and growth. Plant Cell Environ 39:1683–1690

Tian C, Kasiborski B, Koul R, Lammers PJ, Bücking H, Shachar-Hill Y (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiol 153:1175–1187

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677

Tisserant E, Kohler A, Dozolme-Seddas P, Balestrini R, Benabdellah K, Colard A, Croll D, Da Silva C, Gomez SK, Koul R, Ferrol N (2012) The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. New Phytol 193:755–769

Toljander JF, Lindahl BD, Paul LR, Elfstrand M, Finlay RD (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. FEMS Microbiol Ecol 61:295–304

Tomè E, Tagliavini M, Scandellari F (2015) Recently fixed carbon allocation in strawberry plants and concurrent inorganic nitrogen uptake through arbuscular mycorrhizal fungi. J Plant Physiol 179:83–89

Tonhauzer K, Tonhauzer P, Szemesová J, Šiška B (2020) Estimation of N_2O emissions from agricultural soils and determination of nitrogen leakage. Atmos 11:552. https://doi.org/10.3390/atmos11060552

Torres-Olivar V, Villegas-Torres OG, Domínguez-Patiño ML, Sotelo-Nava H, Rodríguez-Martínez A, Melgoza-Alemán RM, Valdez-Aguilar LA, Alia-Tejacal I (2014) Role of nitrogen and nutrients in crop nutrition. J Agric Sci Techol B 4:29–37

Toussaint JP, St-Arnaud M, Charest C (2004) Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an *in vitro* compartmented system. Can J Microbiol 50:251–260

Trap J, Bonkowski M, Plassard C, Villenave C, Blanchart E (2016) Ecological importance of soil bacterivores for ecosystem functions. Plant Soil 398:1–24

Ueda MU, Kachina P, Marod D, Nakashizuka T, Kurokawa H (2017) Soil properties and gross nitrogen dynamics in old growth and secondary forest in four types of tropical forest in Thailand. Forest Ecol Manag 398:130–139

Valentine AJ, Osborne BA, Mitchell DT (2002) Form of inorganic nitrogen influences mycorrhizal colonisation and photosynthesis of cucumber. Sci Hortic 92:229–239

Vallorani L, Polidori E, Sacconi C, Agostini D, Pierleoni R, Piccoli G, Zeppa S, Stocchi V (2002) Biochemical and molecular characterization of NADP-glutamate dehydrogenase from the ectomycorrhizal fungus *Tuber borchii*. New Phytol 154:779–790

Van Aarle IM, Olsson PA (2008) Resource partitioning between extraradical and intraradical AM fungal mycelium. In: Varma A (ed) Mycorrhiza: state of the art, genetics and molecular biology, eco-function, biotechnology, ecophysiology, structure and systematics, 3rd edn. Springer, Heidelberg, pp 321–336

Vangelisti A, Natali L, Bernardi R, Sbrana C, Turrini A, Hassani-Pak K, Hughes D, Cavallini A, Giovannetti M, Giordani T (2018) Transcriptome changes induced by arbuscular mycorrhizal fungi in sunflower (*Helianthus annuus* L.) roots. Sci Rep 8:1–4

Varinderpal-Singh SS, Kunal GSK, Choudhary R, Singh R, Adholeya A, Bijay-Singh (2020) Synergistic use of plant growth-promoting rhizobacteria, arbuscular mycorrhizal fungi, and spectral properties for improving nutrient use efficiencies in wheat (*Triticum aestivum* L.). Commun Soil Sci Plant Anal 5:14–27

Verbruggen E, van der Heijden MG, Rillig MC, Kiers ET (2013) Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197:1104–1109

Veresoglou SD, Sen R, Mamolos AP, Veresoglou DS (2011) Plant species identity and arbuscular mycorrhizal status modulate potential nitrification rates in nitrogen-limited grassland soils. J Ecol 99:1339–1349

Veresoglou SD, Chen B, Rillig MC (2012) Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol Biochem 46:53–62

Vergara C, Araujo KEC, Souza SR, Schultz N, Saggin Junior OJ, Sperandio MVL, Zilli JE (2019) Plant-mycorrhizal fungi interaction and response to inoculation with different growth-promoting fungi. Pesq Agropec Bras 54:1–24

Verzeaux J, Hirel B, Dubois F, Lea PJ, Tétu T (2017) Agricultural practices to improve nitrogen use efficiency through the use of arbuscular mycorrhizae: basic and agronomic aspects. Plant Sci 264:48–56

Wang G, Sheng L, Zhao D, Sheng J, Wang X, Liao H (2016) Allocation of nitrogen and carbon is regulated by nodulation and mycorrhizal networks in soybean/maize intercropping system. Front Plant Sci 7:1901. https://doi.org/10.3389/fpls.2016.01901

Wang Y, Wang M, Li Y, Wu A, Huang J (2018) Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress. PLoS One 13:e0196408. https://doi.org/10.1371/journal.pone.0196408

Whiteside MD, Digman MA, Gratton E, Treseder KK (2012a) Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest. Soil Biol Biochem 55:7–13

Whiteside MD, Garcia MO, Treseder KK (2012b) Amino acid uptake in arbuscular mycorrhizal plants. PLoS One 7:e47643. https://doi.org/10.1371/journal.pone.0047643

Wu F, Zhang H, Fang F, Liu H, Tang M (2017) Arbuscular mycorrhizal fungi alter nitrogen allocation in the leaves of *Populus× canadensis* 'Neva'. Plant Soil 421:477–491

Wu F, Fang F, Wu N, Li L, Tang M (2020) Nitrate transporter gene expression and kinetics of nitrate uptake by *Populus× canadensis* 'Neva' in relation to arbuscular mycorrhizal fungi and nitrogen availability. Front Microbiol 11:176. https://doi.org/10.3389/fmicb.2020.00176

Xu L, Cheng S, Fang H, Xin X, Xu X, Tang H (2019) Soil inorganic nitrogen composition and plant functional type determine forage crops nitrogen uptake preference in the temperate cultivated grassland, Inner Mongolia. Soil Sci Plant Nutr 65:501–510

Yang H, Zhou J, Weih M, Li Y, Zhai S, Zhang Q, Chen W, Liu J, Liu L, Hu S (2020) Mycorrhizal nitrogen uptake of wheat is increased by earthworm activity only under no-till and straw removal conditions. Appl Soil Ecol 155:103672. https://doi.org/10.1016/j.apsoil.2020.103672

Yu Z, Xu J, Liu S, Hu L, Ren M, Liu Y, Tang J, Chen X (2020) Adult plants facilitate their conspecific seedlings by enhancing arbuscular mycorrhizae in a saline soil. Plant Soil 447:333–345

Zarea MJ, Karimi N, Goltapeh EM, Ghalavand A (2011) Effect of cropping systems and arbuscular mycorrhizal fungi on soil microbial activity and root nodule nitrogenase. J Saudi Soc Agri Sci 10:109–120

Zhang H, Wang X, Gao Y, Sun B (2020) Short-term N transfer from alfalfa to maize is dependent more on arbuscular mycorrhizal fungi than root exudates in N deficient soil. Plant Soil 446:23–41

Zhu X, Song F, Liu S, Liu F (2016) Arbuscular mycorrhiza improve growth, nitrogen uptake, and nitrogen use efficiency in wheat grown under elevated CO_2. Mycorrhiza 26:133–140

Chapter 22
Nitrogen Fixing Fungi for Development of Biofertilizer and Future Strategies

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Abstract Urbanization, population explosion, pollution and deforestation lead to negative impact on the environment and disturb its natural cycles. With that comes the natural calamity like uneven rainfalls which causes drought or flooding, extreme high/low temperature, and degradation of quality of air, water, and soil. The ultimate looser is agriculture system. More and more land is becoming unsuitable for agriculture with each passing year. Demands for global food is on rise and will need a 70% increase in production capacity to meet the demand of population by 2050. But with current scenario it looks unreachable and unsustainable. Innovative solution based practices, eco-friendly pest control strategies with biofertilizers, and increased nutritional values, higher yields, and productivity with no harm to environment are the need of the hour. Use of fungi and bacteria based Nitrogen fixation strategies focuses on improving nutrient usage by plants and reduction in dependence of chemical fertilizer. This chapter focuses upon the nitrogen fixing fungi for development of biofertilizer and future strategies.

Keywords Nitrogen fixation · Biofertilizers · Ecofriendly · Fungi · Plant nutrients

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22.1 Introduction

With the rapidly increasing population, the environment is getting disturbed due to urbanization and industrialization. It is difficult to feed the entire population of the world which is significantly increasing day by day (Mahanty 2017; Bhargava et al. 2019a). The natural systems have been modified by humans due to the production of food globally. The use of fertilizers has made it possible to meet the demands of world's food supply. But due to this practice of excessive utilization of fertilizers, the environment has been affected to a great extent. It has led to the contamination of ground water, affected the quality of soil and biodiversity (Weber 2014; Kumar et al. 2020b). Further, plants face certain environmental stresses, both biotic and abiotic, which have a negative impact on the yield and growth of crops. Abiotic stresses which include temperature of environment, salinity, smog, pH of the soil, etc., have always affected agriculture (Wang 2015; Bhargava et al. 2019b). Therefore, instead of utilizing chemical fertilizers, an alternative method to deal with these situations is to elevate the use of biofertilizers (Weber 2014; Kumar et al. 2020a). These biofertilizers are microbial inoculants used in sustainable agriculture. The uptake of nutrients such as phosphorous and nitrogen by plants is made more effective as a result of their usage (Xiong 2017; Ajmani et al. 2019). The capacity to retain nutrients by plants is also increased (Srivastava 2019; Bhargava et al. 2020). Microorganisms like fungi, protozoa, bacteria, etc., can be used for the preparation of biofertilizers (Malusà et al. 2016; Bhargava et al. 2017). Some of these microorganisms are considered to perform functions of the ecosystem for the soil and plants (Maheshwari 2012; Goel et al. 2017). Nowadays, emphasis is given towards meeting the food requirements across the globe but with minimum impact on the environment and this approach is termed as "sustainable intensification" (Davis 2016; Gupta et al. 2018).

In agricultural systems, an essential requirement in the production of crop is to maintain the health of soil. The factors which govern the health of soil are recycling of nutrients, transmission of disease, resistance, etc., which are associated with the microorganisms present in the soil. The microorganisms present in the soil are affected by the fertilizers and other inputs given to the soil (Shen 2015; Jain et al. 2011). If chemical fertilizers are used for a longer period of time, it reduces the amount of bacteria in the soil and microbiota is also imbalanced. Hence, the biological properties of the soil are affected. Studies have found an effective way to regulate the microbiota of soil by mixing the chemical fertilizers with biofertilizers. This way the good bacteria are promoted in the soil and the harmful pathogens are snuffed out (Li 2017; Kaur et al. 2010).

Biofertilizers contribute directly towards the increase in the concentration of nitrogen and phosphorous in the soil. These nutrients can be directly taken up by the plants. Studies on chickpea and sorghum have shown a rise in the nutrients of soil due to the combined effect of *Rhizobium* species, *Glomus fasciculatum* and *Bacillus megaterium* (Khan et al. 2010; Maurya et al. 2013). For productivity and growth of plants, nitrogen is one of the most crucial nutrients (Mahanty 2017; Maurya et al.

2014). "Nitrogen cycle" is referred to the transition of nitrogen in and out of the soil. The availability of nitrogen affects the biomass and the yield of crops as it is the limiting nutrient. It regulates various processes of the plant and helps to produce certain chemicals which fight against several pathogens. A large amount of nitrogen present in the atmosphere is unavailable to the plants until it is made to be of use. Legumes are capable of fixing the nitrogen present in the atmosphere. They also set up a symbiotic relationship with various fungi for providing proper nutrition to the plants. Planting of leguminous crops in nutrient deficient soil is an effective approach towards development of sustainable agriculture (Mia 2010; Negi and Vats 2013).

Depending upon the deficiency in the soil and the type of plant, different types of biofertilizers are available such as nitrogen fixing, phosphorous solubilizing, phosphate mobilizing, plant growth promoting, and enriched compost biofertilizers. Fungal biofertilizers if either inoculated alone or in combination are beneficial to the plants in terms of growth, crop yield, etc. With the plants, a symbiotic relationship is established by mycorrhiza. There are two types of mycorrhizal fungi which play crucial roles in the development of plants. The first one, namely, Ectomycorrhizal (ECM) fungi assist in the growth of several trees by colonizing with the roots. They help in better absorption of nutrients such as nitrogen, phosphorous, etc. ECM also protect the plants from several environmental stresses, e.g. drought, high temperature, etc. One example of ECM fungus is *Piriformospora indica* which makes the plant tolerant to both biotic as well as abiotic stress along with promoting its growth. The other type of fungi is Endomycorrhizae which develop a symbiotic relationship with the roots of the plant. It is also termed as Arbuscular mycorrhizal (AM) fungi (Li 2017; Ojha et al. 2013). In both, organic and inorganic forms, AM fungi can take up nitrogen. They can fulfil up to 50% of plant's requirement of nitrogen especially in the arid and semi-arid climatic conditions (Malusà et al. 2016; Painuly et al. 2019). They are considered as one of the important components of sustainable agriculture. They assist the plant in the following ways. (1) Enhance the rate of photosynthesis, (2) Increase the rate of nitrogen fixation either by associative or symbiotic nitrogen fixing bacteria, (3) Help fight against pests, (4) Provide resistance against environmental stress. In case of most of the legumes, a symbiotic relationship is formed with AM fungi as well as with nitrogen fixing rhizobia. Such a case is considered beneficial where rhizobia and AM fungi both interact with identical legume. This tripartite relationship is not just responsible for providing proper nutrition of nitrogen to the plant but also improves the yield. Mycorrhizae are considered a prerequisite for the formation of nodules in legumes. It has been observed that there was improper nodulation without the formation of mycorrhizae. With the inoculation of plants with AM fungi, that can be in the form of biofertilizers, an elevation in the rate of nitrogen fixation in *Leucaena leucocephala* and *Medicago sativa* has been observed. In addition to this, improvement was also observed in terms of yield and growth of plants (Khan et al. 2010; Saxena et al. 2019).

22.2 Biofertilizer: A Potential Approach for Sustainable Agriculture Development

22.2.1 Biofertilizers and Their Necessity

In the rhizosphere, there is large number of microorganisms present in the soil. There is interaction between plants and microorganisms in the rhizosphere. Some of these microorganisms have a functional relationship with plants. This relationship is beneficial for the plants in different ways such as enhancing their growth and providing resistance to environmental stress which include contamination of heavy metal, deficiency of nutrient and water, etc. (Wu 2005; Sharma et al. 2018).

Biofertilizers are a combination of organic matter and useful microorganisms. Xiong (2017) and Sharma et al. (2014) reported that the organic matter acts as a niche for the microorganisms and also helps in recycling. The elements which are important in terms of nutrition are made available by these microorganisms through different biological processes. Now, biofertilizers are an important part of the system which supplies nutrients and have a crucial role to play in improving the yield of crops (Wu 2005; Singh and Vats 2019). They are gaining popularity in different parts of the world as they are found to protect the roots of the plant from pathogens present in the soil along with the enhancement in the fertility of soil. The microbiota of the bulk soil is modified by the addition of biofertilizers (Shen 2015; Tandon and Vats 2016).

For the improvement of biological fertility of soil, biofertilizers are considered more superior in comparison to the chemical fertilizers used (Li 2017; Vats 2017). Due to huge demand of food supplies, the chemical fertilizers are used frequently which has polluted and damaged the habitats of useful microorganisms and insects. This has made the crops more susceptible to diseases and has also impaired the fertility of soil. To meet the growing demand of food supplies, the productivity has to be increased in a sustainable and environment friendly manner. Therefore, the practice of using biofertilizers is supposed to be adopted. Biofertilizers need to be preferred over chemical fertilizers as they are cost-effective, secrete growth hormones, uplift crop yield, improves nitrogen fixation, and does not cause harm to the environment (Mahanty 2017; Vats and Bhargava 2017a, 2017b).

22.2.2 Carriers for the Preparation of Biofertilizers

Biofertilizers are prepared using carrier composed of microorganisms (Wang 2015; Vats and Kumar 2015). It is considered as a transporter of biofertilizers from the manufacturing unit to the field. There are different carriers for different biofertilizers (Mahanty 2017; Vats and Miglani 2011). Generally, peat is used as a carrier for biofertilizers in several countries. A favourable carrier should have the following characteristics:

1. Powdery or in the form of granules.
2. Help the microbes to grow and survive.
3. Be able to absorb moisture.
4. Eco-friendly.
5. Cost-effective.
6. Be able to easily discharge the useful microorganisms into the soil.

The organic waste obtained from several industries such as food and agriculture has characteristics of a biofertilizer carrier. Making use of this organic waste is beneficial for the environment. Perlite is also an eco-friendly biofertilizer carrier used which is composed of aluminium silicate and some amount of water. It is also porous and light weight. In order to store the biofertilizer carrier for a long period of time, its sterilizations is crucial. One of the methods to sterilize the carrier material is through gamma irradiation. This technique is accessible and causes limited losses during storage. Biofertilizers on carriers are preferred over free-cell as carrier provides protection to the microorganisms from environmental stress. Thereby, providing shelter to the microbes. During adverse conditions also, carrier helps in the survival of microorganisms (Wang 2015; Vats and Mishra 2016).

22.2.3 Classification of Biofertilizers

In the production of crops, nowadays, biofertilizers have a crucial role to play in terms of productivity and nutrient regulation. Biofertilizers are composed of diverse microorganisms which when administered to plants, seeds stimulate their growth. In this, nitrogen fixation and phosphorous solubilization are important biological processes (Singh 2016; Vats and Negi 2013). Biofertilizers also prevent the plants from getting infected by several diseases, e.g. Fusarium wilt disease (Li 2017; Vats and Bhargava 2017a, 2017b). Several biofertilizers are made available, listed in Fig. 22.1.

For proper growth of plants, phosphorous is an important macronutrient. Due to reduced levels of soluble phosphate in the soil, the plant is not able to grow properly. To overcome this issue, P-fertilizers were commonly made into use. In spite of this, a very small quantity of phosphorus is used up by plants and most of it is precipitated in the form of complexes of aluminium, iron, and calcium. The use of these fertilizers impacted the environment by causing eutrophication of water. Phosphorous present in the soil cannot be always taken up by the plants easily. Microorganisms, which can solubilize phosphorous, act as biofertilizers. They convert the phosphorous which is insoluble into soluble form which is readily taken up by the plants. This approach of using phosphorous biofertilizers is responsible for improving the yield of agriculture in several countries (Wang 2015; Vats and Kumar 2015).

There is a symbiotic relationship between Arbuscular mycorrhizal fungi and most of the species of plants. This symbiotic relationship acts as a mediator in the mobilization of nutrients. The fungus penetrates into the roots of the host. Firstly

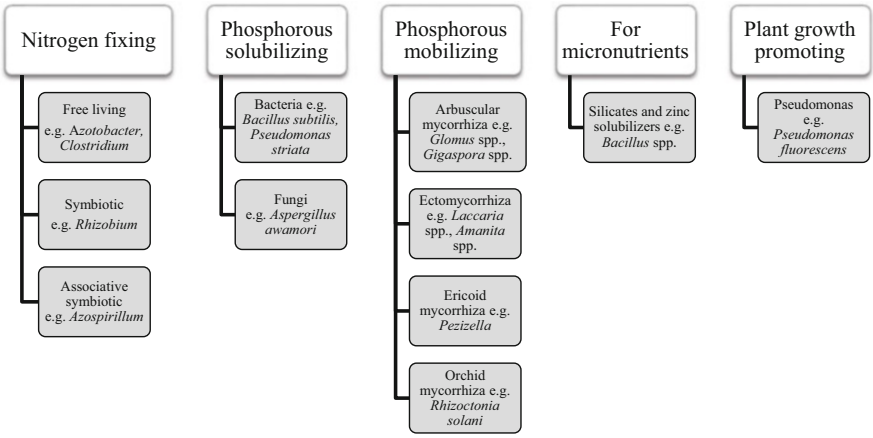

Fig. 22.1 Classification of biofertilizers on the basis of function and nature (Singh 2016)

the fungus is observed to grow between the cortical cells, then escalate towards the cell wall through hyphae and then finally into the cell. The hyphae are responsible for increasing the surface area and mobilizing the nutrients for the soil upwards. Therefore, mycorrhizal fungi are capable of transporting nutrients like zinc, phosphorous, and copper (Singh 2016; Vats and Negi 2013).

Nitrogen is one of the vital nutrients utilized by plants. To fulfil the demand of nitrogen for the growth and development of plants, nitrogen fertilizers are used which are hazardous to the environment if utilized in excess quantities. Therefore, as a substitute, biofertilizers are used in order to satisfy the requirements (Mia 2010; Vats et al. 2019). Plants cannot directly take up the atmospheric nitrogen. Therefore, it is first converted into ammonia by the process of nitrogen fixation and then the assimilation takes place. The nitrogen fixing microorganisms fix nitrogen with the help of nitrogenase enzyme. These microorganisms are present in the biofertilizers (Mahanty 2017; Vats and Miglani 2011). They also assist in the water and mineral absorption (Mia 2010; Vats et al. 2012). Examples of nitrogen fixing biofertilizers are given in Fig. 22.1.

22.2.4 Plant Growth Promoting Fungi (PGPF)

The microbes present in the rhizosphere help in the improvement of productivity of plants. These microorganisms are now considered as a substitute for chemical fertilizers. They improve the physiochemical properties of soil in terms of its health, growth, development of plants and their productivity. The population of microbes beneficial in agriculture is rhizobacteria, mycorrhiza, cyanobacteria, etc. (Bhardwaj 2014; Vats and Negi 2013; Vats et al. 2014).

In certain aspects, fungi are considered more valuable as compared to bacteria. Fungi have better tolerance towards acidic conditions, better mobility of phosphates, phytohormones like Indole-3-acetic acid (IAA), gibberellins, etc., are produced by fungi (Kour 2019; Vats and Bhargava 2017a, 2017b). The beneficial effects of fungi present in the rhizosphere in the growth and development of plants have been identified. Plant Growth Promoting Fungi (PGPF) are non-pathogenic fungi which assist in the growth and development of plants in a sustainable manner. The genera of the species which come under PGPF are *Phoma, Penicillium, Trichoderma, Fusarium, Aspergillus*, etc. PGPF interact in the same way as Plant Growth Promoting Rhizobacteria (PGPR) with the host plant. PGPF are capable to protect the plant against various pathogens. They enhance the ability of plants to perform photosynthesis, development of the roots and the shoot, crop yield, etc. When comes in contact with the host plant, there is an exchange in the signals between both, which enables to choose the correct partner and develops a beneficial relationship (Hossain et al. 2017). Researchers have started to recommend PGPF as bioinoculants because of the beneficial properties they exhibit (Kour 2019). Many PGPF are responsible to prevent the host plant from various diseases of which the mechanism of action can be more than one (Tuzun 2006). The functions of a few PGPF depending upon the host plant are shown in Table 22.1.

22.2.5 Relationship Between Biofertilizers and Bioremediation of Metals

Many metals are a component of soil and are needed by plants as micronutrients. Due to industrialization and swift increase in the agricultural practices much of the toxic waste like heavy metals and other contaminants are released in the environment. Due to the fact that heavy metals are soluble in water along with being nonbiodegradable get collected in the biosphere of soil. Metals such as arsenic, cadmium, nickel, mercury, etc., exist in different valence states. Uneven accumulation of metals in soil is hazardous though in proportionate amounts are useful to the plants. When the concentration of metal ions increases in the soil, they are taken up by the roots via shoot to the leaves. This translocation is responsible for disrupting the metabolism and growth of plants by creating stress. Due to the elevated levels of metals, the microbiota and fertility of soil are also affected. By altering the oxidation state, detoxification can be done and hence, remediation of heavy metals. In the reduced form, most heavy metals are less harmful. Out of the various strategies employed for removing heavy metal pollutants, bioremediation is considered to be the finest (Mahanty 2017). Microorganisms are sensitive towards heavy metals and hence are useful in bioremediation (Ilyas and Bano 2012). We have discussed the role of AMF in the process of bioremediation. Many researchers tried to evaluate the reason for the tolerance of AMF towards heavy metals in soil. Studies have shown that AMF utilizes plants for the process of remediation of soil. AMF reduces the

Table 22.1 Function of different PGPF and their host plant

PGPF	Host plant	Function	Reference
Phoma	*Cucumber (Cucumis sativus)*	Fights against bacterial Angular leafspot disease	Koike (2001)
Penicillium simplicissimum (isolate GP17–2)	*Cucumber (Cucumis sativus)*	Protects against anthracnose disease	Chandanie et al. (2006)
Non-sporulating fungus (isolate GU23–3)	*Wheat (Triticum)*	Suppression of common root rot	Shivanna et al. (1996)
Trichoderma longibrachiatum T6	*Wheat (Triticum)*	Promotes growth of plant along with providing resistance against parasitic nematodes	Zhang et al. (2016)
Pythium sp.	*Pearl millet (Pennisetum glaucum)*	Provides resistance against mildew disease	Murali (2012)
Piriformospora indica	*Arabidopsis thaliana*	Increases the number of root hair	Hossain et al. (2017)
Fusarium equiseti (isolate GF18–3) and *Glomus mosseae* (AM fungi) interaction	*Cucumber (Cucumis sativus)*	Fights against "cucumber mosaic virus"	Elsharkawy (2012)
Phoma sp. (isolate GS8–3)	*Tobacco (Nicotiana tabacum)*	The volatile compounds enhance the growth of the plant	Naznin (2013)
Trichoderma harzianum and AM fungi	*Tomato (Solanum lycopersicum)*	Enhances the growth of seedling	Nzanza et al. (2011)
Interaction *Fusarium oxysporum*	*Watermelon (Citrullus lanatus)*	Production of antimicrobial compounds	Bent (2006)

accumulation of heavy metals in roots and in shoots. The translocation of metals from root to the shoot is inhibited. The reason for this could be that AMF is able to filter these heavy metal ions at the time of nutrient uptake. The accumulation of these metals also depends upon the characteristics of soil, fungi, density of the roots, and several other factors. AMF also plays a crucial role in the bioremediation of radionuclides (Leyval 2002). Studies have shown that an association of few AMF and PGPR increases the efficiency of phytoremediation in soil which is contaminated with iron. This symbiotic relationship between AMF and PGPR is not just responsible to bring about some physiological changes but also alter the structure of the roots (Mishra 2016).

22.2.6 *Biofertilizers and Environmental Stress Management in Plants*

Plants have to deal with different kinds of environmental stresses (biotic and abiotic) as shown in Fig. 22.2. The microorganisms which are a part of the rhizosphere interact with plants which lead to the development of a beneficial relationship between the two (Ahmad and Rasool 2014). The symbiotic relationship of plants with fungi can be with fungal endophytes or with mycorrhizal fungi. The fungal endophytes stay in the tissues of the plants while mycorrhizal fungi stay only in the roots (Rodriguez et al. 2004). Ample amount of emphasis is being given to the association developed with AMF. It gives the plant the ability to tolerate both biotic as well as abiotic stress. The fungi involved in the association include families Glomaceae, Acaulosporaceae, Paraglomaceae, Archaeosporaceae, and Gigasporaceae. AMF induces the expression of certain genes which make the host plant tolerant to drought stress. AMF develops certain biochemical and genetic mechanisms which protect the plants from pathogens. AMF alone or in combination of other microorganisms can perform the task. For example, in tobacco plant, blank shank disease can be cured by inoculating *Trichoderma harzianum* and *Glomus fasciculatum* (Ahmad and Rasool 2014). The root-rot disease can be prevented due to the combination of *Pseudomonas fluorescens* and arbuscular mycorrhiza. The multiplication of various root pathogens is also hindered. In basil, *Glomus mosseae*

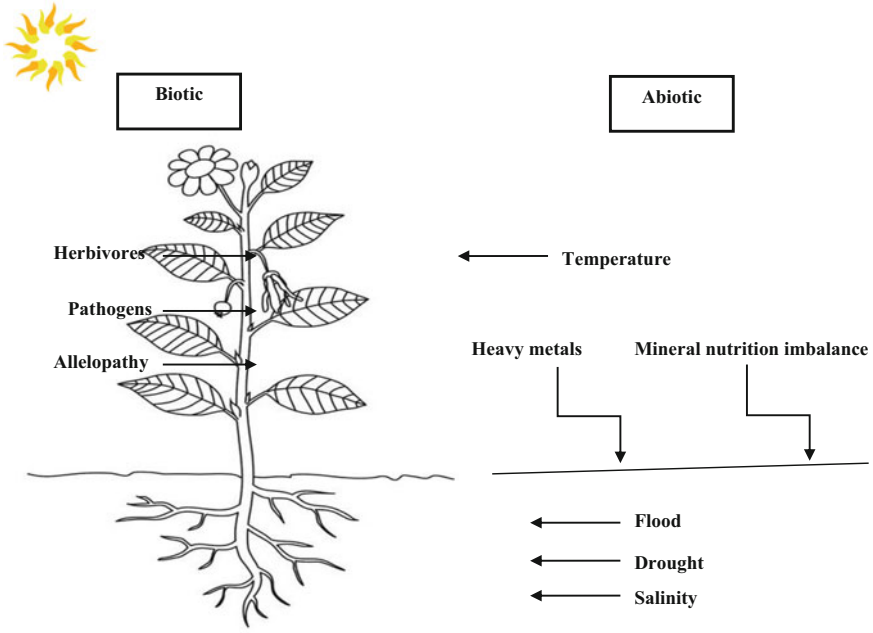

Fig. 22.2 Biotic and abiotic stress experienced by plants (Ahmad and Rasool 2014)

fights against *Fusarium oxysporum* f. sp. Basilica which is responsible for root-rot disease (Bhardwaj 2014). Many researchers have found that AMF is tolerant towards saline stress. AMF is capable of forming various plant growth hormones along with enhancing the uptake of nutrients. They can also make certain changes in the biochemical properties of the host plant (Evelin et al. 2009). A number of biotic factors are also responsible for creating stress in plants. The interaction between plants and endophytic fungi improves the health of the plant, enables it to fight against various biotic stresses, increases the productivity, and plays a crucial role in sustainable agriculture (Chadha 2015).

22.3 Nitrogen Fixing Biofertilizer: Mechanism and Growth Promotion

Nitrogen fixation through the use of nitrogenase complex has been confined as the potential of bacteria. Nitrogen being a critical compound in various molecules including vitamins, amino acids, proteins, and nucleic acids Apart from bacteria, eukaryotic organisms can utilize fixed nitrogen by the symbiotic association with prokaryotes that have the ability to fix nitrogen. The potential of prokaryotes to fix nitrogen and that of eukaryotes to utilize the fixed nitrogen by symbiotic association has enhanced the nitrogen bioavailability. Varying organisms like fungi, animals, protists, and plants act as host for symbiosis (Kneip 2007).

Fixation of molecular N_2 is an extensive process which involves varying organisms and biosphere and thus acts as a medium for the relationship among distinct species and their co-evolution including utmost evolutionary transformation and habituation which led to the creation of organelles within the cells of eukaryotic organisms, for e.g., mitochondria, plastids, chloroplasts, etc.

Origination of mitochondria was from an α-proteobacterial predecessor, which reduced in size in the course of evolution (Andersson 1998; Martin and Müller 1998). A merge between the cyanobacteria and a eukaryote has led to the descend of plastid, photoautotrophic eukaryotic cell organelle (Bhattacharya and Medlin 1995). Synthesis of ATP by the α-proteobacterialsymbiote has led to the co-evolution of mitochondria among the symbiotic partners while the demand for photosynthetic outcomes by plastids has led to the association among organisms. Acquisition of nitrogen from biomolecules as nutrition happens heterotrophically by fungi (Kneip 2007).

22.3.1 Association of Bacteria with Fungi

The host plant, nitrogen fixing bacterium, and Arbuscular Mycorrhizal fungi exhibit a tripartite symbiosis which impacts the nitrogen uptake by the plant. Since the N

and P are provided through the micro-symbionts of the symbiotic association to the plant, therefore the interaction among each micro-symbiont with the host plant as well as with each other affects their association with each other (Miransari 2011).

Association of symbionts with their hosts enables them to exist as endosymbionts intracellularly or to reside either extracellularly (Kneip 2007).

Mycorrhizal symbiotic association is affected by the architecture of roots, plant with deep roots led to an increased symbiotic rate with the fungi under low levels of P in comparison with plant having shallow roots, although at high levels of P nodulation was higher. These variations depict that the levels of N and P greatly impact the tripartite symbiotic association occurring within the plant and each micro-symbiont. In soybean plants, two micro-symbionts were co-inoculated resulting in increased growth of plant and formation of modules at lower levels of nitrogen and phosphorus while the elevated levels of fertilization negatively affected the growth of plant associated with the interaction between the micro-symbionts (Wang 2011; Miransari 2011).

In lichen symbioses, a mycobiont, i.e. fungal counterpart is associated with an extracellular photobiont. The photobionts are generally discrete photosynthetic algae, however, in lichens the cyanobacteria exist as photobionts either in association with algae as tripartite symbiosis or alone as bipartite symbiosis (Kneip 2007).

Photobionts supply carbon metabolites derived from photosynthesis to the fungal partner which acts as an advantage in this association. In addition to carbon, cyanobionts supply fixed N to the host. The morphological and physiological revamping of the association of lichen and cyanobacteria is exhibited by the fixed molecular nitrogen which includes an enhanced quantity of nitrogen fixing heterocysts in comparison with the free living, in symbiotic association with *Nostoc* sp. Tripartite symbiosis exhibits another adaptation where concentration of cyanobacteria in specific areas is seen which is called as Cephalodias, nitrogen fixation occurs in those areas as well as protects against high concentrations of oxygen. The photosynthesis within this tripartite symbiosis is confined to algal photobionts, and thus provides fixed carbon components to their partners (Honegger 2001). In particular, these cyanobionts are not transferred vertically and are considered to be free living in nature thus they are not considered obligate symbionts, therefore independent of the host metabolism (Kneip 2007).

The efficacy of the tripartite association among Rhizobium, Arbuscular mycorrhizal fungi and plants depends largely on the strife among these symbionts for carbon (Geneva 2006). Jakobsen and Rosendahl (1990) evaluated that approximately 20% of the overall fixed 14CO2 in young plants can be used by AM.

In the rhizospheric interactions, the most common is the arbuscular mycorrhizal (AM) symbiosis between fungi and plant roots (Smith et al. 1997). The fungus provides water and nutrients including phosphate to the plants while plants supply carbohydrates, generated through photosynthetic activity to the fungus. Bacteria belonging to the genus *Burkholderia* shelter intracellularly in the AM fungus *Gigaspora margarita* and thus provide fixed nitrogen (Bianciotto 1996; Minerdi 2001; Kneip 2007). The AM fungi integrated with legumes is a vital link for effective nutrition of phosphorus, thereby enhancing nitrogen fixation which favours

growth of root and mycorrhiza. In pea plants, a specific compatibility between AM fungi and *Rhizobium* was reported in pea plants (Geneva 2006).

In the mountains of Spessart, an enlightening discovery was made where at the hyphal tips of *Geosiphon pyriformis,* a fungal related to the AM fungi developed unicellular multinucleated "bladders". These bladders harboured *Nostoc punctiforme,* which played role in fixing carbon dioxide as a vital function of cyanobacterium in this symbiotic association. A heterocyst is also formed by the symbiont which hints that fixation of nitrogen also occurs (Kluge 2002).

The higher quantity of AM structures might be due to the colonization of *G. mosseae* and *G. intraradices* without and with *Rh. Leguminosarum*, in comparison to the variants which were inoculated with N-fixing bacteria only. The status manifestation of mycorrhizal fungi can be done in the variants having phosphorus at both levels and dual inoculation of *Rh. Leguminosarum* and *G. mosseae*, which corresponds to the evaluated N-fixing variables of plants grown without supplementary phosphorus. It was statistically observed that the variants exhibiting tripartite symbiotic association with pea plant, Arbuscular mycorrhizal fungi, *Rh. Leguminosarum*, and *G. mosseae* had maximum values (Geneva 2006).

22.3.2 Mechanism

Emergence of AM fungus mycelia from the root system enables acquisition of minerals and nutrients from the soil which are rather unreachable to roots (Smith et al. 2004; Berruti et al. 2016). However, it is the extra-radical hyphae of AM fungi that regulates the uptake of nutrients specifically N and P to the plants (George et al. 1995). The hyphae are very thin as compared to the roots of plants and thus they easily penetrate into minute pores (Allen 2011). The exchange of minerals, nutrients, and carbohydrates within the roots occurs at the interface of host plant and the fungal hyphae (Berruti et al. 2016).

The colonization of mycorrhizal fungi in turn modifies the characteristics of plants which play a role in nutrient uptake and thus, it impacts the accumulation of nutrients by the plants associated with mycorrhiza. The modifications include enhanced nutrition of phosphorus to mycorrhizal plants or might also be Phosphorus independent. However, the most common impact post colonization of mycorrhiza is modification of the ratio of root and shoot (George 1994a, 1994b) and the specific length of roots (George et al. 1995).

Colonization of the root cortex by the AM fungal hyphae and formation of highly branched edifices within the cells, i.e., arbuscules, which are deemed to be the operative site of exchange of nutrients (Balestrini 2015; Berruti et al. 2016). The colonization of mycorrhiza enhanced the percentage of amino acids and organic acids within the roots and the shoots of *Phleum pratense* (Clapperton and Reid 1992) while few amino acid concentrations were increased in *Plantago lanceolata* (Gange and West 1994; George et al. 1995).

The physiological as well as the molecular prospects of high propinquity phosphate transporters in AM fungi (Harrison and van Buuren 1995) were extensively examined on the basis of nutritional sense after their characterization (Harrison et al. 2002; Paszkowski 2002; Nagy 2005). AM fungi are capable of remarkably ameliorating the acquisition of mineral nutrients by plants, specifically under low-nutrient situations and it has been evidently depicted that plants exhibit an inorganic phosphorus uptake symbiotic pathway (Berruti et al. 2016).

It has been evident that the symbiotic association of AM fungi induces PI transporters expression in plants (Xie 2013; Walder et al. 2015). Apart from the enhanced acquisition and increase in plant Pi acquisition, regulation of arbuscule morphogenesis as well as sustenance of symbiosis has been depicted (Javot 2007; Yang 2012).

Accompanied with the Pi transporters, which play role in uptake via arbuscles (Harrison et al. 2002; Paszkowski 2002; Nagy 2005), mycorrhizal induced Ammonium transporters (AMT) are also recognized (Gomez 2009; Kobae 2010). The last step of mineral nutrient transport takes place at a membrane which is plant-derived, i.e. the periarbuscular membrane, which envelops the arbuscle. The transporters situated at this membrane can acquire nutrients from the apoplast of periarbuscule and are transported to the cortical cells (Javot 2011; Bapaume and Reinhardt 2012).

In *Medicago truncatula* and soybean, the AMTs are situated in the periarbuscular membrane, like the Pi transporters, MtPT4, initially shown in Medicago (Harrison et al. 2002), thereby depicting an important role in transport of ammonium to the cortical cells (Kobae 2010; Breuillin-Sessoms 2015; Berruti et al. 2016). The transport of nitrogen and other minerals is restricted from the fungi towards the plant due to the bio-membranes that divide the tissues of the plant and the fungi (George et al. 1995).

However, it was shown in cucumber that amino acid metabolizing enzymes were not exactly variating among the mycorrhizal zones and the non-mycorrhizal zones (Rosendahl 1992) and thus it was considered that N metabolism was not directly influenced by the colonization of mycorrhiza (George et al. 1995). In 2010, JW et al. showed that the association of mycorrhiza with the plant root system greatly contributed to greater than 50% of the nitrogen needs of the plant. Nitrogen being an essential macronutrient and plays a crucial role in growth of plant as well as the product yield, so it was appropriate to consider the involvement of AM fungi in uptake of N by the plant (Miransari 2011).

Arbuscular fungi have the ability to utilize nitrogen released from organic as well as inorganic sources. The AM fungi enter the plant tissues by means of vascular bundles during mineralization and thus utilize Ni liberated by the soil microorganisms (Aristizábal et al. 2004; Miransari 2011).

22.3.3 Nitrogen Fixation and Uptake

Mycorrhizal roots exhibit symbiotic association with bacteria having role in phosphorus and free-living bacteria having nitrogen fixing ability, thus they can increase

the uptake of P and N in cases where the soil has reduced supply of nutrients (George et al. 1995). A combined impact on uptake of nitrogen and fixation and growth due to the dual root colonization, in pea plant and soybean plant, between AM fungi and bacterium *Rhizobium* was relayed (Xavier and Germida 2003). In comparison with the roots lacking mycorrhizal association those with AM fungi acquire approx 4–20% more photosynthates (Geneva 2006).

In mycorrhizal legumes, under reduced P substrates, the increase in fixation of N is consistent as compared to control ones, i.e. non-mycorrhizal plants. The increase in fixation of N is generally related to the enhanced uptake of P by mycorrhizal plants (Reinhard 1994) and it has been quite strenuous to establish a direct link between colonization of mycorrhiza and formation of nodule or its functioning. Although the uptake of nutrients present in the soil but which are distant from the roots, through the extra-radical hyphae is the most efficient direct involvement of AM fungi to the growth of plant. The hyphal length to roots ratio of AM fungi in the soil is around 100: 1 and can be higher (George et al. 1995).

The plants with mycorrhizal association have increased ratio of P/N (Cuenca and Azcón 1994; Tobar et al. 1994b) as well as C/N (Gange and West 1994) as compared to non-mycorrhizal plants. The uptake of nitrogen that is distant from the roots through AM hyphae was first described by Ames (1983) using boxes which were divided into zones having growing roots and hyphae.

Several findings indicated that when nitrogen is provided in the form of ammonium and nitrate can be absorbed and translocated by the AM hyphae. Frey and Schüepp (1993) evaluated that 30% of the total uptake of N by the maize plant grown in chambered boxes was due to the uptake of nitrogen by the AM fungal hyphae. The hyphal soil chamber was provided with $(15NH_4)SO_4$ as N source which was absorbed up to 40% by the hyphae (George et al. 1995).

In few experiments, it was observed that soil containing ammonium and nitrates were depleted effectively by the hyphae of AM fungi (George 1992; Johansen et al. 1992). Experiments conducted in chambered boxes also led to the quantification of the N supplied to "receiver" plants from "donor" plants, like legumes that fix N, by AM fungal hyphae.

Tobar et al. (1994a) and Azcón et al. (2001); Azcón (2008) examined the efficacy of AM fungi on the % uptake of N upon fertilization of N at varying levels of N in soil. In comparison to the fertilization with P, AM fungi increased the concentration of N in plant, Nitrate reductase activity, content of proline in plants. At moderate levels of fertilization with N (6 mmol N), higher uptake of N was seen by the AM fungi with respect to the lower levels (3 mmol N) and higher levels (9 mmol N) which on the other hand reduced the uptake of N upon fertilization with N. These outcomes depicted that mycorrhizal plants can modulate the uptake of N in plants in relation to the quantity of N available in the soil (Miransari 2011).

Another scientist Tian (2010) showed that AM fungi has the ability to absorb organic as well as inorganic N. Amino acid, arginine are synthesized within the extra-radical hypha and are transported to the intra-radical hypha where N is released for absorption by the plant. Consequently, 11 fungal genes associated with

absorption pathway of nitrogen have been recognized and sequencing of six out of 11 have been done (Miransari 2011).

22.3.4 Plant Growth and Development

Interactions among the fungi and the host plant regulate the modification of the architectural and topological characteristics of the roots by the AMF which leads to extended or enhanced branching of the roots in turn increasing the efficiency of nutrient absorption (Gamalero et al. 2009). Inoculation of *Rh. Leguminosarum* into Pea plant and supply of enhanced levels of phosphorus evinced a considerable increase in N-fixation, number of nodules, and fresh biomass. Due to the enhanced levels of phosphorus, there was stimulation in dry biomass of plant and rate of photosynthesis (Geneva 2006).

However, Jia et al. (2004) described that inoculation of Arbuscular mycorrhizal fungi in *Vicia faba* enhanced the production of biomass and rate of photosynthesis due to the increased supply of P because of the inoculation of AM fungi. Few authors (Saxena et al. 1997) researched that the tripartite symbiotic association among varying cultures of AM fungi, green grams and *Bradyrhizobium japonicum* exhibited increase in growth, nodule formation, and significant levels of ARA for all the varying combinations of the association. Due to increase in the content of carbohydrate in roots through feedback effect led to the decrease in P concentration thereby reducing the rate of photosynthesis (Rychter and Randall 1994; Geneva 2006).

Apart from plant growth promotion, AMF also influences tolerance of plants to varying environmental stress conditions, especially tolerance to heavy metals (Leyval 2002). This enhancement of stress tolerance in the plants is due to the ameliorated supply of nutrients, to the association complex and to the unclear interactions among the AMF and the host plant. Presence of heavy metals at high concentrations lead to reduction, delay, or elimination of AM colonization thereby hampering the beneficial impact the association of mycorrhiza–plant has on the host plants (Gamalero et al. 2009).

The efficiency of mycorrhizal colonization in roots in regions of excessive pollution of heavy metals depends on the specific metals, like the colonization efficiency is not affected by Cd in 3 varying genotypes of pea (Rivera-Becerril 2002). Similarly, colonization of mycorrhiza in poplar plants and in *Pteris vittata,* by 2 AM fungi species, i.e. *Glomus mosseae and Gigaspora margarita,* is not influenced by copper and arsenic, respectively (Todeschini 2007; Trotta 2006).

22.4 Conclusion

It has been estimated that by 2050, the global population will increase by 9 billion; therefore, the agricultural sectors have to counter the situations of doubling the food production rate as well as minimizing the dependency on agrochemicals, such that the health standards of humans and environment are not hampered. This estimated increase in yield is much higher than the present capacity of food production global, thus emphasizing on the necessity to execute or revitalize eco-friendly techniques, namely biofertilizers based on AMF. AMF biofertilizers hold enormous potential still its implementation is not been completely carried out by the agricultural sectors. Inoculation of AMF has led to positive results on the production of plants specifically due to the benefits associated with nutrition provided to the host plants by this fungal symbiont class. AM fungi affect the cycling of nitrogen, growth of plants, and functioning of the ecosystem by influencing the dynamics of nitrogen in the soil system and the uptake of nitrogen by the plants. The N uptake structures of AMF have huge affinity for N uptake through the soil but the regulation of the expression of N transporters of AMF is still unspecified. However, the transcription of few transporters is induced by substrate and regulation by the supply of NH_4^+.

The stable implication of AMF in agricultural practices in order to implement large-scale and multiple location trials with cost-effective procedures and analysis, thus creating increased awareness of the benefits of AMF inoculation as biofertilizer among the prospective end-users. Additionally, these native AM fungi have shown to perform better as compared to the commercial ones as well as from the cultured isolates which has encouraged the farmers to independently culture their AMF inoculum from the indigenous soil. Thus, making the bio-fertilization technique much more affordable, reliable, and fruitful for farmers, especially in the developed countries which prefer highly sustainable cropping methods.

References

Ahmad P, Rasool S (2014) Emerging technologies and management of crop stress tolerance: Volume 2-A sustainable approach. Academic Press

Ajmani A, Kumar R, Bhargava P, Vats S (2019) Mathematically optimized production, purification and characterization of Penicillin G Acylase from AA17A and AA17B: an industrial biocatalyst for production of aminopenicillanic acid a core moiety of different penicillins. Indian J Biotechnol 18:260–268

Allen MF (2011) Linking water and nutrients through the vadose zone: a fungal interface between the soil and plant systems. 3(3):155–163

Ames RN (1983) Hyphal uptake and transport of nitrogen from two 15N-labelled sources by Glomusmosseae, a vesicular-arbuscular mycorrhizal fungus. New Phytol 95(3):381–396

Andersson SGE (1998) The genome sequence of rickettsia prowazekii and the origin of mitochondria. Nature 396(6707):133–140

Aristizábal C, Rivera EL, Janos DP (2004) Arbuscular mycorrhizal fungi colonize decomposing leaves of Myricaparvifolia, M. pubescens and Paepalanthus sp. Mycorrhiza 14(4):221–228

Azcón R (2008) Uptake and metabolism of nitrate in mycorrhizal plants as affected by water availability and N concentration in soil. Eur J Soil Sci 59(2):131–138
Azcón, R., J. M. Ruiz-Lozano, and R. Rodriguez (2001). "Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (15N) under increasing N supply to the soil." Can J Bot 79.10 (2001): 1175–1180
Balestrini R (2015) Plant-soil biota interactions. Soil Microbiol Ecol Biochem:311–338
Bapaume L, Reinhardt D (2012) How membranes shape plant symbioses: signaling and transport in nodulation and arbuscular mycorrhiza. Front Plant Sci 3:223
Bent E (2006) Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). In: Multigenic and induced systemic resistance in plants. Springer, Boston, pp 225–258
Berruti A et al (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front Microbiol 6:1559
Bhardwaj D (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Factories 13(1):1–10
Bhargava P, Gupta N, Vats S, Goel R (2017) Health issues and heavy metals. Austin J Environ Toxicol 3(1):3018
Bhargava P, Khan M, Verma A, Singh A, Singh S, Vats S, Goel R (2019a) Metagenomics as a tool to explore new insights from plant-microbe interface. In: Plant microbe interface. Springer, Cham, pp 271–289
Bhargava P, Vats S, Gupta N (2019b) Metagenomics as a tool to explore Mycorrhizal fungal communities. In: Mycorrhizosphere and pedogenesis. Springer, Singapore, pp 207–219
Bhargava P, Gupta N, Kumar R, Vats S (2020) Plants and microbes: bioresources for sustainable development and biocontrol. In: Plant microbe symbiosis. Springer, Cham, pp 153–176
Bhattacharya D, Medlin L (1995) The phylogeny of plastids: a review based on comparisons of small-subunit ribosomal RNA coding regions. J Phycol 31(4):489–498
Bianciotto V (1996) An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Appl Environ Microbiol 62(8):3005–3010
Breuillin-Sessoms F (2015) Suppression of arbuscule degeneration in Medicago truncatula phosphate transporter4 mutants is dependent on the ammonium transporter 2 family protein AMT2; 3. Plant Cell 27(4):1352–1366
Chadha N (2015) An ecological role of fungal endophytes to ameliorate plants under biotic stress. Arch Microbiol 197(7):869–881
Chandanie WA, Kubota M, Hyakumachi M (2006) Interactions between plant growth promoting fungi and arbuscular mycorrhizal fungus Glomusmosseae and induction of systemic resistance to anthracnose disease in cucumber. Plant Soil 286(1–2):209–217
Clapperton MJ, Reid DM (1992) Effects of low-concentration Sulphur dioxide fumigation and vesicular–arbuscularmycorrhizas on 14C-partitioning in Phleumpratense L. New Phytol 120 (3):381–387
Cuenca G, Azcón R (1994) Effects of ammonium and nitrate on the growth of vesicular-arbuscular mycorrhizal Erythrina poeppigiana OI cook seedlings. Biol Fertil Soils 18(3):249–254
Davis KF (2016) Meeting future food demand with current agricultural resources. Glob Environ Chang 39:125–132
Elsharkawy MM (2012) The plant growth-promoting fungus Fusarium equiseti and the arbuscular mycorrhizal fungus Glomusmosseae induce systemic resistance against cucumber mosaic virus in cucumber plants. Plant Soil 361(1–2):397–409
Evelin H, Kapoor R, Giri B et al (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104(7):1263–1280
Frey B, Schüepp H (1993) Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with Zea mays L. New Phytol 124(2):221–230
Gamalero E et al (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55(5):501–514

Gange AC, West HM (1994) Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in Plantagolanceolata L. New Phytol 128(1):79–87
Geneva M (2006) The effect of inoculation of pea plants with mycorrhizal fungi and rhizobium on nitrogen and phosphorus assimilation. Plant Soil Environ 52(10):435
George E (1992) Water and nutrient translocation by hyphae of Glomusmosseae. Can J Bot 70 (11):2130–2137
George E (1994a) VA mycorrhiza: benefits to crop plant growth and costs. In: Expanding the production and use of cool season food legumes. Springer, Dordrecht, pp 832–846
George E (1994b) Contribution of mycorrhizal fungi to micronutrient uptake by plants. Biochem Micronutr Rhizosph:93–103
George E, Marschner H, IverJakobsen (1995) Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit Rev Biotechnol 15(3–4):257–270
Goel R, Bhargava P, Gupta N, Vats S (2017) Health issues and heavy metals. Austin J Environ Toxicol 3(1):1018
Gomez SK (2009) Medicago truncatula and Glomus intraradices gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. BMC Plant Biol 9(1):10
Gupta N, Vats S, Bhargava P (2018) Sustainable agriculture: role of metagenomics and metabolomics in exploring the soil microbiota. In: In silico approach for sustainable agriculture. Springer, Singapore, pp 183–199
Harrison MJ, van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus Glomus versiforme. Nature 378(6557):626–629
Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from Medicago truncatula involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14 (10):2413–2429
Honegger R (2001) The symbiotic phenotype of lichen-forming ascomycetes. In: Fungal associations. Springer, Berlin, pp 165–188
Hossain MM, Sultana F, Islam S (2017) Plant growth-promoting fungi (PGPF): phytostimulation and induced systemic resistance. In: Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore, pp 135–191
Ilyas N, Bano A (2012) Potential use of soil microbial community in agriculture. In: Bacteria in agrobiology: plant probiotics. Springer, Berlin, pp 45–64
Jain P, Miglani K, Vats S (2011) Aptamers-potential applications in diagnostics and therapeutics. Everyman's Sci 361, volume XLV no. 6, Feb-Mar 11
Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115(1):77–83
Javot H (2007) A Medicago truncatula phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci 104(5):1720–1725
Javot H (2011) Medicago truncatula mtpt4 mutants reveal a role for nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal symbiosis. Plant J 68(6):954–965
Jia Y, Gray VM, Straker CJ (2004) The influence of rhizobium and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by Viciafaba. Ann Bot 94(2):251–258
Johansen A, Jakobsen I, Jensen ES (1992) Hyphal transport of 15N-labelled nitrogen by a vesicular—arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol 122(2):281–288
Kaur A, Vats S, Rekhi S, Bhardwaj A, Goel J, Tanwar RS, Gaur KK (2010) Physico-chemical analysis of the industrial effluents and their impact on the soil microflora. Procedia Environ Sci 2:595–599
Khan MS, Zaidi A, Musarrat J (2010) Microbes for legume improvement. Springer, Vienna
Kluge M (2002) The nostoc-geosiphonendocytobiosis. In: Cyanobacteria in symbiosis. Springer, Dordrecht, pp 19–30
Kneip C (2007) Nitrogen fixation in eukaryotes–new models for symbiosis. BMC Evol Biol 1(55):7
Kobae Y (2010) Localized expression of arbuscularmycorrhiza-inducible ammonium transporters in soybean. Plant Cell Physiol 51(9):1411–1415

Koike N (2001) Induction of systemic resistance in cucumber against several diseases by plant growth-promoting fungi: lignification and superoxide generation. Eur J Plant Pathol 107 (5):523–533

Kour D (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Recent advancement in white biotechnology through fungi. Springer, Cham, pp 1–64

Kumar N, Balamurugan A, Shafreen MM, Rahim A, Vats S, Vishwakarma K (2020a) Nanomaterials: emerging trends and future prospects for economical agricultural system. In: Biogenic Nano-particles and their use in agro-ecosystems. Springer, Singapore, pp 281–305

Kumar N, Srivastava P, Vishwakarma K, Kumar R, Kuppala H, Maheshwari SK, Vats S (2020b) The rhizobium–plant Symbiosis: state of the art. In: Plant microbe symbiosis. Springer, Cham, pp 1–20

Leyval C (2002) Potential of arbuscular mycorrhizal fungi for bioremediation. In: Mycorrhizal technology in agriculture. Birkhäuser, Basel, pp 175–186

Li R (2017) Chemical, organic and bio-fertilizer management practices effect on soil physicochemical property and antagonistic bacteria abundance of a cotton field: implications for soil biological quality. Soil Tillage Res 167:30–38

Mahanty T (2017) Biofertilizers: a potential approach for sustainable agriculture development. Environ Sci Pollut Res 24(4):3315–3335

Maheshwari DK (2012) Bacteria in agrobiology: plant probiotics. Springer Science & Business Media, Berlin

Malusà E, Pinzari F, Canfora L (2016) Efficacy of biofertilizers: challenges to improve crop production. In: Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 17–40

Martin W, Müller M (1998) The hydrogen hypothesis for the first eukaryote. Nature 392 (6671):37–41

Maurya DP, Vats S, Rai S, Negi S (2013) Optimization of enzymatic saccharification of microwave pretreated sugarcane tops through response surface methodology for biofuel. Indian J Exp Biol 51:992–996

Maurya DP, Singh D, Vats S (2014) Cellulase production and utilization. In: Jian A (ed.) Category-chemical technology, Publishing House-LAP LAMBERT Academic Publishing. p 80

Mia MAB (2010) Rhizobacteria as bioenhancer and biofertilizer for growth and yield of banana (Musa spp. cv.'Berangan'). Sci Hortic 126(2):80–87

Minerdi D (2001) Nitrogen fixation genes in an endosymbiotic Burkholderia strain. Appl Environ Microbiol 67(2):725–732

Miransari M (2011) Arbuscular mycorrhizal fungi and nitrogen uptake. Arch Microbiol 193 (2):77–81

Mishra V (2016) Synergistic effects of Arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in bioremediation of iron contaminated soils. Int J Phytoremediation 18 (7):697–703

Murali M (2012) Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease. J Phytol

Nagy R (2005) The characterization of novel mycorrhiza-specific phosphate transporters from Lycopersicon esculentum and Solanum tuberosum uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. Plant J 42(2):236–250

Naznin HA (2013) Analysis of volatile organic compounds emitted by plant growth-promoting fungus Phoma sp. GS8-3 for growth promotion effects on tobacco. Microbes Environ 28 (1):42–49

Negi S, Vats S (2013) Pine forest litter based bio-refinery for biofuels and value-added phytochemicals. In: Singh RS, Pandey A, Larroche C (eds) Advances in industrial biotechnology. IK International Publishing House Pvt Ltd., pp 98–116

Nzanza B, Marais D, Soundy P (2011) Tomato (Solanum lycopersicum L.) seedling growth and development as influenced by Trichoderma harzianum and arbuscular mycorrhizal fungi. Afr J Microbiol Res 5(4):425–431

Ojha AK, Forster S, Kumar S, Vats S, Negi S, Fischer I (2013) Synthesis of well–dispersed silver nanorods of different aspect ratios and their antimicrobial properties against gram positive and negative bacterial strains. J Nanobiotechnol 11:42

Painuly AS, Gupta R, Vats S (2019) Bio-accumulation of arsenic (III) using Nelumbo Nucifera Gaertn. J Health Pollut 9:190902

Paszkowski U (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci 99(20):13324–13329

Reinhard S (1994) Influence of phosphorus supply and light intensity on mycorrhizal response in Pisum-Rhizobium-Glomus symbiosis. Experientia 50(10):890–896

Rivera-Becerril F (2002) Cadmium accumulation and buffering of cadmium-induced stress by arbuscularmycorrhiza in three Pisum sativum L. genotypes. J Exp Bot 53(371):1177–1185

Rodriguez RJ, Redman RS, Henson JM (2004) The role of fungal symbioses in the adaptation of plants to high stress environments. Mitig Adapt Strateg Glob Chang 9(3):261–272

Rosendahl S (1992) Influence of three vesicular-arbuscular mycorrhizal fungi (Glomaceae) on the activity of specific enzymes in the root system of Cucumis sativus L. Plant Soil 144(2):219–226

Rychter AM, Randall DD (1994) The effect of phosphate deficiency on carbohydrate metabolism in bean roots. Physiol Plant 91(3):383–388

Saxena AK, Rathi SK, Tilak KVBR (1997) Differential effect of various endomycorrhizal fungi on nodulating ability of green gram by Bradyrhizobium sp.(Vigna) strain S24. Biol Fertil Soils 24 (2):175–178

Saxena P, Srivastava J, Pandey S, Srivastava S, Maurya N, Kaushik NC, Mishra S, Asthana G, Bhargava P, Kumar R, Vats S (2019) Plants for biocontrol and biological control of plant pathogens. In: Plant biotic interactions. Springer, Cham, pp 147–179

Sharma KM, Kumar R, Siddharth V, Gupta A (2014) Production, partial purification and characterization of alkaline protease from Bacillus aryabhattai K3. Int J Adv Pharm Biol Chem 3:290–298

Sharma D, Javed S, Arshilekha PS, Babbar P, Shukla D, Srivastava P, Vats S (2018) Food additives and their effects: a mini review. Int J Curr Res 10(6):69999–70002

Shen Z (2015) Effect of biofertilizer for suppressing Fusarium wilt disease of banana as well as enhancing microbial and chemical properties of soil under greenhouse trial. Appl Soil Ecol 93:111–119

Shivanna MB, Meera MS, Hyakumachi M (1996) Role of root colonization ability of plant growth promoting fungi in the suppression of take-all and common root rot of wheat. Crop Prot 15 (6):497–504

Singh M (2016) Role of biofertilizers in conservation agriculture. In: Conservation agriculture. Springer, Singapore, pp 113–134

Singh M, Vats S (2019) Mathematically designed bioprocess for release of value added products with pharmaceutical applications from wastes generated from spices industries. Int J Pharm Sci Res 10:130–138

Smith SE, Read DJ, Last FT (1997) Mycorrhizal symbiosis. Ann Bot 5(701):80

Smith SE, Andrew Smith F, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytol 162(2):511–524

Srivastava S (2019) Biotechnological role of fungal microbes in sustainable agriculture. Plant Arch 19(1):107–110

Tandon S, Vats S (2016) Microbial biosynthesis of cadmium sulfide (Cds) nanoparticles and their characterization. Eur J Pharm Med Res 3:545–550

Tian C (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiol 153 (3):1175–1187

Tobar R, Azcón R, Barea JM (1994a) Improved nitrogen uptake and transport from 15N-labelled nitrate by external hyphae of arbuscularmycorrhiza under water-stressed conditions. New Phytol 126(1):119–122

Tobar RM, Azcón R, Barea JM (1994b) The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscularmycorrhizae. Mycorrhiza 4(3):105–108

Todeschini V (2007) Responses to copper of two registered poplar clones inoculated or not with arbuscular mycorrhizal fungi. Caryologia 60(1–2):146–155

Trotta A (2006) Arbuscularmycorrhizae increase the arsenic translocation factor in the as hyperaccumulating fern Pteris vittata L. Chemosphere 65(1):74–81

Tuzun S (2006) In: Tuzun S, Bent E (eds) Multigenic and induced systemic resistance in plants. Springer, New York

Vats S (2017) Methods for extractions of value-added Nutraceuticals from Lignocellulosic wastes and their health application. Ingredients Extraction by Physicochemical Methods in Food. pp 1–64

Vats S, Bhargava P (2017a) Alternate energy: fuel for “Modi’s India” and “smart cities.”. Int J Curr Res 9:49090–49097

Vats S, Bhargava P (2017b) Alternate energy: fuel for “Modi's India” and “smart cities”. Int J Curr Res 9:49090–49097

Vats S, Gupta N, Bhargava P (2019) Vulnerability of soil micro biota towards natural and anthropogenic induced changes and loss of pedospheric functionality. In: Mycorrhizosphere and pedogenesis. Springer, Singapore, pp 191–205

Vats S, Kumar R (2015) Amylolytic-extremoenzymes: saviour of environments. Eur J Biomed Pharm Sci 2:694–702

Vats S, Kumar R, Negi S (2012) Natural food that meet antibiotics resistance challenge: in vitro synergistic antimicrobial activity of Azadirachta indica, Terminalia chebula, Piper nigrum and photoactivated cow urine. Asian J Pharm Biol Res (AJPBR) 2(2)

Vats S, Miglani K (2011) Synergistic antimicrobial effect of cow urine and Azadirachta indica on infectious microbes. Int J Pharm Sci Res 2:1781

Vats S, Mishra A (2016) Soil agro-ecological management by Vermicompost a potential organic nutrient source for the state of Uttar Pradesh. Eur J Pharm Med Res 3:604–609

Vats S, Negi S (2013) Use of artificial neural network (ANN) for the development of bioprocess using Pinus roxburghii fallen foliages for the release of polyphenols and reducing sugars. Bioresour Technol 140:392–398

Walder F, Brulé D, Koegel S, Wiemken A, Boller T, Courty P-E (2015) Plant phosphorus acquisition in a common mycorrhizal network: regulation of phosphate transporter genes of the Pht1 family in sorghum and flax

Wang X (2011) Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza 21 (3):173–181

Wang H-y (2015) Preparation and utilization of phosphate biofertilizers using agricultural waste. J Integr Agric 14(1):158–167

Weber OB (2014) Biofertilizers with arbuscular mycorrhizal fungi in agriculture. In: Mycorrhizal Fungi: use in sustainable agriculture and land restoration. Springer, Berlin, pp 45–66

Wu SC (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125(1–2):155–166

Xavier LJC, Germida JJ (2003) Selective interactions between arbuscular mycorrhizal fungi and rhizobium leguminosarum bv. Viceae enhance pea yield and nutrition. Biol Fertil Soils 37 (5):261–267

Xie X (2013) Functional analysis of the novel mycorrhiza-specific phosphate transporter AsPT1 and PHT1 family from Astragalus sinicus during the arbuscular mycorrhizal symbiosis. New Phytol 198(3):836–852

Xiong W (2017) Bio-fertilizer application induces soil suppressiveness against Fusarium wilt disease by reshaping the soil microbiome. Soil Biol Biochem 114:238–247

Yang S-Y (2012) Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the phosphate transporter1 gene family. Plant Cell 24(10):4236–4251

Zhang S, Gan Y, Xu B (2016) Application of plant-growth-promoting fungi Trichoderma longibrachiatum T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Front Plant Sci 7:1405

Chapter 23
Iron Toxicity and Its Relation to Nitrogen and Phosphorus Availability in Ectomycorrhizal Fungi

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Abstract Iron (Fe) is essential for the maintenance of processes such as respiration and photosynthesis, thus being an important element for the plant production of cultivated or natural species. However, the combination of Fe and the acidity of acid rain creates favourable environments for the occurrence of oxy-reduction reactions, which transform Fe^{3+} into soluble forms (Fe^{2+}), increasing the availability of this metal in the soil, causing direct and indirect effects on plant growth, development, and productivity. Fe can also affect the availability of other nutrients in the soil like nitrogen (N) and phosphorus (P), directly influencing plant growth. At an optimum concentration, Fe can increase the availability of N, while in high concentrations, it does not alter N content. However, Fe has a high affinity for P and in acid soils this metal is commonly found in high concentrations, thus Fe usually binds to P, making this macronutrient unavailable for uptake by the plant, thereby negatively affecting the plant's metabolism. To promote the recovery of environments contaminated by toxic levels of Fe, the use of mycorrhizal associations, such as ectomycorrhizae, is considered a promising practice. Ectomycorrhizal (ECM) fungi developed three Fe absorption mechanisms to prevent its insolubility and toxicity: acidification of the medium, reduction of ferric to ferrous form, and secretion of iron-chelating molecules (melanins and siderophores). However, for host plants to obtain the benefits of

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fungi under conditions of Fe toxicity, it is necessary to carry out in vitro studies, using several sources of this metal. It is also extremely important to analyse the relationship of Fe with other nutrients in the medium, mainly N and P, since ECM fungi have intra and interspecific variations, and their growth is influenced by several abiotic factors.

Keywords Ectomycorrhizal fungus · Nutrient · Heavy metal · Iron sources · Mechanisms · Bioavailability

23.1 Introduction

Soils contaminated by metals have become a global environmental problem due to anthropogenic activities, with the highest emissions occurring during the production of steel and iron, and the combustion of fossil fuels (Sharma and Reddy 2004). Some heavy metals such as iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), cobalt (Co), and manganese (Mn) are essential micronutrients for life in ecosystems, but in high concentrations can be extremely toxic to different organisms (Ott et al. 2002). Thus, emissions of air pollutants by Fe industries can affect the surrounding vegetation at various levels of organization, from isolated individuals to entire populations of various species (Kuki et al. 2008; Neves et al. 2009).

Fe is one of the most abundant elements in the soil and one of the main pollutants emitted by mining and ore processing activities (Neves et al. 2009). In higher plants, excess Fe can cause ecophysiological damage and various nutritional disorders (Chen et al. 2015; Krohling et al. 2016), and depending on the magnitude of the impact, the intensity and frequency exposure, as well as the susceptibility and stage of development of plant species, it can reduce or even eliminate them from ecosystems close to polluting sources (Wong et al. 1978; Lopes et al. 2000; Rocha et al. 2014). In these conditions, the acidity of the soil caused by acid rain makes Fe highly available for root absorption (Hell and Stephan 2003; Ricachenevsky and Sperotto 2014), which can lead to its accumulation in phytotoxic levels in plant tissues (Pugh et al. 2002; Silva and Oliva 2006).

One of the major problems caused by high Fe concentrations in the soil is the nutritional imbalance that can be caused in plants, inducing the deficiency of some essential minerals such as nitrogen (N), phosphorus (P), and magnesium (Mg), compromising plant growth and development (Rengel 2015). This problem can be aggravated if this metal precipitates around the plant roots, forming a layer of Fe oxide that is commonly called “iron plate”, that physically limits the nutrient uptake, causing even more physiological problems for plants (Audebert and Fofana 2009).

In soil aerobic conditions, Fe is present in the form of oxides and hydroxides, in the form of organic chelates or in the form of ferric ion (Fe^{3+}) which are insoluble (Neubauer et al. 2013). Therefore, the concentration of Fe in the soil solution is extremely low in most soils and, consequently, plants and microorganisms have developed mechanisms to mobilize it and allow its uptake (Hell and Stephan 2003;

Philpott 2006; Kim and Lou 2007). Among the microorganisms with several mechanisms of Fe mobilization, ectomycorrhizal (ECM) fungi stand out, due to their capacity of increase plant growth in the presence of toxic metals, accumulating these contaminants in their hyphae and reducing the uptake by the plant (Ray et al. 2005; Khade and Adholeya 2008). Thus, the introduction of these microorganisms in contaminated soils is a promising strategy to increase the plant's tolerance to high Fe availability and recover areas impacted by mining activities (Landeweert et al. 2001; Crane et al. 2010; Lermen et al. 2015).

Ectomycorrhizae are beneficial associations between soil fungi and the roots of forest plants. In this relationship, the plant provides the fungus with carbon, an essential element for fungal development, and the fungus increases the uptake of water and nutrients, especially P and N (Smith and Read 2008). In addition to improving soil structure, mycorrhizae may offer resistance to soil pathogens (Chakravarty and Unestam 1987), to abiotic stresses such as drought (Osonubi et al. 1991), or to the accumulation of toxic elements such as metals (Colpaert and Van Assche 1993). The effectiveness of a diversity of associations between fungi and plants, in relation to their growth on substrates containing metals, has been tested and proven for several metals, which has been related to a series of extra and intracellular fungal mechanisms (Gadd 1993).

The plant-mycorrhizal fungus relationship is specific and can be influenced by several factors such as competition with other microorganisms, availability of nutrients, pH, humidity, and temperature (Augé 2001; Alves et al. 2001; Tagu et al. 2002). Therefore, these factors must be studied in order to obtain a more efficient plant-fungus relationship. In addition, the mechanisms involved in conferring increased tolerance to heavy metals have proved difficult to resolve, as different species or isolates of the same fungal species may present several responses to various types of contaminants (Krpata et al. 2008; Bojarczuk and Kieliszewska-rokicka 2010; Reis et al. 2012). For this reason, the use of different sources of a contaminant can elucidate the mechanisms used by ECM fungi to absorb nutrients and avoid toxicity of metals.

23.2 Iron in the Soil and in Plants

Fe is the second most abundant transition metal in the lithosphere and the fourth most abundant element in the Earth's crust after oxygen, silicon, and aluminium (Pantopoulos and Hentze 2000; Johnson et al. 2012).

In the environment, Fe can exist in several oxidation states, but the main forms that occur naturally in the soil are ferrous (Fe^{2+}) and ferric (Fe^{3+}), which are highly dependent on the redox state of the system (Ilbert and Bonnefoy 2013). The ferric form is generally the most abundant, but it has low solubility, so is less available for absorption. The bioavailability of Fe sources is mainly influenced by the pH, whereas soluble forms predominate in acidic pHs, and insoluble forms predominate in basic pHs (Kim and Lou 2007; Ricachenevsky and Sperotto 2014). The

bioavailability of Fe is also limited due to the formation of oxides and hydroxides, which are insoluble in aerobic conditions (Arantes and Milagres 2007; Marschner et al. 2011).

Despite the low availability in most soils, in some environments, such as wetlands and soils with high influence from mining plants, soluble Fe can be highly available, becoming toxic. Excess free Fe is harmful to living cells as it generates cytotoxic hydroxyl radicals, and only small amounts of essential metals are tolerated within the cell as free ions. This can be applied to all metals, but especially for redox-ions of active metals such as Cu and Fe, which are capable of causing the formation of hydroxyl radicals and other types of oxidative damage (Jeong and Guerinot 2009; Ravet et al. 2009).

This metal has a high biological demand and is essential for both eukaryotic and prokaryotic beings (Johnson 2008; Kobayashi and Nishizawa 2012), since it is directly involved in fundamental processes such as photosynthesis, respiration, nitrogen fixation, DNA, and hormone synthesis (Gurzau et al. 2003; Vigani 2012). Fe homeostasis is extremely important and is influenced by plant species or genotype, the location (type of soil), time of year, age of the plant and the organ or tissue of the plant (Buchanan et al. 2000). Therefore, the balance of Fe must be strictly controlled, as both deficiency and toxicity affect the development (Kuki et al. 2008; Briat et al. 2010) and several physiological process of plants (Pereira et al. 2013).

Plants have specific mechanisms for Fe uptake in order to supply its demand for growth and development. Under normal conditions, with a sufficient concentration of Fe in the soil, plants reduce Fe^{3+} to Fe^{2+} through the plasma membrane via a low affinity transporter (Curie and Briat 2003). Under aerobic conditions, the concentration of Fe^{2+} in the soil solution is usually low, thus, in conditions of Fe deficiency, plants have developed different strategies to increase the uptake of this nutrient. According to Römheld (1987) and Marschner and Rohmëld (1994) these strategies were called Strategy I (reduction of Fe^{3+} to Fe^{2+}) and Strategy II (chelation of Fe^{3+}) (reviewed in Krohling et al. 2016).

However, in acidic soils, where the low pH increases the availability of Fe^{2+} (Lemanceau et al. 2009; Fodor et al. 2012), this metal ceases to be an essential nutrient to become a toxic element, being potentially harmful to the cell due to the formation of free radicals generated by the Fenton reaction (Ravet et al. 2009; Kobayashi and Nishizawa 2012; Pereira et al. 2013). The excess of Fe can also cause nutritional imbalances in plants, inducing deficiency of some essential minerals such as P, calcium (Ca), potassium (K), Mg, and Zn, it can damage the photosynthetic apparatus, reduce the chlorophyll content, cause oxidative stress due to the production of reactive oxygen species, chlorosis in old leaves, browning of the roots and inhibition of plant growth (Connolly and Guerinot 2002; Suh et al. 2002; Chatterjee et al. 2006; Sahrawat 2005; Audebert and Fofana 2009).

The concentrations of Fe in the plant vary between 10 and 1500 mg kg^{-1} of dry matter, depending on the part of the plant and the species, considering concentrations between 50 and 100 mg kg^{-1} as adequate for normal plant growth (Dechen and Nachtigall 2006). However, to achieve toxic levels, the concentrations can depend on the species, cultivar, physiological state and growth conditions (Mahender et al.

2019). In plants of *Solanum tuberosum* L., values between 61 and 134 mg of Fe kg^{-1} of dry matter are considered toxic (Chatterjee et al. 2006), but in *Eugenia uniflora* L., the toxicity symptoms were only detected in the concentrations from 120 to 229 mg of Fe kg^{-1} of dry matter (Jucoski et al. 2016).

In order to survive in conditions of Fe toxicity, plants developed morphological and physiological mechanisms to prevent or even tolerate such conditions, which are: strategy I, a mechanism for the exclusion of Fe^{2+} at the root level, by oxidation and formation of the ferric plaque in the rhizosphere; strategy II, mechanisms of inclusion, sequestration in vacuoles, apoplast, and ferritin proteins; strategy III, mechanism of tolerance or inactivation of Fe in plant tissues, detoxification by action of antioxidant enzymes; strategy IV, symbiotic association with microorganisms (Becker and Asch 2005; Majerus et al. 2007, 2009).

23.3 Mechanisms of Iron Tolerance by Ectomycorrhizal Fungi

Contaminated soils can be remedied by physical (soil removal and treatment) or biological techniques (Khan et al. 2000; Ali et al. 2013). Both are widely used, however, the biological technique preserves useful microorganisms such as solubilizing bacteria and mycorrhizal fungi, as well as the existing fauna, maintaining the ecological balance and soil functionality (Altieri 1999; Finlay 2008; Kuki et al. 2008). The use of microorganisms associated with vascular plants can be a promising alternative in the recovery of environments contaminated by this pollutant (Landeweert et al. 2001; Crane et al. 2010; Lermen et al. 2015).

In this context, one of the factors that allow tolerant plants to survive in contaminated areas is the colonization of their roots by mycorrhizal fungi. During this symbiosis, the host plant has increased growth, productivity, greater capacity to absorb water and nutrients from the soil and reduced damage caused by pathogens, and in exchange the fungus receive carbon originated from the photosynthetic process (St-Arnaud and Vujanovic 2007; Smith and Read 2008; Ismail and Hijri 2012). About 80% of plants exposed to conditions of environmental stress are colonized by mycorrhizal fungi (Smith and Read 2008), because the fungus-plant association considerably reduces the uptake of heavy metals by plant cells (Weissenhorn et al. 1995; Leyval et al. 1997; Ouziad et al. 2005; Hildebrandt et al. 2007).

There are several types of mycorrhizae and among them, ECM fungi have stood out due to their ability to alleviate metal toxicity for a host plant, being crucial for the sustainability of terrestrial ecosystems, not only because of their presence, but because of their genetic and functional diversity (Domínguez-Núñez and Albanesi 2019). The mechanisms involved in increasing this tolerance are diverse and have a high specificity between different types of metals, their concentration in the soil, the associated fungi and the growth of plants (Hartley et al. 1997; Hildebrandt et al.

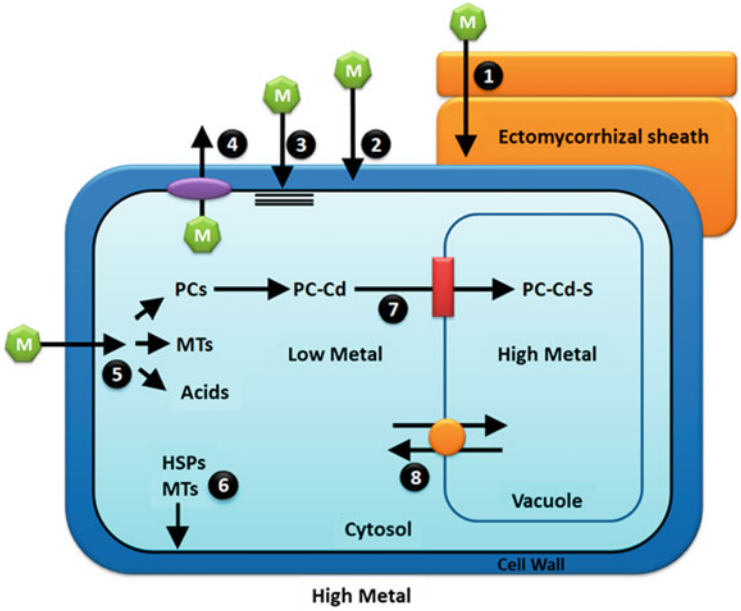

Fig. 23.1 Summary of potential detoxification mechanisms of heavy metals in a fungal cell. (1) Mantle acting as a physical barrier, (2) biosorption of the cell wall and root exudates, (3) decreased flow through the plasma membrane, (4) efflux of metals through membrane proteins, (5) chelation of metals in the cytosol by compounds such as phytochelatins (PCs), metallothioneins (MTs) or acids, (6) repair and protection of plasma membrane under stress conditions, (7) transport of the PC-metal complex into the vacuole, and (8) transport and accumulation of metals in the vacuole. HSPs: Heat shock proteins. (Adapted from Hall 2002)

2007). It is possible that the protection of ECM fungi against heavy metals is due to the extracellular fungal mycelium acting as a physical protective barrier preventing the metal uptake by the host plant (Khan et al. 2000). There are also biological mechanisms, which include extracellular precipitation processes, cell wall biosorption through ion exchange, adsorption, complexation and crystallization, and internal processes in fungal cells, where metals can be complexed, compartmentalized or volatilized (Gadd 1993; Colpaert et al. 2011). All of these mechanisms are summarized in Fig. 23.1.

Due to the redox properties of Fe, microorganisms have developed mechanisms for the maintenance and preservation of Fe homeostasis to prevent both a deficiency (Quatrini et al. 2005; Legay et al. 2012) and possible oxidative stress (Touati 2000; Philpott 2006; Haas et al. 2008; Adeleke et al. 2012). Fungi developed three distinct mechanisms for solubilization and Fe uptake: (1) acidification of the medium, (2) reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which is the most soluble form, and (3) the secretion of soluble Fe-chelators molecules (siderophores). Each fungal species employs these strategies to different degrees and in different ways

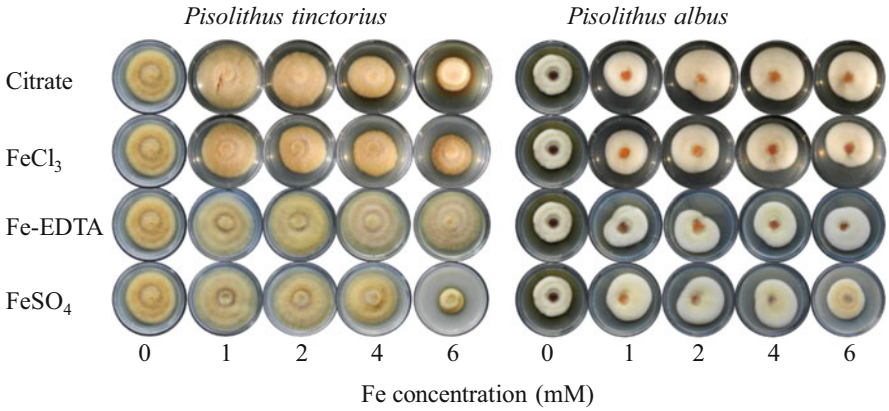

Fig. 23.2 Visual growth of the fungi *Pisolithus tinctorius* and *Pisolithus albus* for 13 days in Modified Merlin Norkrans (MMN) medium (Marx 1969) at different concentrations of Fe sources

(Yun et al. 2000; Kosman 2003; Machuca et al. 2007; Arantes and Milagres 2007; Kornitzer 2009).

The formation of chelates (siderophore or organic acid) is an important process both for Fe resistance and for its uptake by ECM fungi (Illmer and Buttinger 2006; Johnson 2008; Haselwandter 2008; Kobayashi and Nishizawa 2012; Pereira et al. 2013). The use of chelating agents like siderophores, benefits the Fe uptake when this element is scarce in the environment and would also act under stress conditions, forming a Fe^{3+}-siderophore complex, thus facilitating the storage of intracellular Fe (Arantes and Milagres 2007; Aliasgharzad et al. 2009; Harrington et al. 2011). Similarly, organic acids, amino acids, metallothioneins, and glycoproteins are also secreted into the soil by ECM fungi, as chelating agents, and bind to Fe, reducing its mobility and bioavailability for assimilation by plants (Shi et al. 2018).

Another pathway possibly used by ECM fungi to complex metals and avoid toxicity is the use of the pigment melanin, as observed when *Pisolithus tinctorius* and *Pisolithus albus* were grown in different sources (Citrate, $FeCl_3$, Fe-EDTA, and $FeSO_4$) and concentrations (0, 1, 2, 4, and 6 mM) of Fe (Fig. 23.2). *P. tinctorius* has a higher production of melanin, thus presenting increased growth in an Fe-amended medium when compared to *P. albus*, except for the concentration of 6 mM (Fig. 23.2).

Melanins are high molecular weight pigments, dark brown or black in colour, formed by the oxidative polymerization of phenolic and indolic compounds (Fogarty and Tobin 1996). This pigment has several carboxylic, phenolic, hydroxy, amine and quinone groups, which provide many potential sites for binding or adsorption of metals (Gadd and De Rome 1988; Gadd 1993). In fungi, they are located on the cell wall or as extracellular polymers, acting as chelators and providing greater rigidity to the wall (Fogarty and Tobin 1996). Grazziotti et al. (2001) showed that an isolate of *Pisolithus tinctorius* had its production of extracellular pigments increased with the addition of contaminated soil to the culture medium, thus demonstrating that metals

can cause an increased melanin production, by stimulating the production of intermediate products in their synthesis pathway or by increasing the activity of key enzymes, such as tyrosinases and laccases, which act on their biosynthesis (McGraw 2003).

Although production of melanin is a mechanism used by fungi to chelate Fe, the use of siderophores is most mentioned in the literature (De Luca and Wood 2000; Kosman 2003; Frey-Klett et al. 2005; Illmer and Buttinger 2006; Aliasgharzad et al. 2009; Harrington et al. 2011). Siderophores are secondary metabolites produced by the biosynthesis of enzymes known as non-ribosomal peptide synthase and polyketide synthase, and their main function is bound to Fe^{3+} forming a chelate complex (Fe^{3+}-FS), which enter the cell through specific transporters known as Yellow Stripe (YS) (Guerinot and Yi 1994; Ishimaru et al. 2007; Marschner et al. 2011). The production of siderophores is important to increase tolerance to Fe toxicity and the type of siderophore produced (hydroxamates, hydroxycarboxylates, ferrichrome, ferricrocin), with greater or lesser affinity for Fe, could explain the differences in Fe tolerance between different species of fungi, like *P. tinctorius* and *P. albus* (Johnson 2008; Haselwandter 2008).

ECM fungi can also differ in their influence on metal tolerance by the host and it is possible to say that this tolerance is based on an efficient host–fungus interaction, so for that, in vitro analyses are of crucial importance. For example, Ray et al. (2005) tested the tolerance level of eight ECM fungi in relation to several concentrations of six different heavy metals (Al, As, Cd, Cr, Ni, and Pb), concluding that only three isolates (*Hysterangium incarceratum*, *Laccaria fraterna,* and *Pisolithus tinctorius*) had considerable tolerance to the metals in question. Also, two ECM fungi, *Thelephora terrestris* and *Suillus bovinus*, protected *Pinus sylvestris* against Cu toxicity, however, the two fungi accumulated different amounts of Cu and showed differences in sensitivity (Van Tichelen et al. 2001).

The mechanisms involved in providing this increased tolerance are difficult to be studied, since different species or isolates of the same fungal species may show different responses to several contaminants, as demonstrated above. Therefore, the use of different sources of the heavy metal could elucidate the mechanisms used by ECM fungi for solubilization and uptake this metal (Philpott 2006; Johnson 2008). For example, two species of ECM fungus, *Pisolithus tinctorius* and *Pisolithus albus*, were exposed to four sources of toxic Fe concentrations and showed different growth and tolerance to the metal (Fig. 23.3). Both fungi grew in the tested Fe sources, however, only the $FeSO_4$ caused mycelial growth inhibition in both species, but to a lesser extent in *P. albus*. $FeSO_4$ is the only source presenting iron in its bioavailable form (Fe^{2+}) and although fungi can absorb Fe in a wide variety of forms such as free ions (Fe^{2+} and Fe^{3+}), iron chelates, and siderophores (Philpott 2006), when in excess, Fe^{2+} is responsible for the formation of free radicals by the Fenton reaction, causing stress in the fungi and consequently an mycelial growth inhibition (Johnson 2008; Shah et al. 2013).

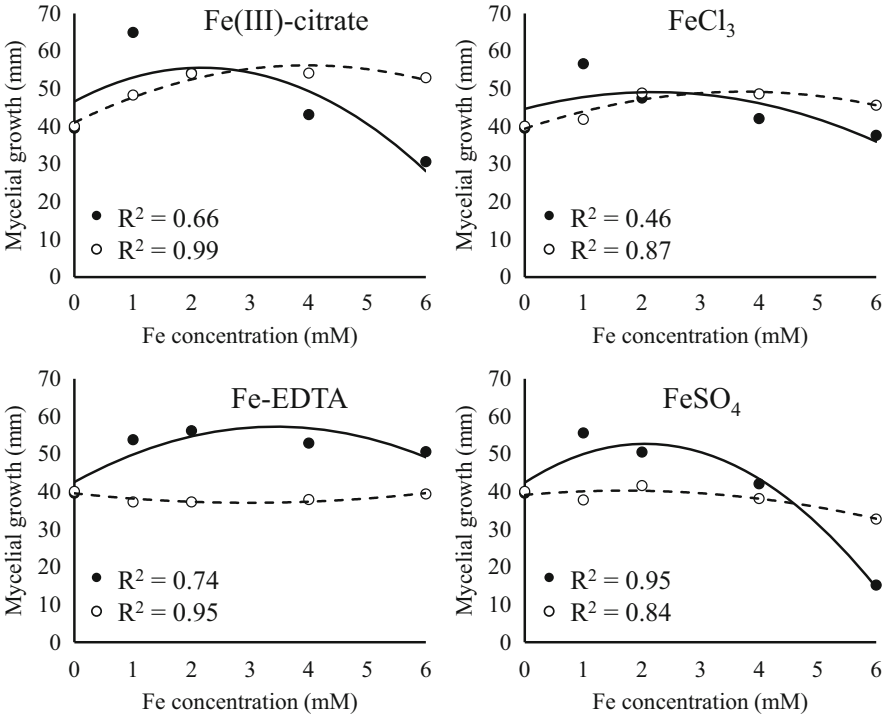

Fig. 23.3 Average mycelial growth of ectomycorrhizal fungi *Pisolithus tinctorius* (●) and *Pisolithus albus* (○) at 13 days in Modified Merlin Norkrans (MMN) medium (Marx 1969) containing different concentrations of Fe sources

23.4 The Relationships between Iron, Phosphorus, and Nitrogen in Ectomycorrhizal Fungi

The excess of Fe in the soil can restrict not only the availability of P to plants, but also the availability of P to microorganisms, since there is a strong positive correlation between their concentrations, suggesting that P is bound to Fe, thus hindering the uptake of this macronutrient (Giesler et al. 2004). The high affinity of Fe oxides for phosphate has driven, for decades, research on the dynamics of adsorption, desorption, and diffusion of P and its availability in soils (Fink et al. 2014, 2016a, 2016b). Using the program Visual MINTEQ, software version 2.53 (Ward et al. 2008) with adaptations by Ramos et al. (2009), we simulated the bioavailability of P in different concentrations of Fe using as reference the ECM fungi growth medium, MMN (Marx 1969). It is evident that with the increasing amount of Fe^{2+} in the culture medium, there is a decrease in the amount of P in absorbable form ($H_2PO_4^-$ and HPO_4^{-2}), with the predominance of P in the form of FeH_2PO^{4+} (Fig. 23.4).

The relationship between Fe and P contents was analysed in two species of ECM fungi, *P. tinctorius* and *P. albus*, grown in vitro, under high concentrations of Fe solid particulate matter (FeSPM) (Fig. 23.5). The content of Fe and P in the

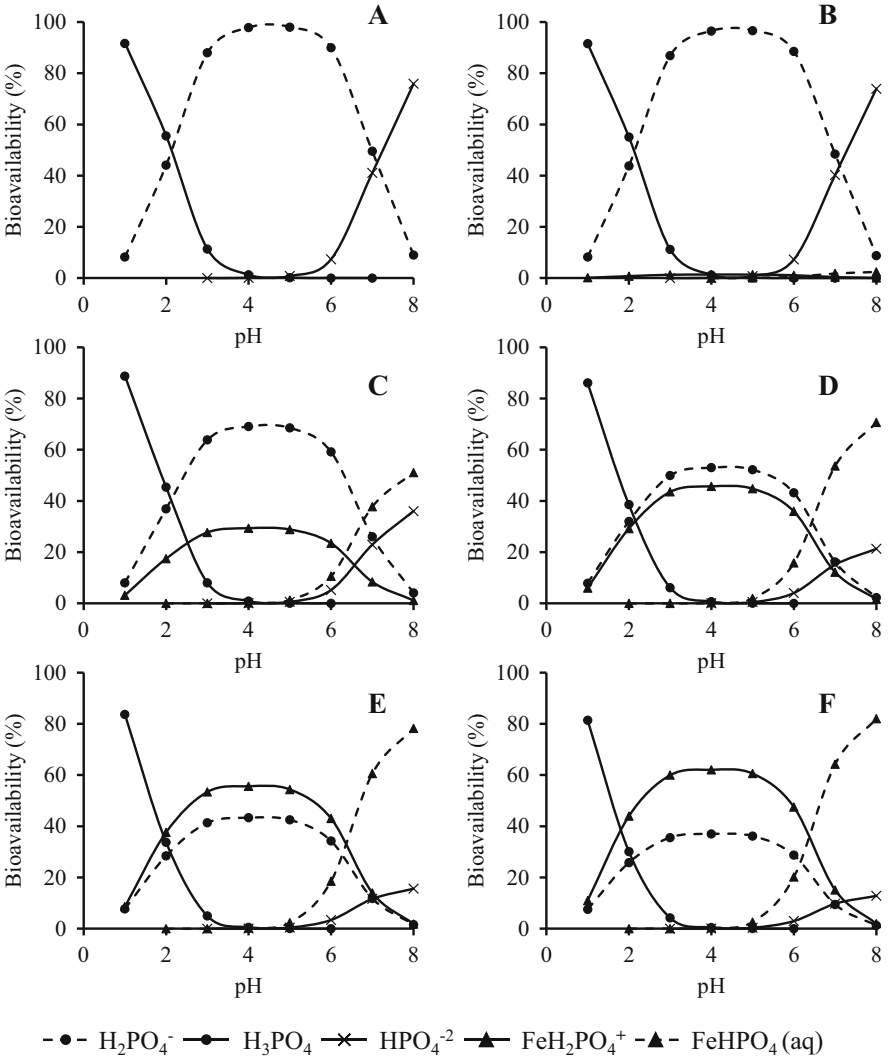

Fig. 23.4 Estimated bioavailability (%) of phosphorus in the Modified Merlin Norkrans (MMN) medium (Marx 1969) with different concentrations of iron ions. (**a**) 0 mM Fe^{2+}; (**b**) 0.07 mM Fe^{2+}; (**c**) 2 mM Fe^{2+}; (**d**) 4 mM Fe^{2+}; (**e**) 6 mM Fe^{2+}; (**f**) 8 mM Fe^{2+} as a function of pH. The estimates were made by Visual Minteq v.2.53

mycelium of *P. albus* increased in the concentrations of 1 and 2 g L^{-1} of FeSPM and became constant at the highest concentrations (Fig. 23.5a, b). However, in the fungus *P. tinctorius*, there were significant increases in the Fe content with the increasing concentrations of FeSPM in the culture medium, but there was a reduction in the content of P with the increasing concentrations of FeSPM, demonstrating a negative correlation between P and Fe (Fig. 23.5a, b).

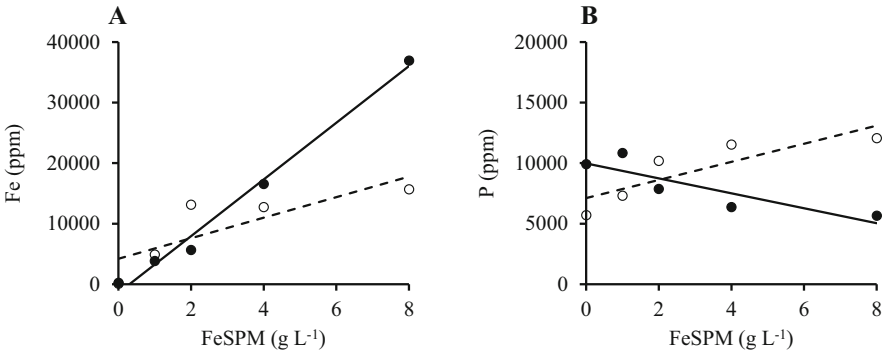

Fig. 23.5 Regression of Fe (**a**) and P (**b**) content in the mycelium of *Pisolithus tinctorius* (●) and *Pisolithus albus* (○) growing under different concentrations of Fe solid particulate matter (FeSPM)

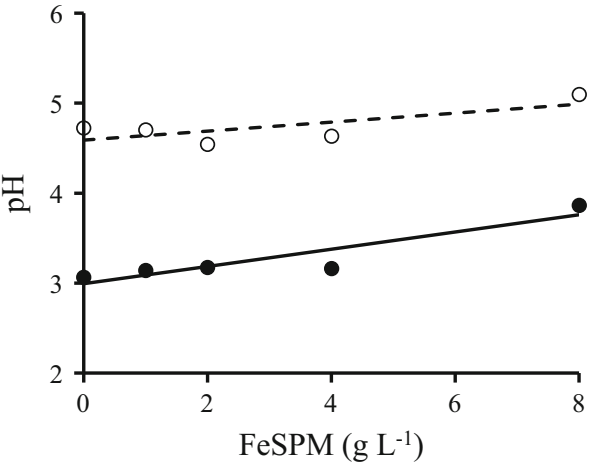

Fig. 23.6 Extracellular medium pH of *Pisolithus tinctorius* (●) and *Pisolithus albus* (○) growing under different concentrations of Fe solid particulate matter (FeSPM)

An interesting parameter to analyse when studying the relationship between Fe and P is the extracellular pH, since it been demonstrated that in high pH soils, P ions are fixed as Ca phosphates, while at low pH, Fe and Al phosphates are prevalent, thus reducing P mobility (Jayachandran et al. 1989). We observed that the *P. tinctorius* extracellular pH was significantly lower than the pH of *P. albus* in all FeSPM concentrations (Fig. 23.6), which would explain the higher content of Fe and, consequently, smaller content of P found in the mycelium of the first fungus. Such medium acidification has been previously demonstrated in an isolate of *P. tinctorius* (Pt715) and was attributed to a high H^+ efflux (Zhang et al. 2014). Also, the higher melanin production of *P. tinctorius* compared to *P. albus*, could be responsible for the chelation of the inorganic Fe-P complex in the fungal cell wall, thus resulting in increasing Fe content without increased P content.

ECM fungal species could vary in their abilities to adapt to the most variated soils conditions, like poor nutrient, drought, and heavy metal toxicity. It is possible to observe, from the results demonstrated in Fig. 23.5, that *P. tinctorius* and *P. albus* have a great variation in Fe and P uptake in medium containing high concentration of FePSM, whereas *P. albus* is more efficient in P uptake than *P. tinctorius*. This could be due to a chelation of Fe through the exudation of several organic acids such as citric, malic, oxalic, malonic, and lactic acid, thereby solubilizing phosphates and increasing the P release (Feng et al. 2011; Zhang et al. 2014; Adeleke et al. 2010), or the synthesis of siderophores, like hydroxamates, that form strong and stable complexes with Fe^{3+}, thus chelating Fe and releasing P from iron phosphate complexes (Haselwandter et al. 2011, 2013).

Among environmental restrictions, the availability of nutrients, mainly P and N, are the most studied since they can limit plant production (Rotaru and Sinclair 2009; Vitousek et al. 2010). In this context, ectomycorrhizae play an important role, because this interaction increases the area of soil explored by the roots and modifies the root architecture, thus enhancing the uptake water and nutrients (Smith and Read 2008; Falandysz and Borovička 2013; Clasen et al. 2018). In forest species most of the N is found in the organic form coupled with organic matter or soil litter, and with a slow rate of mineralization. ECM fungi have an ability to decompose these organic complexes, absorbing it in the form of ammonium (NH^{4+}), thus contributing in a quantitative and qualitative way to the N nutrition of plants (Lindahl et al. 2007; Lindahl and Tunlid 2015; Shah et al. 2016; Makarov 2019).

N can also be absorbed by fungi from proteins complexed in the soil's organic matter. When the fungus switch from NH^{4+} to protein as the main N source, the production of $^{\bullet}OH$ is induced, as well as the release of proteolytic enzymes. From the release of $^{\bullet}OH$, proteins complexed in organic matter are oxidized, and the extracellular proteolytic activity is enhanced (Fig. 23.7). This happens because, oxidized protein substrates allow greater proteolytic activity when compared to unmodified proteins. This entire process is directly regulated by NH^{4+} concentrations, whose depletion activates the signalling for protein-N nutrition and is only possible due to

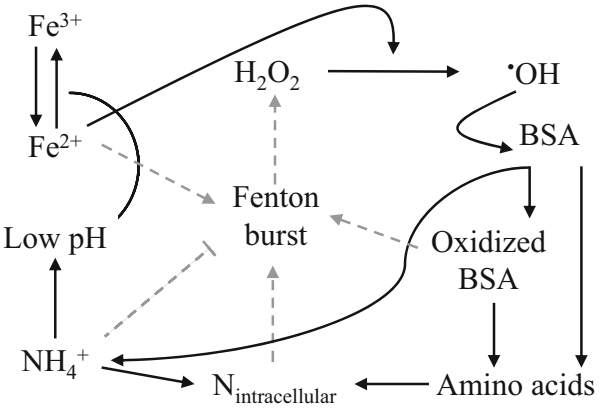

Fig. 23.7 Dynamic modelling of the induction of the Fenton reaction in *Paxillus involutus*. Arrows, positive interactions (conversion, induction); arrows with bars, negative interactions (inhibition) between model components; dashed grey lines, the regulation of the Fenton reaction through H_2O_2 production (adapted from Op De Beeck et al. 2018)

the presence of Fe^{2+}, which via Fenton reaction generates $^{\bullet}OH$ (Fig. 23.7). Thus, Fe in an optimal concentration is important for the N nutrition of ECM fungi and consequently for plants (Op De Beeck et al. 2018).

The N biogeochemical cycle is interfered by anthropogenic activities (Elser et al. 2007), which can increase N concentrations and interfere with P deficiency (Liu et al. 2013; Du and Fang 2014; Tarvainen et al. 2016). Environments with a high concentration of Fe directly affect the availability of P, however, it does not interfere with N bioavailability regardless of the Fe source (Table 23.1).

Nevertheless, the uptake of N could be altered by Fe concentrations in ECM fungi. For instance, both *P. tinctorius* and *P. albus* showed an increased N^{15} content with the increasing Fe ($FeSO_4$) concentrations in their growth medium (Fig. 23.8). It has been reported that depending on the concentration, Fe can stimulate the acidification of the extracellular medium, through the activation of the cells primary transporters (H^+-ATPases), generating a H^+ electrochemical gradient, which activates the secondary transporters, thus increasing the uptake of Fe and other nutrients like N (Shi et al. 2018; Zhang et al. 2018).

Table 23.1 Estimated bioavailability (%) of nitrogen (NH^{4+}) in different sources of iron used in the Modified Merlin Norkrans (MMN) medium (Marx 1969) with different concentrations of iron ions. The estimates were made using the software Visual Minteq v.2.53

	N (NH_4^+) bioavailability (%)			
Fe mM	Fe-citrate	$FeCl_3$	Fe-EDTA	$FeSO_4$
0	99.57	99.57	99.57	99.57
1	99.56	99.58	99.57	98.99
2	99.56	99.59	99.57	98.51
4	99.56	99.61	99.58	97.73
6	99.56	99.63	99.59	97.09

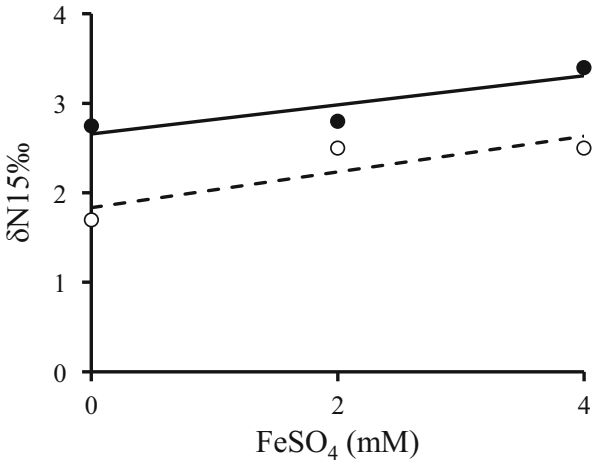

Fig. 23.8 Total N^{15} content in the biomass of *Pisolithus tinctorius* (●) and *Pisolithus albus* (○) grown in different concentrations of Fe ($FeSO_4$)

In the plant-fungus association, the bioavailability of N can be affected by the rhizosphere pH (Rotaru and Sinclair 2009). In acidic soils, plants absorb N in the form of nitrate (NO_3^-) and exchange it for anions (OH^-), increasing the pH and the availability of some nutrients in the rhizosphere. If the pH increases above 5.5, it can cause a reduction in the availability of metallic nutrients such as Fe (which may prevent possible toxicity). However, the uptake of N in the form of NH^{4+} by the plant, results in the acidification of the rhizosphere, increasing the availability of P and Fe (Ariz et al. 2018).

The growth resulting from the symbiotic N_2 fixation has a higher P requirement than the growth supported by NO_3^- assimilation, via reduction in atmospheric N_2 by the nitrogenase system (Liu et al. 2013). A higher concentration of N in the plant would require more P available to avoid deficiencies that would limit the growth of the plant (Huang et al. 2012). The biological fixation of N_2 in legumes also leads to H^+ extrusion, which can increase the availability of nutrients necessary for this process, such as P and Fe (Raven et al. 1990). Liu et al. (2012) analysed the effect of N and P addition to the soil on microbial biomass and showed that the addition of N decreased the microbial biomass of the soil in the short term, due to acidification of the soil resulting from the uptake of NH^{4+} by plants and the nitrification of NH^{4+} in the soil. However, the addition of P in the soil increased microbial biomass, indicating that the availability of P is a limiting factor for microbial growth.

23.5 Conclusion and Future Prospects

The availability of nutrients, mainly P and N, as well as heavy metals, such as Fe, can limit plant production, however, the low soil fertility contributes to an effective mycorrhization, thus favouring plant growth. In soils contaminated by Fe, the association of forest species with ECM fungi allows the development of the plant, because these fungi have mechanisms to avoid the uptake of excess metal by the plant. The ability of ECM fungi to withstand the stress induced by toxic concentrations of Fe in their environment is related to their ability to immobilize, sequester, and compartmentalize this metal. These tolerance mechanisms include adsorption by the cell wall or cell wall compounds such as melanin, precipitation in the extracellular matrix, chelation by compounds such as organic acids and siderophores, and intracellular processes of chelation by metallothioneins or sequestration by phytochelatins inside the vacuoles.

Fe is an essential element for plant and fungi growth and development, existing in several forms ($FeSO_4$, $FeCl_3$, Fe-EDTA, Fe-citrate, FePSM), with an availability strongly dependent on the environmental pH and directly influencing the uptake of important nutrients like P and N. However, there are few studies regarding the interaction of Fe with the main macro and micronutrients and its effect on the growth of ECM fungi. Fungi of the genus *Pisolithus* stand out for their tolerance to high concentrations of Fe and for presenting differences in tolerance between species,

becoming a promising model in studies of the interaction of Fe with the fungal and plant ionome and its relationship with tolerance to this heavy metal.

ECM fungi are capable of mobilize and store Fe in toxic concentrations, however, this ability can vary according to the species of fungus, the host plant, the concentration and source of the metal. High concentrations of Fe can influence the uptake of nutrients essential to the ectomycorrhizae physiology. When in excess, Fe binds to P making it unavailable for uptake by plants and microorganisms, however, the same does not happen with N, because it depends on the form that this nutrient will be absorbed, in which excess Fe contributes to the uptake of NH_4 while decreasing uptake of NO_3^-. Despite not influencing the availability of N in high concentrations, Fe in optimal concentrations can be very important for N nutrition of plants and fungi, participating in the demineralization process of N trapped in the soil's organic matter.

In this context, the use of software that allow the predictions of nutrients interaction and bioavailability, and in vitro studies with different Fe sources are extremely important to eliminate variables, better understand the mechanisms used by ECM fungi to tolerate toxic metals and how the difference in tolerance between species works.

References

Adeleke RA, Cloete TE, Bertrand A, Khasa DP (2010) Mobilisation of potassium and phosphorus from iron ore by ectomycorrhizal fungi. World J Microbiol Biotechnol 26:1901–1913

Adeleke RA, Cloete TE, Bertrand A, Khasa DP (2012) Iron ore weathering potentials of ectomycorrhizal plants. Mycorrhiza 22:535–544

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals-concepts and applications. Chemosphere 91:869–881

Aliasgharzad N, Shirmohamadi E, Oustan S (2009) Siderophore production by mycorrhizal sorghum roots under micronutrient deficient condition. Soil Environ 28:119–123

Altieri MA (1999) The ecological role of biodiversity in agroecosystems. Agric Ecosyst Environ 74:19–31

Alves JR, Souza O, Podlech PAS, Giachini AJ, Oliveira VL (2001) Efeito de inoculante ectomicorrízico produzido por fermentação semi-sólida. Pesquisa Abropecuária Brasileira 36:307–313

Arantes V, Milagres AMF (2007) Identification of iron-regulated cellular proteins, Fe^{3+}-reducing and -chelating compounds, in the white-rot fungus Perenniporia medulla-panis. Can J Microbiol 53:1323–1329

Ariz I, Boeckstaens M, Gouveia C, Martins AP, Sanz-Luque E, Fernández E, Soveral G, von Wirén N, Marini AM, Aparicio-Tejo PM, Cruz C (2018) Nitrogen isotope signature evidences ammonium deprotonation as a common transport mechanism for the AMT-Mep-Rh protein superfamily. Sci Adv 4:eaar 3599

Audebert A, Fofana M (2009) Rice yield gap due to iron toxicity in West Africa. J Agron Crop Sci 195:66–76

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal. Mycorrhiza 11:3–42

Becker M, Asch F (2005) Iron toxicity in rice: conditions and management concepts. J Plant Nutr Soil Sci 168:558–573

Bojarczuk K, Kieliszewska-rokicka B (2010) Effect of ectomycorrhiza on cu and Pb accumulation in leaves and roots of silver birch (Betula pendula Roth.) seedlings grown in metal-contaminated soil. Water Air Soil Pollut 207:227–240

Briat JF, Duc C, Ravet K, Gaymard F (2010) Ferritins and iron storage in plants. Biochim Biophys Acta 1800:806–814

Buchanan BB, Gruissen W, Jones RL (2000) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, p 1367

Chakravarty P, Unestam T (1987) Differential influence of ectomycorrhizae on plant growth and disease resistance in Pinus sylvestris seedlings. J Phytopathol 120:104–120

Chatterjee C, Gopal R, Dube BK (2006) Impact of iron stress on biomass, yield, metabolism and quality of potato (Solanum tuberosum L.). Sci Hortic 108:1–6

Chen X, Zhou Z, Teng M, Wnag P, Zhou L (2015) Accumulation of three different sizes of particulate matter on plant leaf surfaces: effect on leaf traits. Arch Biol Sci 67:1257–1267

Clasen BE, Silveira ADO, Baldoni DB, Montagner DF, Jacques RJ, Antoniolli ZI (2018) Characterization of ectomycorrhizal species through molecular biology tools and morphotyping. Sci Agric 75:246–254

Colpaert JV, Van Assche JA (1993) The effects of cadmium on ectomycorrhizal Pinus sylvestris L. New Phytol 123:325–333

Colpaert JV, Wevers JHL, Krznaric E, Adriaensen K (2011) How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. Ann For Sci 68:17–24

Connolly EL, Guerinot ML (2002) Iron stress in plants. Genome Biol 3:1024.1–1024.4

Crane S, Dighton J, Barkay T (2010) Growth responses to and accumulation of mercury by ectomycorrhizal fungi. Fungal Biol 114:873–880

Curie C, Briat JF (2003) Iron transport and signaling in plants. Annu Rev Plant Biol 54:183–206

De Luca N, Wood P (2000) Iron uptake by fungi: contrasted mechanisms with internal or external reduction. Adv Microb Physiol 43:39–74

Dechen AR, Nachtigall GR (2006) Micronutrientes. In: Fernandes MS (ed) Nutrição mineral de plantas. Sociedade Brasileira de Ciência do Solo, Viçosa, pp 326–354

Domínguez-Núñez JA, Albanesi AS (2019) Ectomycorrhizal fungi as biofertilizers in forestry. In: Biostimulants in plant science. IntechOpen

Du E, Fang J (2014) Weak growth response to nitrogen deposition in an old growth boreal forest. Ecosphere 5:1–9

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142

Falandysz J, Borovička J (2013) Macro and trace mineral constituents and radionuclides in mushrooms: health benefits and risks. Appl Microbiol Biotechnol 97:477–501

Feng MH, Ngwenya BT, Wang L, Li W, Olive V, Ellamd RM (2011) Bacterial dissolution of fluorapatite as a possible source of elevated dissolved phosphate in the environment. Geochim Cosmochim Acta 75:5785–5796

Fink JR, Inda AV, Bayer C, Torrent J, Barrón V (2014) Mineralogy and phosphorus adsorption in soils of south and central-West Brazil under conventional and no-tillage systems. Acta Sci Agron 36:379–387

Fink JR, Inda AV, Bavaresco J, Barrón V, Torrent J, Bayer C (2016a) Adsorption and desorption of phosphorus in subtropical soils as affected by management system and mineralogy. Soil Tillage Res 155:62–68

Fink JR, Inda AV, Bavaresco J, Sánchez-Rodríguez AR, Barrón V, Torrent J, Bayer C (2016b) Diffusion and uptake of phosphorus, and root development of corn seedlings, in three contrasting subtropical soils under conventional tillage or no-tillage. Biol Fertil Soils 52:203–210

Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. J Exp Bot 59:1115–1126

Fodor F, Kovács K, Czech V, Solti A, Tóth B, Lévai L, Bóka K, Vértes A (2012) Effects of short term iron citrate treatments at different pH values on roots of iron-deficient cucumber: a Mössbauer analysis. J Plant Physiol 169:1615–1622

Fogarty RV, Tobin JM (1996) Fungal melanins and their interactions with metals. Enzym Microb Technol 19:311–317

Frey-Klett P, Chavatte M, Clausse M-L, Courrier S, Le Roux C, Raaijmakers J, Martinotti M, Pierrat J-C, Garbaye J (2005) Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytol 165:317–328

Gadd GM (1993) Interactions of fungi with toxic metals. New Phytol 124:25–60

Gadd GM, De Rome L (1988) Biosorption of cooper by fungal melanin. Appl Microbiol Biotechnol 29:610–617

Giesler R, Satoh F, Ilstedt U, Nordgren A (2004) Microbially available phosphorus in boreal forests: effects of aluminum and iron accumulation in the humus layer. Ecosystems 7:208–217

Grazziotti PH, Siqueira JO, Moreira FM, Carvalho D (2001) Tolerância de fungos ectomicorrízicos a metais pesados em meio de cultura adicionado de solo contaminado. Rev Br de Ciências do Solo 25:839–848

Guerinot ML, Yi Y (1994) Iron: nutritious, noxious, and not readily available. Plant Physiol 104:815–820

Gurzau ES, Neagu C, Gurzau AE (2003) Essential metals - case study on iron. Ecotoxicol Environ Saf 56:190–200

Haas H, Eisendle M, Turgeon BG (2008) Siderophores in fungal physiology and virulence. Annu Rev Phytopathol 46:149–187

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53366:1–11

Harrington JM, Winkelmann G, Haselwandter K, Crumbliss AL (2011) Fe(III)-complexes of the tripodal trishydroxamate siderophore basidiochrome: potential biological implications. J Inorg Biochem 105:1670–1674

Hartley J, Cairney JWG, Meharg AA (1997) Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment. Plant Soil 189:303–319

Haselwandter K (2008) Structure and function of siderophores produced by mycorrhizal fungi. Mineral Mag 72:61–64

Haselwandter K, Haninger G, Ganzera M (2011) Hydroxamate siderophores of the ectomycorrhizal fungi Suillus granulatus and S. luteus. Biometals 24:153–157

Haselwandter K, Haninger G, Ganzera M, Haas H, Nicholson G, Winkelman G (2013) Linear fusigen as the major hydroxamate siderophore of the ectomycorrhizal Basidiomycota Laccaria laccata and Laccaria bicolor. Biometals 26:969–979

Hell R, Stephan UW (2003) Iron uptake, trafficking and homeostasis in plants. Planta 216:541–551

Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139–146

Huang W-J, Zhou G-Y, Liu J-X (2012) Nitrogen and phosphorus status and their influence on aboveground production under increasing nitrogen deposition in three successional forests. Acta Oecol 44:20–27

Ilbert M, Bonnefoy V (2013) Insight into the evolution of the iron oxidation pathways. Biochim Biophys Acta (BBA)-Bioenerg 1827:161–175

Illmer P, Buttinger R (2006) Interactions between iron availability, aluminium toxicity and fungal siderophores. Biometals 19:367–377

Ishimaru Y, Masuda H, Suzuki M, Bashir K, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2007) Overexpression of the OsZIP4 zinc transporter confers disarrangement of zinc distribution in rice plants. J Exp Bot 58:2909–2915

Ismail Y, Hijri M (2012) Arbuscular mycorrhisation with Glomus irregulare induces expression of potato PR homologues genes in response to infection by Fusarium sambucinum. Funct Plant Biol 39:236–245

Jayachandran K, Hetrick BAD, Schwab AP (1989) Mycorrhizal mediation of phosphorus availability: synthetic iron chelate effects on phosphorus solubilization. Soil Sci Soc Am J 53:1701–1706

Jeong J, Guerinot ML (2009) Homing in on iron homeostasis in plants. Trends Plant Sci 14:280–285

Johnson L (2008) Iron and siderophores in fungal – host interactions. Mycol Res 112:170–183

Johnson DB, Kanao T, Hedrich S (2012) Redox transformations of iron at extremely low pH: fundamental and applied aspects. Front Microbiol 3:1–13

Jucoski GO, Cambraia J, Ribeiro C, Oliveira JA (2016) Excess iron on growth and mineral composition in Eugenia uniflora L. Rev Ciênc Agron 47:720–728

Khade SW, Adholeya A (2008) Effects of heavy metal (Pb) on arbuscular mycorrhizal fungi in vitro. World J Microbiol Biotechnol 24:1663–1668

Khan A, Kuek C, Chaudhry T, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Kim S, Lou GM (2007) Mining iron: iron uptake and transport in plants. FEBS Lett 581:2273–2280

Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation in higher plants. Annu Rev Plant Biol 63:131–152

Kornitzer D (2009) Fungal mechanisms for host iron acquisition. Curr Opin Microbiol 12:377–383

Kosman DJ (2003) Molecular mechanisms of iron uptake in fungi. Mol Microbiol 47:1185–1197

Krohling CA, Eutrópio FJ, Bertolazi AA, Dobbss LB, Campostrini E, Dias T, Ramos AC (2016) Ecophysiology of iron homeostasis in plants. Soil Sci Plant Nutr 62:39–47

Krpata D, Peintner U, Langer I, Fitz WJ, Schweiger P (2008) Ectomycorrhizal communities associated with Populus tremula growing on a heavy metal contaminated site. Mycol Res 112:1069–1079

Kuki KN, Oliva MA, Pereira EG (2008) Iron ore industry emissions as a potential ecological risk factor for tropical coastal vegetation. Environ Manag 42:111–121

Landeweert R, Hoffland E, Finlay RD, Kuyper TW, Breemen N (2001) Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol Evol 16:248–254

Legay S, Guignard C, Ziebel J, Evers D (2012) Iron uptake and homeostasis related genes in potato cultivated in vitro under iron deficiency and overload. Plant Physiol Biochem 60:180–189

Lemanceau P, Bauer P, Kraemer S, Briat J-F (2009) Iron dynamics in the rhizosphere as a case study for analyzing interactions between soils, plants and microbes. Plant Soil 321:513–535

Lermen C, Mohr FBM, Alberton O (2015) Growth of Cymbopogon citratus inoculated with mycorrhizal fungi under different levels of lead. Sci Hortic 186:239–246

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153

Lindahl BD, Tunlid A (2015) Ectomycorrhizal fungi-potential organic matter decomposers, yet not saprotrophs. New Phytol 205:1443–1447

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol 173:611–620

Liu L, Gundersen P, Zhang T, Mo J (2012) Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. Soil Biol Biochem 44:31–38

Liu L, Zhang T, Gilliam FS, Gundersen P, Zhang W, Chen H, Mo J (2013) Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. PLoS One 8:e61188

Lopes AS, Oliva MA, Martinez CA (2000) Impacto das emissões de dióxido de enxofre e deposição de material particulado de ferro em espécies vegetais de restinga: avaliação ecofisiológica. In: Espíndola ELG, Paschoal CMR, Rocha O, Bohrer MBC, Oliveira Neto AL (eds) Ecotoxicologia - Perspectivas para o século XXI. RiMa Artes e Textos, São Carlos, pp 53–71

Machuca A, Pereira G, Aguiar A, Milagres a MF (2007) Metal-chelating compounds produced by ectomycorrhizal fungi collected from pine plantations. Lett Appl Microbiol 44:7–12

Mahender A, Swamy B, Anandan A, Ali J (2019) Tolerance of iron-deficient and -toxic soil conditions in rice. Plan Theory 8:1–31

Majerus V, Bertin P, Lutts S (2007) Effects of iron toxicity on osmotic potential, osmolytes and polyamines concentration in the African rice (Oryza glaberrima Steud). Plant Sci 173:96–105

Majerus V, Bertin P, Lutts S (2009) Abscisic acid and oxidative stress implications in overall ferritin synthesis by African rice (Oryza glaberrima Steud.) seedlings exposed to short term iron toxicity. Plant Soil 324:253–265

Makarov MI (2019) The role of mycorrhiza in transformation of nitrogen compounds in soil and nitrogen nutrition of plants: a review. Eurasian Soil Sci 52:193–205

Marschner H, Rohmëld V (1994) Strategies of plant for acquisition of iron. Plant Soil 165:375–388

Marschner P, Crowley D, Rengel Z (2011) Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis - model and research methods. Soil Biol Biochem 43:883–894

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

McGraw KJ (2003) Melanins, metals, and mate quality. Oikos 102:402–406

Neubauer E, Schenkeveld WDC, Plathe KL, Rentenberger C, von der Kammer F, Kraemer SM, Hofman T (2013) The influence of pH on iron speciation in podzol extracts: iron complexes with natural organic matter, and iron mineral nanoparticles. Sci Total Environ 461-462:108–116

Neves NR, Oliva MA, Centeno DC, Costa AC, Ribas RF, Pereira EG (2009) Photosynthesis and oxidative stress in the Restinga plant species Eugenia uniflora L. exposed to simulated acid rain and iron ore dust deposition: potential use in environmental risk assessment. Sci Environ 407:3740–3745

Op De Beeck M, Troein C, Peterson C, Persson P, Tunlid A (2018) Fenton reaction facilitates organic nitrogen acquisition by an ectomycorrhizal fungus. New Phytol 218:335–343

Osonubi O, Mulongoy K, Awotoye OO, Atayese MO, Okali DUU (1991) Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. Plant Soil 136:131–143

Ott PH, Secchi ER, Moreno IB, et al. (2002) Report of the working group on fishery interactions. Report of the working group on fishery interactions. In: Secchi ER (ed.). Special issue on the biology and conservation of Franciscana. The Latin American Journal of Aquatic Mammals 1: 1-192

Ouziad F, Hildebrandt U, Schmelzer E, Bothe H (2005) Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. J Plant Physiol 162:634–649

Pantopoulos K, Hentze MW (2000) Nitric oxide, oxygen radicals and iron metabolism. Section II-principles of biology. Academic Press:293–313

Pereira EG, Oliva MA, Rosado-Souza L, Mendes GC, Colares DS, Stopato CH, Almeida AM (2013) Iron excess affects rice photosynthesis through stomatal and non-stomatal limitations. Plant Sci 201-202:81–92

Philpott CC (2006) Iron uptake in fungi: a system for every source. Biochim Biophys Acta 1763:636–645

Pugh RE, Dick DG, Fredeen AL (2002) Heavy metal (Pb, Zn, Cd, Fe, and Cu) contents of plant foliage near the anvil range lead/zinc mine, Faro, Yukon territory. Ecotoxicol Environ Saf 52:273–279

Quatrini R, Lefimil C, Holmes DS, Jedlicki E (2005) The ferric iron uptake regulator (fur) from the extreme acidophile Acidithiobacillus ferrooxidans. Microbiology 151:2005–2015

Ramos AC, Lima PT, Dias PN et al (2009) A pH signaling mechanism involved in the spatial distribution of calcium and anion fluxes in ectomycorrhizal roots. New Phytol 181:448–462

Raven JA, Franco AA, de Jesus EL, Jacob-Neto J (1990) H^+ extrusion and organic acid synthesis in N_2-fixing symbioses involving vascular plants. New Phytol 114:369–389

Ravet K, Touraine B, Boucherez J, Briat J-F, Gaymard F, Cellier F (2009) Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. Plant J 57:400–412

Ray P, Tiwari R, Reddy UG, Adholeya A (2005) Detecting the heavy metal tolerance level in ectomycorrhizal fungi in vitro. World J Microbiol Biotechnol 21:309–315

Reis FS, Ferreira ICFR, Martins A (2012) Effect of the mycorrhizal symbiosis time in the antioxidant activity of fungi and Pinus pinaster roots, stems and leaves. Ind Crop Prod 35:211–216

Rengel Z (2015) Availability of Mn, Zn and Fe in the rhizosphere. J Soil Sci Plant Nutr 15:97–409

Ricachenevsky FK, Sperotto RA (2014) There and back again, or always there? The evolution of rice combined strategy for Fe uptake. Front Plant Sci 5:189

Rocha DI, Silva LC, Pereira EG, Sant'Anna BF, Gontijo ER, Oliva MA (2014) Early detection of injuries in leaves of Clusia hilariana Schltdl (Clusiaceae) caused by particulate deposition of iron Revista Árvore, 38:423–432

Römheld V (1987) Different strategies for iron acquisition in higher plants. What's new in plant physiology. Physiol Plant 70:231–234

Rotaru V, Sinclair TR (2009) Interactive influence of phosphorus and iron on nitrogen fixation by soybean. Environ Exp Bot 66:94–99

Sahrawat KL (2005) Managing iron toxicity in lowland rice: the role of tolerant genotypes and plant nutrients. In: Toriyama K, Heong KL, Hardy B (eds.) Rice is life: scientific perspectives for the 21st century, Tsukuba

Shah F, Rineau F, Canbäck B, Johansson T, Tunlid A (2013) The molecular components of the extracellular protein-degradation pathways of the ectomycorrhizal fungus Paxillus involutus. New Phytol 200:875–887

Shah F, Nicolás C, Bentzer J, Ellström M, Smits M, Rineau F (2016) Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. New Phytol 209:1705–1719

Sharma HD, Reddy KR (2004) Geoenvironmental engineering: site remediation, waste containment, and emerging waste management technologies. Wiley, New Jersey, p 992

Shi W, Zhang Y, Chen S, Polle A, Rennenberg H, Luo ZB (2018) Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. Plant Cell Environ 42:1087–1103

Silva LC, Oliva MA (2006) Responses of Restinga plant species to pollution from an iron pelletization factory. Water Air Soil Pollut 175:241–256

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, New York, p 815

St-Arnaud M, Vujanovic V (2007) Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. In: Hamel C, Plenchette C (eds) Mycorrhizae in crop production. Haworth Press, Binghampton, pp 67–122

Suh HJ, Kim CS, Lee JY, Jung J (2002) Photodynamic effect of iron excess on photosystem II function in pea plants. Photochem Photobiol 75:513–518

Tagu D, Lapeyrie F, Martin F (2002) The ectomycorrhizal symbiosis genetics and development. Plant Soil 244:97–105

Tarvainen L, Lutz M, Räntfors M, Näsholm T, Wallin G (2016) Increased needle nitrogen contents did not improve shoot photosynthetic performance of mature nitrogen-poor scots pine trees. Front Plant Sci 7:1–17

Touati D (2000) Iron and oxidative stress in bacteria. Arch Biochem Biophys 373:1–6

Van Tichelen KK, Colpaert JV, Vangronsveld J (2001) Ectomycorrhizal protection of Pinus sylvestris against copper toxicity. New Phytol 150:203–213

Vigani G (2012) Discovering the role of mitochondria in the iron deficiency-induced metabolic responses of plants. J Plant Physiol 169:1–11

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol Appl 20:–5e15

Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG (2008) The effect of iron on the primary root elongation of Arabidopsis during phosphate deficiency. Plant Physiol 147:1181–1191

Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy-metal uptake by maize (Zea mays L.) in pot culture with contaminated soil. Mycorrhiza 5:245–251

Wong MH, Chan KC, Choy CK (1978) The effect of the iron ore tailing on the costal environment of Tolo Harbor, Hong Kong. Environ Res 15:342–356

Yun CW, Tiedeman JS, Moore RE, Philpott CC (2000) Siderophore-iron uptake in Saccharomyces cerevisiae: identification of ferrichrome and fusarinine transporters. J Biol Chem 275:16354–16359

Zhang L, Wang MX, Li H, Yuan L, Huang JG, Penfold C (2014) Mobilization of inorganic phosphorus from soils by ectomycorrhizal fungi. Pedosphere 24:683–689

Zhang X, Chen L, Wu H, Liu L, Wan X (2018) Root plasma membrane H^+-ATPase is involved in low pH-inhibited nitrogen accumulation in tea plants (Camellia sinensis L.). Plant Growth Regul 86:423–432

Part VII
Algae in Nitrogen Fixation

Chapter 24
Role of Algae in Soil Nitrogen Fixation

Anamika Singh

Abstract Microorganisms help to increase the soil nutritional quality, as they are able to decompose organic matter. From soil, plants get nutrition for their growth and development. Along with microorganism, many fungi and algae also perform such functions. Algal cells are natural fertilizer and nowadays it is used worldwide, without any side effect, Algal cells have specific cells called heterocyst and are the site of nitrogen fixation. Algae are able to convert unavailable dinitrogen into bioavailable ammonia. *Anabaena*, *Nostoc*, and many other cyanobacteria (blue green algae, BGA) are able to fix atmospheric nitrogen.

Keywords Nitrogen fixation · Cyanobacteria · Nitrogenase · Nitrogen cycle · Heterocyst

24.1 Introduction

Nitrogen is the most abundant gas in the earth's atmosphere, it is nearly 79%. In atmosphere it is present in the form of di-nitrogen (N_2). It is one of the most important elements for survival of life on Earth. Proteins, nucleic acids and chlorophyll (Ferguson 1998; Smil 2004) are the few most important Nitrogen containing biological molecules. These biological molecules are required for need to build, cytochromes, alkaloids, phytohormones, and many of the vitamins (Bray 1983). The dinitrogen form of nitrogen is highly inaccessible to most organisms, because both the nitrogen atoms (N=N) linked to each other by a triple bonds, that make the molecule almost inert (Jia and Quadrelli 2014; MacKay 2004). About 225 kcal of energy is required to break this triple bond, and it is difficult to achieve. Primary producers are using nitrogen only in the form of ammonia (NH_3), so conversion of dinitrogen gas into ammonia (NH_3) is required. Agronomic, economic, and

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ecological impact of the world depends upon the agriculture production and it is directly linked to availability of fixed nitrogen. Ammonia (NH_3) or nitrate (NO_3^-) (Canfield et al. 2010; Cheng 2008) are the two important fixed forms of nitrogen, utilized by organisms and are continuously sequestered into sediments, rendering them unavailable for metabolism. Nitrification and denitrification are the two combined processes related to nitrogen metabolism and is the conversion of N_2 to NH_3 (Cheng 2008; Thamdrup 2012). The process of conversion of molecular and elemental free nitrogen into nitrogenous compounds, which can be easily absorbed by the plants, is called as nitrogen fixation. It is carried out by physicochemical and biological means. About 10% of natural nitrogen fixation takes place by physicochemical methods and 90% by biological methods. In nature nitrogen fixation is a complex process and takes place by two ways: (1) non-biological nitrogen fixation or physical nitrogen fixation or geochemical processes like lightning (Gruber and Galloway 2008) and (2) biological nitrogen fixation (Burk et al. 1934; McGlynn et al. 2013; Dos Santos et al. 2012).

Non-biological nitrogen fixation mainly includes natural nitrogen fixation and industrial nitrogen fixation (Fig. 24.1). Industrial is through the Haber–Bosch process (Smil 2004; Haber 1992, 1923). Haber–Bosch process is widely used for the production of nitrogen fertilizers and almost half of the human population

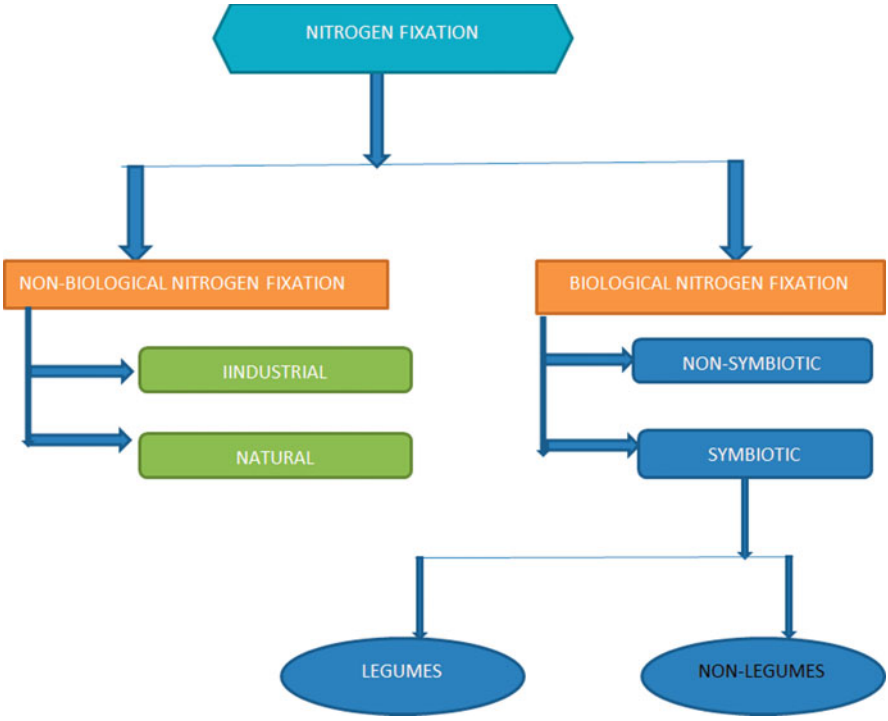

Fig. 24.1 Showing different ways of atmospheric nitrogen fixation

depends upon its applications (Smil 2004; Canfield et al. 2010). In nitrogen cycle few microorganisms like bacteria play important role in the conversion of N_2 to ammonia, other bacteria convert ammonia to nitrate and few nitrates to nitrogen gas.

24.2 Non-biological Nitrogen Fixation or Physical Nitrogen Fixation

It contains natural as well as industrial nitrogen fixation.

24.2.1 Natural Nitrogen Fixation

Under the influence of lightning (i.e., electric discharge in the clouds) and thunder, N_2 and O_2 of the air react to form nitric oxide (NO). The nitric oxides are again oxidized with oxygen to form nitrogen peroxide (NO_2).

The reactions are as follows

$$N_2 + O_2 \text{ Lightning} \rightarrow \text{Thunder } 2NO \text{ (Nitric Oxide)}; 2NO + O_2 \rightarrow 2NO_2 \overset{\text{Oxidation}}{} \text{ (Nitrogen peroxide)}$$

During rains, NO_2 combines with rain water to form nitrous acid (HNO_2) and nitric acid (HNO_3). The acids fall on the soil along with rain water and react with the alkaline radicals to form water soluble nitrates (NO_3-) and nitrites (NO_2^-).

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \; HNO_3 + \text{Ca or K salts} \rightarrow \text{Ca or K nitrates}$$

The nitrates are soluble in water and are directly absorbed by the roots of the plants.

24.2.2 Industrial Nitrogen Fixation

Ammonia is produced industrially by direct combination of nitrogen with hydrogen (obtained from water) at high temperature and pressure. Later, it is converted into various kinds of fertilizers, such as urea, etc.

24.3 Biological Nitrogen Fixation

Biological nitrogen fixation is a process mediated by living organism, and they are actually fixing the nitrogen into the nitrogenous. It is carried out by two main types of microorganisms: (1) symbiotic microorganisms and (2) non-symbiotic microorganisms. Symbiotic association involves interaction of plants and microorganisms. Biological nitrogen fixation (BNF) involves reduction of atmospheric nitrogen into ammonia.

Diazotrophy
Biological nitrogen fixation through the agency of microorganism occurs in the presence of nitrogenize enzyme. Thiss enzyme is actually a biological catalyst present in few symbiotic microorganisms like *Rhizobium* and *Frankia* or the free-living *Azospirillum* and *Azotobacter* and blue green algae (BGA). These microorganisms are able to reduce inert gaseous dinitrogen (N_2) into ammonia (NH_3). This converted ammonia is easily absorbed by roots of plants and it is known as biological nitrogen fixation or diazotrophy.

It is a complex biochemical reactions and can be summarized as:

$$N_2 + 8H_2 + 16\text{ATP nitrogenase} \rightarrow 2\,NH_3 + 2H_2 + 16\text{ADP} + 16\,P_i$$

In this equation one molecule of nitrogen gas (N_2) combines with eight hydrogen ions (also known as protons) ($8H^+$) to form two molecules of ammonia ($2NH_3$) and two molecules of hydrogen gas ($2H_2$). This reaction occurs in the presence of nitrogenase enzyme. The 16 molecules of ATP (ATP = adenosine triphosphate, an energy storing compound) represent the energy required for the biological nitrogen fixation (BNF) reaction to take place. 16 ATPs are biochemically a large amount and 'expensive' to the plant in terms of energy usage. Energy of sun through photosynthesis is utilized directly. As ammonia (NH_3) is formed in this reaction, it is converted to glutamine. The nitrogen in amino acids can be used by the plant to synthesize proteins for its growth and development.

Nitrogen Fixers
These are mainly bacteria and cyanobacteria (blue green algae, BGA), called as diazotrophs. They are able to fix almost 95% of the total global nitrogen. Diazotrophs may be symbiotic (free living) or symbiotic.

1. Free-living nitrogen fixing bacteria
2. Symbiotic nitrogen fixing bacteria
3. Free-living nitrogen fixing algae
4. Symbiotic nitrogen fixing algae

(1) *Free-living nitrogen fixing bacteria*: Free-living nitrogen fixing bacteria adds 10–30 kg of nitrogen/hectare/annum. *Rhodospirillum*, *Chromatium*, *Rhodopseudomonas* are the examples of Photoautotrophic bacterium while *Clostridium* (anaerobic), *Beijerinckia* and *Azotobacter* are saprophytic in nature are able to fix nitrogen. Few chemotrophic bacteria like *Desulphovibrio* are also able to fix nitrogen biologically.
(2) *Symbiotic nitrogen fixing bacteria*: In soil many species of *Rhizobium* are present and only few species are able to fix nitrogen who comes in contact with leguminous planets, as they form a symbiotic association in the form of root nodule. *Rhizobium* is aerobic, gram negative, and symbionts of Papilionaceous roots. Rhizobium is found in roots nodules of *Sesbania rostrata* while *Aerorhizobium* in stem nodules. In non-leguminous plants like *Casuarina* and *Alnus, Frankia* is found in root nodules. Leaves of few family members of Rubiaceae and Myrsinaceae (e.g., *Ardisia*) form symbiotic association with *Xanthomonas* and *Mycobacterium*.
(3) *Free-living nitrogen fixing blue green algae (Cyanobacteria)*: *Anabeaena*, *Nostoc*, *Cylindrospermum*, *Trichodesmium*, and *Aulosira* are the most common blue green algae that help in nitrogen fixation. They almost fix 20–30 kg nitrogen per hectare per annum. *Aulosira fertilissima* is found in rice fields, whereas in sugarcane fields Cylindrospermum is found.

(4) *Symbiotic nitrogen fixing blue green algae (cyanobacteria)*: *Anabaena* and *Nostoc* are the most common algal species found in symbiotic association in lichens (Fig. 24.2). Other important associations are *Cycas* roots with Anthoceros and *Azolla*. A water fern *Azolla pinnata* found in rice fields as *Anabaena azollae* in its fronds helps in nitrogen fixation.

24.4 Cyanophycean Algae and Nitrogen Fixation

Algae is playing an important role in nitrogen fixation especially cyanobacteria (blue green algae, BGA). Cyanobacteria or blue green algae are a diverse group of prokaryotes that mostly form a complex association with bacteria and green algae, and this structure is known as cyanobacterial mats (Rodrigo and Novelo 2007). Cyanophyceae or myxophyceae are algal group having xanthophyll, carotenes, and chlorophyll A and are photosynthetic in nature. They also have phycocyanine, phycoerythrin, and phycobilin pigments. They are known as Cyanophyta (Cyanophyceae, Myxophyta), now popularly called as Cyanobacteria (According to the "Bergey's Manual of Systematic Bacteriology" Vol. 3 1989). There are different types of blue green algae that help differently in soil nitrogen fixation. Cyanobacteria are classified in Bergey's Manual and has five orders (subsections). These five orders (subsections) include three for non-heterocystous types algae and two for heterocystous type algae (Castenholz 2001; Castenholz and Waterbury

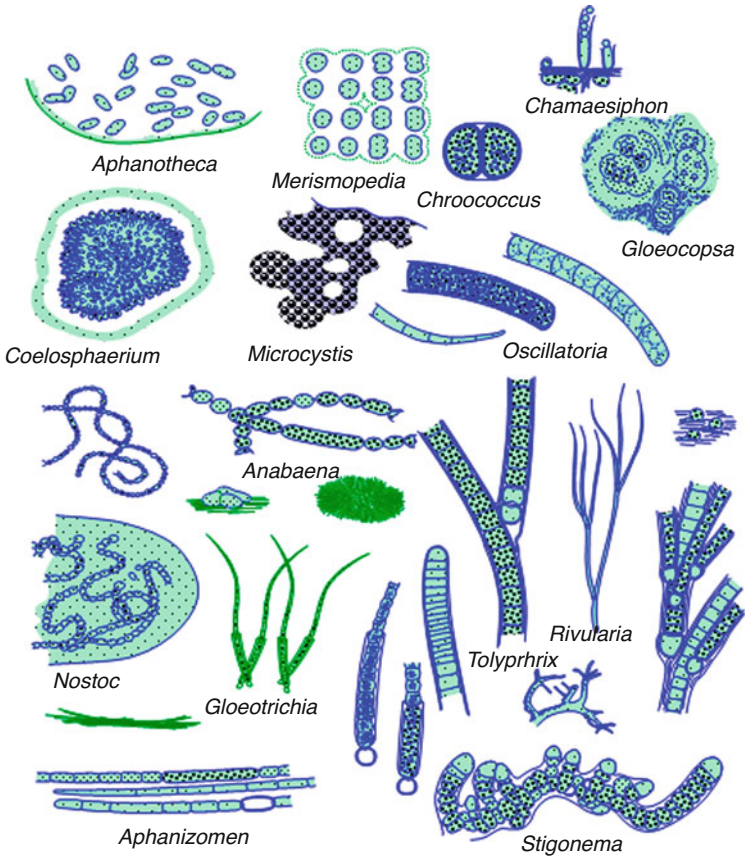

Fig. 24.2 Different blue green algae help in biological nitrogen fixation (Image taken from Issa et al. 1997)

1989). The non-heterocystous algae belongs to order *Chroococcales*, *Pleurocapsales*, and *Oscillatoriales*, while heterocystous algae belongs to order Nostocales and Stigonematales and they all are able to fix atmospheric (Bergman et al. 1997).

From Table 24.1 it is clear that cyanophycean algae may have heterocyst or may not have but in both the cases they are able to fix atmospheric nitrogen. Totally, more than 150 species of 33 genera have been reported to be able to fix nitrogen (Li et al. 1983). There are many unicellular blue green algae, able to fix nitrogen like Gloeocapsa, almost five different strains have been reported that can fix nitrogen aerobic situation (Wyatt and Silvey 1969; Rippka and Stanier 1978; Rippka et al. 1979). Three strains of Synechococcus perform N_2 fixation under anaerobic conditions. Studies on the nitrogen fixing BGA are in different heads as per the presence and absence of heterocyst in the cell and are known as heterocystous and non-heterocystous algae. Non-heterocystous filamentous blue green algae are able to fix nitrogen under aerobic conditions (Bergman et al. 1997). Non-heterocystous

Table 24.1 Cyanophycean algae and its classifications

Cyanophyceae	Order	Features
Non-heterocystous	1. Chroococcales	Unicellular cyanobacteria and reproduce by binary fission, e.g., Merismopedia Gloeocapsa, *Trichodesmium*, and *Microcystis*
	2. Pleurocapsales	Unicellular cyanobacteria and produces daughter cells smaller than the parent *eg. Pleurocaps*a
	3. Oscillatoriales	They are filaments form of algae and cells are known as trichomes, e.g., *Oscillatoria Phormidium*, *Microcoleus*, *Lyngbya*, and *Planktothrix*
Heterocystous	1. Nostocales	Trichomes present with vegetative cells are also divided into heterocysts. few species have false branching in some species *Anabaena*, *Aphanizomenon*, *Nostoc*, *Calothrix*, and *Tolypothrix*
	2. Stigonematales	Trichomes is present with heterogeneous cellular composition as well as heterocysts and akinetes vegetative cells. Filament multiseriated with true branching, e.g., Stigonema, Mastigocladus, and *Fischerella*

cyanobacteria are able to maintain a nitrogenase level sufficient for photoautotrophic growth at the expense of N_2 under aerobic conditions in both unicellular and multicellular forms. Non-heterocystous algae are having ability to synthesize nitrogenase. *Plectonema boryanum* (Stewart et al. 1969; Lex 1970) can fix atmospheric nitrogen only under microaerobic conditions. Similarly, Phormidium, Raphidiopsis (Singh 1961), Oscillatoria, Lyngbya, and Plectonema were shown to fix N_2 under microaerobic conditions only. Rippka and Stanier (1978) analysed that in strict anaerobic conditions almost 50% non-heterogenous blue green algae are able to synthesis nitrogenase enzyme. Intense anaerobic situations were created by blocking the photosynthetic O2-evolution by 3,4-dichlorophenyl-1,1-dimethylurea (DCMU).

Heterocystous blue green algae are able to fix N_2 aerobically and microaerobically. Among heterocystous *Anabaena*, *Aulosira*, *Calothrix, Cylindrospermum*, *Nostoc*, *Scytonema*, *Tolypothrix*, *Fischerella*, *Mastigocladus*, and *Stigonema* are the most common blue green algae. These algae have specialized cells different from vegetative cell. These special cells are known as heterocyst. Normal vegetative cells change to heterocysts when grown in the absence of combined nitrogen. Fogg (1949) suggested that heterocysts are the actual sites of nitrogen fixation in algae cells. Further it was reported that nitrogenase is located in the heterocyst under aerobic growth conditions (Stewart et al. 1969; Fleming and Haselkorn 1973; Peterson and Wolk 1978a, b). Nitrogenase is an oxygen sensitive enzyme and heterocyst is a suitable site for its function as it lacks photosystem II, which is responsible for oxygen evolution in cells. So heterocysts show nitrogenase activity under anaerobic conditions (Smith and Evans 1971; Rippka and Stanier 1978).

Symbiotic blue green algae are heterocystous and few are unicellular blue green algae able to develop symbiosis. Different groups of cyanophycean algae were found which can fix nitrogen (Table 24.2). Blue green algae are having vast host range and

Table 24.2 Different Cyanophycean algae and its groups

S. No	Algal group	Features
1.	Unicellular group	Unicellular strains successfully grow on BG II medium in the absence of nitrogen (broth is a universal medium for cultivation of blue green algae)
2.	*Anabaena* group	Heterocystous algae having thin sheath, no branches, non-mucilaginous colonies with definite shape *(Anabaena, Nodularia, Cylindrospermum, Anabaenopsis,* etc.)
3.	Nostoc group	Heterocystous mucilaginous strains without branching, but thick sheath and well-defined shape *(Nostoc)*
4.	*Aulosira* group	Heterocyst containing strains, having thick sheath, without branching, does not form diffuse colonies on agar medium *(Aulosira)*
5.	*Scytoneina* group	False branched algae with heterocyst, no polarity. Velvet like patched colonies found when grown in agar medium *(Scytonema)*
6.	*Calothrix* group	Same as Scytonema group *(Calothrix, Tolypothrix, Hassalia*)
7.	*Gloeotrichia* group	Definite shape mucilaginous colony with heterocyst, with polarity *(Gloeotrichia, Rivularia*)
8.	*Fischerella* group	True branching with heterocyst *(Fischerella, Westiellopsis, Stigonema*)

found in different parts of plant. They are found in close association with diatoms, lichens, fungi, bryophytes, gymnosperms, and angiosperms. Two most important characteristics of blue green algae are presence of heterocyst and formation of short motile fragment known as hormogonia. This hormogonium makes algal cells motile otherwise immotile. *Nostoc*, *Calothrix*, *Scytonema*, *Fischerella*, and *Gloeocapsa* are commonly found in symbiosis with fungi and form lichens. In bryophytes *Azolla* and *Anthoceros* are symbiotically associated with *Anabaena* and *Nostoc*, respectively. Macrozamia (gymnosperms) is symbiotically found with Nostoc and Gunnera (angiosperms) is found with Nostoc (Stewart et al. 1980). *Azolla* mainly used as green manure to improve the nitrogen balance in rice fields. Global nitrogen fixation is highly influenced by blue green algae of lichens and liverworts (Stewart et al. 1980). Different algal groups of blue green algae are summarized in Table 24.2.

24.5 Heterocyst and Nitrogen Fixation

Heterocysts (Gr. Hetero = different; Cyst = swollen and encapsulated cell) are found in many species of filamentous blue-green algae (Fay and Fogg 1962). Heterocysts are anaerobic factories for nitrogen fixation. These cells are slightly larger size as compared to vegetative cells, and it also develops from vegetative cells and may be solitary, or in pairs, or several in a row (Fogg 1949). It is a colourless, enlarged, thick walled cell without chlorophyll (Fig. 24.3). It is the site for nitrogen fixation, and it has nitrogenase enzyme (Stewart et al. 1980). The cell is maintained in its anoxic condition as it lacks photosynthetic activity (produce oxygen) and thick

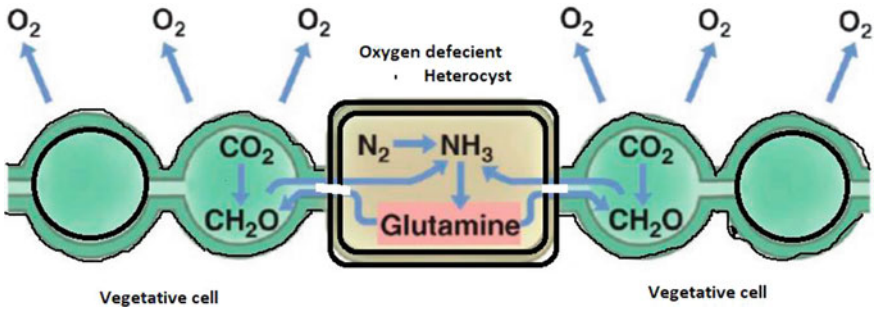

Fig. 24.3 Algal cells showing vegetative cell and heterocyst cells

wall, these conditions are essential for the proper functioning of nitrogenase. Heterocyst contains only photosystem1 for ATP production, Photosystem II is completely absent. Heterocysts cells are linked with other cells through plasmodesmata and get nutrients from surrounding cells. The shape of the heterocysts and its position in the trichomes are determined genetically. The number of heterocysts in trichomes of any algal populations depends on the nitrogen supply in the environment. As the level of NH_4^+ or NO_3^- increases, the number of heterocyst decreases within a trichome.

At low intensity of light and at high concentration of phosphate in medium, the number of heterocyst in filament increases in algae (Fay and Fogg 1962). Heterocyst's fixes atmospheric nitrogen under anaerobic condition due to the presence of nitrogenase, which is maintained within the heterocyst. Heterocyst can be at the tip of trichome, i.e., terminal (*Cylindrospermum*), intercalary (*Anabaena*) or metameric, i.e., roughly at regular distances from one another, or in pairs (*Anabaenopsis*). During development, the thylakoid apparatus degrades and specific DNA rearrangements (e.g., *nif* genes) occur.

Nif Gene

The atmospheric nitrogen fixation is an energy intensive process. If there is no nitrogen fixation by cell all the enzymes must be tightly controlled and not to fix nitrogen at any cost. So the genetic control of nitrogen fixation in bacteria must have turn off and turn on mechanism whenever required. These are a specific gene working for nitrogen fixation in cells. The nif gene codes proteins that are actually able to fix atmospheric nitrogen. It is also found in few nitrogen fixing bacteria (free-living nitrogen fixing bacteria and in symbiotic bacteria) and cyanobacteria. The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen along with this it also encodes few regulatory proteins involved in nitrogen fixation. Primarily nif genes encode nitrogenase complex, which can convert atmospheric nitrogen to ammonia like nitrogen forms.

(continued)

Nitrogen fixation is regulated by nif regulon, which has both positive and negative regulators and a set of seven operons which includes 17 nif genes. Nif genes have both positive and negative regulators. Some nif genes are: Nif A,D, L,K, F,H,S,U,Y,W,Z.

Nitrogen sensitive NifA protein activates nif genes transcription. NtrC, which is an RNA polymerase is used by plants when there is not enough fixed amount of nitrogen factor, and it triggers NifA's expression. Further NifA activates the transcription for the nif genes. NifL protein gets activated whenever there is sufficient amount of reduced nitrogen or oxygen present in the cell. In such situation NifL inhibits activity of NifA activity that causes inhibition of nitrogenase formation in cell. Then NifL is regulated by other proteins, and this protein is sensitive for O_2 and ammonium concentration of the surrounding environment. The nif genes are also present in bacterial cells and are associated with plasmids along with few other genes responsible for nitrogen fixation in bacterial cell.

Nitrogenase Nitrogenase term was given by Burk 1934 for the enzyme that catalyses the conversion of atmospheric unavailable nitrogen to a bio-accessible nitrogen (Burk et al. 1934). Nitrogenase is playing very vital and meaningful role in heterocyst. Nitrogenase is two soluble protein components: (1) Component I or Mo-Fe protein also known as dinitrogenase consists of 2 Mo atoms, 28–34 Fe atoms, and 26–28 acid-labile sulphides, also known as iron-molybdenum cofactor (FeMoco). Component I has two copies each of two subunits α and β (Fig. 24.4). (2) Component II or electron-transfer Fe protein, this unit is known as dinitrogenase reductase. Component II contains two copies of a single subunit (Fig. 24.4). This protein has four non-heme Fe atoms and four acid-labile sulphides (4Fe-4S). A reducing source and catalysis MgATP is required. Substrate binding and reduction takes place on component I, which binds to ATP and ferredoxin or flavodoxin proteins (Fdx or Fld). The hydrolysis of ATP supplies the energy for the reaction, while the Fdx/Fld proteins supply the electrons and this reduction reaction electron must be added to

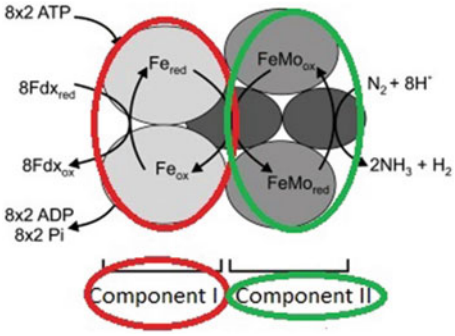

Fig. 24.4 Structure of nitrogenase enzyme components I dinitrogenase and component II dinitrogenase reductase

the nitrogen (N_2) to reduce it to NH_4. In short component II simply supplies electrons to component I, one at a time. ATP is not hydrolysed to ADP until component II transfers an electron to component I. 21–25 ATPs are required for each N_2 to be fixed.

Damage Control Mechanism of Nitrogenase

Enzyme nitrogenase is oxygen and cold sensitive, so cannot perform functions in the presence of O_2. Photosystem II is also absent in heterocysts, which is responsible for water photolysis and generates oxygen, this is the reason why PSII is absent. PSI helps to generate ATP, i.e., assimilatory power helps in nitrogen fixation. Absence of PSII maintains oxygen free environment and hydrogen rich inside the cell and nitrogenase acts perfectly in this condition. Oxidase enzymes is a very importanat enzymes and it is found in heterocystous cells, by chance if oxygen molecules enters inside heterocyst through polar plug, It converts it into water molecule as hygrogen gas is already in cells. Due to this it mainatins a reducing and non-oxygenating environment within heterocystous cells.

Mechanism of Protection of Nitrogenase

Blue green algae is having very important enzymes that helps in nitrogen fixation within algala cell. This enzyme is Nitrogenase and it is sensitive to oxygen. As it is oxygen sensitive so blue green algae have evolved different mechanism of protection. Among them first one is the presence of thick mucilaginous coating around blue green algae and it protects algal cells from oxygen diffusion. Other important modification is that PS II is absent and there is no oxygen evolved during photosynthesis. Heterocyst cells act as a separate compartment. Respiratory activity is higher in heterocyst as compared to vegetative cells. Oxygen from air binds to special glycolipids found in laminated layer of heterocyst envelope. In case of Gloeothece an intensive internal protective compartment is found. The cells manage to perform little photosynthesis, reserve of fixed nitrogen, and later more photosynthesis without nitrogen fixation activity. This mechanism generates an intensive system of internal membranes which actually represent intracellular protective compartment. In case of *Trichodesmium* (Carpenter and Price 1976), it filament forms a bundle like structure and outsider filaments perform photosynthesis while the filaments placed at inner side perform nitrogen fixation.

Under nitrogen fixing conditions hydrogenase produces along with nitrogenase and it helps in formation of H_2 in blue green algae. Hydrogen formation stimulated in light and proceeds in dark, and low concentration of oxygen present for respiration. ATP-dependent H_3O^+-reduction catalysed by nitrogenase enzymes and finally production of Hydrogen occurs. This hydrogen is used by cell in two different ways and both ways were catalysed by hydrogenases. First it is used as oxygen dependent reaction in respiratory chain and helps in supply of extra ATP production. Second it may be used in light-requiring reaction.

24.6 Significance of Algal Nitrogen Fixation

Globally fixed nitrogen ultimately affects the productivity of the major ecosystems, especially agriculture fields are highly affected. Throughout world there are different regions and variety of cyanophycean algae is able to fix atmospheric nitrogen. These regions are different due to climatic conditions and difference in soil texture. It has been observed that in temperate soil heterocystous blue green algae increases nitrogen content in soil. Reports show that blue green algae fixes nitrogen up to 51 or 94 kg N/ha per year. In cold dominant ecosystem, blue green algae fixes atmospheric nitrogen in the form of Lichens and algal moss association, while few free-living algae were also reported. Nostoc commune is the most common specie reported in tundra and Antarctic soils either as free-living or as photobiont in lichens. Blue green algae are widely found in tropical and temperate region but most abundant in tropics, highly active in submerged soil. In weed free maize fields *Cylindrospermum licheniforme* grows successfully and fix atmospheric nitrogen. In freshwater environment nitrogen fixation is very critical due to uneven distribution of algae in water (Fogg 1949), while in marine system blue green algae are less common. Trichodesmium (Oscillatoriaceae) forms large aggregated biomass in the form of bundles of filaments. These aggregates are able to fix nitrogen and can develop large biomass and fix N_2 (Fogg 1949). Few species of Calothrix were found to colonize large areas of sand (P. Roger unpub.). *Anabaena cylindrica* was isolated from an aquatic habitat and there is no report of its presence in freshwater. Anabaena few species are found in freshwater and considered as important nitrogen fixer. In modern agriculture system cyanobacteria (BGA) is widely used as biofertilizer.

24.7 Conclusion

Algae play important roles in many fields like medicine, food, agriculture, and in biological research. Cyanobacteria are ubiquitous in the world soils and are primary photosynthetic agents of the soil, play important role in soil ecology like soil fertility and reclamation. Cyanobacteria have special feature of nitrogen fixation and make them an important biofertilizer. As the continuous use of chemical fertilizer leads to soil health degradation so cyanophycean algae is a boom for soil and environment. Cyanobacterial (BGA) biofertilizers lead to soil enrichment and are compatible with long-term sustainability. Most interestingly it is eco-friendly and not at all dangerous to the environment. Other important feature is its adaptation for extreme environments.

References

Bergman B, Gallon JR, Rai AN, Stal LJ (1997) N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol Rev 19:139–185

Bray CM (1983) Nitrogen metabolism in plants. Longman Group Ltd, Harlow

Burk D, Lineweaver H, Horner CKJ (1934) The specific influence of acidity on the mechanism of nitrogen fixation by azotobacter. Bacteriology 27:325

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of earth's nitrogen cycle. Science 330:192

Carpenter EJ, Price CC (1976) Marine Oscillatoria (Trichodesmium): explanation for aerobic nitrogen fixation without heterocysts. Science 191(4233):1278–1280

Castenholz RW (2001) Cyanobacteria. In: Boone DR, Castenholz RW (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 473–487

Castenholz RW, Waterbury JB (1989) Group I. Cyanobacteria. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams & Wilkins, Baltimore, pp 1710–1728

Cheng QJ (2008) Perspectives in biological nitrogen fixation resesarch. Integr Plant Biol 50:786

Dos Santos PC, Fang Z, Mason SW, Setubal JC (2012) Dixon R. Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics 13:162

Fay P, Fogg GE (1962) Studies on nitrogen fixation by blue-green algae. III. Growth and nitrogen fixation in Chlorogloea fritschii Mitra. Arch Mikrobiol 42:310–321

Ferguson S (1998) Nitrogen cycle enzymology. J Curr Opin Chem Biol 2:182

Fleming H, Haselkorn R (1973) Differentiation in Nostoc muscorum: nitrogenase is synthesized in heterocysts. Proc Natl Acad Sci U S A 70:2727–2731

Fogg GE (1949) Growth and heterocyst production in Anabaena cylindrica Lemm. II. In relation to carbon and nitrogen metabolism. Ann Bot 13:241–259

Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. Nature 451:293

Haber F (1923) Bemerkung zu vorstehender Notiz. Naturwissenschaften 11:339–340

Haber F (1992) About the representation of ammonia from nitrogen and hydrogen. Nat Sci 10:1041

Issa AA, Alla MHA, Ohyama T (1997) Nitrogen fixing cyanobacteria: future prospects, 2014. In: Advances in biology and ecology of nitrogen fixation. InTech Open, London

Jia H-P, Quadrelli E (2014) Mechanistic aspects of dinitrogen cleavage and hydrogenation to produce ammonia in catalysis and organometallic chemistry: relevance of metal hydride bonds and dihydrogen. Chem Soc Rev 43:547

Lex M (1970) Nitrogenase activity in the blue-green alga *Plectonema boryanum* strain 594. Arch Mikrobiol 73:250–260

Li S, Ley SH, Qianlin W (1983) Nitrogen fixing blue-green algae, a source of biofertilizer. In: Tseng CK (ed) Proc of the joint China-US phycology symposium. Science Press, Beijing, p 479

MacKay BA (2004) Dinitrogen coordination chemistry: on the biomimetic borderlands. Chem Rev 104:385

McGlynn SE, Boyd ES, Peters JW, Orphan VJ (2013) Classifying the metal dependence of uncharacterized nitrogenases. Front Microbiol 3:419

Peterson RB, Wolk CP (1978a) Localization of an uptake hydrogenase in Anabaena. Physiology 61:49

Peterson RB, Wolk CP (1978b) High recovery of nitrogenase activity and of 55Fe-labeled nitrogenase in heterocysts isolated from Anabaena variabilis. Proc Natl Acad Sci U S A 75:6271–6275

Rippka R, Stanier RY (1978) The effect of anaerobiosis on nitrogenase synthesis and heterocyst development by nostocacean cyanobacteria. J Gen Microbiol 105:83–94

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories, and properties of pure cultures of cyanobacteria. J Gen Microbial 111:l61

Rodrigo V, Novelo E (2007) Seasonal changes in periphyton nitrogen fixation in a protected tropical wetland. Biol Fertil Soils 43:367–372
Singh RN (1961) Role of blue-green algae in nitrogen economy. In Indian Council Agric. Res. New Delhi, India
Smil V (2004) Enriching the earth. In: Haber F, Bosch C (eds) The transformation of world food production. MIT Press, Cambridge
Smith RV, Evans MCW (1971) Nitrogenase activity in cell free extracts of the blue-green alga Anabaena cylindrica. J Bacteriol 105:913–917
Stewart WDP, Haystead A, Pearson HW (1969) Nitrogenase activity in heterocysts of blue-green algae. Nature 224:226–228
Stewart WDP, Rowell P, Rai AN (1980) Symbiotic nitrogen-fixing cyanobacteria. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 239–277
Thamdrup B (2012) New pathways and processes in global nitrogen cycle. Annu Rev Ecol Evol Syst 43:407
Wyatt JT, Silvey JKGS (1969) Nitrogen fixation by Gloeocapsa. Science 165:908–909

Chapter 25
Role of Cyanobacteria in Rhizospheric Nitrogen Fixation

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Abstract An innumerable group of microorganisms are associated with plants and present in the phyllosphere, rhizosphere, and endosphere. They produce different metabolites—such as amino acids, proteins, polysaccharides, and vitamins—that affect plant growth and help to improve soil fertility and crop production naturally. Furthermore, among all inhabitants of the rhizosphere, cyanobacteria are among the foremost organisms, with potential to contribute to biological nitrogen fixation. They contain a specialized type of cell (a heterocyst), which is considered the actual site of dinitrogen fixation in cyanobacteria. Bryophyte–cyanobacterial associations are significant sources of nitrogen fixation in terrestrial ecosystems such as boreal forests. Therefore, cyanobacterial inoculants are influential biofertilizers in sustainable agriculture. This chapter describes beneficial aspects of nitrogen fixation by cyanobacteria in the rhizosphere and the role of cyanobacteria in increasing soil fertility, which leads to improved crop productivity.

Keywords Cyanobacteria · N_2 fixation · Heterocyst · Bryophyte–cyanobacteria association

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25.1 Introduction

The plant rhizosphere is enriched with very high abundance and varying population fractions of the microbiota. One such microbial fraction comprises different cyanobacteria sustained in associative or free-living symbiosis with a variety of plant roots. Cyanobacteria, which are popularly known as diazotrophic blue-green algae, are considered to be one of the oldest and most pioneering forms of photosynthetic microbes in the evolutionary history of life (Demoulin et al. 2019). In fact, they are often described as early colonizers and initiators of primary ecological succession on bare land, in association with fungi (lichens) (Lan et al. 2012). Members of cyanobacterial communities are chlorophyll-containing, simple thalloid, autotrophic, aerobic, Gram-negative, unicellular or multicellular, filamentous or nonfilamentous prokaryotic cells, ranging between 0.5–1 and 40 μm in diameter, and they build ecological niches in terrestrial and aquatic ecosystems (Whitton and Potts 2012). In terrestrial soil ecosystems, some free-living forms (*Anabaena*, *Calothrix*, *Oscillatoria*, *Tolypothrix*, and *Scytonema*, among others) can be found inhabiting and colonizing the rhizospheric soil of various members of the plant kingdom, ranging from bryophytes to angiosperms (Adams et al. 2013). Some members, such as those belonging to the genus *Nostoc*, have been found to establish endosymbiotic relationships with coralloid roots of pteridophytes (Chang et al. 2019). The sole role of these microalgal communities in the rhizosphere is to convert atmospheric nitrogen into ammonia, thus making nitrogen bioavailable to plants and hence enhancing plant growth. In nutrient-rich aquatic ecosystems, they form dense green free-floating colonial clusters by aggregation of millions of cyanobacteria, resulting in algal blooms and thus causing eutrophication.

Colonies of different members dwelling in the rhizosphere or in any other habitat show various types of morphological, physiological, and biochemical adaptations to cope with the selective pressures operating within their ecological niche (Galhano et al. 2011). Some of them can produce filaments, sheets, or even hollow spheres (Sand-Jensen 2014). To protect their thalloid body from desiccation and mechanical stress, they secrete mucilage. Their potential to differentiate into multiple cells performing specialized functions within the same filament is important in terms of the physiological and biochemical aspects of cyanobacteria with respect to survival. Under favorable conditions, the primary thalloid body is composed of vegetative cells and photosynthetic cells. However, in adverse conditions, some cells in the filament differentiate to form climate-resistant spores called akinetes (Sukenik et al. 2015). Moreover, the majority of cyanobacterial species develop several thick-walled cells, called heterocysts, after every 9–15 cells at repeated intervals within the filament (Kumar et al. 2010). These special cells harbor the oxygen-sensitive nitrogenase (the enzymatic complex that enables nitrogen fixation) and serve as the principal anaerobic site most suitable for nitrogen fixation. It is important to note that free-living cyanobacteria such as *Nostoc* can supply large amounts of nitrogen to plant roots after they die, followed by lysis of the filament along with the ammonia-rich heterocyst cells (Meeks and Elhai 2002). However, endosymbiont

cyanobacteria can survive in dark conditions in plant tissues and supply plants with nitrogen in the form of ammonia in their living state (Issa et al. 2014a, 2014b).

Their metabolic flexibility means that cyanobacteria principally obtain nutrients by performing photosynthesis, which makes them self-sufficient for their own food production and one of the major sources of oxygen evolution (Hamilton et al. 2016). In fact, it is believed that their photosynthetic metabolism, contributing to oxygen production, set the foundation for emergence of aerobic life-forms on Earth millions of years ago (Schirrmeister et al. 2011). Also, the biochemical diversity and metabolic potential of various members of this group has enabled them to interconnect with an extensive network of ecosystem services in synergism with other biotic components of nature (Kumar et al. 2015). Some of the important ecosystem services they provide include their abilities to carry out nitrogen and carbon fixation, produce oxygen, promote plant growth, shape plant rhizosphere microbiomes, and perform nutrient cycling by linking several biogeochemical cycles. The potent plant growth–promoting properties of cyanobacterial inoculants can be commercially exploited as biofertilizers with promising results (Chittora et al. 2020). Ongoing research and genome decoding of several species of cyanobacteria have firmly proved their multidimensional benefits in plant productivity and crop improvement.

25.2 Diversity and Ecological Distribution of Cyanobacteria in the Plant Rhizosphere

Cyanobacteria (blue-green algae) are photosynthetic prokaryotic free-living bacteria from an ancient lineage, and they are established in most, though not all, types of environment. They are globally responsible for much of the primary production and nitrogen fixation that occurs globally. Global ecosystems have been immeasurably impacted by cyanobacteria, and their photosynthetic actions support oxygen requirements for procreation of aerobic life-forms. Additionally, cyanobacteria are acknowledged as the abundant primary manufacturer on Earth. Furthermore, cyanobacteria can be found in marine habitats, hot habitats, cold habitats, freshwater habitats, and terrestrial habitats. Cyanobacteria also commonly enter into symbiotic relationships with plants, eukaryotic algae, and fungi. A considerable contribution of cyanobacteria is the plant chloroplast, in which a cyanobacterium lives inside the plant's cells and survives by taking in food that the plant makes for itself. In the early Cambrian period or in the late Proterozoic period, cyanobacteria took up residence in some eukaryote cells, conducting an endosymbiotic relationship by providing food for them in return for shelter (Issa et al. Issa et al. 2014a, 2014b). Cyanobacteria require only light, oxygen, inorganic substances, carbon dioxide, and water to manufacture their food. Photosynthesis is still considered to be their prime food-manufacturing process, but some cyanobacteria can survive in complete darkness. Cyanobacteria are considered to be the first plants to form outposts in bare areas, such as on soil and rocks. Cyanobacteria have evolved over time by increasing their

adaptability to comparatively exposed terrestrial environments and developing ultraviolet (UV) light–absorbing sheath pigments. A variety of these organisms are found in soil and different habitats mainly on land, where they are helpful in the functioning of ecosystems and nutrient cycling (Severin et al. 2010). In addition, marine and limnic environments are considered cyanobacterial extrusive habitats. They grow in fresh/brackish water, in salty water, in hot and cold springs, and in environments where other microalgae cannot survive. Most marine cyanobacteria flourish along the shore as seafloor vegetation in the zone between the low and high tide marks. A large proportion of cyanobacteria are globally distributed in marine plankton. Cyanobacteria are halotolerant; they can tolerate saline environments rather than being halophilic. They can survive in salt concentrations as high as 2–3% and are found in salty areas because they can colonize euryhaline environments (Moisander et al. 2002). Fresh water with a distinct trophic environment is an extrusive habitat for cyanobacteria. Many different species instinctively inhabit and sporadically influence both epilimnic lake water (close to the surface) and hypolimnic lake water (the deeper layer close to the lake bed). Some survive at the surface by forming mats, which float on the water surface, and some adhere to sediments or rocks (Herrero et al. 2001). Cyanobacteria not only survive in fertile environments but also have an ability to survive on and colonize infertile substrates such as rock or sediments, desert soil, and volcanic ash. Cyanobacteria have an exceptional ability to colonize all ecosystem environments on Earth, such as extraordinary cold and hot environments, mountain streams, katharobic waters and polysaprobic zones, and Antarctic and Arctic lakes (Alvarenga et al. 2015).

This shows the successful evolution of cyanobacteria to survive in almost all ecological habitats on Earth. This successful evolution of cyanobacteria to survive at the limits of life in the environment has helped to inspire the notion that life could once have existed on Mars. The ability of cyanobacteria to adapt to extreme abiotic stresses includes tolerance of freezing, drought, extreme light exposure (including high UV-B flux), and low-nutrient oligotrophic conditions.

Cyanobacteria also play major roles in marine ecosystems and in the global carbon cycle. The marine environment is dominated by *Synechococcus* and *Prochlorococcus*. These two photoautotrophic picoplankton genera are prevalent throughout the world's oceans, where they hold a prime position at the base of the marine ecosystem food web, potentially controlling carbon flow through the system. The tiny cyanobacterium *Prochlorococcus*, which is predominant in the central Pacific Ocean off Hawaii, may contribute up to 40% of chlorophyll production and 30% of living carbon, forming a microbial loop, which helps recycle mineral elements. The larger cyanobacterium *Synechococcus*, which is predominant in the Sargasso Sea, causes formation of large granules, improving the efficiency of carbon export to higher trophic levels (Andreeva et al. 2020).

Cyanobacteria are also known for their ability to fix atmospheric nitrogen. These species are heterocystous biofertilizers in filamentous forms and belong to the Nostocales and Stigonematales orders. Eighty percent of rhizosphere isolates are *Anabaena* and *Nostoc*. Their oxygen photosynthesis and nitrogenase activity differ

according to their size, shape and nitrogenase activity, and are generally light dependent (Syiem et al. 2017). Nitrogen-fixing cyanobacteria are mostly found in the rhizospheres of rice and wheat, and they help promote growth of crops and other plants. This helps explain the roles of cyanobacteria in marine ecosystem communities, carbon cycling, and climate modulation. It also highlights the importance of cyanobacteria in nitrogen fixation and balancing of elements (Prasanna et al. 2009).

25.3 The Heterocyst: A Potent Diazotrophic Cyanobacterial Cell

Organisms that contain diverse cell types acquire various structures, functions, and behaviors, and are more capable than unicellular organisms. Among multicellular cyanobacteria, the formation of heterocysts provides an example of their cellular and multicellular pattern arrangements. Many cyanobacteria with distinctive heterocysts are able to carry out nitrogen fixation under anaerobic conditions. Heterocysts are functional cells that fix atmospheric nitrogen, and they are formed in conditions of nitrogen deprivation by filamentous cyanobacteria (Kumar et al. 2010). The structure of a heterocyst includes thick cell envelopes and cyanophycin granules at the poles. At the poles, there is an outer polysaccharide layer (HEP) and a heterocyst-specific glycolipid (HGL) layer, which are "laminated" together, and a narrow cytoplasmic membrane is visible. It has also been noted that formation of a neck at a heterocyst pole occurs only when the pole is adjacent to vegetative cells, so terminal heterocysts have only one neck (Herrero et al. 2016). Cyanobacteria forming heterocysts have highly differentiated specialized cells in which the heterocyst has a symbiotic relationship with vegetative cells, providing fixed nitrogen to adjacent vegetative cells inside the filament in return for photosynthetic products. This differentiation of cellular functions plays an important role because cyanobacteria can perform photosynthesis in conditions of oxygen deprivation, but nitrogenase is unstable in aerobic environments. Heterocysts can be distinguished from vegetative cells as they are bigger, rounder, and less pigmented, and they have heavy tegumentary layers, thylakoid membranes, and granules at the poles, adjacent to vegetative cells (Bergman et al. 2007). When a source of combined nitrogen is present, *Anabaena* cyanobacteria multiply in a long filament form with hundreds of photosynthetic vegetative cells. Heterocysts are formed in conditions of nitrogen deprivation. Heterocyst differentiation is provoked by absence of a fixed nitrogen source, which acts as an environmental indicator. In this differentiation, comprehensive alteration of cellular metabolism takes place, changing the earlier structure. Hence, the mother vegetative cell, with phototrophic metabolism, is reconstructed in a change to photoheterotrophic metabolism, which promotes the activity of nitrogenase. All of the functional changes and modifications result from formation of an evolved pattern of genetic expiration (Falcón et al. 2002). The heterocyst pattern is controlled by differentiation into fresh heterocysts through cell division at the center

of the vegetative cells. In some cyanobacteria, the frequency of heterocyst formation can be highly variable. Extreme cellular differentiation is also represented by heterocysts and akinetes (produced only by heterocyst cyanobacteria) in cyanobacteria, but research has shown that nonheterocystous cyanobacteria have a more specialized cellular composition for survival in conditions of desiccation and for nitrogen fixation. Interfilamentous signaling is used by many different cyanobacteria for cellular differentiation (Adams and Duggan 1999).

25.4 Role of Rhizospheric Cyanobacteria in Nitrogen Fixation

The term *rhizosphere* was coined by Lorenz Hiltner (a plant physiologist and German agronomist) in 1904. The rhizosphere encompasses the plant roots and nurtures a legion of microbes, which benefit the plant in multitudinous ways, such as biocontrol and promotion of plant growth and health (Mendes et al. 2013). The microbial community is governed by both biotic and abiotic determinants. The anatomy and the population size of the microbial community depend on the plant and its exudates (Olanrewaju et al. 2019). The associated microbes may or may not directly influence the plant and may not always benefit the plant; they can also be pathogenic (Vives-Peris et al. 2020). Along with the above benefits, the rhizosphere also acts as an agent for bioremediation and soil aggregation (Yu et al. 2019). The abundance of the microbiota can be boosted and optimized with good understanding of the microbiota and their ecology, which is determined by the chemical constitution and structure of the rhizosphere. The microbiota establish contact with plant roots, fix a multitude of inorganic forms of macronutrients and micronutrients, and take the lead in regulation of various ecological and mineral cycles (Zanardini et al. 2019).

From urea to essential and vital proteins and nucleic acids, nitrogen plays a significant role and is thus essential for survival (Gu et al. 2018). It is a prime constituent of chlorophyll and is crucial for plant growth and production (Bhattacharyya and Jha 2012). Dinitrogen is abundant in the atmosphere, but plants can utilize nitrogen only when it is in a reduced or oxidized form. These forms are produced by various microbes, which convert dinitrogen into bioavailable forms (Kant 2018). At a industrial level, the Haber–Bosch process is used for ammonia production. Microbial nitrogen fixation is, however, more efficient than the Haber–Bosch process (Kandemir et al. 2013), as it is eco-friendly, conserves energy, and occurs in much less optimal conditions. In addition to converting nitrogen into a bioavailable form for uptake by plants, microbes benefit plants and their surrounding soil in various ways. One of the most prominent genera known for nitrogen fixation is *Rhizobium*. Rhizobia are able to form nodules on plants roots as well as in their stems in certain cases (Bhattacharyya and Jha 2012; Dicenzo et al. 2019). Efficiency in biology refers to effective utilization of energy in a process at minimal cost under

specific conditions. Nitrogen fixation via a symbiotic process (van Rhijn and Vanderleyden 1995) also helped meet the need for energy in 1970 during the world's energy crisis, which occurred due to dramatic increases in oil prices all over the world. Scientists therefore utilized solar energy to enhance nitrogen production for plant growth, and since then it has become an essential part of research on feeding the world's human population by fulfilling the goals of sustainable development, agriculture, and production. This has led to replacement of fossil fuels with biological nitrogen fixers, which has proved to be more effective and efficient than the Haber–Bosch process, which depends upon fossil fuels. This is referred to as *community level efficiency* (Masclaux-Daubresse et al. 2010; Zanardini et al. 2019).

Apart from rhizobia, *Frankia* and cyanobacteria symbiotically fix nitrogen in plants. Unlike rhizobia, which can fix nitrogen for Leguminosae, Parasponia, etc., cyanobacteria can fix nitrogen for a number of other plant species and families. They are capable of fixing nitrogen aerobically (like *Anabaena*), anaerobically, or microaerobically (like *Plectonemaboryanum*) (Feng et al. 2010). However, *Frankia* fixes nitrogen for actinorhizal plants only by forming nodules (Sellstedt and Richau 2013).

25.5 Significance of Cyanobacteria in Nitrogen Fixation

Cyanobacteria (Haselkorn 2009) are competent at fixing nitrogen and are known to promote healthy growth in plants. Nitrogen fixation involves conversion of atmospheric dinitrogen into ammonia, the assimilatory form of nitrogen, with the help of enzymes. The process is mediated by microbes and provides nitrogen in water bodies that are nitrogen deficient. This is majorly accomplished by cyanobacterial species (Zehr 2011). Often, cyanobacterial species face a paradoxical situation, as they produce oxygen in photosynthesis but the anaerobic process of nitrogen fixation is highly sensitive to oxygen. For this, diazotrophic taxa and some other taxa of cyanobacteria have specialized morphological, biochemical, and ecological adaptations. They also have special mechanisms for shielding nitrogenase from inactivation by oxygen (Ohki et al. 2008), such as restricting the process of nitrogen fixation at night (as photosynthesis does not takes place at that time), forming aggregates and colonies to decrease illumination, forming microzones with low levels of oxygen, acting as endosymbionts in certain biological associations, and forming heterocysts (in taxa that are filamentous), which allow them to fix nitrogen, while photosynthesis is also performed by the same species, reducing the oxygen problem (Berman-Frank et al. 2003).

Trichodesmium lacks heterocysts, yet it is capable of fixing nitrogen during daytime or the light phase. This fact is currently under investigation; however, it is estimated that nitrogenase is restricted to the subset of cells in the filament, and these cells are not known to produce oxygen (Inomura et al. 2019).

Nitrogen fixation is a process that requires high energy input (Silsbury 1977), as 16 adenosine triphosphate (ATP) molecules are required to fix a single molecule of

dinitrogen. However, unlike other nonphotosynthetic nitrogen-fixing bacteria, which require a redox reaction and organic matter to meet this high energy need for nitrogen fixation, cyanobacteria are capable of meeting this energy need by photosynthesis.

Cyanobacteria form symbioses with multiple hosts and, unlike rhizobia and other nitrogen-fixing microbes, these are not confined to plant roots. Cyanobacteria can form symbiotic relationships with other parts of plants, and they are not necessarily located inside the host plant's cells. Cyanobacteria are also capable of fixing carbon in nonphotosynthetic hosts (Eigemann et al. 2019). Since they are facultative heterotrophs, they are not confined to the parts of the plant that receive sunlight. Two paramount attributes of these plant symbionts are that they can differentiate into specialized nitrogen-fixing cells (heterocysts) and briefly into motile filaments (hormogonia) which lack the heterocyst but provides dispersal pathway for other nonmotile cyanobacteria (Pang et al. 2018; Schuergers et al. 2017).

Cyanobacteria that are symbiotically associated with bryophytes (Adams and Duggan 2008) achieve higher rates of nitrogen fixation than the same cyanobacteria in a free-living state (Salemaa et al. 2019). This is due to a significantly increased frequency of heterosis, resulting in a nitrogen fixation rate 6–10 times that achieved by cyanobacteria in a free-living state. In this state, only 20% of the fixed nitrogen is retained by the cyanobacteria and the remainder is transferred to the host in the form of ammonia.

Often, *Azolla* forms an association with *Anabaena* as its nitrogen-fixing partner (Peters 1977). Its upper lobe contains a central cavity, which accommodates *Anabaena* filaments. *Azolla* draws nitrate from water, as well as the ammonia secreted by *Anabaena* in the cavities of *Azolla* leaves, and this plays a crucial role in production of rice (Bidyarani et al. 2015). Together, they are widely used effectively and have been confirmed as a great source of green manure, especially in Asian countries, where they are majorly used as a fertilizer, increasing production of rice by up to 58%. This has made it possible to produce rice year after year and has led to a decline in dependency on crop rotation, with increments in productivity of several other crops as well.

25.6 Metabolic Interconnections Established by Cyanobacteria in Biogeochemical Cycles Operating in the Rhizosphere

Cyanobacteria are one of the most ancient groups of photosynthetic microbes found in inland waters. They have major effects on water quality and aquatic ecosystem function in that they can produce taste and odor compounds, toxins, and noxious blooms. In inland waters, groups of cyanobacteria form mats and, in particular, they form polysaccharide crusts, films, and thick layers on rocks, plants, and sediments. Bloom-forming species of some microalgae are mainly present in eutrophic water

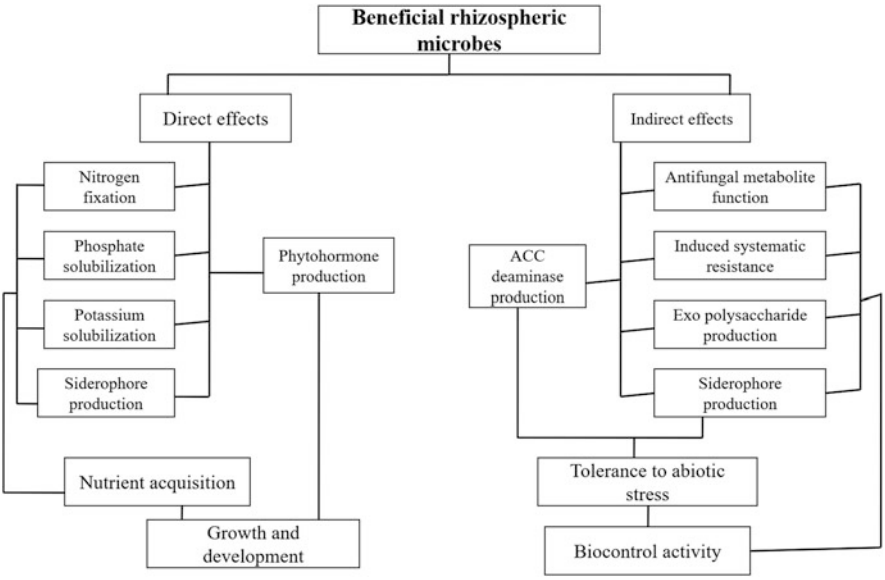

Fig. 25.1 Effects of beneficial rhizospheric microbes

bodies, disrupting the food web and producing toxins and surface scum. Unicellular picocyanobacteria perform photosynthesis in oligotrophic lakes, where they are part of the microbial food web. These bacteria can also be used as sources of food, animal feed, fertilizers, and health-related products. They are Gram-negative bacteria and can cause acute illness, such as gastroenteritis, when cyanobacteria-contaminated water is consumed.

Cyanobacteria have the ability to store essential nutrients and metabolites in their cytoplasm (Fig. 25.1). The depletion of nitrogen in the medium increase lipid production in cyanobacterial cells. The fundamental metabolic process of cyanobacteria is dinitrogen fixation, and because of this feature, they are considered the simplest nutritional suppliers of all living organisms. Through use of nitrogenase, nitrogen is directly converted into ammonium (NH_4) and thereby enters the food chain.

25.6.1 Nitrogen-Fixing Cyanobacteria

Among filamentous forms of cyanobacteria, there are many heterocyst-forming genera such as *Anabaena*, *Trichodesmium*, and *Nostoc* (Stewart 1973). Heterocysts are the main site of dinitrogen fixation in cyanobacteria. The process of nitrogen fixation by these organisms is increased when sufficient nutrients are available, which leads to rapid growth and production of these organisms. Mass development

of such forms is known as a bloom and is commonly seen in eutrophic lakes and other marine environments.

25.6.2 Nitrogen-Fixing Ecostrategists

Many species that belong to genera such as *Anabena*, *Cylindrospermopsis*, *Aphanizomenon*, *Nodularia*, and *Nostoc* are able to fix nitrogen in nitrogen-limited conditions, such as in deep and narrow aquatic systems. These organisms adopt this type of strategy especially when levels of dissolved nitrogen are low. However, it has been found that various water bodies (mostly lakes) with limited nitrogen have no dominance of nitrogen-fixing cyanobacteria. This could be due to less availability of light, because huge amounts of energy are required for the nitrogen fixation process. Zevenboom and Mur (1980) noted that in turbid lakes, less light is available for effective performance of the nitrogen fixation process. A number of nitrogen-fixing species may form colonies and possess gas vesicles, making them capable of regulating their buoyancy. The reduction of phosphate and nitrogen simultaneously can be strengthen pre-bailing nitrogen limiting environment promotes growth of many nitrogen-fixing cyanobacterial species. These species may use nitrogen sources such as nitrate, nitrite, and ammonium for their growth (Guerrero and Lara 1987). Reduction of molecular nitrogen and growth at the expense of ammonium is observed (Liotenberg et al. 1996). Via an active transportation system, ammonium ions (whenever they are externally supplied) enter the cell, whereas unprotonated ions get into the cell by a process of diffusion and are trapped via protonation. Nitrate is taken up by cyanobacterial cells via an active transport system and is sequentially reduced to nitrite by nitrate reductase and then to ammonium by nitrite reductase. The glutamine synthetase–glutamate synthase (GS-GOGAT) enzyme system is the most important pathway for assimilation of ammonium, whether it is supplied exogenously or generated internally. All nitrogen sources converge in production of ammonium and glutamine; any physiological differences are related to the nitrogen source (which depends on the level of ammonium within the cell) or to a direct effect of the nitrogen source on the regulation of cell metabolism.

Cyanobacteria accumulate different types of reserves that can be used as sources of nitrogen, carbon, or both (Allen 1984). The cyanobacterial nitrogen reserve is unique to cyanobacteria but not universally present among them (Liotenberg et al. 1996). These nonribosomally synthesized polypeptides consist of equimolar quantities of arginine and aspartic acid, which get assembled into granules with a molecular mass ranging from 25,000 to 100,000 kDa (Simon 1971, 1973, 1976). Cyanophycin usually accumulates in the cell when growing in excess of other essential nutrients, such as phosphorus and sulfur, and it decreases under nitrogen-deficient growing conditions, where it serves as a nitrogen source.

According to Carr (1988), the cyanophycin granule polypeptide is a more dynamic nitrogen reserve then phycobiliproteins. Glycogen is a major carbon and

energy reserve compound accumulated by cyanobacteria during photoautotrophic growth (Smith 1982). Accumulation of this glucose polymer can occur as a result of nitrogen-limited growth conditions in light or in the presence of an excess of a utilizable carbon source (Lehmann and Wöber 1976). Growth under suboptimal temperature conditions (van Eykelenburg 1980) or with a sudden increase in light energy input without any changes in nitrogen metabolism can also lead to glycogen accumulation. In darkness or in light, once the conditions for balanced growth are re-established, glycogen reserves are rapidly depleted to yield energy and carbon for cell metabolism. Glycogen may therefore act as a reserve with the dual functions of product storage and acting as a buffer substance between carbon fixation and carbon consumption in other biosynthetic pathways.

The supply of assimilable nitrogen in the environment depends on phycobiliprotein synthesis, and these proteins can serve as a nitrogen reserve (Tandeau de Marsac and Houmard 1993). Cyanobacteria that are incapable of fixing molecular nitrogen respond to nitrogen deprivation by degrading phycobiliproteins and linker polypeptides that form phycobilisomes, leading to rapid cell bleaching (Liotenberg et al. 1996). As was originally postulated by Allen and Smith (1969) with regard to *Anacystis nidulans* (*Synechococcus* sp. PCC 6301), phycocyanin acts as a nitrogen storage compound in *Spirulina platensis*. In the marine cyanobacterium *Synechococcus* sp. strain DC2, free phycoerythrin is also a pool of stored nitrogen (Wyman et al. 1985) with importance in marine environments, where nitrogen is frequently limited. The filamentous cyanobacterium *Calothrix* PCC 7601 has lost the capacity to differentiate between functional of heterocysts and to fix dinitrogen (Kallas et al. 1985).

25.6.3 Importance of the Carbon/Nitrogen Balance

A balance between carbon and nitrogen is necessary for each and every biological system because all cellular components—proteins, genetic materials, pigments, energy-carrying molecules, etc.—are derived from these activities. Carbon and nitrogen metabolism are tightly coupled in very different living organisms. The coupling mechanisms in prokaryotes and plants depend on two factors. The first is that two elements (carbon and nitrogen), which are most abundant in cell and increases the coordination mechanism requirement and the second is that nitrogen assimilation is dependent upon carbon skeleton availability for biosynthesis. Therefore, C/N stoichiometry in different organisms varies within a relatively narrow range; for example, the mass ratio of C/N is 31/4 in phytoplankton because it is the proper balance for metabolism of carbon and nitrogen and is important for optimal growth. Different regulation levels exist in different cells to control uptake and assimilation of different nitrogen and carbon sources, whose supply may vary under different environment conditions (Chellamuthu et al. 2013; Herrero et al. 2004; Burnap et al. 2015). Such regulation can occur at various levels of control, ranging from allosteric modulation of the activity of nutrient assimilation proteins to

a variety of mechanisms controlling expression of the genes encoding these structural proteins. The latter include a very rich variety of transcriptional and posttranscriptional mechanisms, some of which are still being experimentally clarified (Klähn et al. 2018; Du et al. 2014; Selim et al. 2018). Recently, tremendous progress has been made toward answering questions involving cyanobacteria by using them as a model for photosynthetic organisms, and toward summarizing the data and highlighting the mechanisms underlying signaling and transcriptional regulation involved in metabolic control of carbon and nitrogen. Plastids are ancestors of cyanobacteria (Ponce-Toledo et al. 2017); therefore, common features such as signaling mechanisms and balancing of C/N metabolism are shared by plants and cyanobacteria.

25.7 Associative and Non-associative Nitrogen-Fixing Symbioses with Terrestrial Vegetation

The nitrogen-fixing cyanobacterium *Nostoc* is a common terrestrial and aquatic cyanobacterium mostly found in symbiosis with a wide range of plant, algal, and fungal species (Bergman et al. 2007). Numerous species of N_2-fixing cyanobacteria can form symbiotic associations with prokaryotes and eukaryotes, providing most with nitrogenous compounds. Their hosts include diatoms, fungi, bryophytes, pteridophytes, gymnosperms (cycads), and angiosperms (Bergman et al. 2007).

25.7.1 Associative Nitrogen Fixation: Root–Nodule Symbiosis

This type of symbiosis has excited biologists across a diversity of disciplines, including microbiological cell and developmental biology and evolution, because it involves a diversity of nodule organ structures with externally triggerable development (Pawlowski and Demchenko 2012; Remigi et al. 2016; Masson-Boivin and Sachs 2018), phylogenetically different endosymbiotic bacteria, and a diversity of infection modes (Pawlowski and Demchenko 2012). Our understanding of the evolution of nodulation has made a significant leap with the discovery that all N_2-fixing root nodule–forming genera belong to a single phylogenetic clade and are much more closely related than previously thought (Soltis et al. 1995).

25.7.2 Biological Nitrogen Fixation

Nitrogen fixation is a dynamic and a very high energy–demanding process (Rosenblueth et al. 2018). Free-living diazotrophs correspond to a small fraction of plant rhizosphere ecosystems and are members of the Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Cyanobacteria (Morris and Schniter 2018). Their presence, function, and importance can be explained by the "Black Queen" hypothesis, which states that in free-living microbial communities, a few "helpers" that perform most of the workload in terms of functions, such as high energy–requiring nitrogen fixation, support the remaining flora and fauna, which are beneficiaries depending on those "helpers" for their nitrogen requirements. In the symbiotic relationship between soil bacteria (collectively known as rhizobia) and legumes, root-derived nodules fix atmospheric nitrogen through the action of nitrogenase (Morris and Schniter 2018). Biological nitrogen fixation by plant–bacterial associations represents a very important natural system for capturing atmospheric nitrogen and processing it into a reactive form of nitrogen through enzymatic reduction. Biological nitrogen fixation is an exceedingly sensitive process affected by nutrients and environmental conditions, and it allows a plant to fulfill all or part of its requirements through interaction with endosymbiotic, associative, and endophytic symbionts, giving it a competitive advantage over a non-nitrogen-fixing plant (Graham 1992; King and Purcell 2005; Zahran 1999). The conservation of nitrogenous complexes in free-living and symbiotic diazotrophs allows them to take part in various types of association with their host plants. Biological N_2 fixation by plant rhizobial symbiotic systems is mediated by endosymbiotic interactions when plants grow root nodules. In legumes and rhizobia, Gram-negative alphaproteobacteria are the most common microbial species associated with legumes in the Fabaceae family (Schultze and Kondorosi 1998; Desbrosses and Stougaard 2011). Nitrogen fixation has also been well documented in actinomycetes such as *Parasponia* species, which associate with a broad spectrum of actinorhizal plants (Santi et al. 2013). Cyanobacteria (*Nostoc* spp.) have also been found to colonize different plant organs either interacellularly in the Gunneraceae family or extracellularly in Cycadaceae, liverworts, and hornworts. Associative N_2-fixing symbiosis is frequently observed between diazotrophs and a large variety of plants, including cereals. Nitrogenase, associated proteins, and non-protein-forming nitrogenous enzymes are sensitive to the presence of O_2 (Burén and Rubio 2018). For extreme sensitivity to oxygen, anaerobes such as *Clostridium pasteurianum* are perfect candidates for nitrogen fixation. Facultative anaerobes, such as *Kiebsiella oxytoca*, also have the ability to fix nitrogen but only in the absence of oxygen. Obligate aerobes, such as Azotobacter vinelandii, can shield nitrogenase from oxygen and fix nitrogen by using oxygen via cytochrome oxidases (Yates and Jones 1974; Poole and Hill 1997).

25.7.3 Nitrogen Fixation by Moss–Cyanobacterial Associations

Biological fixation of atmospheric nitrogen is an important pathway for entry of available nitrogen into an ecosystem. In boreal forests with limited nitrogen, N_2 fixation is performed by cyanobacteria in association with mosses. In these associations, nitrogen fixation is inhibited by nitrogen input; thus, nitrogen fixation takes place only in areas with low nitrogen deposition. Laboratory and field studies have shown that artificial addition of nitrogen inhibits N_2 fixation by moss–cyanobacterial associations. N_2 fixation is affected in different ways by the type and quantity of nitrogen that enters the system, and by the moisture level in the plant host; moist conditions promote N_2 fixation. Nitrogen is probably leaked by cyanobacteria, but it is not known whether the nitrogen moves into the soil, how quickly that occurs, and for how long.

25.7.4 Moss–Cyanobacterial Associations in Boreal Forests

Little background nitrogen deposition occurs in boreal forests (Karlsson et al. 2009). Their soils are characterized by low inorganic nitrogen concentrations, low pH values, and low temperatures, which contribute to the limitation of nitrogen in these system. Mosses play a critical role in boreal forest ecosystems because of their contribution to habitat heterogeneity (Longton 1988) and their effects on the hydrology, temperature, and chemistry of boreal forest soil (Bonan 1991). By buffering abiotic factors, they can demonstrate high water retention capacity (Dickson 2000). Mosses can provide a solid and beneficial habitat for cyanobacterial colonizers, which promote N_2 fixation in nitrogen-limited ecosystems (DeLuca et al. 2002). Various genera of cyanobacteria (*Nostoc* and *Calothrix*) have been found living epiphytically on feather mosses. There is a linear relationship between the numbers of cyanobacteria cells and nitrogen fixation rates in feather mosses (DeLuca et al. 2007), showing that the cyanobacteria are responsible for nitrogen fixation, whereas methanotrophs probably contribute little to nitrogen fixation in feather mosses (DeLuca et al. 2007; Ininbergs et al. 2011).

Moss biomass and the activity of cyanobacteria (DeLuca et al. 2007, 2002; Sorensen et al. 2012) are sensitive to nitrogen input, which dramatically reduces the abundance of dominant moss species and significantly reduces or eliminates nitrogen fixation in moss–cyanobacterial associations (DeLuca et al. 2007). The amount of nitrogen input determines the form in which nitrogen enters the ecosystem, either as organic nitrogen via the moss layer when there is little nitrogen deposition and high nitrogen fixation, or as inorganic nitrogen when nitrogen deposition is greater and bypasses the moss layer. Mosses absorb nutrients and water from atmospheric deposition; thus, they are very sensitive to increased input of nutrients. Unlike addition of nitrogen, which probably only lowers nitrogen

fixation rates, addition of other nutrients to the moss layer has the potential to raise nitrogen fixation rates. Addition of soluble phosphates to Arctic mosses has been reported to increase nitrogen fixation rates.

25.8 Use of Cyanobacteria Inoculants as Important Biofertilizers in Sustainable Agriculture

25.8.1 Uses in Organic Farming

Soil is a natural system, which helps to grow ecosystems, develops communities, and affects the lives of the human population. Soil organic matter is always a target for improving the quality of food, so, from time to time, novel technologies have been developed to increase soil productivity. Soil organic content can be increased in various ways, which may have tremendous effects. Natural enhancers of soil productivity and soil health have advantages over chemicals and fertilizers. Organic farming has been practiced for a long time, but in more recent times, excessive use of fertilizers and chemicals has caused loss of soil health. There are many different types of organic farming, which greatly help to restore natural ecosystems and natural soil resources. Use of biofertilizers and green manure increases the organic matter and nitrogen content of soil, reduces soil pollution, and also helps to maintain soil health. Unlike use of synthetic pesticides, which pollute groundwater and cause major losses, organic farming does not pose risks to soil systems (Stolze et al. 2000).

Some common organic farming practices and their benefits are discussed in the following subsections.

25.8.1.1 Crop Rotation

Crop rotation helps to maintain soil nutrient levels and enhances soil quality. It also disrupts weed and insect growth, interrupts the life cycles of pests, sequesters carbon and nitrogen, and diversifies crop production.

25.8.1.2 Green Manure Application

Green manure application causes an increase in soil organic matter and enhances soil quality and sequestration of carbon and nitrogen, improving crop productivity.

25.8.1.3 Cover Cropping

A cover crop is a specific crop that is planted to maintain soil nutrient values and to benefit the growth of other crops. Cover crops are used to suppress weeds, reduce erosion, enhance soil quality, and improve the nutrient content, quality, and fertility of soil.

25.8.1.4 Avoidance of Synthetic Fertilizers and Pesticides

Use of synthetic fertilizers causes surface contamination and groundwater contamination. Use of synthetic pesticides mainly causes loss of biodiversity, as they threaten the survival of small microorganisms that are needed for soil fertility. Avoidance of synthetic fertilizer and pesticide use enhances biodiversity, improves water quality, enhances soil quality, assists in effective pest management, prevents disruption of pollinators, and reduces the costs of chemical inputs.

25.8.1.5 Planting of Habitat Corridors

Planting of habitat corridors enhances biodiversity, supports biological pest management, and provides wildlife habitats. Use of buffer areas improves water quality, enhances biodiversity, and prevents wind erosion.

25.8.2 Multidimensional Role of Cyanobacteria in Organic Farming

Cyanobacteria use solar energy to fix carbon and nitrogen in soil. They do not require a host cell for physiological activities such as growth, development, and production of valuable organic products. In rice paddy fields, the association of *Azolla* with *Anabaena* is an example of symbiotic nitrogen fixation and helps enrich the fields' nutrient content. These biofertilizers have been widely used in cultivation of radishes, lettuces, maize, barley, oats, tomatoes, sugarcane, chillies, and cotton (Thajuddin and Subramanian 2005). The World Health Organization has projected that a 50% increase in global food production will be needed by 2029. "Green revolution" practices are now being used worldwide to increase agricultural productivity and minimize the risks posed by chemical-based fertilizer use, which ultimately affects human health as well as the environment. These "green technologies" are eco-friendly and involve utilization of microbes. Green technologies include many different applications using cyanobacteria to improve soil fertility and crop productivity. Cyanobacteria are able to degrade a wide range of soil pollutants and also help maintain soil ecosystems and soil fertility (Subramanian and Uma 1996).

Cyanobacteria are considered promising microorganisms for use in sustainable agricultural development (Rangel-Yagui et al. 2004; Singh et al. 2017a; Singh et al. 2017b). Among them, diazotrophs such as cyanobacteria are useful as eco-friendly biofertilizers and are both cost effective and easily available. Some very important benefits of cyanobacteria are that they improve the water-holding capacity and aeration of soil, and they also add vitamin B_{12} (Paumann et al. 2005; Malik et al. 2001; Song et al. 2005).

The most commonly used and most highly effective cyanobacteria include Anabaena variabilis, *Nostoc linkia*, *Aulosira fertilisima*, *Tolypothrix* spp., *Calothrix* spp., and *Scytonema* spp. (Prasad and Prasad 2001). *Anabaena* and *Nostoc* can survive on rock, as well as on soil surfaces, and are able to fix up to 20–25 kg/ha of atmospheric nitrogen. *Anabaena* enriches soils with organic matter and can fix up to 60 kg/ha of nitrogen (Moore 1969). Because of this useful property of cyanobacteria, they are widely used in sustainable agriculture and also for large-scale biofertilizer production. A few important factors such as temperature, water, light, pH, carbon dioxide content, and supplementation with nutrients (C, N, P, S, K, Fe, etc.) affect the success of cyanobacterial growth (Pulz 2001; Flynn et al. 2010; Meena et al. 2017a; Meena et al. 2017b). In rice fields, *Aulosira fertilissima*, *Anabaena* spp., *Nostoc* spp., and *Scytonema* spp. are most abundant, while *Gloeotrichia* spp., *Cylindrospermum* spp., and *Rivularia* spp. are common in deep-water rice cultivation. Cyanobacteria are the most inexpensive sources of natural biofertilizers for rice-based cropping systems (Omar 2000).

25.9 Advantages of Biofertilizers over Chemical Fertilizers

In the modern era, increases in crop productivity have depended upon use of chemical fertilizers and pesticides. Pests and other pathogens greatly affect agricultural productivity. These chemicals are mainly used to increase soil health and productivity, but they actually disturb plant–microbe associations within the soil system. Plant cells and tissues get softened as a result of chemical reactions and thus are more prone to pathogenic attacks. As a result of leaching of chemicals, nutrients are easily lost and decomposition of soil is increased, causing soil acidification or alkalinization, and a consequent reduction in soil fertility. Collectively, soil exposure to fertilizers and pesticides leads to loss of natural ecosystems and damages soil health overall.

Sustainable agriculture includes high production of food for better human health and can also restore natural resources, ensure economic viability, and protect ecosystems. Continuous use of these chemicals and pesticides affects soil health and can also impair productivity, as it increases loss of nutrients from soil; thus, important steps must be taken to conserve natural resources and, at the same time, increase production. Use of cyanobacterial biofertilizer is one of the first steps. These small microorganisms are abundant worldwide and can improve the growth and development of plants. They also produce some highly active substances of

biological origin that assist absorption of heavy metals, which is very important for bioremediation (Ibraheem 2007). Moreover, these biofertilizers are natural, eco-friendly, productive, efficient, and more easily accessible to small farmers than chemical fertilizers (Mishra et al. 2013).

25.10 Conclusion and Future Perspective

Nitrogen-fixing cyanobacterial strains improve soil nitrogen content. Cyanobacterial species in the rhizosphere have potential to fix dinitrogen; therefore, use of such strains in farming is beneficial for sustainable agricultural practice and is also eco-friendly. Furthermore, use of cyanobacterial biomass could be promising for production of food, energy, and biofertilizers. Large-scale cultivation of nitrogen-fixing cyanobacteria strains is required for their use in soil inoculation, leading to formation of biocrusts and greatly enhancing soil fertility. Optimization of cyanobacterial farming is desirable. There is a need for further research on the pertinency of nitrogen-fixing cyanobacteria species that can be utilized for inoculation in diverse ecosystems with different soil types.

References

Adams DG, Duggan PS (2008) Cyanobacteria–bryophyte symbioses. J Exp Bot 59:1047–1058

Adams DG, Bergman B, Nierzwicki-Bauer SA, Duggan PS, Rai AN, Schüßler A (2013) Cyanobacterial–plant symbioses. In: Rosenberg E, DeLong EF, Stackebrandt E, Lory S, Thompson F (eds) The prokaryotes: prokaryotic biology and symbiotic associations. Springer, Berlin, pp 359–400

Adams DG, Duggan PS (1999) Tansley review no. 107. Heterocyst and akinete differentiation in cyanobacteria. New Phytol 144:3–33

Issa AA, Abd-Alla MH, Ohyama T (2014a) Nitrogen fixing cyanobacteria: future prospect. In: Ohyama T (ed) Advances in biology and ecology of nitrogen fixation. InTech Open Science, London, pp 23–48

Allen MM, Smith AJ (1969) Nitrogen chlorosis in blue-green algae. Arch Mikrobiol 69:114–120

Allen MM (1984) Cyanobacterial cell inclusions. Annu Rev Microbiol 38:1–25

Alvarenga DO, Rigonato J, Branco LHZ, Fiore MF (2015) Cyanobacteria in mangrove ecosystems. Biodivers Conserv 24:799–817

Andreeva NA, Melnikov VV, Snarskaya DD (2020) The role of cyanobacteria in marine ecosystems. Russ J Mar Biol 46:154–165

Bergman B, Rai AN, Rasmussen U (2007) Cyanobacterial associations. In: Elmerich C, Newton WW (eds) Associative and endophytic nitrogen fixing bacteria and cyanobacterial associations. Springer, Dordrecht, pp 257–301

Berman-Frank I, Lundgren P, Falkowski P (2003) Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Res Microbiol 154:157–164

Bhattacharyya PN, Jha DK (2012) Plant growth–promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Bidyarani N, Prasanna R, Chawla G, Babu S, Singh R (2015) Deciphering the factors associated with the colonization of rice plants by cyanobacteria. J Basic Microbiol 55:407–419

Bonan GB (1991) A biophysical surface energy budget analysis of soil temperature in the boreal forests of interior Alaska. Water Resour Res 27:767–781

Burén S, Rubio LM (2018) State of the art in eukaryotic nitrogenase engineering. FEMS Microbiol Lett 365:fnx274

Burnap RL, Hagemann M, Kaplan A (2015) Regulation of CO_2 concentrating mechanism in cyanobacteria. Life 5:348–371

Carr NG (1988) Nitrogen reserves and dynamic reservoirs in cyanobacteria. In: Rogers LJ, Gallon JR (eds) Biochemistry of the algae and cyanobacteria. Clarendon, Oxford, pp 13–21

Chang ACG, Jun D, Li N (2019) Perspectives on endosymbiosis in coralloid roots: association of cycads and cyanobacteria. Front Microbiol 10:1888

Chellamuthu VR, Alva V, Forchhammer K (2013) From cyanobacteria to plants: conservation of PII functions during plastid evolution. Planta 237:451–462

Chittora D, Meena M, Barupal T, Swapnil P, Sharma K (2020) Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochem Biophys Rep 22:100737

DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152:121–130

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419:917–920

Demoulin CF, Lara YJ, Cornet L, François C, Baurain D, Wilmotte A, Javaux EJ (2019) Cyanobacteria evolution: insight from the fossil record. Free Radic Biol Med 140:206–223

Desbrosses GJ, Stougaard J (2011) Root nodulation: a paradigm for how plant–microbe symbiosis influences host developmental pathways. Cell Host Microbe 10:348–358

Dicenzo GC, Zamani M, Checcucci A, Fondi M, Griffitts JS, Finan TM, Mengoni A (2019) Multidisciplinary approaches for studying *Rhizobium*–legume symbioses. Can J Microbiol 65:1–33

Dickson LG (2000) Constraints to nitrogen fixation by cryptogamic crusts in a polar desert ecosystem, Devon Island, NWT, Canada. Arct Antarct Alp Res 32:40–45

Du J, Förster B, Rourke L, Howitt SM, Price GD (2014) Characterisation of cyanobacterial bicarbonate transporters in *E. coli* shows that SbtA homologs are functional in this heterologous expression system. PLoS One 9:e115905

Eigemann F, Vogts A, Voss M, Zoccarato L, Schulz-Vogt H (2019) Distinctive tasks of different cyanobacteria and associated bacteria in carbon as well as nitrogen fixation and cycling in a late stage Baltic Sea bloom. PLoS One 14:e0223294

Falcón LI, Cipriano F, Chistoserdov AY, Carpenter EJ (2002) Diversity of diazotrophic unicellular cyanobacteria in the tropical North Atlantic Ocean. Appl Environ Microbiol 68:5760–5764

Feng X, Bandyopadhyay A, Berla B, Page L, Wu B, Pakrasi HB, Tang YJ (2010) Mixotrophic and photoheterotrophic metabolism in *Cyanothece* sp. ATCC 51142 under continuous light. Microbiology 156:2566–2574

Flynn KJ, Greenwell HC, Lovitt RW, Shields RJ (2010) Selection for fitness at the individual or population levels: modelling effects of genetic modifications in microalgae on productivity and environmental safety. J Theor Biol 263:269–280

Galhano V, De Figueiredo DR, Alves A, Correia A, Pereira MJ, Gomes-Laranjo J, Peixoto F (2011) Morphological, biochemical and molecular characterization of *Anabaena*, *Aphanizomenon* and *Nostoc* strains (cyanobacteria, Nostocales) isolated from Portuguese freshwater habitats. Hydrobiologia 663:187–203

Graham PH (1992) Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Can J Microbiol 38:475–484

Gu J, Li Z, Mao Y, Struik PC, Zhang H, Liu L, Wang Z, Yang J (2018) Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. Plant Sci 274:320–331

Guerrero MG, Lara C (1987) Assimilation of inorganic nitrogen. In: Fay P, van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 163–186

Hamilton TL, Bryant DA, Macalady JL (2016) The role of biology in planetary evolution: cyanobacterial primary production in low-oxygen proterozoic oceans. Environ Microbiol 18:325–340

Haselkorn R (2009) Cyanobacteria. Curr Biol 19:R277–R278

Herrero A, Muro-Pastor AM, Flores E (2001) Nitrogen control in cyanobacteria. J Bacteriol 183:411–425

Herrero A, Muro-Pastor AM, Valladares A, Flores E (2004) Cellular differentiation and the NtcA transcription factor in filamentous cyanobacteria. FEMS Microbiol Rev 28:469–487

Herrero A, Stavans J, Flores E (2016) The multicellular nature of filamentous heterocyst–forming cyanobacteria. FEMS Microbiol Rev 40:831–854

Ibraheem IB (2007) Cyanobacteria as alternative biological conditioners for bioremediation of barren soil. Egypt J Phycol 8:99–117

Ininbergs K, Bay G, Rasmussen U, Wardle DA, Nilsson MC (2011) Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. New Phytol 192:507–517

Inomura K, Wilson ST, Deutsch C (2019) Mechanistic model for the coexistence of nitrogen fixation and photosynthesis in marine *Trichodesmium*. MSystems. https://doi.org/10.1128/mSystems.00210-19

Issa AA, Abd-Alla MH, Ohyama T (2014b) Nitrogen fixing cyanobacteria: future prospect. Adv Biol Ecol Nitr Fixat 2:24–48

Kallas T, Coursin T, Rippka R (1985) Different organization of *nif* genes in nonheterocystous and heterocystous cyanobacteria. Plant Mol Biol 5:321–329

Kandemir T, Schuster ME, Senyshyn A, Behrens M, Schlögl R (2013) The Haber–Bosch process revisited: on the real structure and stability of "ammonia iron" under working conditions. Angew Chem 52:12723–12726

Kant S (2018) Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. Semin Cell Dev Biol 74:89–96

King CA, Purcell LC (2005) Inhibition of N_2 fixation in soybean is associated with elevated ureides and amino acids. Plant Physiol 137:1389–1396

Klähn S, Bolay P, Wright PR, Atilho RM, Brewer KI, Hagemann M, Hess WR (2018) A glutamine riboswitch is a key element for the regulation of glutamine synthetase in cyanobacteria. Nucleic Acids Res 46:10082–10094

Kumar K, Mella-Herrera RA, Golden JW (2010) Cyanobacterial heterocysts. Cold Spring Harb Perspect Biol 2:a000315

Kumar M, Singh DP, Prabha R, Sharma AK (2015) Role of cyanobacteria in nutrient cycle and use efficiency in the soil. In: Rakshit A, Singh HB, Sen A (eds) Nutrient use efficiency: from basics to advances. Springer, New Delhi, pp 163–171

Lan S, Wu L, Zhang D, Hu C (2012) Successional stages of biological soil crusts and their microstructure variability in Shapotou region (China). Environ Earth Sci 65:77–88

Lehmann M, Wöber G (1976) Accumulation, mobilization and turn-over of glycogen in the blue-green bacterium *Anacystis nidulans*. Arch Microbiol 111:93–97

Liotenberg, S., Campbell, D., Rippka, R., Houmard, J., de Marsac, N.T. (1996) Effect of the nitrogen source on phycobiliprotein synthesis and cell reserves in a chromatically adapting filamentous cyanobacterium. Microbiology, 142: 611–622

Longton RE (1988) Biology of polar bryophytes and lichens. Cambridge University Press, Cambridge

Malik FR, Ahmed S, Rizki YM (2001) Utilization of lignocellulosic waste for the preparation of nitrogenous biofertilizer. Pak J Biol Sci 4:1217–1220

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann Bot 105:1141–1157

Masson-Boivin C, Sachs JL (2018) Symbiotic nitrogen fixation by rhizobia—the roots of a success story. Curr Opin Plant Biol 44:7–15

Meeks JC, Elhai J (2002) Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. Microbiol Mol Biol Rev 66:94–121
Meena M, Swapnil P, Upadhyay RS (2017a) Isolation, characterization and toxicological potential of *Alternaria*-mycotoxins (TeA, AOH and AME) in different *Alternaria* species from various regions of India. Sci Rep 7:1–19
Meena M, Swapnil P, Zehra A, Dubey MK, Upadhyay RS (2017b) Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. Arch Phytopathol Plant Protect 50:629–648
Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663
Mishra D, Rajvir S, Mishra U, Kumar SS (2013) Role of bio-fertilizer in organic agriculture: a review. Res J Recent Sci 2:39–41
Moisander PH, McClinton EIII, Paerl HW (2002) Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microb Ecol 43:432–442
Moore AW (1969) *Azolla*: biology and agronomic significance. Bot Rev 35:17–34
Morris JJ, Schniter E (2018) Black queen markets: commensalism, dependency, and the evolution of cooperative specialization in human society. J Bioecon 20:69–105
Ohki K, Kamiya M, Honda D, Kumazawa S, Ho KK (2008) Morphological and phylogenetic studies on unicellular diazotrophic cyanobacteria (cyanophytes) isolated from the coastal waters around Singapore. J Phycol 44:142–151
Olanrewaju OS, Ayangbenro AS, Glick BR, Babalola OO (2019) Plant health: feedback effect of root exudates–rhizobiome interactions. Appl Microbiol Biotechnol 103:1155–1166
Omar HH (2000) Nitrogen-fixing abilities of some cyanobacteria in sandy loam soil and exudate efficiency on rice grain germination. Egypt J Phycol 1:157–167
Pang K, Tang Q, Chen L, Wan B, Niu C, Yuan X, Xiao S (2018) Nitrogen-fixing heterocystous cyanobacteria in the Tonian period. Curr Biol 28:616–622
Paumann M, Regelsberger G, Obinger C, Peschek GA (2005, 1707) The bioenergetic role of dioxygen and the terminal oxidase(s) in cyanobacteria. Biochim et Biophys Acta (BBA)-Bioenerg:231–253
Pawlowski K, Demchenko KN (2012) The diversity of actinorhizal symbiosis. Protoplasma 249:967–979
Peters GA (1977) The *Azolla–Anabaena* azzolae symbiosis. Basic Life Sci 9:231–258
Karlsson GP, Akselsson C, Hellsten S, Karlsson PE, Malm G (2009) Övervakning av luftföroreningar i Stockholms län. Resultat hydrologiskt år 2008/09 resp. kalenderår 2008. IVL rapport B 1851. Svenska Miljöinstitutet, Göteborg
Ponce-Toledo RI, Deschamps P, López-García P, Zivanovic Y, Benzerara K, Moreira D (2017) An early-branching freshwater cyanobacterium at the origin of plastids. Curr Biol 27:386–391
Poole RK, Hill S (1997) Respiratory protection of nitrogenase activity in *Azotobacter vinelandii*—roles of the terminal oxidases. Biosci Rep 17:303–317
Prasad RC, Prasad BN (2001) Cyanobacteria as a source biofertilizer for sustainable agriculture in Nepal. J Plant Sci Bot Orient 1:127–133
Prasanna R, Nain L, Ancha R, Srikrishna J, Joshi M, Kaushik BD (2009) Rhizosphere dynamics of inoculated cyanobacteria and their growth-promoting role in rice crop. Egypt J Biol 11:26–36
Pulz O (2001) Photobioreactors: production systems for phototrophic microorganisms. Appl Microbiol Biotechnol 57:287–293
Rangel-Yagui C, Danesi EDG, de Carvalho JCM, Sato S (2004) Chlorophyll production from *Spirulina platensis*: cultivation with urea addition by fed-batch process. Bioresour Technol 92:133–141
Remigi P, Zhu J, Young JPW, Masson-Boivin C (2016) Symbiosis within symbiosis: evolving nitrogen-fixing legume symbionts. Trends Microbiol 24:63–75

Rosenblueth M, Ormeño-Orrillo E, López-López A, Rogel MA, Reyes-Hernández BJ, Martínez-Romero JC, Reddy PM, Martínez-Romero E (2018) Nitrogen fixation in cereals. Front Microbiol 9:1794

Salemaa, M., Lindroos, A.J., Merilä, P., Mäkipää, R., Smolander, A. (2019) N_2 Fixation associated with the bryophyte layer is suppressed by low levels of nitrogen deposition in boreal forests. Sci Total Environ, 653: 995–1004

Sand-Jensen K (2014) Ecophysiology of gelatinous *Nostoc* colonies: unprecedented slow growth and survival in resource-poor and harsh environments. Ann Bot 114:17–33

Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot 111:743–767

Schirrmeister BE, Antonelli A, Bagheri HC (2011) The origin of multicellularity in cyanobacteria. BMC Evol Biol 11:45

Schuergers N, Mullineaux CW, Wilde A (2017) Cyanobacteria in motion. Curr Opin Plant Biol 37:109–115

Schultze M, Kondorosi A (1998) Regulation of symbiotic root nodule development. Annu Rev Genet 32:33–57

Selim KA, Haase F, Hartmann MD, Hagemann M, Forchhammer K (2018) PII-like signaling protein SbtB links cAMP sensing with cyanobacterial inorganic carbon response. Proc Natl Acad Sci 115:E4861–E4869

Sellstedt A, Richau KH (2013) Aspects of nitrogen-fixing actinobacteria, in particular free-living and symbiotic *Frankia*. FEMS Microbiol Lett 342:179–186

Severin I, Acinas SG, Stal LJ (2010) Diversity of nitrogen-fixing bacteria in cyanobacterial mats. FEMS Microbiol Ecol 73:514–525

Silsbury JH (1977) Energy requirement for symbiotic nitrogen fixation. Nature 267:149–150

Simon RD (1971) Cyanophycin granules from the blue-green alga Anabaena cylindrica: a reserve material consisting of copolymers of aspartic acid and arginine. Proc Natl Acad Sci USA 68:265–267

Simon RD (1973) Measurement of the cyanophycin granule polypeptide contained in the blue-green alga Anabaena cylindrica. J Bacteriol 114:1213–1216

Simon RD (1976) The biosynthesis of multi-L-arginyl-poly (Laspartic acid) in the filamentous cyanobacterium Anabaena glindrica. Biocbim Biophys Acta 422:407–418

Singh R, Parihar P, Singh M, Bajguz A, Kumar J, Singh S, Singh VP, Prasad SM (2017a) Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: current status and future prospects. Front Microbiol 8:515

Singh SP, Pathak J, Sinha RP (2017b) Cyanobacterial factories for the production of green energy and value-added products: an integrated approach for economic viability. Renew Sust Energ Rev 69:578–595

Smith AJ (1982) Modes of cyanobacterial carbon metabolism. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell, Oxford, pp 47–85

Soltis, D.E., Soltis, P.S., Morgan, D.R., Swensen, S.M., Mullin, B.C., Dowd, J.M., Martin, P.G. (1995) Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. Proceedings of the National Academy of Sciences, 92: 2647–2651

Song T, Mårtensson L, Eriksson T, Zheng W, Rasmussen U (2005) Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. FEMS Microbiol Ecol 54:131–140

Sorensen PL, Lett S, Michelsen A (2012) Moss-specific changes in nitrogen fixation following two decades of warming, shading, and fertilizer addition. Plant Ecol 213:695–706

Stewart WDP (1973) Nitrogen fixation by photosynthetic microorganisms. Annu Rev Microbiol 27:283–316

Stolze, M., Piorr, A., Häring, A.M., Dabbert, S. (2000) Environmental impacts of organic farming in Europe. Universität Hohenheim, Stuttgart-Hohenheim

Subramanian G, Uma L (1996) Cyanobacteria in pollution control. J Sci Ind Res 55:685–692

Sukenik A, Maldener I, Delhaye T, Viner-Mozzini Y, Sela D, Bormans M (2015, 1067) Carbon assimilation and accumulation of cyanophycin during the development of dormant cells (akinetes) in the cyanobacterium *Aphanizomenon ovalisporum*. Front Microbiol:6

Syiem MB, Singh AK, Rai AN (2017) N_2-fixing cyanobacterial systems as biofertilizer. Agro-Environ Sustain 1:43–61

Tandeau de Marsac N, Houmard J (1993) Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol Rev 10:119–189

Thajuddin N, Subramanian G (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci 89:47–57

Van Eykelenburg C (1980) Ecophysiological studies on *Spirulina platensis* effect of temperature, light intensity and nitrate concentration on growth and ultrastructure. Antonie Van Leeuwenhoek 46:113–127

Van Rhijn P, Vanderleyden J (1995) The *Rhizobium*–plant symbiosis. Microbiol Rev 59:124–142

Vives-Peris V, de Ollas C, Gómez-Cadenas A, Pérez-Clemente RM (2020) Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep 39:3–17

Whitton BA, Potts M (2012) Introduction to the cyanobacteria. II, Ecology of Cyanobacteria, pp 1–13

Wyman MRPF, Gregory RPF, Carr NG (1985) Novel role for phycoerythrin in a marine cyanobacterium, *Synechococcus* strain DC2. Science 230:818–820

Yates MG, Jones CW (1974) Respiration and nitrogen fixation in *Azotobacter*. Adv Microb Physiol 11:97–135

Yu K, Pieterse CMJ, Bakker PAHM, Berendsen RL (2019) Beneficial microbes going underground of root immunity. Plant Cell Environ 42:2860–2870

Zahran HH (1999) *Rhizobium*–legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989

Zanardini E, May E, Purdy KJ, Murrell JC (2019) Nutrient cycling potential within microbial communities on culturally important stoneworks. Environ Microbiol Rep 11:147–154

Zehr JP (2011) Nitrogen fixation by marine cyanobacteria. Trends Microbiol 19:162–173

Zevenboom W, Mur LR (1980) N_2-fixing cyanobacteria: why they do not become dominant in Dutch, hypertrophic lakes. In: Barica J, Mur LR (eds) Hypertrophic ecosystems. Springer, Dordrecht, pp 123–130

Chapter 26
Molecular Aspects and Oxygen Relations of Nitrogen Fixation in Cyanobacteria

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Abstract The biological reduction of nitrogen is catalyzed by nitrogenase enzyme which is irreversibly inhibited by molecular oxygen. Cyanobacteria are nitrogen-fixing organisms that produce oxygen as a by-product of the process of photosynthesis, and which must negotiate the presence of molecular oxygen with an essentially anaerobic enzyme. This chapter draws together an international group of leading cyanobacterial investigators' experience and excitement to include a state-of-the-art review of the area and addresses problems around cyanobacterial life. On our planet, for quite a long time there have been cyanobacteria. They are mostly widespread, making them ideal model organisms for the study of microbial biogeography as problems of evolution. Aerobic nitrogen fixation is localized in heterocysts and these heterocysts protect nitrogenase from inactivation by atmospheric oxygen. Cyanobacteria contribute greatly to the primary production of oceans and are one of the most important groups that release molecular nitrogen. We confirm that their pigmentation is due to the strength and sometimes even to the color of the light available. Others display remarkable life tolerance under anaerobic conditions; several forms of cyanobacteria thrive at extreme temperatures; other organisms can endure adverse conditions for long periods in salinity and pH, including when growth conditions are not acceptable. Many forms of cyanobacteria are simple to grow in the laboratory, and those in axenic culture have been collected and examined. Cyanobacteria are the most broadly dispersed community of photosynthetic

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prokaryotes present in virtually every region of the world and play an important role in the nitrogen and carbon cycle of the planet. Research efforts in the past 10 years have revealed a range of O_2 sensitivity of nitrogenase in different strains of cyanobacteria and a variety of adaptations for the protection of nitrogenase from damage by both atmospheric and photosynthetic sources of O_2. The most complex and apparently most efficient mechanisms for the protection of nitrogenase are incorporated in the heterocysts, the nitrogen-fixing cells of cyanobacteria. Several genetic studies have shown that the controls of heterocyst development and nitrogenase synthesis are closely interrelated and that the expression of nitrogen fixation (nif) genes is regulated by pO_2. The incremental transition from decreasing environment to oxidizing atmosphere has become a turning point in the Earth's evolutionary past which has made possible the conditions for present life types.

Keywords Cyanobacteria · *Heterocyst* · Pigmentation · Photosynthetic prokaryotes · Nitrogen fixation · Carbon cycle

26.1 Introduction

Speaking about the editors' invitation for writing this chapter under the title "Cyanobacteria—Genetics, ecology, and evolution" never has an introductory chapter covered all aspects. But it has been decided to highlight a number of the subjects relating to the cyanobacteria life that fascinate us the most.

Cyanobacteria are an incredibly biologically significant community of interesting and functional bacteria. The bluish pigment phycocyanin that they use to gather light for photosynthesis gives cyanobacteria their name. Chlorophyll A, the same dye used by plants, is also found. The chloroplast is originally a symbiotic cyanobacterium of plants picked up from a Precambrian green algal ancestor of the plants. However, all "blue-green" bacteria are not blue; some different types of phycoerythrin are red or pink. These bacteria are sometimes present on greenhouses or around sinks and drains. The Red Sea is named for the occasional flowering of a reddish Oscillatoria species and the roses of *Spirulina* in African flamingos (https://ucmp.berkeley.edu/bacteria/cyanointro.html).

Cyanobacteria are among the first species to colonize the planet; these bacteria are the chloroplast photosynthetic relatives of eukaryotes, such as plants and algae. They also remove oxygen, live symbiotically in very aggressive conditions, have circadian cycles, exhibit gliding mobility, and can be differentiated by different cell types called heterocysts. This makes them perfect model structures to research in simple processes including nitrogen fixation and photosynthesis. Furthermore, cyanobacteria develop a variety of bioactive substances, some of which can become novel antimicrobial agents, anticancer medications, UV protection agents, etc. Throughout recent years the remarkable resilience of cyanobacteria has drawn tremendous scientific attention. Twenty-four genome sequences have been published and several further studies are still ongoing. An immediate need to outline and study the latest molecular biology, genomics, and evolution of these essential species has been met (Herrero and Flores 2008).

26.1.1 The Cyanobacteria

The "day of blue-green algae" (Schopf 1974) is known as the Precambrian, and Schopf and Walter (Schopf and Walter 1982) called the Proterozoic era – the era of the cyanobacteria that changed from oxidized to oxygenated by oxygen photosynthesis from 2.5 and 0.54 billion years ago, although there is still some debate about the actual presence of the cyanobacteria on Earth. They are really mature creatures. It has been demonstrated that the Archean age had oxygen photosynthesis (Knoll 1979; Olson 2006), maybe also more than 3.7 Ga before (Rosing and Frei 2004). The Precambrian sedimentary past is full of microfossils that look like various forms of present-day cyanobacteria, and the cyanobacteria are usually considered to be originated long before 2.5 Ga (Schopf and Barghoorn 1967; Schopf 1993, 1970, 2012). The contemporary stromatolites found in Shark Bay (Logan 1961) in the late 1950s are often used as counterparts of the Precambrian fossil stromatolites. The latter are also called hypersaline marine lagoons (Logan 1961). The detailed study of the stromatolites was carried out by Bauld and Stal (Bauld 1984; Stal 1995, 2012), but the degree to which the populations of Shark Bay are identical to the kind of systems created before oxygenic phototrophs first colonized the earth and began to emit oxygen into the atmosphere is still not clear. Cyanobacteria descriptions began to appear in botanical literature in the late eighteenth century. In the early days, the group was commonly named "Schizophytae"; in 1874, Sachs introduced the name "Cyanophyceae" and in 1938 Smith adopted the name "Cyanophytae" (Smith 1938). The earliest described genus is possibly Rivularia (Roth 1797), and Vaucher, who oddly placed these species in an animal kingdom, published Oscillatoria and Nostoc in 1803 (Fogg et al. 1973). Many cyanobacterial species were portrayed in the algae monographs of the first and third century, which were splendid depictions of Lyngbye (Agardh 1824; Kutzing 1849; Lyngbye 1819). These and other books in the 19th century are the forerunners of newer morphological taxonomical treatises on cyanophyceae/cyanophyta (Desikachary 1959; Geitler 1932). The group of prokaryotes, Bacteria and Archaea, is morphologically much more complex than any other group, such that taxonomical arrangements appear to rely primarily on morphologic characteristics. In the classification of cyanobacteria, however, molecular sequence data are increasingly relevant (Wilmotte and Herdman 2001; Wilmotte 2001). Ferdinand Cohn had already in the second half of the nineteenth century (Cohn 1897, 1872, 1875) classified "blue- green algae" with bacteria instead of other classes of algae (Oren 2013).

They are microscopic, but their colonies or mats are very noticeable. Cyanobacteria's ecosystems are very diversified because of their unusual adaptability to a range of temperature environments from ice, sea, and lake to deserts. They are a precautionary organism of the biomass, maybe after the first bacteria, which formed on earth, thousands of years ago far before humanity. They are complex species that exist in the world's most extremes and can quickly be transported through the air to new avenues. The complexities of morphology include independent, filamentous colonies. Cyanobacteria cells are larger than regular bacterial cells.

The structure of the cell wall consists of peptidoglycan, and the outer portion of protoplast consists of photosynthetic pigments. The filament is filled with mucin, and no locomotive device has been recorded, although there are some forms which display oscillatory movement (Tiwari 2014). The majority of cyanobacteria are aerobic photoautotroph and photosynthesis is their principal mode of energy metabolism. Cyanobacteria are essential environmental and plant growth prokaryotes. Although few species are free living, they live with other eukaryotic diseases symbiotically and play important new roles that are necessary for the environment. Certain cyanobacteria show a distinct ability for heterotrophic nutrition (Fay 1965). They are the normal nitrogen fasteners, which is very important for the biological system as a whole. Bio-nitrogen fixation is classified as the ability to transform atmospheric nitrogen to organic ammonia, nitrite, or nitrate and is important for plant growth. The abilities of some cyanobacteria to generate nitrogen fixing allow them to survive in low concentrations of nitrogen, which is an important gain in terms of environmental survival and adaptability. Cyanobacteria are found in a wide range of natural ecosystems and are widespread in air, sea, and terrestrial environments (Fogg et al. 1973). Some families include heterocyst cyanobacteria, including algae, mushrooms, liverwort, ferns, and higher plants, which have unique symbiotic connections. The endosymbiotic cyanobacteria were modified to a large degree in these variations, resulting in very strong nitrogen fixation and the transfer of much of the fixed nitrogen to the host organism, including biochemical properties and metabolic activity (Stewart et al. 1983). For the survival and development of many plants, cyanobacteria are very essential species. They are one of the few species able to transform neutral nitrogen from the atmosphere into organic material, such as nitrate or ammonia. The plants need to expand and receive from the soil certain "locked" sources of nitrogen. In addition, fertilizers produce other fixed nitrogen which can be consumed by plants via the roots. Nitrification in the presence of oxygen cannot occur, but in specialist cells known as heterocysts nitrogen is regulated (Oren 2013). These cells have an extremely thickened wall in an anaerobic environment. Cyanobacteria have special defensive mechanisms for nitrogenase, an enzyme that fixes nitrogen to O_2 and nitrogen regulates N_2-fixing machinery expression, induces symbiosis, and is thus essential to modern research on N_2 fixation. They have evolved multiple specialized cells called heterocysts which provide the microoxic environment required for nitrogen fixation.

26.1.2 Nitrogen Fixation

The fixation of cyanobacterial N_2 was used for fertilization purposes since ancient times in agriculture. Various types of cyanobacteria are also diazotrophic. One such symbiosis is mentioned in Chinese literature for years. In Europe, the cyanobacterial N_2 fixation is also a long-standing tradition, which dates back to more than 100 years. And we are now expanding on that and setting up the first organized European N_2-setting cyanobacterial interdisciplinary program. Cyanobacterial N_2

fixation is a field that draws many scientists from chemical engineering to biochemistry, genetics and ecology, agronomy, and agricultural disciplines. More than thirty leading scientists from 11 countries have shared their wishes. Teams and areas that are ideally suitable for joint study have been defined to be included in this initiative. We foresee a synergistic impact on scientific development to pool research funding that currently takes place through individual laboratories in Europe. The different groups complement each other and put core laboratories and skills together in numerous multiple styles: symbiotic and free living. Expertise involves host plants and the cyanobacterial cell symbiosis examined, although groups researching free-living cyanobacteria include all forms of N_2 fixers from all different plant groups. The groups also research various facets of N_2 fixation: modulation, variability, signal transduction, and N_2 fixation affecting particular genes and proteins (European Science Foundation 1999).

The definition of common nitrogen-fixing origin corresponds to the physical and chemical properties of the enzyme system of aerosols which in species otherwise unlike (Cohn 1897; European Science Foundation 1999; Mortenson and Thorneley 1979) are remarkably identical in nature. The complex enzyme consists of two elements of the protein. One is a protein called dinitrogenase, a Mo-Fe protein, and the other a Fe protein called dinitrogenase reductase. It is a naturally occurring protein. The tetrarch is composed of two pairs (2 to 2 lb), 4 (4Fe-4S) clusters, and two cofactor molecules. Dinitrogenase comprises two pairs (2 to2 Lb). The Mo-Fe cofactor is an integral part of the dinitrogenase clusters, comprising 8 fe and 6 s atoms, with atom Mo, and without 4Fe-4S. The molecular weight of dinitrogenase is about 245 kDa. The dimer contains two identical subunits with one unit and a molecular weight of around 64 kDa (4Fe-4S) (Emerich and Burris 1978).

The Mo-Fe cofactor is intended to bind dinitrogen and reduce it to ammonia:

$$N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$$

Dinitrogenase reductase is supplied with electrons for the reduction of N_2. It requires a very endergonic reaction, which lowers roughly 12–15 mol ATP per mol of N_2. A variety of other compounds, including acetylene, hydrogen azide, hydrogen cyanide, and nitrous oxide, can be reduced by nitrogenase. These compounds molecules involve a threefold relation such as N_2. Of both, acetylene reduced to ethylene is of special interest, as gas chromatography can quickly and accurately detect both acetylene and ethylene. The procedure for acetylene reduction is now commonly used to measure the activity of nitrogenase. A varying volume of proton reduction and production of H_2 is often followed by a fixation of nitrogen even at a 50 atm N_2 (5065 kPa) pressure (Bothe et al. 1980; Simpson and Burris 1984). This development of nitrogenase catalysis of H_2 results in a loss of energy and of reduction, partly recuperated by the activity of an absorption hydrogenase in many species that bind nitrogen. It catalyzes the oxidation of H_2 in a reaction between oxidation hydrogen and Knallgas. For most of the organisms which are set to N_2, ferredoxin or flavodoxin is the physiological electron donor for iron deficiency, and

the donor, for its part, derives electrons from intermediate metabolism-shaped reduction structures. Ammonia, the portion of the nitrogen fixation, is similarly assimilated to exogenous ammonia (Microbiology and Molecular Biology 1969; Tempest et al. 1970), primarily through the glutamine synthase glutamate synthase pathway. Other enzymes can play a minor role in the incorporation of ammonia, for example, alanine dehydrogenase and glutamate dehydrogenase. Even though bio-based fixation of nitrogen is restricted to prokaryotic microorganisms, its capability of fixing nitrogen in the population is by no means universal. While it is present in otherwise unrelated members the property can be limited within families to just a few genera or species, families of gram-positive, gram-negative, heterotrophic, obligatory anaerobic, facultative, and aerobic bacteria (Postgate 1978). The erratic thing in the prokaryotes is the presence of nitrogen fixation that may seem to indicate that a bacterial emergence of early nitrogen fixation (Broda and Peschek 1983; Cloud 1976; Margulis 1982; Sprent and Raven 1985) may have occurred. A possible explanation of the dispersion of nitrogen fixation based on the horizontal genetic probability in the light of the findings that indicate that the genes of nitrogenase developed in combination with the bacteria that bear them (Hennecke et al. 1985) is that transmission has been recently assumed to be feasible (Postgate and Eady 1988; Silver and Postgate 1973). Every nitrogen-fixing organism or diazotroph assimilates preferentially ammonia or other types of fixed nitrogen (nitrate, urea, amino acid), only when nitrogenase is lacking from these sources. In certain cells that fix nitrogen, ammonia can easily inhibit the production of nitrogenase. This reversible ammonia inhibition is attributed to one subunit of the reductase of nitrogenase (Gotto and Yoch 1982; Reich and Boeger 1989). Ammonia is mainly involved in the management of nitrogen synthetization in all nitrogen-fixing species by glutamine synthetase at the transcriptional level (Malin and Pearson 1988; Shanmugam et al. 1978; Tyler 1978). The synthesis of its ingredient proteins is repressed by oxygen which inactivates nitrogenase irresistibly (Eady et al. 1978; St. John et al. 1974). Regulation of ammonia and oxygen nitrogenase synthesis prevents energy, reducing waste and offsetting the high costs of nitrogen fixation. The genetic nitrogen-fixing regulation and N_2-fixing genes have a basic similarity between species which fix N_2 but have different patterns that may lead to the needs of unique groups or human strains of N_2-fixing genes (Haselkorn 1986). The genes of nitrogen fastening or *nif* genes are normally located in the chromosome of the bacteria. In the commonly studied bacterium *Klebsiella pneumoniae*, 17 *nif* genes were known. The components of the nitrogenase complex are encoded by three of those genes, *nip*I, *nif*D, and *nif*K. The codes *nif*D and *nif*K of the dinitrogenase subunits ot and c are encoded by *ni*Jf, while the nitrogenase reductase is encoded by *ni*Jf. Many of the organisms examined have been well preserved and identified. However, there is more heterogeneity in the classification of related genetic genes. Environmental signals seems to be affected by control genes nif nitrogen fastening conditions are beneficial or unfavorable (Gordon 1981). The genes of nifL and nifA are the operon of nifLA. Its performance tracks the expression of all other nif genes (Gussin et al. 1986; Kennedy et al. 1981). The nifA product (positive check) is necessary for expression, and its expression in the presence of oxygen is inhibited by

nifL gene product (negative control). However, the activation of two other regulatory nitrogen genes ntrA and ntrC by drugs requires the expression of NifLA operon. The result of the *Nif*L gene is known to inactivate the *nif*A protein in the presence of ammonium or oxygen (Haselkorn 1986).

26.1.2.1 Effects of Oxygen on Bio-Nitrogen fixation

A variety of important studies have provided true knowledge of the unique susceptibility of the nitrogen-fixing enzyme mechanism to oxygen. The first was the long-suffering definitive proof that fixing nitrogen is a method of reduction and ammonia is the main component of nitrogen fixation (Bergersen 1965; Mortenson et al. 1962). It was also no longer shocking that oxygen interferes with this reaction. Secondly, it was found that the synthesis of nitrogenase could only occur under aerobic and microaerobic conditions in facultative bacteria such as *Kiebsiella pneumoniae* (Klucas 1972; Pengra and Wilson 1938) or *Bacillus polymyxa*, as well as in microaerophilic species such as *Mycobacterium flavum* (Biggins and Postgate 1969; Grau and Wilson 1963), when supplied with a suitable supply of combined nitrogen (Hino and Wilson 1958). Then it was shown that also in aerobic *Azotobacter*, oxygen inhibits the function of nitrogenase. Burk suggested that the nitrogen fixation efficiency (N_2 fixed by ingested O_2) improved by 10- to 20-fold as the oxygen partial pressure (PO_2) reduced from 0.21 to 0.01 atm (21.27–1.01 kPa) (Bulen et al. 1965). A normal bell-shaped curve (Dalton and Postgate 1969), which indicates that the rate of nitrogen fixation is still suboptimal under environmental PO_2 values, was obtained when the activities in nitrogenase were compared with PO_2. The *Azotobacter* spp. is known to rely on oxidative metabolism to support nitrogen fixation. Finally, strong evidence of nitrogenase oxygen exposure comes from experiments performed in pure enzyme preparations, all derived from a required anaerobic such as *Clostridium pasteurianum* (Carnahan et al. 1960) and distinctly *Azotobacter vinelandii* (Bulen and LeComte 1966; Bulen et al. 1965) being similarly killed by oxygen. In early experiments of freely living nitrogen-fixing species as well as symbiotic structures (Bergersen 1962; Bond 1961; Hamilton and Wilson 1955; Meyerhof and Burk 1928; Parker and Scutt 1960), the inhibitor effect of atmospheric oxygen on the fixation of nitrogen was observed. The end of the nitrogen fastening mandatory anaerobic and facultative bacteria is often due to the general inherent vulnerability of certain species to oxidation in the absolute absence or at very low oxygen concentrations.

In strict anaerobic conditions, most photosynthetic bacteria trap nitrogen in light only. An exception is *Rhodobacter capsulatus*, which is capable of sustaining nitrogenase action in air penetration (Meyer et al. 1978; Willison et al. 1983), but at a very limited scale. In heterocyst cyanobacteria, the nitrogenase activity is dramatically increased with reduced dissolved oxygen concentrations in culture medium (Mortenson and Thorneley 1979). Nitrogenase isolated from cyanobacteria was found to be extremely sensitive to oxygen (Fay and Cox 1967; Haystead et al. 1970). In a chemoautotrophic metabolism, *Xanthobacter autotrophicus* can fix

nitrogen under the air both as the energy source for H_2CO_2 reduction and when it is heterotrophically cultivated in sugar as a carbon and energy source. The relation between nitrogen-fixing behavior and PO_2 is, however, close in the latter case to that with *Azotobacter* species (Dalton 1980).

Oxidation in the nitrogenase proteins would most likely be oxidized by reactivating oxygen species, such as superoxides, hydroxyl peroxide, or singlet oxygen, formed during oxygen reduction (Fridovich 1986; Hassam and Fridovich 1984; Krieg and Hoffman 1986; Robson and Postgate 1980). The changes in protein structure and redox status of the 4Fe-4S clusters (Robson and Postgate 1980) seem to cause a sequence which makes them unable to accept or donate electrons. Oxygen can also cause protease activity, leading to rapid nitrogenase protein degradation (Roberts and Brill 1981). Not only does oxygen inactivate and disable nitrogenase, but nitrogenase synthesis is also inhibited. Minimum PO_2 is needed to repress *Azotobacter* spp. nitrogenase synthesis. However, it is twenty times better than the nitrogenase inactivator (Postgate et al. 1981). During oxygen intake, chroococcum is used (Robson 1979). It was not because of the relentless degradation of newly synthesized enzymes, but because of their absence. The separate kinetics from repression by ammonia and oxygen caused by synthesis of nitrogenase oxygen, respectively, is suggested for repression.

The synthesis of nitrogenase could also be inhibited by oxygen in an ammonia-depressed mutant K pneumoniae (Fredriksson and Bergmann 1997). The synthesis of chroococcus is much quicker than that of ammonia (Robson 1979). This finding indicates that all coercive systems may operate independently through ammonia and oxygen (Yates et al. 1980). Oxygen suppression of synthesis with nitrogenase avoids the excessive development of harmful enzymes. Structural gene products (*nif*I, *nif*D, and *nif*K) are all oxygen regulated, and the *nif*AL operon seems to be part of the regulation. The *nif*L gene product functions as a negative regulator, blocking the absorption of oxygen in *nif* operons (Ditta et al. 1987; Kennedy et al. 1981)

Symbiotic process experiments have supported the data obtained from free-living species that release nitrogen. The 5N fixation with excised nodules of soybeans increased to 0.5 atm (50.6 kPa) and, at higher oxygen pressures, then decreased suddenly (Bergersen 1962; Burms et al. 1955). Similarly, 15N integration was found to be impaired at higher concentrations of oxygen in non-leguminous root nodules (Bond 1961; Silver 1969). An aerobic life is thus more associated with nitrogen fixation than with aerobic metabolism. It is fair to say that this inference may support the hypothesis that during the early history of the Earth when oxygen was most successful, the nitrogenase system evolved (Broda and Peschek 1983; Schopf and Walter 1982; Sprent and Raven 1985). The two elements of the nitrogenase complex are irreversibly inactivated by the reaction to oxygen (Burris et al. 1980; Mortenson and Thorneley 1979; Robson and Postgate 1980). Fe proteins are especially vulnerable to oxygen, as seen by their rapid air destruction; the half-life of dinitrogenase reductase is only 30 s–2 min. MOP is significantly larger, with a half-life in the air between 4.5 and 10 min. But the Mo-Fe cofactor is much more labile to oxygen than the reductase to dinitrogenase (Eady et al. 1980; Shah and Brill 1977). Recent studies of Rhodobacter capsulate *nif* regulatory mutants have shown that combined nitrogen

and oxygen controls work independently and at different amounts through molecular mechanisms that sense the signal state the oxygen concentration of cellular nitrogen (Rippka et al. 1979; Roberts and Brill 1981). The first degree of regulation requires nitrogen sensing and signaling. Second-stage oxygen regulation genes can only be activated under conditions of nitrogen deficiency. The role of DNA-topoisomerase I enzymes and DNA-topoisomerase II enzymes (DNA gyrase), the supercoiling sum by the cell O_2 is known. Anaerobic conditions induce intense DNA gyrase, leading to a healthier conformation of the DNA negative in the spin and activating nif gene transcripts. The *nif*LA promoter is believed to be able to detect the chromosome supercoiling condition (Fay 1992; Kranz and Foster-Hartnett 1990).

26.1.3 Different Strategies: Cyanobacterial Nitrogen Fixation

As one of the main classes of prokaryotes capable of fixing gaseous nitrogen, cyanobacteria are very important in the global nitrogen cycle. It is achieved in several forms with and without heterocysts, both single and filamentous (Stewart 1980). Although a link between cyanobacteria and biological nitrogen fixation has been suspected for a long time, the first concrete confirmation of nitrogen fixation is the unicellular representation which was only published in 1969 (Silver 1969). Nitrogenase was identified in the same year in heterocysts (Stewart et al. 1969). Nitrogenase is irreversibly inactivated by molecular oxygen, the result of oxygen photosynthesis, in the complex of enzymes that convert the nitrogen to the ammonium ions. The two systems are thus fundamentally different. Different techniques for solving this issue have emerged in various cyanobacteria communities. The second technique is temporal separation: the development of oxygen in the daytime and the fixation of nitrogen in the night. It has been recently discovered that certain unicellular marine cyanobacteria cannot fully produce oxygen and are able to hold an active nitrogenase system. Even now, it is not entirely understood whether pathways in various forms of cyanobacteria use a combination of nitrogen fixation and aerobic lifestyles.

In the filaments of pigmented cells, the work of heterocysts is like empty spray cells and has for a long time perplexed scientists, who term these structures “botanic enigmas” (Fritsch 1951). In 1968, it was already possible to take into consideration the role of the heterocyst as a site of nitrogen fixation (Fay et al. 1968). Spores are sleeping cells that could theoretically germinate under acceptable circumstances (Wolk 1965). By 1853, however, Ferdinand Cohn explicitly reported that these unique cells were not able to grow into new filaments (Cohn 1853): According to these observations the entire multi-cell thread seems to be expanding at Anabaena—not at *Nostoc verrucosum*, after Thuret—but every vegetative cell of the same cell to a new cluster of thread, whereas the permanent cells are not ready for further growth. (From these findings it has been apparent that not the whole multicellular filament in

Anabaena, in contrast with *Nostoc verrucosum* (Thuret), but each vegetative cell will replicate and grow into a new mass of filaments while the resistant cells cannot evolve any further (Cohn 1853).)

We now realize that nitrogenase is present in heterocyst and that the oxygen photosynthesis and carbon dioxide fixation of these cells has been damaged, but photosystem I is retained to supply the requisite nitrogen fixation energy. Breathing and phosphorylation are also made, so that the fixation of nitrogen does not depend solely on the sun. In Anabaena a small diffusible peptide guides the classification of heterocyst (Yoon and Golden 1998). Heterocysts are contained in subsections IV and V (see the morphological variation section above). The symbiotic relationships between cyanobacteria and eukaryotic cells, depending on the nitrogen-fixing capacity of the prokaryotic host, frequently present heterocyst forms. It is a well-known example of the Azolla–Anabaena symbiosis described at the start of this chapter. Very extraordinarily, *Richelia intracellularis* occur in large aquatic pennate diatoms, the small filaments with a large terminal heterocyst *Rhizosolenia* and the genus *Hemiaulus*.

Not all cyanobacteria that fix nitrogen develop special differentiated cells that contain nitrogenase. Another technique, used by filamentous and single-cell organisms, is the temporary division of the development of photosynthetic oxygen and the fixation of nitrogen. While it was noted that nitrogen fixation is always correlated with the atmosphere of the tropics more than half a century ago, the properties of this organism are indeed large filamentous non-heterocystous cyanobacterium *Trichodesmium* (Dugdale et al. 1961): heterocyst missing but only in the light repairs nitrogen (Capone and Carpenter 1982; Zehr et al. 1999). The blooms of Trichodesmium contains long filaments that organize into various morphological aggregates. These aggregates can travel large distances through the water column and their booming behavior is regulated by the presence of gas vesicles. It had been historically believed that the aggregates permitted the indoor cells to act as "heterocyst," but that does not seem to be the case. It is suggested that a trichome subgroup called "diazocytes" includes nitrogenase (Fredriksson and Bergmann 1997). There are several signs whether certain diazocytes are terminally distinct cells, such as heterocysts, is unknown. These cells are more likely to become temporary cells that replace nitrogen. This approach was then treated as a mixture of spatial and temporal differences (Berman-Frank et al. 2001). At the end of the night, nitrogen fixation begins and lasts until noon. Nitrogenase production decreases afterward and the enzyme is inactivated in the dark easily and irreversibly. An oxidation of nitrogen-fixing cells has a fast-respiratory rhythm. The low oxygen solubility in warm seas can restrict Trichodesmium's geographical distribution to oceanic areas with water temperatures above 25 °C. Heterocysts are not necessary under these conditions (Staal et al. 2003).

Particularly surprising is the latest finding in tropical and subtropical ocean of unicellular cyanobial bacteria with an excess of nitrogen fastening over the day. In the subtropical North Pacific Ocean, they were first recorded to occur in 2001 (Zehr et al. 2001). These photosystem II cells are absent and thus are unable to oxygenate photosynthesis. Examination of their own genome reveals that the Calvin enzymes,

the tricarboxylic acid cycle, and the biosynthesis process of any amino acid are not yet isolated. These species apparently rely on some sort of organic carbon supplied by other marine ecosystem members that have not already been described. In fact, most cyanobacteria are obligatory photoautotrophs (Smith et al. 1967), but some species have demonstrated optional photoheterotrophy, including chemoheterotrophy. Heterotrophic cyanobacterial development, at least in laboratory culture, is thus precedented. We are also completely ignorant of the interrelation between this new aquatic way of life and the other species found in the sea (Bothe et al. 2011; Tripp et al. 2010; Zehr et al. 2008).

26.1.4 Different Strategies for Moving to Areas with the Most Favorable Conditions

Many cyanobacteria may be put within a column of water or inside a benthic microbial mat to provide light conditions; the best attainable are nutrients and other environmental parameters. Two methods for achieving this goal are essentially available: passive movement cell booster control and active motility at energy cost.

Some cyanobacteria were already recognized in the nineteenth century for the existence of "carbon vacuoles"—gas vesicle clusters. The first findings were possibly from 1895, when three publications reported the floodplain of numerous water bacteria and the resistance to strain of gas vesicles (Ahlborn 1895; Klebahn 1895; Strodtmann 1895). Gas vesicles are hollow, protein-built tubes. The principal protein that makes up *Gvp*A is highly hydrophobic because water is actively repelled by the inner side of the vesicle wall. The arrangement of the vesicles on the exterior is reinforced with the second protein, *Gvp*C. Gases flow easily across the membrane of the vesicles to represent the concentration of gases absorbed in the liquid water in a gas mix. Gas vesicles can be regulated per cell to increase or decrease cell buoyancy (Walsby 1987, 1981, 1994). Among the genres, the distribution of Microcystis and Anabaena, which also form dense flowers at nutrient-rich lakes, is highly dependent on the cell's gas vesicle content and Trichodesmium is one of the principal aquatic species that capture nitrogen. The vast amount of gas vesicles in the Trichodesmium cell periphery and the presence of broad bundles of booster filaments permit masses of bright, phycoerythrin-rich cyanobacterial patches to migrate to the sea surface. Cyanobacterial productive activity is also possible. Many organisms demonstrate a process of gliding: rapid spreading over or over a firm substrate or along with the trichomes. The acceleration of gliders is sluggish, normally 1–10 μm/s in size. Oscillatoria and Spirulina, which follow the rotation of the trichoma around its axis, are known for their remarkable motions. The motion direction can be caused by the gradients of environmental parameters and particularly the available light intensity and efficiency, but other factors, including the medium's salinity, may also be significant. Therefore, in the intertidal hypersaline microbial mat (Kohls et al. 2010), a "halotaxis," where cells migrate toward maximum salt concentrations, was

found in the cyanobacterial group recently. The action of gliders relies solely on the strong substratum being present. The most popular mode of active movement is flagella; the prokaryote universe, for cyanobacteria, has yet to be known. It was therefore a major surprise to discover marine single-cell Synechococcus which swim in the water actively, without the need for solid surface conditions (Waterbury et al. 1985). The presence of flagella or other cell structure associated with locomotion was not disclosed by electron microscopy.

26.2 Effect of Oxygen on Nitrogen Fixation

Early experiments with free-living nitrogen-fixing organs as well as with symbiotic structures have demonstrated the inhibitory influence of atmospheric oxygen on nitrogen fixation (Bergersen 1962; Bond 1961; Hamilton and Wilson 1955; Meyerhof and Burk 1928; Parker and Scutt 1960). A variety of important results have contributed to a clear understanding of the unique intense susceptibility of the enzyme-fixing mechanism to oxygen. The first was the long-seeking definitive proof that a significantly reduction mechanism is nitrogen fixation and that ammonia is the main component of nitrogen fixation (Bergersen 1965; Dugdale et al. 1961). It was also no longer shocking that oxygen interferes with this reaction. Secondly, it was noted that the synthesis of nitrogenase could only occur in anaerobic and microaerobic environments with facultative bacteria, including *Klebsiella pneumoniae* (Klucas 1972) or *Bacillus polymyxa* and microaerophilic organics (Grau and Wilson 1963), such as *Mycobacterium flavum*, whereas, when supplied with an adequate supply of combined nitrogen (Biggins and Postgate 1969), the same bacteria may expand with the aid of air (Hino and Wilson 1958). There was more proof that also in aerobic *Azotobacter* bacteria, oxygen inhibits nitrogenase activity. In Burk the efficiency of the nitrogen fixation (N_2 fixed by O_2 consumed) improved between 10 and 20 times by 0.21 to 0.01 (21.27 to 1.01 kPa) with reduced partial oxygen pressure (PO_2) (Burk 1930). The standard bell-shaped curve was obtained when nitrogenase activity was plotted against PO_2, and this suggests that the rate of nitrogen fixation is currently suboptimal under ambient PO_2 values (Dalton and Postgate 1969). All of this was particularly unexpected for *Azotobacter* spp., recognized in support of nitrogen connection to dependent oxidative metabolism. Finally, oxygen exposure is specifically proven by experiments with pure enzyme preparation; oxygen, be it a necessary anaerobe, for example, *Clostridium pasteurianum* (Carnahan et al. 1960) or a simply aerobic *Azotobacter* vinelandii, has been eliminated in the same way (Bulen and LeComte 1966; Bulen et al. 1965).

Nothing less susceptible to oxygen is found in autotrophic nitrogen-fixing species (Gallon 1980). In extreme anaerobic conditions, the most photosynthetic bacteria are nitrogen fixed only in the sun. One exception to this is Rhodobacter capsulate that is able to sustain nitrogenase Rhodopseudomonas capsulate exposed to air (Meyer et al. 1978; Willison et al. 1983), but at a very low dose. Continuous spreading using an anaerobic gas mixture to avoid the inactivation of nitrogenase by photosynthetic

oxygen includes *Plectonema boryanum* (Stewart and Lex 1970). The activity of nitrogenase is greatly increased with decreased concentrations in the culture medium of dissolved oxygen in all heterocyst cyanobacteria (Stewart 1971). Nitrogenase isolated from cyanobacteria has been shown to be extremely susceptible to oxygen (Fay and Cox 1967; Haystead et al. 1970). In the chemical metabolic process, *Xanthobacter autotrophicus* will fix nitrogen under air when CO_2 is reduced with H_2 as an energy source and when sucrose is heterotrophically produced as a carbon and energy source. The nitrogen-fixing behavior relation is nevertheless close to that of the *Azotobacter* species in the above case (Dalton 1980). Results produced with free-living nitrogen-fixing species were verified by experiments of symbiotic structures. 5N fixation by excised nodules of soybean was reported as rising to 0.5 atm (50.6 kPa) with PO_2 rise and, at elevated oxygen pressures, decreasing suddenly (Bergersen 1962; Burms et al. 1955). In the same way, it was shown that 15N was inhibited at higher oxygen concentrations in non-leguminous root nodules (Bond 1961; Silver 1969). Therefore, an anaerobic life is more consistent with nitrogen fastening than is rational to assume. The hypothesis may support the theory that during the early years of the earth's existence the nitrogenase mechanism evolved as in the simple atmosphere oxygen was most likely missing (Broda and Peschek 1983; Schopf and Walter 1982; Sprent and Raven 1985). The application of oxygen to all portions of the nitrogenase complex is unstable irreversibly (Burris et al. 1980; Golden et al. 1985; Robson and Postgate 1980). Dinitrogenase (Fe protein) reductions are highly vulnerable to oxygen due to the increased oxidation of air and the half-life of dinitrogenase. Mo-fe protein dinitrogenase is less prone to a half-life in the air of 4.5–10 min. Mo-Fe is, however, much more oxygen-labile than reducing dinitrogenase (Eady et al. 1980; Shah and Brill 1977). Oxidation of nitrogenase proteins is likely to lead to a more reactive oxygen compound, including superoxide, hydrogen peroxide, hydroxyl radicals, or single oxygen (Fridovich 1986; Hassam and Fridovich 1984; Kranz and Haselkorn 1986; Robson and Postgate 1980). Protein oxygen is usually unable to accept or donate electrons in a sequence of modifications in the protein structure and redox state in the 4Fe-4S (Robson and Postgate 1980) clusters (Bothe et al. 2011). Protease production can also be caused by oxygen and a fast degradation of nitrogenase defense (Roberts and Brill 1981). Oxygen inactivates not only nitrogenase but also inactivates and stops nitrogenase replication. The lowest PO_2 is required in *Azotobacter* spp. to suppress nitrogenase synthesis. Yet, it is twenty times stronger than nitrogenase inactivation (Postgate et al. 1981). The cells of *K. pneumoniae* were not present with both dinitrogenase and dinitrogenase reductase (St. John et al. 1974). After oxygen absorption, chroococcum is used. The different kinetics of ammonia and oxygen suppression have shown that their loss can help not only to degrade new synthesized enzyme but also to inhibit the synthesis by oxygen. Synthesis of nitrogenase in an ammonia mutation even oxygen can be blocked from *K. pneumoniae* (Eady et al. 1978). In *A. chroococcum* the oxygen repressed synthesis of nitrogenase even quicker than ammonia did (Robson 1979). These results tend to indicate that the two pathways of repression will work separately through ammonia and oxygen (373). Repression of oxygen nitrogenase synthesis inhibits the excessive production of harmful enzymes.

Oxygen is regulated for all structural gene products (*nif*I, *nif*D, and *nif*K), and *nif*AL tends to participate in a manipulation of the membrane. The *nif*L gene product serves to regulate *nif*l and inhibits the accumulation of oxygen from other nif operons (Ditta et al. 1987; Kennedy et al. 1981). Studies with *nif* Rhodobacter capsular regulatory mutants found that transcriptional controls are controlled independently and on various levels by combined nitrogen and oxygen. The first stage of tracking includes nitrogen sensing and signaling. The nifLA promoter is believed to be able to feel the chromosome supercoiling condition (Eady et al. 1978; Kranz and Foster-Hartnett 1990; Kranz and Haselkorn 1985, 1986).

26.2.1 Nitrogen with Oxygen Defense

While in both nitrogen-fixing agents the biochemical properties and the extremely oxygen-dependent function of a nitrogenase were studied, the mechanisms for shielding the enzyme system from oxygen-related adverse effects are very different. There may be more than one process in certain diazotrophs and a wide variety of instruments appearing to work in an organized way in cyanobacteria to conserve nitrogenase from ambient and intracellular supplies of oxygen. Cyanobacterial defense processes are the key focus in this analysis and will be further discussed below. A short survey can, however, provide a valuable framework for a comparison of the different adaptive processes functioning in other diazotrophs. Obligatory anaerobics like *Clostridium pasteurianum* and *Desulfovibrio desulfuricans* appear to be deprived of any unique oxygen deleterious effects mechanism to shield their nitrogenase or even any additional cell constituents. Thus, they can only survive and fix nitrogen without oxygen and are restricted to oxygen-free conditions in their natural distribution. In both the life and absence of oxygen, available bacteria such as *Klebsiella pneumoniae*, *Bacillus polymyxa,* and *Rhodospirillum rubrum* may release nitrogen anaerobically. The choice of sub-atmospheric oxygen levels in nitrogen connection was demonstrated by microaerophilic bacteria such as *azospirillum* genus. They cannot fix nitrogen under heavy oxygen pressures or anaerobic conditions. In the end, aerosol bacteria are capable of growth in air on dinitrogen, as evidenced by *Azotobacter* species. However, oxygen sensitivity can be seen in certain strains during nitrogenase synthesis induction. The last three classes of nitrogen-fixing bacteria have shown defense mechanisms.

26.2.2 Diffusion of Controlled Oxygen

The key mechanism for nitrogenase defense in bacteroid root nodule is oxygen flow regulation. Leghemoglobin is now well known for low oxygen transport in leguminous root nodules' contaminated cells (Hill 1971). The system is improved by membrane limitations that provide barriers to diffusion and minimize oxygen flow,

and even by hydrogenase activity the mechanic is also improved (Bergersen 1980; Layzell et al. 1990; Sheehy and Bergersen 1986).

26.2.3 Respiratory Safety Measures

The criteria for nitrogenase activities are defined in oxygen-dependent breathing in aerobic nitrogen-fixing species under conditions occurring to be in conflict with the oxygen lability of nitrogenase. The efficiency of nitrogen fixation in *Azotobacter* spp. was noted by Meyerhof and Burk (Meyerhof and Burk 1928). PO_2 is shaped differently. Bacteria spp. A rise in dissolved concentrations and other aerobic diazotrophs may react to the breath volume; oxygen maintains low intracellular oxygen levels and preserves the nitrogenase against inactivation (Dalton and Postgate 1969; Hochman and Bums 1981). This adaptation to PO_2 shifts is due to the oxygen-sensor mechanism (Peterson 1989) and can involve various components of the respiratory system, such as dehydrogenase NADH/NADPH and cytochrome a2 (Robson and Postgate 1980). The respiratory reaction becomes nonlinear at higher oxygen pressures (Post et al. 1983), suggesting additional defensive mechanisms (Dingler and Oelze 1987; Kuhla and Oelze 1988). However, under natural conditions even with a limited amount of dissolved oxygen concentrations, breathing protection can be ideal for scaling up surplus oxygen and for maintaining nitrogenase in a practically oxygen-free cellular atmosphere (Hochman and Bums 1981).

26.2.4 Hydrogenase Activity

As described previously, nitrogen fixation is consistent with a variable hydrogen evolution in a nitrogen-catalyzed reaction in both free-living species and symbiotic structures (Robson and Postgate 1980). Without a proper substrate, like N_2, nitrogenase releases protons to produce H_2 and absorb all ATP and reducing agents (Bulen et al. 1965). H_2 formation is typically an intrinsic trait of nitrogenase reaction, which continues at a low level (1 mol H_2/mol N_2) even though nitrogenases (acetylene, cyanide, and even azide) are strongly oxygenated even in alternative substrates. However, H_2 evolution is absent in ammonia or nitrate-grown cells, which often inhibit the synthesis of nitrogenase (Robson and Postgate 1980). The use of H_2 for oxyhydrogen reaction partially compensates for H_2 depletion. This is catalyzed by the one-way absorption of hydrogenase which takes place uniformly in aerobic species that fix nitrogen. Its function relies solely on oxygen. The oxyhydrogen reaction rate can be similar to endogenous breathing (Bothe et al. 1980). Dixon notes that hydrogenase absorption is multifold (Dixon 1972): it destroys N_2 inhibitory H_2 (Mortenson 1978), functions as oxygen scavenging mechanism, and increases breathing protection.

26.2.5 *Protecting Enzymes from Reactive Oxygen Sources*

The development of reactive oxygen compounds, such as superoxide radicals (O_2), but also hydrogen peroxide (H_2O_2) and hydroxyl radical (HO^-), results in a univalent reduction of O_2. These organisms are specifically connected to PO_2 in the cell. The reactive sources of oxygen have a very toxic impact on biological processes and will significantly affect the degradation of not only nitrogenase but also many other important cell constituents. The primary defensive mechanism to prevent possible oxygen toxicity is superoxide dismutase (Henry et al. 1978), which catalyzes the elimination of superoxide radicals. Catalases, which mediate their conversion to H_2O and O_2, can dissolve hydrogen peroxide. The use of other devices to shield nitrogenase from oxygen inactivation in aerobic and microaerophilic diazotrophics seems to play a significant role for antioxidant enzyme (Dingler and Oelze 1987; Tel-Or et al. 1986; Toezuem and Gallon 1979).

26.3 Incompatibility Photosynthesis in Cyanobacteria D Nitrogen Fixation

A remarkable evolutionary accomplishment appears to be the coexistence of oxygen developmental photosynthesis and oxygen responsive acid fixation in diazotrophic cyanobacteria, particularly when one sees both antagonistic processes occurring not only in a single organism but also in the same cell, actually. Owing to their photosynthetic metabolism, the species had to acquire effective devices to defend a nitrogenase, first against oxygen and later even from external nitrogenase heat of air. When photo evolution of oxygen happens next to a nitrogenase operation, it may be much more important for this. The primary defensive mechanism to prevent possible oxygen toxicity is superoxide dismutase (Henry et al. 1978), which catalyzes the elimination of superoxide radicals. Catalases, which mediate their conversion to H_2O and O_2, can dissolve hydrogen peroxide. The use of other devices to shield nitrogenase from oxygen inactivation in aerobic and microaerophilic diazotrophics seems to play a significant role for antioxidant enzyme (Dingler and Oelze 1987; Tel-Or et al. 1986; Toezuem and Gallon 1979).

26.3.1 *Unique Problem in Cyanobacteria with Nitrogen Fixation*

Cyanobacteria have developed a number of nitrogen detection strategies in the past 15 years of in-depth studies to make sure they are oxygen protected (Becana and

Rodriguez-Barrueco 1989; Gallon 1981; Hallenbeck 1987; Kallas et al. 1983). The complicated nitrogen-fixing cell, the heterocyst, explains diverse and efficient mechanisms from reasonably simple, largely unicellular cyanobacterial strategies. Indirect transition evidence is possible, according to the evolutionary picture, because of the steady growth in oxygen content of the atmosphere between simple and increasingly complex processes (Broda and Peschek 1983; Giovannoni et al. 1988; Holland 1990; Schopf and Packer 1987; Schopf and Walter 1982).

26.3.2 Detection Step of Cyanobacteria N_2-Fixing Potential

The slow advance of N_2 fixation capacity between various members of the community over 50 years indicates a slow evaluation of nitrogenase extreme oxygen sensitivities and the numerous abilities of diazotrophic cyanobacteria to defend their nitrogenase against oxygen inactivation. Frank first proposed (Frank 1889), however, that cyanobacteria could correct N_2. In 1928, proof was presented 40 years later of N_2 association of two cyanobacteria forming heterocyst in pure crops (Drewes 1928). More than 20 heterocyst species, all of them N_2, were found to be able to stabilize N_2, but there is no evidence of N_2 fixation in more than 15 unicellular and filamentous non-heterocysts in critical experiments in the mid-1950s. During testing under traditional conditions of batch cultivation stresses were obtained (Fogg and Wolfe 1954). This finding, along with the clear relation between the presence of heterocysts and N_2-fixing capacity, has led to the belief that N_2-fixing capacities are confined to heterocyst-forming organisms (Fogg 1949). It was only in 1968, however, that Stewart and coworkers made a definite proposal (Krieg and Hoffman 1986), and shortly afterward, experimental data were submitted that proposed that heterocysts be used as potential sites in some cells for N_2 fixation. It has been demonstrated that it is essential to distinguish a vegetative cell into a heterocyst for the transmission and defense of nitrogenase from inactivation from ambient and photosynthetic oxygen sources.

In 1961, the N_2 fixation of the marine non-heterocystous cyanobacterium of Trichodesmium (Oscillatoria) sp. was mentioned by Dugdale et al. (Dugdale et al. 1961). In the same era, the assertion could not be proven because of failure to isolate the strain in axenic culture, although $15N_2$ incorporation was seen in the field data. In 1969, Wyatt and Silvey were the first to prove N_2 fixation in atmospheric environments with the special cyanobacterium of the Gloeocapsa genus (later referred to as Gloeothece) (Wyatt and Silvey 1969). Stewart and Lex in 1970 showed that the filamentous non-heterocyst *Plectonema boryanum* can synthesize nitrogenase under microaerobic conditions under the sun (Stewart and Lex 1970), i.e., if a continuous stream of N_2-CO_2 has been flushed with the crops to remove the oxygen provided during photosynthesis. About ten years later, aerobic N_2 fixation of the non-heterocyst filamentous Microcoleus chthonoplastes was confirmed (Pearson et al. 1979).

The system of "anaerobic nitrogenase induction" was developed by Rippka and Waterbury (Rippka and Waterbury 1977) enabling the production of nitrogenase when the oxygen is absent. It requires a provisional nitrogen therapy (Hunger, Cox and Fay 1969) which has an effect upon the accumulation of carbohydrate stores and the anaerobic gas mixture (AR-CO_2) was continuously flushed in order to remove photosynthetic oxygen development through the 3-(4,4-dichlorophenyl)-1,1-dimethylurea (DCMU) seed suspension. Cyclic photophosphorylation can further create ATP when the nitrogenase reducing agent is produced during the experimental treatment by the breakup of storage glycogen. Through using the abovementioned procedure, 52 of 131 (40%) synthesized nitrogenases were screened for a significant number of non-heterocystous cyanobacteria for nitrogenase activity.

Therefore, the genetic capacity for N_2 fixation among non-heterocyst species is far more common than previously presumed. Objects that impede the expression of nif genes in many non-heterocystous cyanobacteria in aerobic contexts are simply the oxygen-dependent abolition of nitrogenase synthesis and the organism's lost or diminished capacity to protect the nitrogenase toward oxygen disabilities.

Non-heterocystous cyanobacteria interact temporally in fixing of N_2 and development of photosynthetic O_2. Currently, they repair N_2 in the shadow, and in the sun, photosynthesis. Now it is evolving, though this is done in numerous forms by multiple non-heterocystous cyanobacteria. Nitrogenase proteins in other cultures are switched over and can only be detected while N_2 is stable, while in others nitrogenase remains active with the transient activation of the Fe protein enzyme. The pattern of N_2 fixation is likewise endogenous in some people because it continues under continuous illumination until established by alternation between light and darkness. In the light phase in the absence of heterocysts, sea cyanobacteria Trichodesmium fixes N_2 but seems to confine nitrogenase to a subset of the filament cells. It is also important to thoroughly analyze the N_2-fixing cells, to ensure that these cells generate O_2. The actual mechanism(s) can be useful when genetics are enough to examine mutants.

26.3.3 N_2 Fixation and Possible Oxygen Photosynthesis

A variety of strains of non-heterocystous cyanobacteria have the ability to adjust their carbon metabolism between standard oxygen photosynthesis and a mode of anoxygenic photosynthesis (Padan and Cohen 1982). This is analogous in the case of photosynthesis. The external electron donor in these strains is sulfide rather than vapor. The first observation came from the sulfide-rich sols Solar Pool of Israel (Cohen et al. 1975) Oscillatoria Limnetica genus. In case of sulfide concentrations between 0.1 and 0.2 mM, oxygenic PS II-driven photosynthesis will be stopped and anoxygenic PS I-dependent photosynthesis which is resistant to DCMU begins after a short period (2-h) of induction. The hydrogenase-catalyzed hydrogen evolution of PS I-driven sulfide oxidation results in a CO_2 absence. When sulfide is extracted, the

organism returns spontaneously to oxygen photosynthesis, which appears to mean that PS II remains constitutive.

26.4 Oxygen Nitrogen Defense in Non-heterocystous Cyanobacteria

Over the last two decades, science has been striving to discover the existence in non-heterocyst cyanobiacin for a universal mechanism to combat nitrogenase inactivation. Instead, a variety of strategies appear, alone or in combination, to protect the complex enzymes from both exogenous (air) and biological (photosynthetic) origins. A selection of oxygen tolerance has been derived from these studies. Interestingly, all the strains analyzed in depth had a specific oxygen reaction under conditions of nitrogen fixing. A brief discussion of these reactions is needed before trying to draw any general conclusions, as exposed by a few representative strains of non-heterocystous nitrogen-fixing cyanobacteria.

26.4.1 Gloeothece

After Wyatt and Silvey (Wyatt and Silvey 1969) first reported that Gloeothece sp. is able to fix aerobic N_2, several attempts were made to clarify, in photosynthesis, that the function of nitrogenase is preserved under air and apparently in O_2 cells (Gallon and Hamadi 1984; Maryan et al. 1986b). Any morphological or structural variations between the cells grown on dinitrogen and those cultivated with combined nitrogen were not observed during light and electron microscopic testing (Gallon et al. 1974). The fact that nitrogenase was slightly more susceptible to O_2 when induced anaerobically than air is clearly suggested by the involvement of defensive mechanisms. The existence of O_2 (Kallas et al. 1983) is needed to articulate these mechanisms. The first proposals on potential temporal separation of photosynthetic and nitrogen fixing were based on the observation that these two processes resulted in growth in continuously illuminated batch cultivations at various stages (Gallon et al. 1974). In batch cultures of different non-N_2-fixing photosynthetic microorganisms certain improvements in the rates of nitrogen assimilation and protein synthesis and carbon metabolism and carbohydrate accumulation are normal. The findings contribute to the conclusion that the primary cause of reducing and energizing N_2 fixation at Gloeothece sp. is aerobic respiration and not photosynthesis in the dim, as well as the light (Eady et al. 1985; Maryan et al. 1986a). The author concludes that the diazotrophy of the body does not involve the fixation of N_2 and photosynthesis and can occur concurrently. The author suggests that it is not mandatory to distinguish the N_2 fixation and photosynthesis. The potential function and relevance of additional pathways that could improve the nitrogenase defense of Gloeothece

against O_2 inactivation remains obscure. Notes on the nature of hydrogenase intake (Van der Oost et al. 1987) are worth mentioning, but not for catalase since the predicted feature is not quantitatively assessed.

26.4.2 Synechococcus

During the times of cell division, intensive photosynthesis, cell elongation, and carb accumulation, little to no nitrogenase activity was observed during these processes. Photosynthesis peaked in light, while in the middle of the dark time the highest activity in a nitrogenase was estimated. In the center of the light period, the cell carbon content was highest; its gradual decrease in darkness correlates with the increased activity of nitrogenase. The reduction in the evolution of photosynthetic O_2 coincided with the rise and degradation of the reservation of carbohydrate. The nitrogen fixation was determined by deterioration of the cellular carbohydrate reserves and energy, reduction and likely nitrogenase defense were supplied by respiration (Mitsui et al. 1987), which finds that temporary separation of photosynthesis and nitrogen fixation within the cycle of cells enables Synechococcus strains to develop on the dinitrogen in the cell cycle The synthesis of nitrogenase needs anaerobic cultural treatment and a high cell suspension density has been promoted. The activity of hydrogenase has coincided with the activity of nitrogenase. Their nitrogenase demonstrated a distinct susceptibility to O_2 with an ideal gas step of about 1 percent ((0.01 atm [1.01 kPa]) O_2 fluence density, and thus reduced photosynthetic evolution of O_2.

When crops are converted into continuous illumination, the rhythmic occurrence of the operation of nitrogenase with cultures suited to the diet was maintained (Gotto and Yoch 1982). Acetylene depletion was comparatively low in continued light crops and nearly threefold when the concentrations of dissolved O_2 were decreased by the addition of N_2 broken cultures and DCMU (Grobbelaar et al. 1986). The activity of nitrogenase was inversely linked to dissolved O_2 and the highest nitrogenase activity was linked to a distinct decrease in net O_2 development as a result of higher breathing rates. This cyclic trend of the expression of nif genes was maintained for several days while a community became continuous (Huang and Chow 1990). When N_2 is incubated in the light under microaerobic conditions, the filamentous non-heterocyst *Plectonema boryanum* is in a position to set N_2, which is constantly sprayed with an O_2-free gas mixture (N_2-CO_2) (Pearson and Howsley 1980; Rogerson 1980; Stewart et al. 1969; Weare and Benemann 1974). The factors that explicitly or indirectly influence the amounts of exogenous and endogenous O_2 depended between moving filament to nitrogen-free medium and the onset of nitrogenase activity.

26.4.3 Plectonema

Plectonema boryanum can be filamented with non-heterocyst filaments to fix N_2 when incubated in the sun under micro-aerobic conditions, as is the case with the sparking of the seed suspension proceeding to an O_2-free gas mixture (N_2-CO_2), which is (Maryan et al. 1986b; Pearson and Howsley 1980; Rogerson 1980; Stewart and Lex 1970; Weare and Benemann 1974). Conditions such as absence of the O_2 (Pearson and Howsley 1980), low photon fluence (Giani and Krumbein 1986), or use of chemical reductors and O_2 absorbers, such as the sulfides and dithionite, have been used for the continuous concurrent photoautotrophic development and N_2 fixation (Pearson and Howsley 1980; Rogerson 1980). The reducing of N_2 fixation is obviously induced by oxidative metabolism under these conditions, while cyclic photophosphorylation can provide the nitrogenase activity requirements for ATP (Pearson and Howsley 1980). In subsequent studies (Giani and Krumbein 1986; Rogerson 1980), the proposal concerning temporal photosynthesis separation and N_2 fixation was not substantiated based on the observation of alternating peaks of photosynthesis and nitrogenase processes, and the rhythmic duration of phycobiliprotein-pigment degradation and synthesis (Weare and Benemann 1974). Five percent or below of the gas phase was adequate not to inactivate nitrogenase but to promote respiratory metabolism. Aerobic ventilation could not only fulfill reduction and energy requirements but could also provide restricted nitrogenase defense by intracellular O_2 stress regulation. On the other hand, in cells that also contained nitrogenase, the main photosynthetic enzyme, Rubisco, was located both in N_2-fixed and in non-fixing filament.

26.4.4 Microcoleus

In an aerobic state (Weare and Benemann 1974), a reduction of acetylene was demonstrated in the filamentous *Microcoleus chthonoplastes* isolated from a sea cyanobacterial layer. The species demonstrates a propensity to multiply, i.e., no signs of cell differentiation under N_2-fixing conditions could be shown by the creation of light and electron microscopic analyses. Reducing ability was shown to be identical in all cells along the trichome when examined by the triphenyltetrazolium chloride reaction (Shindler et al. 1980). Under ambient atmospheric conditions, chthonoplastes were able to stabilize N_2, decreased O_2 pressures improved its nitrogenase activity in light. However, O_2 stopped a drop in acetylene in the dark (Weare and Benemann 1974). This and the dependency on carbon reserves created during the previous light incubation (Westberry and Siegel 2006) in the dark and the nitrogenase activity mean that *M. chthonoplastes* must have effective photosynthesis safety systems.

26.4.5 Oscillatoria

Surveys performed by Stal and Krumbein and their associates of the N_2 fixation of marine strain Oscillatoria limosa 23 have identified some characteristics that may be important to cyanobacteria survival in the particular case. This strain has been isolated from the intertidal mat benthic system (Stal and Krumbein 1981). There was no sign of cell division in the close analysis of the morphology and pigmentation of the trichomes. Latest Stal and Bergman experiments have shown that the protein is equally spread across the cytoplasm and is present through an immunogold electron microscopy in all trichome cells under N_2-fixing conditions in the Fe protein position (Stal and Bergman 1990). Therefore, the organism's ability to sustain nitrogenase activity under air can be due to those pathways that might shield the enzyme from inactivation by O_2. Nitrogenase activity did not occur at O_2 concentrations up to 0.15 atm (15.19 kPa), but the O_2 voltages were higher and triggered inactivation of nitrogenase (Stal and Krumbein 1985), apparently because the O_2-scavenging machines have restricted space. The reaction to decreased ambient and endogenous O_2 pressures showed a distinct sensitivity of nitrogenase to O_2. Thus, the nitrogenase activity was increased 4.5 times when filaments had been incubated under helium, 6 times when DCMU was added to the culture suspension, and 5 times during the transmission of cultures in the dark (Stal and Krumbein 1981). The presence of DCMU, a combination of low photon fluence rate, and anaerobic incubation supported the best levels for the elimination of acetylene.

26.4.6 Trichodesmium

Trichodesmium genus planktonic cyanobacteria are known as main actors. Female members have always been stabilized in the Atlantic, Pacific, and Indian Oceans in tropical and subtropical segments. They can produce massive accumulations of surfaces ("blooms") that can be seen with the naked eye. The Trichodesmium helps preserve aquatic life by releasing essential nutrients like carbon/nitrogen and death and decay, for example (Capone and Carpenter 1982; Capone et al. 1998; Davis and McGillicuddy 2006; Tyrrell et al. 2003). The worldwide input through Trichodesmium N_2 fixation has been originally estimated at around 5 Tg N per year. The O_2 nitrogenase N_2 fixation enzyme is rapidly inactivated, so either fix N_2 in the night (to prevent photosynthetically produced oxygen) or discriminate between a photosystem II thread-wall-deficient heterocystous form that supports daytime N_2 fixation. N_2 fixation is a diazotrophic cyanobacteria (Carpenter and Capone 2008; Westberry and Siegel 2006). The most important agents of N_2 fixation in the marine pelagic system are known to be Trichodesmium species. In the depth of 15–25 m below the surface, populations were observed to peak. The flowering of the gas vesicles causes species to position themselves favorably in the euphotic region. In Trichodesmium spp. physiology studies, the repeated loss of separation and

organism development in axenic culture has been impeded. Evidence of the capacity to species of Trichodesmium. The detected light would be borne out to fix N_2 and Nitrogenase activity enhancement.

Through the use of radioautograph 14C, Carpenter and Price detected the high degree of nitrogenase activity of some cells in the central area of the trichomes. The cells have been suggested to specializes with heterocystic cyanobial bacteria for N_2 fixation in a similar way. The authors proposed that the O_2 content in those cells may be sufficiently low to allow nitrogenase to work. Regular prototrophic bacteria could further facilitate the protection of microaerobic conditions in close association with the central center of bundles (Brusca et al. 1989; Paerl et al. 1989a). The evidence is consistent with an obvious nitrogenase sensitivity. Intracellular operation of O_2 voltages elevated (Bryceson and Fay 1981). The activity in the enzyme increased rapidly during the early morning period under clear conditions but remained measurable throughout the nocturnal period. It is possible that the increased activity is through an ample supply of ATP, possibly through an apparent increased photoproduction of ATP but suppressed enzyme activity, probably by increased phtocells. The isolations and conservation of Trichodesmium spp. were effective (Ohki and Fujita 1982). In laboratory culture, by removing, grasses and other microorganisms associated with them and taking note of the organism's remarkable susceptibility to heavy metals and its comparatively high CO_2 concentrations. Single trichomes have been taken forth clonal cultures. The axenic existence of the bacteria Trichomes has been observed in these cultures. Apparently, during colony's late growth, it is not appropriate to separate the N2 cultures during the early development stage and to form small bundle-formed colonies. The operation of nitrogenase was, however, reversely related to the size of the colony (Ohki and Fujita 1988). For immunological tests, an epifluorescent microscopy in the pigment content of the trichomes might be used to detect a trichome or to be limited to around 10 to 40% (Paerl et al. 1989b).

In cells fastening N_2 two improvements were observed to the Fe protein with apparent molecular masses of 38 and 40 kDa. In cells raised with nitrate or ammonium, either of its higher-molecular form was present; none was detectable in cells cultivated with urea. The operation of Trichodesmium spp. can be achieved by nitrogenase. The reversible alteration of the protectant protein controls and preserves the enzyme, as this shift occurs from exposure O_2 When trichomes are burned under N_2, the enzyme is completely changed and is likely to be more active (Zehr et al. 1991). The organization, as in other non-heterocystous N_2-setting cyanobacteria, of the Trichodesmium nif genes has been shown to be contiguous and populous (Zehr et al. 1991).

26.5 Nitrogenic Preservation of Oxygen in Heterocystous Systems

The heterocysts are complex cellular structures, which include Anabaena, Nodulana, Cylindrospermum, Nostoc, Scytonema, Calothrix, Fischerella, and Chlorogloeopsis, formed by transformation to cyanobacteria of group sections IV and V (Rippka et al. 1979). For the successful functioning and the defense of nitrogenase, heterocysts have a finely regulated anaerobic microenvironment. Production of the heterocyst affects spatial isolation of oxygen photosynthesis and oxygen-relevant nitrogen fixation from the two opposing metabolic activities. In monographs and review papers, there is extensive material on identifying (Adams and Carr 1981; Fay 1980; Fogg et al. 1973; Haselkorn 1978; Wolk 1982) morphology and patterning and cellular organization and heterocyst function. While this analysis concentrates on discussing multiple mechanisms to prevent nitrogenase in heterocysts from oxygen harm, it may nevertheless be useful to readers who are less aware of the biology of our current knowledge of the heterocyst cyanobiology.

Heterocyst separation includes deep structural and biochemical differences including mobilizing granular inclusions and reserve components, the accumulation of an external envelope, the creation of a little crossroads between the cell wall and the heterocyst, vegetative cell, intracytoplasmic membrane system disintegration and new development and degradation of protein, as well as new proteins synthesis (Fleming and Haselkorn 1973, 1974; Kulasooriya et al. 1972; Lang and Fay 1971). The cellular stores of nitrogen are quickly reduced during a cycle of nitrogen shortage that contribute to heterocyst differentiation and nitrogenase synthesis, which increase the cellular carbon / nitrogen ratio to around 4.5 by 8.1 over the normal value. The formation of heterocysts appears to start with a ratio above 6.1. Nitrogenase production is not completely mature in trichomes of so-called pro-heterocysts. Only as the carbon/nitrogen ratio grows to around 8.1 would events be measurable. The first ripening heterocysts appear this way (Kulasooriya et al. 1972). The simple prerequisite for nitrogen-fixing ability is to complete structural and biochemical changes, so that nitrogenase can work effectively and without obstructing oxygen in the transformed cell. In Anabaena sp. Strain PCC7120, Buikema and Haselkorn identified the hetR gene recently, one of many genes that regulate heterocyst production (Bulen et al. 1965). The existence of extra copies of this gene contributes to several heterocysts being produced. Although it may be possible to sense C/N ratios in the cells, the function of the hetR agent in the differentiating heterocyst has yet to be identified. The normal isolation of the heterocysts in the trichome is a reasonable tool for fostering a consistent and effective distribution of fixed nitrogen in a linear multicellular system from the heterocysts on vegetative cells. In the opposite direction carbon compounds are used to integrate nitrogen fixed in a heterocyst into the substrates for breathing and carbohydrated skeletons (Wolk 1968). Fogg's early hypothesis that fixed nitrogen resists the development of heterocysts and that the origin of the inhibitor is

heterocystic is based on the trend of heterocyst differentiation (Fogg 1949). The inhibitor's personality is expected to disperse.

26.5.1 Molecular Biology of Cyanobacterial N_2 Fixation

Because of nitrogenase sensitivity to O_2, scientists have been intrigued by the interrelations between N_2 fixation and O_2. Diazotrophic cyanobacteria are capable of simultaneously producing O_2 and fixing N_2 and the pathways used by N_2 fixers can be especially well developed in cyanobacteria to shield nitrogenase from O_2. Heterocyst cyanobacteria spatially distinguish oxygen (vegetative cell) and N_2 (non-photosynthetic heterocysts) from oxygen (vegetative cells). Some filamentous cyanobacteria launch a differentiation program after hungering for nitrogen, which leads to heterocyst creation. These cells form the positions of N_2 fixation in a semiregular interval in the filaments. Nitrogenase is preserved by heterocysts by different pathways, including elevated breath rates and reduced permeability to O_2 from inactivation. Heterocysts do not grow O_2 and cannot repair CO_2 so they rely on adjacent vegetative cells for a carbon source. Recent developments in the genetics of Anabaena are being greatly encouraged by studies on heterocyst growth. Genes implicated in the formation of heterocysts will now be tagged by mutagenesis with transposon. A physiological characterization of mutants, sequence analyses of tagged genes, and biochemical analysis of the genetic products may clarify the mechanisms that manage heterocyst differentiation.

Two proteins, Mo-Fe and Fepro, are contained in a nitrogenase. The Fe protein of nitrogenase undergoes some other diazotrophs with a reversible covalent modification (ADP-ribose attachment), leaving the Fe protein inactive. Covalent nitrogenase alteration is triggered by the transition of cultures to darkness or exposure to ammonium or sources of O_2. Fe protein can also be solved in a variety of cyanobacteria; there is no covalent alteration of two elements, although there is no proof to date. Therefore, further study on this phenomenon would be beneficial. Another complicated element to research is the existence of fixed nitrogen. Many cyanobacteria with N_2 fixation release fixed nitrogen. For example, the Gloeothece single-cell cyanobacterium fixes N_2 in the dark under alternating light and darkness and releases some nitrogen into its external capsule. The subsequent light stage reassimilates this nitrogen and it can be blocked by cumulative nitrogen by nitrogenase action. In certain cases, the release and transportation of amino acids are very complex. The respective genes have been represented and identified.

Nitrogen release from the host fixation plant N_2 is necessary if the symbiotic relationship of symbiotic cyanobacteria is to survive (typically Nostoc). However, it is mostly unclear how the plant continues to receive fixed nitrogen from the Nostoc N_2 fixing. It is an important objective to recognize certain pathways, which can unlock directions for biotechnological applications. Cyanophycin is a nitrogen-rich reservoir of arginine and aspartate in the N_2-fixing cyanobacteria. Latest developments in cyanophycin biosynthesis enzymology must be followed by an

understanding of cyanophycin's turnover of dynamic nitrogen supply available. There is much interest in more research on this subject (European Science Foundation 1999).

26.5.2 Genetic Regulation of Cyanobacterial Nitrogen Fixation

In other N_2 fixers, cyanobacteria vary. Cyanobacteria are normally prohibited from adding N_2, owing to the presence of a common nitrogen supply such as nitrate or ammonium in the atmosphere. This activity is biologically important because it is less costly to assimilate decreased nitrogen types. Since nitrogenase is grown in a nitrate or ammonium state, cyanobacteria do not synthesize, and it is clear that nitrogen synthesis in cyanobacteria has been controlled by genetically modified nitrogen, for example, expression of genes for nitrogen assimilation, including gene for the separation of heterocyst and assimilation of ammonia (e.g., hetR and glnA)-earliest NtcA-responsive heterocyst differentiation gene. Is NtcA essential to control N_2 fixation in non-heterocyst and symbiotic cyanobacteria? The PII (encoded with glnB) protein used to coordinate C and N assimilation was also identified in some N_2-fixing cyanobacteria. The fascinating probability of PII being active in nitrogen signals in cyanobacteria may also be lifted. However, since PII can directly bind molecules that are important for the conditions of nitrogen stress, it can act both as a signal transducer and as a sensor (European Science Foundation 1999).

26.6 Genetics (Cyanobacteria)

One fascinating paradox of N_2 cyanofixation is that our understanding of the molecular genetics of certain free-living heterocystic strains (especially of the Anabaena) has now surpassed our physiological knowledge and biochemistry, with little details about other cyanobacteria's genetics being available. We would also extend our knowledge of genetics of some previously very obscure and symbiotic species (e.g., Gloethece, Trichodesmium, Nostoc, and Calothrix). Any of these were immune to genetic transformation and DNA insulation. In this type of cyanobacteria, the development of an effective genetic approach is a significant priority research in the project. There are recent reports that certain heterocyst cyanobacteria possess two separate expression profiles of molybdenum-dependent nitrogenases, nif1 and nif2. In non-heterocyst cyanobacteria, vegetative cell nitrogenase (Nif2) only fixes N_2 anaerobically. In addition, certain cyanobacteria may generate model "alternative" (V or Fe) nitrogenases, encoded by genes encoding vnf and anf. This is a subject which can be paired with biotechnology (European Science Foundation 1999).

26.6.1 16S rRNA Gene

Due to 16S-rRNA gene universal distribution in prokaryote, functional constancy, existence of vector and retained regions, and high content of details, this 16S-rRNA gene has all the characteristics of a phylogenetic marker gene (Woese 1987).

Other features in 16S rRNA are: (1) the appropriate longitude of the gene of around 1500 bp, (2) the occurrence of long, strongly preserved areas useful in the calculation of distant phylogenetic relationships, (3) the existence of ample regions of variable intensity in order to calculate closeness, (4) not appropriate for rapid sequence changes, and (5) (Tiwari 2014).

A similarity of 16S rDNA of 96–97% and DNA: 70% RB DNA hybridization with ident 5°C reflects lower boundaries between the distinct bacterial species (Wayne et al. 1987). Similarly, the relationship between RB and 16S rDNA sequences differed considerably within the same subphylus, and the similarity between the two was found by Keswani and Whitman 2001. In spite of this, the upper and lower RB values for species borders in prokaryotes tend to need to be altered. Rosselló-Mora and Amann proposed a 50% RB value at a uvT m 7 °C to differentiate organisms, because the overall DNA homology was as low as 10–40% RB even if the 16S rDNA sequence similitude was 98–99% (Rosselló-Móra and Amann 2001). Their conclusion was that 16S rRNA sequence identification does not have appropriate guidelines for maintaining the identification of species based on the sequences of 16S rRNA genes (Woese 1987). The sequence of the DNA: DNA hybridization experiments was 5% 16S rRNA but distinct from each other. Studies of this molecule created an immense public library. There have been a number of attempts to change the lower limits for species delineation of bacteria.

The alignment of 16S rRNA gene sequences is simple to compare between strains. In phylogenetic analyses, this is a critical step (Fig. 26.3). Alignment takes place by adding holes, such that homologous sequence locations are located in the same data matrix columns. For alignment, for instance, a variety of computer programs were used: EBI, Ribosomal Database Initiative, Antwerp, or GenBank (European Bioinformatics Institute). In addition, individual software tools are available from different software packages for sequence editing, alignment, and phylogenetic analyses. In sequence alignment by primary structure data, the program is helpful, with processed aligned sequences. It can be connected to the Internet through local or global networks. When homologues have several features, gene function prediction approaches are used to distinguish between top hits. Relevant amino or nucleotide positions in multiple genes are a positional homology. The gene that is marked as the most heavily influenced by a test for similarities is assigned to the uncharacterized gene. For the uncharacterized gene, top 10 + hits are listed. The query sequence is assigned a specific function depending on the degree of consensus of the top hits.

The sequence of evolution in the form of a phylogenetic tree is present. It is a graphical representation of the ancestral history of genes or animals. The pattern of a branching tree demonstrates evolutionary relations between the strains (topology).

The nodes and edges of a tree are formed. The nodes fit the organisms and the boundaries indicate their relationships. For the terminal nodes, there is only one link edge that corresponds to species. These are commonly referred to as taxonomic operating units (OTUs). Three connecting edges have internal nodes which correspond to a possible ancestor of one collection of species. The root of the tree, the common ancestor to all taxa, is a special inner node with two edges only.

Phylogenetic trees may be reflected as rooted or unrooted. Rooted or unrooted trees are identical, with the exception of having just two edges connected while they are absent in the latter (Fig. 26.4). The two approaches proposed for tree building have been divided into two different categories: algorithm alternatives and optimum require. The first steps may create a specific tree while the latter assess all feasible trees and pick one to better meet those criteria. In algorithmic methods the algorithm plays a key role, but it is just a means for optimally testing parameters. Algorithmic methods are referred to as distance methodologies since a tree computes the distance between pairs of sequences using a distance matrix. An algorithm based on distance is simply a process to construct a tree that is matrix dependent. If the tree is to be additive, all distances should be followed by three rules: (1) only zero if two points with the same points must be distances, (2) there are symmetrical distances, and (3) no tree shortcuts occur, i.e., a–c cannot surpass the a–b and b–c combined distances. There are symmetrical distances between two points. A tree point restriction of distance a–b from the third point cannot be extended in addition (maximum distance b–c and a–c). Unweighted pair group approach (UPGMA) is an ultra-metric tree construction algorithm that adheres to all rules listed before. By entering an ancestral sequence per stage, UPGMA is carried out. UPGMA chooses the least remote pair of sequences in the first round (or one); the distance is outlined as the branches of a new tree and the pair of the whole matrix as one person recalculates the distance (with mean distances taking). The matrix will be reduced to just one element after *N*-1 steps (where *N* is the sequence number). The last deduced ancestor is the tree root. Distance methods such as neighbor link (NJ) from alignment sequences and typically two pairs were developed (i.e., the number of essential discrepancies between two sequences). The NJ tree does not describe the root. The root is very popular when a sequence is introduced to the set and is considered to be more distinct than the rest of the considered category. The outer category location on the tree lists the root. For instance, a chimney outgroup was used for the identification of the root of human mitochondria (Tiwari 2014).

26.6.2 Symbiotic Cyanobacteria

In order to broaden the N_2-fixing list to include commercially interesting plants like cereals, symbiotically competent cyanobacteria have excellent features which make them particularly valuable. Unlike rhizobia, often symbiotic cyanobacteria have a mechanism of their own to resist oxygen inactivation by nitrogenase (heterocysts). They have an unrivaled host diversity (angiosperms to fungi) and are not restricted to

Table 26.1 Nitrogenase distribution amongst cyanobacteria non-heterocysts[a]

Section[b]	Basic morphology	Reproduction	Order (family)[c]	Typical genera
I	Unicellular or colonial	Binary fission	*Chroococcales*	*Gloeobacter*, *Synechococcus*, *Synechocystis*, *Microcystis*, *Gloeothece*, *Gloeocapsa*, *Chroococcus*
II	Unicellular or colonial	Budding, multiple fission	*Chamaesiphonales* *Pleurocapsales*	*Chamaesiphon*, *Dermocarpa*, *Dermocarpella* *Xenococcus*, *Myxosarcina*, *Pleurocapsa*
III	Filamentous nondifferentiated	Trichome fragmentation, hormogonia	*Nostocales* (*Oscillatoriaceae*)	*Oscillatoria*, *Microcoleus*, *Spirulina*, *Pseud-anabaena*, *Plectonema*, *Lynghya*, *Phormidium*
IV	Filamentous hetero-cystous	Trichome fragmentation, hormogonia, akinetes	(*Nostocaceae*)	*Anahaena&$$$;*, *Aphanizomenon*, *Nostoc*, *Nodularia*, *Anabaenopsis*
			(*Rivulariaceae*)	*Calothrix*, *Gloeotrichia*, *Rivularia*
			(*Scytonemaceae*)	*Scytonema*, *Tolypothrix*
V	Branched filamentous heterocystous	Trichome fragmentation, hormogonia, akinetes	*Stigonematales*	*Fischerella*, *Mastigocladus*, *Stigonema*, *Westiella*

[a]From Fay (Golden et al. 1988)
[b]From Rippka et al. (Horne and Fogg 1970)
[c]From Fritsch (Helber et al. 1988)

roots, but can form a symbiosis with numerous seedlings and must not be situated intracellularly within the hosts. However, it may be important to extend our comprehension of the current ones until we can create new N_2-fixing symbiosis. The coordination of symbionts by chemical signals becomes increasingly evident. As well, it is possible that genes would be caused by both partners, during the creation of a stable N_2-fixing cyanobacterial symbiosis which will assist the symbiosis of the items. In symbiosis, we will research the existence and products of certain genes as well as contact between symbionts. Both facets of biologic research are important in the transfer of fixed nitrogen to the host plant and in reciprocal carbon flow to a cyanobacterium (European Science Foundation 1999)

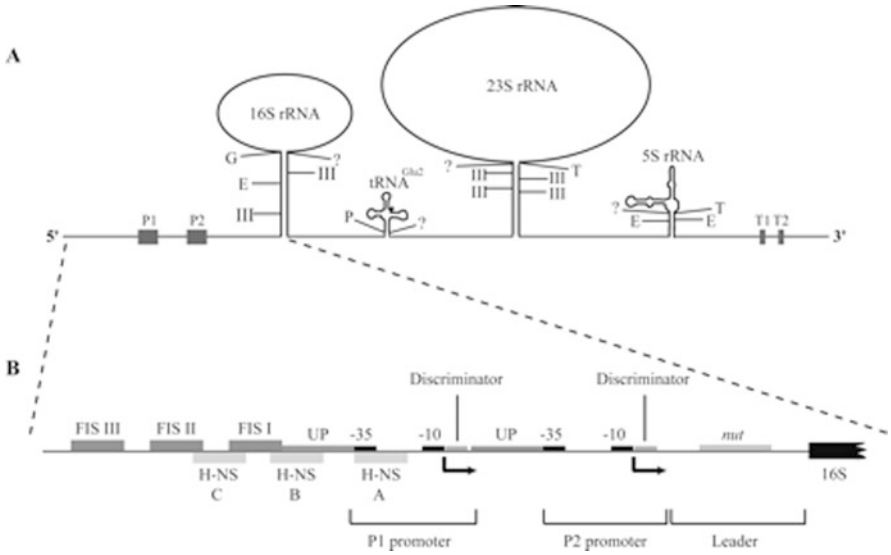

Fig. 26.1 RnB operon diagrammatic painting. (a) Key rrnB transcript nucleolytic processing. The rRNAs, tRNA organisms, P1 and P2 promoters, T1 and T2 terminators, and the RNase-III, RNase G (G), and RNase E (E), P(P), T(T) RNase and unknown RNases?) (processing sites shall also be indicated. (b) RRNB operon field promoter. (b) Locations and the UP, discriminators, and nut sequences of the FIS and H-NS sites are labeled. The arrows show the transcription starting sites (Microbiology and Molecular Biology 1969)

26.6.3 Stress of Oxygen and Nitrogenase Defense in Nature

While N_2 attachment is largely based on energy and power reduction formed in luminous reactions in heterocyst-forming cyanobacteria, the nitrogenase reaction itself is light independent (Cox 1966; Cox and Fay 1969). Several strains are able to use heterotrophic metabolism to generate organic substrates (mainly sugars) and to steadily, however, set N_2 in the darkness (Allison et al. 1937; Fay 1965; Khoja and Whitton 1975). And in obligatory photoautotrophs, including A. The N_2 cylinders will continue to be fixed in the dark until endogenous carbon reserves formed in the preceding photosynthesis are available (Bottomley and Stewart 1977; Cox and Fay 1969; Fay 1976). The degree and duration of the passage of N_2 in the dark (Fay 1976; Horne and Fogg 1970) is due to the amount of light incident absorbed during this period. Up to 30% of the cumulative daily fixed volume of N_2 can be blurred (Paerl and Kellar 1979) and night concentrations among planktonic populations of heterocystous cyanobacteria will amount to 25% of the full daily time (Storch et al. 1990). In general, but, even in heterotropically competent species, nitrogenase activity concentrations in the dark are significantly lower than in the sun. The intrinsic lack of energy and/or reduction to sustain the full fastening of N_2 (Bottomley and Stewart 1977; Fay 1965, 1976) will result from this circumstance. In natural waters, the diazotrophic cyanobacterial plant communities are exposed to

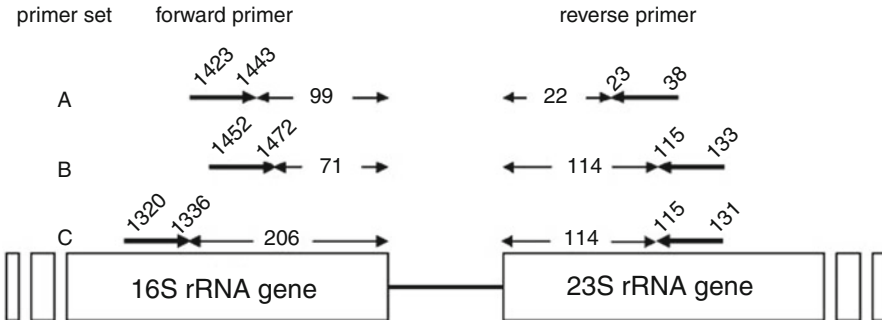

Fig. 26.2 rRNA operon E (not drawn to scale). Coli displaying unique 16S rRNA regions which are typically amplified in prokaryote recognition. There are six other 23S rRNA regions that are also used for this purpose (Cardinale 2004)

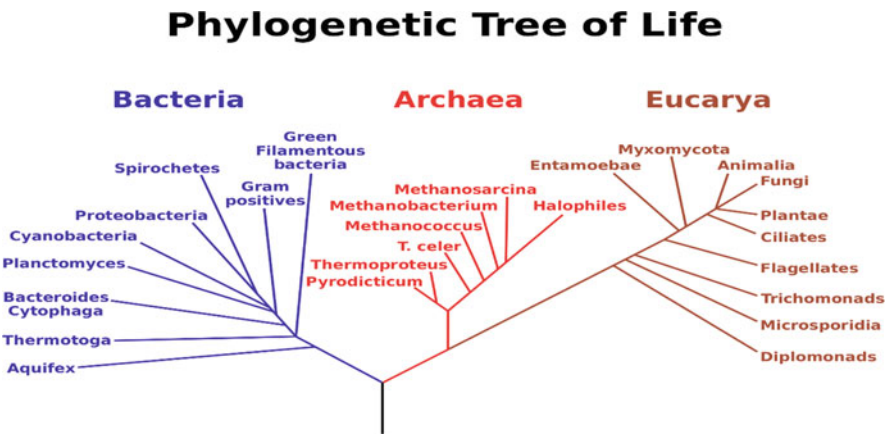

Fig. 26.3 Based on 16S rRNA sequencing, three domain classifications of the tree of life (Fact Sheet 2020)

a diverse and constantly evolving climate, which is largely influenced by regular changes in the incidence of the sun. Modifications in light amounts change photosynthesis rates and can contribute to major differences in the dissolved O_2 and inorganic carbon concentrations. Under open skies, solar light rapidly peaks in the morning, reaches a high in the middle of the day, and then falls slowly into darkness at night. Photosynthesis can be prevented by midday photon fluence concentrations in surface waters, disrupting cell structures and inducing photooxidative death (Abeliovich and Shilo 1972). By decreasing the pigment concentration, in particular the relative carotenoid concentration, considered to prevent the photooxidation of the cells (Kellar and Paerl 1980; Wyman and Fay 1986), cyanobactin may react to certain conditions. In comparison with low inorganic carbon concentrations and supersaturating dissolved levels of O_2, high irradiance facilitates photorespiration. The oxidization of 1,5-bisphosphate ribulose is light-stimulated, catalyzed by

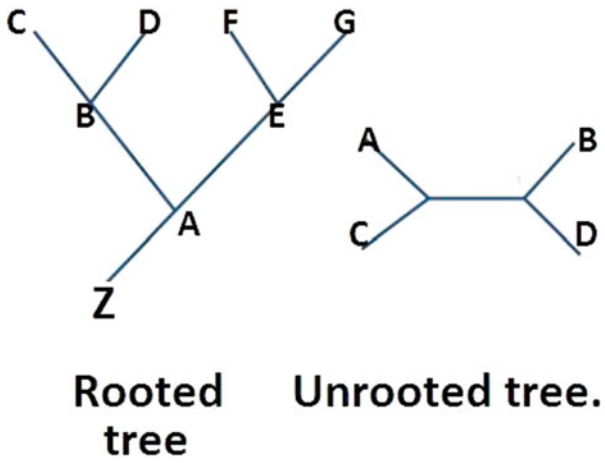

Fig. 26.4 Phylogenetic trees that are rooted and unrooted (https://brainly.in/question/3769990)

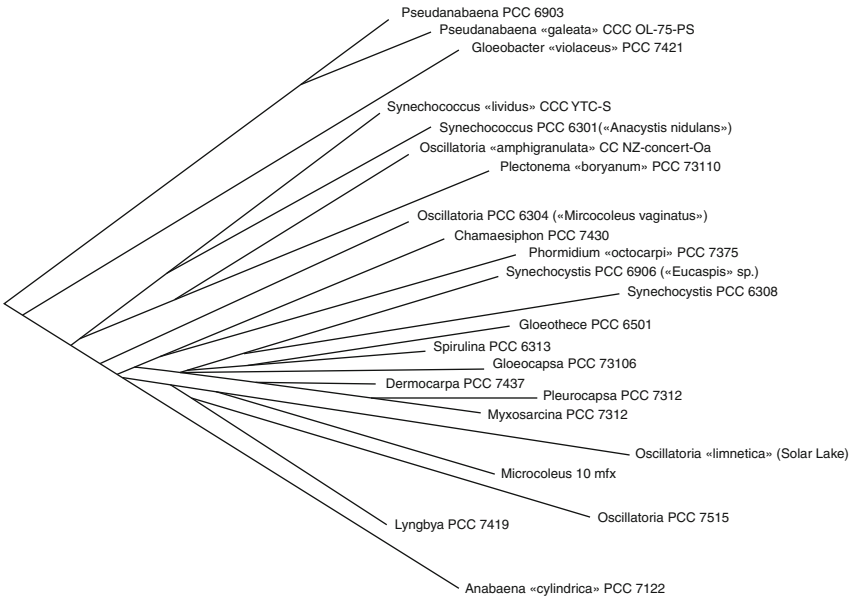

Fig. 26.5 Topological roots of the trees demonstrate innovations within 16S rRNAs of heterocyst cyanobacteria (Giovannoni et al. 1988)

oxygenase-reduced carboxylase ribulose-1,5-bisphosphate. The impact does not have a short-term influence on the nitrogenase operation but may be a drain on reducing cellular resources (Gallon et al. 1975; Lex et al. 1972).

The photosynthetic O_2 production by phytoplankton will easily, under calm circumstances, increase the concentration of dissolved O_2 by midday (up to 150–200%) to supersaturation levels (Paerl 1980; Paerl and Kellar 1979; Peterson et al. 1977). It is seen that photosynthesis is more likely than N_2 fixation to O_2 and can be blocked from the action of nitrogenase before major impact (Paerl and Kellar 1979; Peterson et al. 1977). Instead of the continued O_2 supersaturation, the activity concentrations of nitrogenase are initially decreased and then bounced down as CO_2 attachments are reduced (Bergersen 1962; Shindler et al. 1980). The fluvial formation or collapse of gas vesicles that change the vertically position of the water column will influence the planktonic cyanobacteria (Walsby 1987). Photoinhibition and photooxidation can be avoidable by migrating to colder, less lit water as irradiance rises (Peterson et al. 1977; Reynolds and Walsby 1975). The fixing values for N_2 can also be higher if O_2 levels are lower, without depleting nitrogenase reserve carbon (Ganf and Horne 1975).

A number of freshwater lakes are calculated by diurnal photosynthesis and N_2 fixation in the heterocyst-forming cyanobacteria populations (Ganf and Horne 1975; Peterson et al. 1977; Stewart et al. 1971; Storch et al. 1990). These statistics show the extreme complexities of the natural situation and affect several physical, chemical, and biological influences. Nevertheless, the diurnal variation patterns, considering variations in frequency and the relative degree of peaking values, are in conformity with two essential considerations: (1) the fixation in CO_2 and the attachment of N_2 have O_2 stress and (2) the nitrogenase depression precedes photosynthesis inhibition. The temporal isolation of the top-down activities has been suggested for heterocyst cyanobacteria to have two main advantages: allowing endogenous carbon stores to be built up in heterocysts and reducing the rivalry between photosynthesis and N_2 fixation. Clearly, the observed isolation of peak photosynthesis and nitrogenase activities is little if any essential for nitrogenase defense against O_2 injuries and heterocysts are fitted with a range of interrelated mechanisms to prevent their nitrogenase being inactivated and killed, even in severe stress conditions O_2.

26.7 Expression of Oxygen Tension and Nif Genes in Cyanobacteria

Klebsiella and Rhizobium spp. experiments of *Azotobacter* have shown that the stress of oxygen induces not only nitrogenase inactivation but also nitrogenase suppression (Robson and Postgate 1980). The result of the nifL gene exists in oxygen deprivation conditions to inhibit the exposition of the nifHDK operon. However, the exact control process of O_2 is still largely unclear. Analyzing the nitrogenase protein variable genes in cyanobacteria has over the past 10 years shown that genes are nearly homologous with genes that repair bacteria in other N_2. The arrangement and vocabulary of the genera heterocysts, however, is noteworthy. The structural gene nitrogenase (nif H, nif J, and nifK), along with those found in

Klebsiella, Azotobacter, or Rhizobium spp., is classified as one HDK-operon contiguous Nif. In non-herozyphal N_2-induced cyanobacteria, for example, Gloeothece, Cyano Technic, Synechococcale, Plectonema, or Pseudanabaena spp. (Kallas et al. 1983, 1985; Singh et al. 1987; Barnum and Gendel 1985) Gloeothece spp.'s strength. Factors (so far unknown) other than the structural genes are clearly regulated for fixation of N_2 in air and its nitrogenase-protection function from O_2 lesion (Haselkorn 1986).

In the most recent Haselkorn and his colleagues' research (Kallas et al. 1985) it has become clear that the structure of the nif genes for heterocyst cyanobacteria as in Anabaena (Rice et al. 1982), Nostoc, and Calothrix spp. is different from the general pattern. In vegetative cell DNA, NifH is adjacent to NifD; however, the NifD element is separated from the NifD sequence by 11 kbp of DNA (Golden et al. 1985). The separation code for nipK and nifD genes of both the dinitrogenase subunits was astonishing, because their active nitrogenase products are also required and cotranscribed in Klebsiella spp. (Golden 1988). Item of 11 kbp of DNA, The chromosome of Anabaena sp. heterocysts forming, however, is excised. Power PCC7120 results in a contiguous nifHDK operon at an advanced stage of distinguishing and causes the three structure nif genes to concurrently be expressed (Golden et al. 1985). The nifD portion includes the xisA gene that generates a recombinase protein that is thought to be responsible for nifD portion excision (Brusca et al. 1989). Excision is prevented if inactivated, no usable nitrogenase is produced, and without a mixture of nitrogen supplies the organism is not able to extend (Golden et al. 1988). For N_2 fixation the xisA gene is important; the xisA gene can be governed by a developmental factor documenting the completion of heterocyst differentiation. A similar rearrangement of nif structural genes occurs during heterocyst development in the Nostoc community (Damerval et al. 1985). In a second rearrangement, a larger DNA sequence (55-kbp) next to the nifS gene is deleted. The gene product is responsible for the maturation of the enzyme complex of nitrogenase (Golden and Wiest 1988). In a late stage of heterocyst development, nipD and nifS rearrangements occur when the morphological transition is obvious and concurring with the nif Mrna (Golden 1988) synthesis. A second nif operon in the DNA upstream of the nifjIDK operon has been identified in the Anabaena sp. strain PCC7120 (Mulligan and Haselkorn 1989). It is made up of four genes, namely, nifB, fdxN, nifS, and nifU, presumably translated as a single operon and only after the 55-kbp portion is excised, Bacterial ferredoxin type fdxN codes. For Fe-Mo cofactor synthesis, the product of nifB may be needed. The function of nifU and nifS products is still uncertain. Possibly transcribed as a single operon only after excising 55-kbp. fdxN codes for a bacterial type ferredoxin. The compound nifB can be used to synthesize the Fe-Mo cofactor. The goods of nifU and nifS are not yet well known.

In heterocystous cyanobacteria, the unusual organization and rearrangement of nif genes tends to work by limiting nif gene expression to completely formed heterocysts, equipped with both the structural and biochemical characteristics that protect oxygen stress nitrogenases. The findings of the Elhai and Wolk study (Elhai and Wolk 1990), which investigated the transcription of nif structural genes through

the fusion of nif gene promoters into luciferase genes using light emissions, clearly support the view that the developmental signal(s) associated with a mechanism of heterocyst distinction are the induction of nif genes and not environmental signal(s). The results also demonstrate that gene expression regulation takes place at the transcriptional level. Studies with Anabaena sp. mutants offered more evidence for the findings. PCC7120 heterocysts envelope deficient strain (Lammers and Ryncarz 1991). The O_2 intracellular stress which was obviously higher did not preclude the diagnosis of DNA in mutant strains. Any experimental results suggest, however, that nif expression in heterocyst-forming cyanobacteria has a fairly complex mechanism for controlling gene expression, when Anabaena sp. communities. Strain PCC7120 had only a 55 kbp excision of the DNA after being incubated in argon (in the absence of O_2, N_2, and CO_2), with neither a morphologic separation nor a nitrogenesis (Golden and Wiest 1988), showing crop activity (25 kbp). In an additional analysis, in the presence of DCMU, *A. variabilis* was incubated under argon, mRNA nitrogenase was synthesized, and after a brief duration of nitrogen starvation nitrogenase activity was observed, but the discrepancy in heterocyst levels had been suppressed. After the exposure of those filaments to O_2, mRNA nitrogenase levels decreased rapidly (Helber et al. 1988). Appropriately, DNA has the contiguous assembly of nif structural genes in the heterocyst strain of the Fischerella family, close to those of non-heterocyst cyanobacteria (Saville et al. 1987). It includes forms with numerous patterns of ultrastructure, heterozytes, and heterozyte growth, compared with those found by members of the order Nostocales (Nierzwicki-Bauer et al. 1984). Fischerella belongs to the Stigonematales order. The Stigonematales orders have been suggested to be a more basic step of cyanobacterial growth, which may be related to coccoid and filamental types (Martin and Wyatt 1974).

26.8 Evolutionary Considerations

Owing to the scarcity of geological and fossil sources and their inconclusive existence, our understanding of primordial environments and the earliest life forms is based mainly on geophysical and geochemical considerations (Bernal 1967; Margulis 1982; Schopf and Walter 1982). The primordial environment possibly had a diminished disposition and then transformed into a redox-neutral state until it got aerobic (Broda and Peschek 1983). According to a broadly reasonable theory, the first living species are widely thought to have been heterotrophic anaerobes reliant on their metabolism of geochemically generated organic matter. Nitrogen in plentiful quantities is often expected to exist in their lowest form, ammonia, which is used by older microorganisms for cell content biosynthesis. As nitrogenase synthesis is suppressed by ammonia and oxygen, the evolution of nitrogen fixation seems logical to suppose that the simultaneous depletion or restricted supply of nitrogen fixation followed it. The power to stabilize N_2, close to the present clostridia, was first obtained by ancient fermenters. Still now anaerobic and voluntary bacteria have a more common ability to be used for N_2 fixation than aerobic bacteria (Postgate

1982). The evolution of chemical and photo-autotrophy was presumably the result of the exhaustion of organic nutrients in the ancient oceans from related reasons. There is compelling indirect proof to support the opinion that the prehistoric atmosphere was nearly anoxic around two billion years ago (Cloud 1974; Holland 1990). The O_2 atmosphere contents were only roughly 0.2% in advance of the evolved oxygenic photosynthesis, with many, if not most, of the old cyanobacteria inherited and transported details on nitrogenase synthesis. The creation of O_2-created cyanobacterium photosynthesis was probably the most significant occurrence in the Precambrian era, and the further path of bio-development was decided more than any other circumstances. The amounts of atmospheric O_2 appear to have shifted dramatically from 1.5 billion years and have been remarkably stable only over Earth's last 350 million years. As a result of the accumulation of cyanobacteria in Precambrian times (Schopf and Walter 1982), the incremental rise in O_2 atmospheric content provided the environment for aerobic breathing and metabolic processes and ways of life to be diversified. In turn, N_2-fixing species have been contained in anaerobic environments in increasingly oxic settings. Selective developmental stresses have demonstrated the obvious biochemical and structural properties of a variety of microaerobic and anaerobic bacteria, including cyanobacteria, to avoid oxygen inactivation or degradation of their nitrogenase, and fix N_2 under high tension conditions of O_2.

Early Precambrian microfossils reports are rare because of the scarcity of non-metamorphosed sedimentary rocks and also because of the absence of rugged, easy-to-use systems in the old microbials. Earlier records of suspected cyanobacterial rock microfossils aged 3.5–3.8 billion years are now regarded as questionable and insufficient proof of cyanobacteria remains (Schopf and Walter 1982). Nevertheless, there are more recent records of early Archean carbonate microfossils from 3.3 to 3.5 billion years ago. Schopf and Packer (Schopf and Packer 1987) tend to retain a colonial, filamentous, and "suggestive" population of cyanobacteria of chroococcus. There has been more compelling evidence of cyanobacterial fossils of ancient, matt-forming cultures, including fine structures like the oscillatorian cyanobacteria of the present period, from stromatolite (laminoid sedimentary rock) between 2.5 and 2.8 billion years old (Schopf and Walter 1982). Given the rare and unknown fossil records, molecular approaches to study into bacterial evolution are increasingly important and detectable. Genetic interaction measurement biochemical approaches include the analysis and processing of amino acids in proteins, nucleotide DNA and RNA processing, analysis of the base content of DNA and genomic size determinations, and hybridization of DNA. A comparative study of 16S rRNAs of oligonucleotide sequences has been one of prokaryotic genealogy as most accurate and relevant. That is primarily because rRNAs are spread uniformly and tend to change very little and preserve their shape and function throughout phylogeny. In recent years, this approach was commonly and successfully used to establish evolutionary connexions between prokaryotes. A variety of significant insights may be drawn from the results of a comprehensive theoretical study undertaken by Fox (Fox et al. 1980). Only anaerobic was the earliest bacterial phenotypes; during the growth of the bacteria, aerobic phenotypes were

identified many times. Second, there appears to be a variety of nonphotosynthetic bacteria which have established a discovery which challenges the notion that heterotrophic, photosynthetic bacteria are the first. Third, cyanobacteria of single cells tend to be a combination of the most widespread and most advanced forms of cyanobacteria. A recent research by Giovannoni et al. 1988 offered further data on cyanobacterial evolutionary relations. Its findings indicate that significant eubacterial lines, including phototrophic anaerobic bacteria, have differed. The discrepancies between cyanobacterial lines have arisen within comparatively short evolutionary time, and the various cyanobacterial divergences are a distinct phylogenetic assembly which has developed considerably later than that of the other cyanobacterial lines verifiable before diversification of cyanobacteria (Fig. 26.5).

The results and analysis above demonstrate clearly that a lot more knowledge is needed to support tentative studies about how cyanobacterial development advances from simplistic unicellular types to heterocyst, colonial, and filamentous structures. Nevertheless, there is no evidence that the incremental rise in O_2 concentrations of the atmosphere is followed by the creation, in ancient cyanobacteria, of more complex structural and physiological property. Proof has been added that the creation of structures and pathways to effectively defend and operate the N_2-fixing enzyme is also involved. Evidence is growing that this advancement has included the creation of architectures and pathways to defend and operate efficiently in the cyanobacteria N_2-fixing enzyme system.

26.9 Conclusion and Future Perspectives

Geological documents and phylogenetic studies on the basis of 16S mRNA indicate that N_2 fixation during cyanobacteria evolution followed oxygen photosynthesis. The primary need to defend nitrogenase from O_2 has therefore been published. The production of oxygen photosynthesis and inhibitory concentrations at atmosphere O_2 may have occurred inside the same cell in ancient cyanobacteria at the same time. We may conclude that certain systemic and/or biochemical mechanisms were established in this early period to avoid intracellular stress of O_2. As the concentration of O_2 in the environment has steadily been raised, there is also a need to protect the nitrogenase from outside O_2 distributed to N_2-fixing cells. Any diazotrophic cyanobacteria acquired additional, effective nitrogenase defense mechanisms. Others, unable to adapt, might only repair N_2 in a microaerobic climate or in oxygen-free environments. Over the past two decades, studies have found a number of oxygen tolerances in response to O_2 stress among diazotrophic cyanobacteria and great diversity. It is tempting to speculate that this difference represents the phases of cyanobacteria's tolerance to O_2 atmosphere enrichment.

In the 10 years since our understanding of biochemistry and genetic stability for cyanobacterial N_2 attachment has improved dramatically in the physiology and ecology of N_2 cyanobacteria, our perception of nitrogenase defense in non-heterocyst cyanobacteria, however, has advanced relatively slowly, from its

adverse effects in endogenous and exogenous O_2. It remains until a persuasive solution to such problems (e.g., the cellular function of nitrogenase activity at photosynthesis site and the transport mechanisms of breathing electrons, distribution of reduced substances and ATP to light and darkness, and a spatial and/or temporal distinction between photosynthesis and activity of nitrogenase within cells and human trichomes) is sought. Latest studies on the presence of conformational defense linked to reversible modification of dinitrogenase reductase show inadequately yet definitely more closely connected to other possible mechanisms including oxygen reaction and enzyme safety, besides light-based O_2 intake or photos piracy.

In symbiotic connectivity with plants with numerous structural and physiological complexities, some heterocystous cyanobacteria grow. Cyanobionts fix N_2 in these interactions at significantly higher concentrations than in isolation and the host organism takes up a large part of the fixed nitrogen (Stewart et al. 1983). N_2 fixation in cyanobiont appears to be inducted in the microaerobic environment of the host organism and efficient transfer of fixed nitrogen. Given recent advancements in bioengineering, there can be no longer an illusion regarding the possibilities of artificially forming stable cyanobacterium–crop plant connexions (Gantar et al. 1991). Such collaborations will be very helpful for growing plants and could minimize requirements for chemical fertilizers.

Acknowledgments The above paragraphs contain major highlights and display some facets of cyanobacteria's interesting community. The topic of selection is entirely personal. However, I hope that the material covered will be sufficiently large for a general overview of the nature of cyanobacteria, molecular aspects, and oxygen relations of nitrogen fixation in cyanobacteria. I would like to thank and I am grateful to the authors cited in the text.

References

Abeliovich A, Shilo M (1972) Photooxidative death in blue-green algae. J Bacteriol 111:682–689

Adams DG, Carr NG (1981) The developmental biology of heterocyst and akinete formation in cyanobacteria. Crit Rev Microbiol 9:45–100

Agardh CA (1824) Systema algarum. Litteris Berlingianis, Lundae

Ahlborn F (1895) Uber die Wasserbl¨ute Byssus flos-aquaeund ihr Verhalten gegen Druck. Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg 3:25–36

Allison FF, Hoover SR, Morris H (1937) Physiological studies with the nitrogen-fixing alga Nostoc muscorum. Bot Gaz 98:433–463

Barnum SR, Gendel SM (1985) Organization of nitrogen fixation genes in a non-heterocystous filamentous cyanobacterium. FEMS Microbiol Lett 29:339–342

Bauld, J. 1984. Microbial mats in marginal marine environments: Shark Bay, Western Australia and Spencer Gulf, South Australia, in Microbial mats: stromatolites (eds Y. Cohen, R.W. Castenholz and H.O. Halvorson), Alan R. Liss, New York, pp. 39–58

Becana M, Rodriguez-Barrueco C (1989) Protective mechanisms of nitrogenase against oxygen excess and partially reduced oxygen intermediates. Physiol Plant 75:429–438

Bergersen FJ (1962) The effect of partial pressure of oxygen upon respiration and nitrogen fixation by soybean root nodules. J Gen Microbiol 29:113–125

Bergersen FJ (1965) Ammonia, an early stable product of nitrogen fixation by soybean root nodules. Aust J Biol Sci 18:1–9
Bergersen FJ (1980) Leghaemoglobin, oxygen supply and nitrogen fixation: studies with soybean nodules. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 139–160
Berman-Frank I, Lundgren P, Chen Y-B (2001) Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium Trichodesmium. Science 294:1534–1537
Bernal JD (1967) The origin of life. Weidenfeld & Nicolson, London
Biggins DR, Postgate JR (1969) Nitrogen fixation by cultures and cell-free extracts of Mycobacterium flavum 301. J Gen Microbiol 56(2):181–193
Bond G (1961) The oxygen relations of nitrogen fixation in root nodules. Z Allg Mikrobiol 1:93–99
Bothe H, Neuer G, Kalbe I, Eisbrenner G (1980) Electron donors and hydrogenase in nitrogen-fixing microorganisms. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 83–112
Bothe H, Tripp HJ, Zehr JP (2011) Unicellular cyanobacteria with a new mode of life: the lack of photosynthetic oxygen evolution allows nitrogen fixation to proceed. Arch Microbiol 192:783–790
Bottomley PJ, Stewart WDP (1977) ATP and nitrogenase activity in nitrogen-fixing heterocystous blue green algae. New Phytol 79:625–638
Broda E, Peschek GA (1983) Nitrogen fixation as evidence for the reducing nature of the early biosphere. Biosystems 16:1–8
Brusca JS, Hale MA, Carrasco CD, Golden JW (1989) Excision of an 11-kilobase-pair DNA element from within the nifD gene in Anabaena variabilis heterocysts. J Bacteriol 171:4138–4145
Bryceson I, Fay P (1981) Nitrogen fixation in Oscillatoria (Trichodesmium) erythraea in relation to bundle formation and trichome differentiation. Mar Biol 61:159–166
Bulen WA, LeComte JR (1966) The nitrogenase system from Azotobacter. Proc Natl Acad Sci U S A 56:979–986
Bulen WA, Burns RC, LeComte JR (1965) Nitrogen fixation: hydrosulfite as electron donor with cell-free preparations of Azotobacter vinelandii and Rhodospirillum rubrum. Proc Natl Acad Sci U S A 53:532–539
Burk D (1930) The influence of oxygen gas upon the organic catalysis of nitrogen fixation by Azotobacter. J Phys Chem 34:1195–1209
Burms RH, Magee WE, Bach MK (1955) The pN2 and P02 function for nitrogen fixation by excised soybean nodules. Ann Acad Sci Fenn Ser 60:190–199
Burris RH, Arp DJ, Benson DR, Emerich DW, Hagerman RV, Ljones T, Ludden PW, Sweet WJ (1980) The biochemistry of nitrogenase. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 37–54
Capone DG, Carpenter EJ (1982) Nitrogen fixation in the marine environment. Science 217:1140–1142
Capone DG, Subramaniam A, Montoya JP, Voss M, Humborg C, Johansen AM, Siefert RL, Carpenter EJ (1998) An extensive bloom of the N(2)-fixing cyanobacterium Trichodesmium erythraeum in the central Arabian Sea. Mar Ecol Prog Ser 172:281–292
Cardinale M (2004) Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities applied and environmental microbiology
Carnahan JE, Mortenson LE, Mower HF, Castle JE (1960) Nitrogen fixation in cell-free extracts of Clostridium pasteurianum. Biochim Biophys Acta 44:520–535
Carpenter EJ, Capone DG (2008) Nitrogen fixation in the marine environment. In: Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (eds) Nitrogen in the marine environment. Academic, San Diego, pp 141–198
Cloud P (1974) Evolution of ecosystems. Am Sci 62:54–66
Cloud P (1976) Beginnings of biospheric evolution and their biogeochemical consequences. Paleobiology 2:351–387

Cohen Y, Padan E, Shilo M (1975) Facultative anoxygenic photosynthesis in the cyanobacterium Oscillatoria limnetica. J Bacteriol 123:855–861
Cohn F (1853) Untersuchungen uber die entwicklungsgeschichte mikroskopischer algen und pilze. Nova Acta Academia Caesarea Leopoldina Carolina 24:103–256
Cohn F (1872) Untersuchungen uber Bakterien. Beitrage Biol Pflanzen 1:127–224
Cohn F (1875) Untersuchungen uber Bakterien. Beitrage Biol Pflanzen 1:141–207
Cohn F (1897) Die Pflanze. Votrage aus dem Gebiete der Botanik, vol 2. J.U. Kern's Verlag, Breslau
Cox RM (1966) Physiological studies on nitrogen fixation in the blue-green alga Anabaena cylindrica. Arch Mikrobiol 53:263–276
Cox RM, Fay P (1969) Special aspects of nitrogen fixation by blue-green algae. Proc R Soc London, Ser B 172:357–366
Dalton H (1980) Chemotrophic nitrogen fixation. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 177–195
Dalton H, Postgate JR (1969) Effect of oxygen on growth of Azotobacter chroococcum in batch and continuous culture. J Gen Microbiol 54:463–473
Damerval T, Franche C, Rippka RM, Cohen-Bazire G (1985) Rearrangement of nif structural genes in Nostoc PCC7906. In: Evans HJ, Bottomley PJ, Newton WE (eds) Nitrogen fixation research progress. Martinus Nijhoff, New York, p 517
Davis CS, McGillicuddy DJ (2006) Transatlantic abundance of the N(2)-fixing colonial cyanobacterium Trichodesmium. Science 312:1517–1520
Desikachary TV (1959) Cyanophyta. Indian Council of Agricultural Research, New Delhi
Dingler C, Oelze J (1987) Superoxide dismutase and catalase in Azotobacter vinelandii grown in continuous culture at different dissolved oxygen concentrations. Arch Microbiol 147:291–294
Ditta G, Virts E, Palomares A, Kim CH (1987) The nifA gene of Rhizobium meliloti is oxygen regulated. J Bacteriol 169:3217–3223
Dixon R (1972) Hydrogenase in legume root nodule bacteroids: occurrence and properties. Arch Mikrobiol 85:193–201
Drewes K (1928) Ueber die Assimilation des Luftstickstoffs durch Blaualgen. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg 76:88–101
Dugdale RC, Menzel DW, Ryther JH (1961) Nitrogen fixation in the Sargasso Sea. Deep-Sea Res 7:298–300
Eady RR, Issack R, Kennedy C, Postgate JR, Ratcliffe HD (1978) Nitrogenase synthesis in Klebsiella pneumoniae: comparison of ammonium and oxygen regulation. J Gen Microbiol 104:277–285
Eady RR, Imam S, Lowe DJ, Miller RW, Smith BE, Thorneley RNF (1980) The molecular enzymology of nitrogenase. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 19–35
Eady RR, Maryan PS, Gallon JR, Chaplin AE (1985) Nitrogen fixation by Gloeothece sp. (PCC6909): respiration and not photosynthesis is the major source of energy for nitrogenase activity in the light, abstr. 252. Abstr. 5th Int. Symp. Photosynth. Prokaryotes, Grindelwald, Switzerland
Elhai J, Wolk CP (1990) Developmental regulation and spatial pattern of expression of the structural genes for nitrogenase in the cyanobacterium Anabaena. EMBO J 9:3379–3388
Emerich DW, Burris RH (1978) Complementary functioning of the component proteins of nitrogenase from several bacteria. J Bacteriol 134:936–943
European Science Foundation (1999) Cyanobacterial nitrogen fixation (CYANOFIX), An ESF scientific programme
Fact Sheet (2020) rRNA in evolutionary studies and environmental sampling. https://microbe.net/simple-guides/fact-sheet-rrna-in-evolutionary-studies-and-environmental-sampling
Fay P (1965) Heterotrophy and nitrogen fixation in Chlorogloea fritschii. J Gen Microbiol 39:11–20
Fay P (1976) Factors influencing dark nitrogen fixation in a blue-green alga. Appl Environ Microbiol 31:376–379

Fay P (1980) Nitrogen fixation in heterocysts, p. 121-165. In: Subba Rao NS (ed) Recent advances in biological nitrogen fixation. Edward Arnold, London
Fay P (1992) Oxygen relations of nitrogen fixation in cyanobacteria. Queen Mary and Westfield College, University of London, London, pp 340–373
Fay P, Cox RM (1967) Oxygen inhibition of nitrogen fixation in cell-free preparations of blue-green algae. Biochim Biophys Acta 143:562–569
Fay P, Stewart WDP, Walsby AE, Fogg GE (1968) Is the heterocyst the site of nitrogen fixation in blue-green algae? Nature 220:810–812
Fleming H, Haselkorn R (1973) Differentiation in Nostoc muscorum: nitrogenase is synthesized in heterocysts. Proc Natl Acad Sci U S A 70:2727–2731
Fleming H, Haselkorn R (1974) The program of protein synthesis during heterocyst differentiation in nitrogen-fixing blue-green algae. Cell 3:159–170
Fogg GE (1949) Growth and heterocyst production in Anabaena cylindrica Lemm. II. In relation to carbon and nitrogen metabolism. Ann Bot 13:241–259
Fogg GE, Wolfe M (1954) The nitrogen metabolism of the blue-green algae (Myxophyceae). In: Fry BA, Peel JL (eds) Autotrophic microorganisms. Cambridge University Press, Cambridge, pp 99–125
Fogg GE, Stewart WDP, Fay P, Walsby AE (1973) The blue-green algae. Academic, London
Fox GE, Stackebrandt E, Hespell RB, Gibson J, Maniloff J, Dyer TA, Wolfe RS, Balch WE, Tanner RS, Magrum LJ, Zablen LB, Blakemore R, Gupta R, Bonen L, Lewis BJ, Stahl DA, Luemsen KR, Chen KN, Woese CR (1980) The phylogeny of prokaryotes. Science 209:457–463
Frank B (1889) Ueber den experimentellen Nachweis der Assimilation freien Stickstoffs durch erdboden-bewohnende Algen. Ber Dtsch Bot Ges 7:34–42
Fredriksson C, Bergmann B (1997) Ultrastructural characterisation of cells specialised for nitrogen fixation in a non-heterocystous cyanobacterium, Trichodesmium sp. Protoplasma 197:76–85
Fridovich J (1986) Biological effects of the superoxide radical. Arch Biochem Biophys 247:1–11
Fritsch FE (1951) The heterocyst: a botanical enigma. Proc Linnean Soc 162:194–211
Gallon JR (1980) Nitrogen fixation by photoautotrophs. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 197–238
Gallon JR (1981) The oxygen sensitivity of nitrogenase: a problem for biochemists and micro-organisms. Trends Biochem Sci 6:19–23
Gallon JR, Hamadi AF (1984) Studies on the effect of oxygen on acetylene reduction (nitrogen fixation) in GCloeothece sp. ATCC27152. J Gen Microbiol 130:495–503
Gallon JR, LaRue TA, Kurz WGW (1974) Photosynthesis and nitrogenase activity in the blue-green alga Gloeocapsa. Can J Microbiol 20:1633–1637
Gallon JR, Kurz WGW, LaRue TA (1975) The physiology of nitrogen fixation by a Gloeocapsa sp. In: Stewart WDP (ed) Nitrogen fixation by free-living microorganisms. Cambridge University Press, Cambridge, pp 159–173
Ganf GG, Horne AJ (1975) Diurnal stratification, photosynthesis and nitrogen fixation in a shallow equatorial lake (Lake George, Uganda). Freshw Biol 5:13–39
Gantar M, Kerby NW, Rowell P (1991) Colonization of wheat (Triticum vulgare L.) by N2-fixing cyanobacteria. II. An ultrastructural study. New Phytol 118:485–492
Geitler L (1932) Cyanophyceae. In: Kolkwitz R (ed) Rabenhorst's Kryptogamen-Flora von Deutschland, Osterreich under Schweiz, vol 14. Akademische Verlag, Leipzig
Giani D, Krumbein WE (1986) Growth characteristics of non-heterocystous cyanobacterium Plectonema boryanum with N2 as nitrogen source. Arch Microbiol 145:259–265
Giovannoni SJ, Turner S, Olsen GT, Barns S, Lane DJ, Pace NR (1988) Evolutionary relationships among cyanobacteria and green chloroplasts. J Bacteriol 170:3584–3592
Golden JW (1988) Genome rearrangement during Anabaena heterocyst differentiation. Can J Bot 66:2098–2102
Golden JW, Wiest DR (1988) Genome rearrangement and nitrogen fixation in Anabaena blocked by inactivation of xisA gene. Science 242:1421–1423

Golden JW, Robinson SJ, Haselkorn R (1985) Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobacterium Anabaena. Nature 314:419–423
Golden JW, Carrasco CD, Mulligan ME, Schneider GJ, Haselkorn R (1988) Deletion of a 55-kilobase-pair DNA element from the chromosome during heterocyst differentiation of Anabaena sp. strain PCC7120. J Bacteriol 170:5034–5041
Gordon YK (1981) Introduction to the nitrogen-fixing prokaryotes. In: Starr MP, Stolp H, Trueper HG, Balows A, Schlegel HG (eds) Prokaryotes, vol I. Springer-Verlag, Berlin, pp 781–794
Gotto JW, Yoch DC (1982) Regulation of Rhodospirillum rubrum nitrogenase activity. Properties and interconversion of active and inactive Fe-protein. J Biol Chem 257:2868–2873
Grau FH, Wilson PW (1963) Hydrogenase and nitrogenase in cell-free extracts of Bacillus polymyxa. J Bacteriol 85:446–450
Grobbelaar N, Huang TC, Lin HY, Chow TJ (1986) Dinitrogen-fixing endogenous rhythm in Synechococcus RF-1. FEMS Microbiol Lett 37:173–177
Gussin GN, Ronson CW, Ausubel FM (1986) Regulation of nitrogen fixation genes. Annu Rev Genet 20:567–591
Hallenbeck PC (1987) Molecular aspects of nitrogen fixation by photosynthetic prokaryotes. Crit Rev Microbiol 14:1–48
Hamilton PB, Wilson PW (1955) Nitrogen fixation by Aerobacter aerogenes. Ann Acad Sci Fenn Ser 60:139–150
Haselkorn R (1978) Heterocysts. Annu Rev Plant Physiol 29:319–344
Haselkorn R (1986) Organization of the genes for nitrogen fixation in photosynthetic bacteria and cyanobacteria. Annu Rev Microbiol 40:525–547
Hassam HM, Fridovich I (1984) Oxygen toxicity in prokaryotes. In: Bannister JV, Bannister WH (eds) Biology and chemistry of active oxygen. Elsevier Science Publishing, Inc., New York, pp 128–138
Haystead A, Robinson R, Stewart WDP (1970) Nitrogenase activity in extracts of heterocystous and nonheterocystous blue-green algae. Arch Mikrobiol 75:235–243
Helber JT, Johnson TR, Yarbrough LR, Hirschberg R (1988) Regulation of nitrogenase gene expression in anaerobic cultures of Anabaena variabilis. J Bacteriol 170:552–557
Hennecke H, Kaluza K, Thoeny B, Fuhrmann M, Ludwig W, Stackebrandt E (1985) Concurrent evolution of nitrogenase genes and 16S rRNA in Rhizobium species and other nitrogen fixing bacteria. Arch Microbiol 142:342–348
Henry LE, Gogotov IN, Hall DO (1978) Superoxide dismutase and catalase in the protection of the proton-donating systems of nitrogen fixation in the blue-green alga Anabaena cylindrica. Biochem J 174:373–377
Herrero A, Flores E (2008) The cyanobacteria molecular biology, genomics and evolution
Hill S (1971) Influence of oxygen concentration on the colony type of Derxia gummosa grown in nitrogen free medium. J Gen Microbiol 67:77–83
Hino S, Wilson PW (1958) Nitrogen fixation by a facultative Bacillus. J Bacteriol 75:403–408
Hochman A, Bums RH (1981) Effect of oxygen on acetylene reduction by photosynthetic bacteria. J Bacteriol 147:492–499
Holland HD (1990) Origins of breathable air. Nature 347:17
Horne AJ, Fogg GE (1970) Nitrogen fixation in some English lakes. Proc R Soc London, Ser B 175:351–366
Huang TC, Chow TJ (1990) Characterization of the rhythmic nitrogen-fixing activity of Synechococcus sp. RF-1 at the transcriptional level. Curr Microbiol 20:23–26
Kallas T, Rippka R, Coursin T, Rebiere MC, Tandeau de Marsac N, Cohen-Bazire G (1983) Aerobic nitrogen fixation by non-heterocystous cyanobacteria. In: Papageorgiou GC, Packer L (eds) Photosynthetic prokaryotes: cell differentiation and function. Elsevier Science Publishing, Inc., New York, pp 281–392
Kallas T, Coursin T, Rippka R (1985) Different organization of nif genes in non heterocystous and heterocystous cyanobacteria. Plant Mol Biol 5:321–329

Kellar PE, Paerl HW (1980) Physiological adaptations in response to environmental stress during an N2-fixing Anabaena bloom. Appl Environ Microbiol 40:587–595
Kennedy C, Cannon F, Cannon M, Dixon R, Hill S, Jensen J, Kumar S, McLean P, Merrick M, Robson R, Postgate J (1981) Recent advances in the genetics and regulation of nitrogen fixation. In: Gibson AH, Newton WE (eds) Proc. 4th Int. Symp. nitrogen fixation. Australian Academy of Science, Canberra, pp 146–156
Keswani J, Whitman WB (2001) Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. Int J Syst Evol Microbiol 51:667–678
Khoja TM, Whitton BA (1975) Heterotrophic growth of filamentous blue-green algae. Br Phycol J 10:139–148
Klebahn H (1895) Gasvakuolen, ein Bestandteil der Zellen des wasserbl¨utebildenden Phycochromaceen. Flora Allgemeine Botanische Zeitung 80:241–282
Klucas R (1972) Nitrogen fixation by Klebsiella grown in the presence of oxygen. Can J Microbiol 18:1845–1850
Knoll AH (1979) Archean photoautotrophy: some alternatives and limits. Orig Life 9:313–327
Kohls K, Abed RMM, Polerecky L (2010) Halotaxis of cyanobacteria in an intertidal hypersaline microbial mat. Environ Microbiol 12:567–575
Kranz RG, Foster-Hartnett D (1990) Transcriptional regulatory cascade of nitrogen-fixation genes in anoxygenic photosynthetic bacteria: oxygen- and nitrogen-responsive factors. Mol Microbiol 4:1793–1800
Kranz RG, Haselkorn R (1985) Transcriptional regulation of nitrogen fixation genes in Rhodopseudomonas capsulata studied using lac fusions. Gene 40:203–215
Kranz RG, Haselkorn R (1986) Anaerobic regulation of nitrogen fixation genes in Rhodopseudomonas capsulata. Proc Natl Acad Sci U S A 83:6805–6809
Krieg NR, Hoffman PS (1986) Microaerophily and oxygen toxicity. Arnu Rev Microbiol 40:107–130
Kuhla J, Oelze J (1988) Dependence of nitrogenase switch-off upon oxygen stress on the nitrogenase activity in Azotobacter vinelandii. J Bacteriol 170:5325–5329
Kulasooriya SA, Lang NJ, Fay P (1972) The heterocysts of blue-green algae. III. Differentiation and nitrogenase activity. Proc R Soc London, Ser B 181:199–209
Kutzing FT (1849) Tabulae phycologicae oder abbildungender tange. Wilhelm Kohne, Nordhausen
Lammers PJ, Ryncarz AJ (1991) Evidence that oxygen does not inhibit Anabaena DNA rearrangements, abstr. 151B. Abstr. 7th Int. Symp. Photosynth. Prokaryotes, Amherst, Mass
Lang NJ, Fay P (1971) The heterocysts of blue-green algae. II. Details of ultrastructure. Proc R Soc London, Ser B 178:193–203
Layzell DB, Hunt S, Palmer GR (1990) Mechanism of nitrogenase inhibition in soybean nodules. Plant Physiol 92:1101–1107
Lex M, Silvester W, Stewart WDP (1972) Photorespiration and nitrogenase activity in the blue-green alga, Anabaena cylindrica. Proc R Soc London, Ser B 180:87–102
Logan BW (1961) Cryptozoon and associate stromatolites from the recent of Shark Bay, Western Australia. J Geol 69:517–533
Lyngbye HC (1819) Tentamen Hydrophytologiae Danicae.Continens Omina Hydrophyta Cryptogama Daniea, Holsatiae, Faeroae, Islandiae, Groenlandiae . . . , Hafniae, In Commissis Librariae Gyldendaliae
Malin G, Pearson HW (1988) Aerobic nitrogen fixation in aggregate-forming cultures of the non heterocystous cyanobacterium Microcoleus chthonoplastes. J Gen Microbiol 134:1755–1763
Margulis L (1982) Early life. Science Books International, Boston
Martin TC, Wyatt JT (1974) Comparative physiology and morphology of six strains of stigonematacean blue-green algae. J Phycol 10:57–65
Maryan PS, Eady RR, Chaplin AE, Gallon JR (1986a) Nitrogen fixation by Gloeothece sp. PCC6909; respiration and not photosynthesis supports nitrogenase activity in the light. J Gen Microbiol 132:789–796

Maryan PS, Eady RR, Chaplin AE, Gallon JR (1986b) Nitrogen fixation by the unicellular cyanobacterium Gloeothece. Nitrogenase synthesis is only transiently repressed by oxygen. FEMS Microbiol Lett 34:251–255
Meyer J, Kelley BC, Vignais PM (1978) Aerobic nitrogen fixation by Rhodopseudomonas capsulata. FEBS Lett 85:224–228
Meyerhof O, Burk D (1928) Ueber die Fixation der Luftstickstoffs durch Azotobakter. Z Phys Chem Ser A 139:117–142
Microbiology and Molecular Biology (1969) Published 31 December
Mitsui A, Cao S, Takahashi A, Arai T (1987) Growth synchrony and cellular parameters of the unicellular nitrogenfixing marine cyanobacterium, Synechococcus sp. strain Miami BG043511, under continuous illumination. Physiol Plant 69:1–8
Mortenson LE (1978) The role of dihydrogen and hydrogenase in nitrogen fixation. Biochemie 60:219–224
Mortenson LE, Thorneley RNF (1979) Structure and function of nitrogenase. Annu Rev Biochem 48:387–418
Mortenson LE, Mower HF, Carnahan JE (1962) Nitrogen fixation by enzyme preparations. Bacteriol Rev 26:42–50
Mulligan ME, Haselkorn R (1989) Nitrogen fixation (nif) genes of the cyanobacterium Anabaena species strain PCC7120. J Biol Chem 264:19200–19207
Nierzwicki-Bauer SA, Balkwill DL, Stevens SE (1984) Heterocyst differentiation in the cyanobacterium Mastigocladus laminosus. J Bacteriol 157:514–525
Ohki K, Fujita Y (1982) Laboratory culture of the pelagic blue-green alga Trichodesmium thiebautii: conditions for unialgal culture. Mar Ecol Prog Ser 7:185–190
Ohki K, Fujita Y (1988) Aerobic nitrogenase activity measured as acetylene reduction in the marine non-heterocystous cyanobacterium Trichodesmium spp. grown under artificial conditions. Mar Biol 98:111–114
Olson JM (2006) Photosynthesis in the Archean era. Photosynth Res 88:109–117
Oren A (2013) Cyanobacteria: biology, ecology and evolution. Wiley, Hoboken
Padan E, Cohen Y (1982) Anoxygenic photosynthesis. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications Ltd., Oxford, pp 215–238
Paerl HW (1980) Ecological rationale for H2 metabolism during aquatic bloom of the cyanobacterium Anabaena. Oecologia 47:43–45
Paerl HW, Kellar PE (1979) Nitrogen-fixing Anabaena: physiological adaptations instrumental in maintaining surface blooms. Science 204:620–622
Paerl HW, Bebout BM, Prufert LE (1989a) Bacterial associations with marine Oscillatoria sp. (Tnchodesmium sp.) populations: ecophysiological implications. J Phycol 25:773–784
Paerl HW, Priscu JC, Brawner DL (1989b) Immunochemical localization of nitrogenase in marine Trichodesmium aggregates: relationship to N2 fixation potential. Appl Environ Microbiol 55:2965–2975
Parker CA, Scutt PB (1960) The effect of oxygen on nitrogen fixation by Azotobacter. Biochim Biophys Acta 38:230–238
Pearson HW, Howsley R (1980) Concomitant photoautotrophic growth and nitrogenase activity by cyanobacterium Plectonema boryanum in continuous culture. Nature 288:263–265
Pearson HW, Howsley R, Kjeldsen CK, Walsby AE (1979) Aerobic nitrogenase activity associated with a nonheterocystous filamentous cyanobacterium. FEMS Microbiol Lett 5:163–167
Pengra RM, Wilson PW (1938) Physiology of nitrogen fixation by Aerobacter aerogenes. J Bacteriol 75:21–25
Peterson JB (1989) Respiratory differences associated with culture aeration in Azotobacter vinelandii. Can J Microbiol 35:918–924
Peterson RB, Friberg EE, Bums RH (1977) Diurnal variation in N2 fixation and photosynthesis by aquatic bluegreen algae. Plant Physiol 59:74–80
Post E, Kleiner D, Oelze J (1983) Whole cell respiration and nitrogenase activities in Azotobacter vinelandii growing in oxygen controlled continuous culture. Arch Microbiol 134:68–72

Postgate JR (1978) Nitrogen fixation. Edward Arnold, London
Postgate JR (1982) The fundamentals of nitrogen fixation. Cambridge University Press, Cambridge
Postgate JR, Eady RR (1988) The evolution of biological nitrogen fixation. In: Bothe H, de Bruijn FJ, Newton WE (eds) Nitrogen fixation: hundred years after. Gustav Fischer, Stuttgart, pp 31–40
Postgate JR, Eady RR, Dixon RA, Hill S, Kahn D, Kennedy C, Partridge P, Robson R, Yates MG (1981) Some aspects of the physiology of dinitrogen fixation. In: Bothe H, Trebst A (eds) Biology of inorganic nitrogen and sulfur. Springer, Berlin, pp 103–115
Reich S, Boeger P (1989) Regulation of nitrogenase activity in Anabaena variabilis by modification of the Fe protein. FEMS Microbiol Lett 58:81–86
Reynolds CS, Walsby AE (1975) Water-blooms. Biol Rev 50:437–481
Rice D, Mazur BI, Haselkorn R (1982) Isolation and physical mapping of nitrogen fixation genes from cyanobacterium Anabaena 7120. J Biol Chem 257:13157–13163
Rippka R, Waterbury JB (1977) The synthesis of nitrogenase in nonheterocystous cyanobacteria. FEMS Microbiol Lett 2:83–86
Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61
Roberts GP, Brill WJ (1981) Genetics and regulation of nitrogen fixation. Annu Rev Microbiol 35:207–235
Robson RL (1979) Repression of nitrogenase synthesis in Azotobacter chroococcum. FEMS Microbiol Lett 5:259–262
Robson RL, Postgate JR (1980) Oxygen and hydrogen in biological nitrogen fixation. Annu Rev Microbiol 34:183–207
Rogerson AC (1980) Nitrogen-fixing growth by non-heterocystous cyanobacterium. Nature 284:563–564
Rosing MT, Frei R (2004) U-rich Archean sea-floor sediments from Greenland – indications of>3700 Ma oxygenic photosynthesis. Earth Planet Sci Lett 217:237–244
Rosselló-Móra R, Amann R (2001) The species concept for prokaryotes. FEMS Microbiol Rev 25:39–67. https://doi.org/10.1111/j.1574-6976.2001.tb00571.x
Roth AG (1797) Catalecta botanica, vol 1–3. G. Muller, Leipzig
Saville B, Straus N, Coleman JR (1987) Contiguous organization of nitrogenase genes in a heterocystous cyanobacterium. Plant Physiol 85:26–29
Schopf JW (1970) Precambrian microorganisms and evolutionary events prior to the origin of vascular plants. Biol Rev 45:319–352
Schopf JW (1974) Paleobiology of the Precambrian: the age of blue-green algae. In: Dobzhansky T, Hecht MK, Steere WC (eds) Evolutionary biology, vol 7. Plenum, New York, pp 1–43
Schopf JW (1993) Microfossils of the early Archean Apex chert: new evidence of the antiquity of life. Science 260:640–646
Schopf JW (2012) The fossil record of cyanobacteria. In: Whitton BA (ed) Ecology of cyanobacteria II: their diversity in space and time. Springer, Dordrecht, pp 15–36
Schopf JW, Barghoorn ES (1967) Algae-like fossils from the early Precambrian of South Africa. Science 156:508–512
Schopf JW, Packer BM (1987) Early Archean (3.3- billion to 3.5-billion-year old) microfossils from Warrawoona Group, Australia. Science 237:70–73
Schopf JW, Walter MR (1982) Origin and early evolution of cyanobacteria: the geological evidence. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications Ltd, Oxford, p 564
Shah VK, Brill WJ (1977) Isolation of an iron-molybdenum cofactor from nitrogenase. Proc Natl Acad Sci U S A 74:3249–3253
Shanmugam KT, O'Gara F, Andersen K, Valentine RC (1978) Biological nitrogen fixation. Annu Rev Plant Physiol 29:263–276

Sheehy JE, Bergersen FJ (1986) A simulation study of the functional requirements and distribution of leghemoglobin in relation to biological nitrogen fixation in legume root nodules. Ann Bot 58:121–136
Shindler DB, Paerl HW, Kellar PE, Lean DRS (1980) Environmental constraints on Anabaena N2- and C02- fixation: effects of hyperoxia and phosphate depletion on blooms and chemostat cultures. In: Barica J, Mur LR (eds) Developments in hydrobiology, vol 2. W. Junk, The Hague, pp 221–229
Silver VS (1969) Biology and ecology of nitrogen fixation by symbiotic associations of non-leguminous plants. Proc R Soc London, Ser B 172:289–340
Silver VS, Postgate JR (1973) Evolution of asymbiotic nitrogen fixation. J Theor Biol 40:1–10
Simpson FB, Burris RH (1984) A nitrogen pressure of 50 atmosphere does not prevent evolution of hydrogen by nitrogenase. Science 224:1095–1097
Singh RK, Stevens SE, Bryant DA (1987) Molecular cloning and physical mapping of the nitrogenase structural genes from the filamentous non-heterocystous cyanobacterium Pseudanabaena sp. PCC7409. FEMS Microbiol Lett 48:53–58
Smith GM (1938) Cryptogamic botany, vol 1. McGraw-Hill, New York
Smith AJ, London J, Stanier RY (1967) Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. J Bacteriol 94:972–983
Sprent JI, Raven JA (1985) Evolution of nitrogenfixing symbioses. Proc R Soc Edinb Ser B 85:215–237
St. John RT, Shah VK, Brill WJ (1974) Regulation of nitrogenase synthesis by oxygen in Klebsiella pneumoniae. J Bacteriol 119:266–269
Staal M, Meysman FJR, Stal LJ (2003) Temperature excludes N2-fixing heterocystous cyanobacteria in the tropical oceans. Nature 425:504–507
Stal LT (1995) Review No. 84. Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol 131:1–32
Stal LJ (2012) Cyanobacterial mats and stromatolites. In: Whitton BA (ed) Ecology of cyanobacteria II. Their diversity in time and space. Springer, Dordrecht, pp 65–125
Stal LJ, Bergman B (1990) Immunological characterization of nitrogenase in the filamentous non-heterocystous cyanobacterium Oscillatoria limosa. Planta 182:287–291
Stal LJ, Krumbein WE (1981) Aerobic nitrogen fixation in pure cultures of the benthic marine Oscillatonia (cyanobacteria). FEMS Microbiol Lett 11:295–298
Stal LJ, Krumbein WE (1985) Oxygen protection of nitrogenase in the aerobically nitrogen-fixing non-heterocystous cyanobacterium Oscillatoria sp. Arch Microbiol 143:72–76
Stewart WDP (1971) Physiological studies on nitrogen-fixing blue-green algae. In: Lie TA, Mulder EG (eds) Biological nitrogen fixation in natural and agricultural habitats. Plant soil. Martinus Nijhoff Publishers, Dordrecht, pp 377–391
Stewart WDP (1980) Some aspects of structure and function in N2-fixing cyanobacteria. Annu Rev Microbiol 34:497–536
Stewart WDP, Lex M (1970) Nitrogenase activity in the blue-green alga Plectonema boryanum strain 594. Arch Microbiol 73:250–260
Stewart WDP, Haystead A, Pearson HW (1969) Nitrogenase activity in heterocysts of blue-green algae. Nature 224:226–228
Stewart WDP, Mague T, Fitzgerald GP, Burris RH (1971) Nitrogenase activity in Wisconsin lakes of differing degrees of eutrophication. New Phytol 70:497–509
Stewart WDP, Rowell P, Rai AN (1983) Cyanobacteria-eukaryotic plant symbioses. Ann Microbiol 134:205–228
Storch TA, Saunders GW, Ostrofsky ML (1990) Diel nitrogen fixation by cyanobacterial surface blooms in Sanctuary Lake, Pennsylvania. Appl Environ Microbiol 56:466–471
Strodtmann S (1895) Die Ursache des Schwebvermogens bei den Cyanophyceen. Biol Centralblatt 15:113–115
Tel-Or E, Hufleit ME, Packer L (1986) Hydroperoxide metabolism in cyanobacteria. Arch Biochem Biophys 246:396–402

Tempest DW, Meers JL, Brown CM (1970) Synthesis of glutamate in Aerobacter aerogenes by a hitherto unknown route. Biochem J 117:405–407
Tiwari A (2014) Cyanobacteria: nature, potentials and applications. Astral International Publishing House, New Delhi
Toezuem SRD, Gallon JR (1979) The effects of methyl viologen on Gloeocapsa sp. LB795 and their relationship to the inhibition of acetylene reduction (nitrogen fixation) by oxygen. J Gen Microbiol 111:313–326
Tripp HJ, Bench SR, Turk KA (2010) Metabolic streamlining in an open-ocean nitrogen-fixing cyanobacterium. Nature 464:90–94
Tyler B (1978) Regulation of the assimilation of nitrogen compounds. Annu Rev Biochem 47:1127–1162
Tyrrell T, Maranon E, Poulton AJ, Bowie AR, Harbour DS, Woodward EMS (2003) Large-scale latitudinal distribution of Trichodesmium spp. in the Atlantic Ocean. J Plankton Res 25:405–416
Van der Oost J, Kanneworff WA, Krab K, Kraayenhof R (1987) Hydrogen metabolism of three unicellular nitrogen-fixing cyanobacteria. FEMS Microbiol Lett 48:41–45
Walsby AE (1981) Cyanobacteria: Planktonic gas-vacuolate forms. In: Starr MP, Stolp H, Truper HG (eds) The prokaryotes. A handbook on habitats, isolation and identification of bacteria. Springer, Berlin, pp 224–235
Walsby AE (1987) Mechanism of buoyancy regulation in planktonic cyanobacteria with gas vesicles. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier/Science Publishers B.V., Amsterdam, pp 377–392
Walsby AE (1994) Gas vesicles. Microbiol Rev 58:94–144
Waterbury JB, Willey JM, Franks DG (1985) A cyanobacterium capable of swimming motility. Science 230:74–76
Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrand E, Starr MP, Trüper HG (1987) Report of the ad-hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464
Weare NM, Benemann JR (1974) Nitrogenase activity and photosynthesis in Plectonema boryanum. J Bacteriol 119:258–265
Westberry TK, Siegel DA (2006) Spatial and temporal distribution of Trichodesmium blooms in the world's oceans. Glob Biochem Cycl 20:4
Willison JC, Jouanneau Y, Colbeau A, Vignais PM (1983) H2 metabolism in photosynthetic bacteria and relationship to N2 fixation. Ann Microbiol 134:115–135
Wilmotte A (2001) Molecular evolution and taxonomy of the cyanobacteria. In: Bryant D (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht, pp 1–25
Wilmotte A, Herdman M (2001) Phylogenetic relationships among the cyanobacteria based on 16S rRNA sequences. In: Boone RD, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, The archaea and the deeply branching phototrophic bacteria, vol 1, 2nd edn. Springer, New York, pp 487–494
Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271
Wolk CP (1965) Heterocyst germination under defined conditions. Nature 205:201–202
Wolk CP (1968) Movement of carbon from vegetative cells to heterocysts in Anabaena cylindrica. J Bacteriol 96:2138–2143
Wolk CP (1982) Heterocysts. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications Ltd., Oxford, pp 359–386
Wolk CP (1988) Heterocysts. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. University of Berkeley Press, Berkeley, pp 359–386
Wyatt JT, Silvey JKG (1969) Nitrogen fixation by Gloeocapsa. Science 165:908–909
Wyman M, Fay P (1986) Underwater light climate and the growth and pigmentation of planktonic blue-green algae (Cyanobacteria). I. The influence of light quantity. Proc R Soc London, Ser B 227:367–380

Yates MG, Partridge CDP, Walker CC, Van der Werf AN, Campbell F, Postgate JR (1980) Recent research in the physiology of heterotrophic non-symbiotic nitrogen-fixing bacteria. In: Stewart WDP, Gallon JR (eds) Nitrogen fixation. Academic, London, pp 161–176

Yoon HS, Golden JW (1998) Heterocyst pattern formation controlled by a diffusible peptide. Science 282:935–938

Zehr JP, Ohki K, Fujita Y (1991) Arrangement of nitrogenase structural genes in an aerobic filamentous nonheterocystous cyanobacterium. J Bacteriol 173:7055–7058

Zehr JP, Dominic B, Chen Y-B (1999) Nitrogen fixation in the marine cyanobacterium Trichodesmium. A challenging model for ecology and molecular biology. In: Peschek GA, Loffelhardt W, Schmetter G (eds) The phototrophic prokaryotes. Kluwer/Plenum, New York, pp 485–500

Zehr JP, Waterbury JB, Turner PJ (2001) Unicellular cyanobacteria fix N2 in the subtropical North Pacific Ocean. Nature 412:635–638

Zehr JP, Bench SR, Carter BJ (2008) Globally distributed uncultivated oceanic N2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112