Chapter 8
Roles of Root Exudates in Different Processes in the Nitrogen Cycle in the Rhizosphere

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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

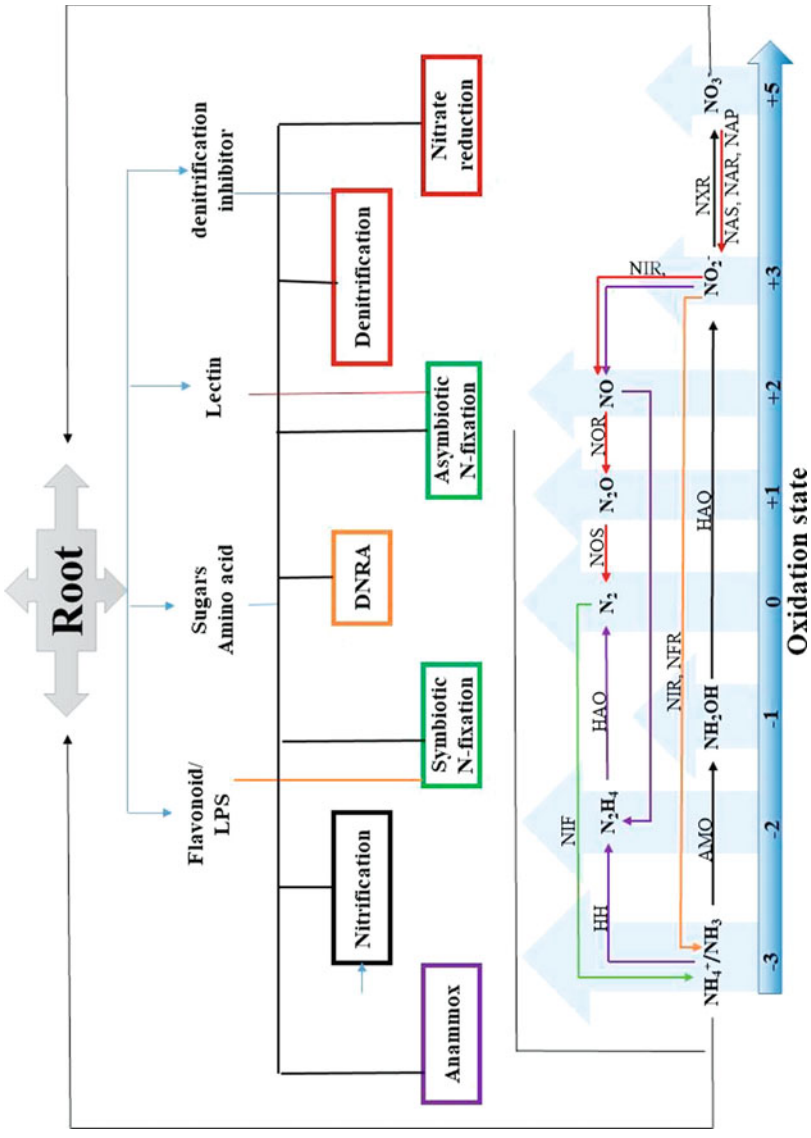

Fig. 8.1 Schematic outline of root exudates involved in different biological processes in the nitrogen cycle. *Colored arrows* show different processes in the nitrogen cycle, which are catalyzed by various enzymes, including nitrate reductases (NAS, NAR, and NAP), nitric oxide reductase (NOR), nitrite 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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