Chapter 2
Role of Microbial Diversity for Soil, Health and Plant Nutrition

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2.1 Introduction

Soil provides the medium for root development, and with the exception of carbon, hydrogen, oxygen and some nitrogen, plants depend on soil for all other nutrients and water. Soils develop by the disintegration of rocks, and minerals therein, through biotic actions of the microbes and the fauna sustained by them. Earlier, only the physical and chemical properties of soil were considered important. However, the role of soil biodiversity in maintaining fertility, and the interdependence of soil biological activities with physical and chemical characteristics is well recognized now (Abbott and Murphy 2003; Fitter 2005; Suzuki et al. 2005; Madsen 2005; Manlay et al. 2007). Physical properties and the amount of soil organic matter (SOM) determine the microbial diversity that varies with depth, and soil health. SOM adds to soil fertility, water retention and has a great influence on the growth of the above ground vegetation. Biological indicators such as microbial biomass, soil respiration, enzyme activities and microbial diversity indicate soil health. Significance of soil biodiversity for sustainability of the farming systems has been discussed at length (Brussard et al. 2007). Microbial diversity is an excellent indicator of soil health (Nielsen and Winding 2002). They report that variation in microbial population or activities precede changes that can be noticed in some cases as early signs of soil degradation or amelioration. Water and nutrient supply from soil, particularly N and P, determine the plant growth both in natural and agro-ecosystems. The above ground vegetation is the ultimate source of C for the microbes in the rhizosphere that, in turn, support the macro-fauna. Thus, the above ground vegetation influences the below ground microbial community structure and soil properties (Orwin and Wardale 2005).

This chapter gives an overview of the role of soil microbial diversity and processes controlled by them to enhance C sequestration into soil and meet the growing needs of increasing crop productivity. The effects of genetically engineered (GE)

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C.S. Nautiyal, P. Dion (eds.) *Molecular Mechanisms of Plant and Microbe Coexistence.* Soil Biology 15, DOI: 10.1007/978-3-540-75575-3

crops and microbes used for biocontrol on soil microbial diversity are examined. Possibilities of introducing peptides and enzymes that interact with the pathogenic soil microbes or enhance nutrient availability are considered. Two major challenges are to effectively manipulate soil microbial and genetic diversity and its interactions with the crop plants to (i) minimize the chemical fertilizer inputs, and enhance their use efficiency without reduction in productivity and (ii) enhance carbon sequestration into soil to reduce atmospheric CO_2 increasing at an alarming rate due to the anthropogenic factors.

2.2 Soil Microbial Diversity

In natural ecosystems C, N, P, K, Ca, Mg, S and all other mineral nutrients are cycled back into soil through litter fall and decay of the organic matter. The soil microbes that include bacteria, fungi, actinomycetes, protozoa and algae play a significant role in the nutrient cycling. Though it is widely accepted that soil biodiversity is vital for maintaining productivity in natural and managed agro ecosystems, the understanding of the microbial communities, soil fauna and their diversity is extremely limited (Buckley and Schmidt 2003; Nannipieri et al. 2003; Lynch et al. 2004; Fitter 2005; Fitter et al. 2005; Nannipieri and Smalla 2006). Of the soil microbes, 99% cannot be cultured; identification, characterization and finding their role are particularly difficult for such organisms. Estimates on the number of microbial species present in the soil vary from few thousands to millions. Lately, the nucleic acid based techniques including analysis of DNA and rRNA molecules from soil samples have revealed enormous diversity (Buckley and Schmidt 2003; Suzuki et al. 2005). The molecular methods used for soil microbial diversity are covered in the reviews by Nannipieri et al. (2003) and Lynch et al. (2004). High throughput DNA sequencing techniques developed for the human genome project are now being used to determine the soil genomes and diversity of sequences (Deutschbauer et al. 2006; Gewin 2006). Soil genome sequencing is reported to be highly complex. Hopefully, as a result of these investigations, in future it may be possible to characterize and quantify the microbial diversity in soil samples, and to follow the effect of plant communities and agronomic practices. The tools of metagenomics where the isolated genes are expressed in *E. coli* are providing new information on specific genes isolated from the DNA extracted from soil samples (Rondon et al. 2000; Gewin 2006).

Torsvik et al. (2002) compared the genome complexity among three terrestrial niches, where the number of prokaryotic cells per cubic centimeter of soil was similar (about 10 billion). The pristine pasture and forest soils contained over 10 times the genome complexity (equivalent to 3500–8800 *E. coli* genomes) compared to that of the agricultural field soils (equivalent to 140–350 *E. coli* genomes). Using improved analytical methods (Gans et al. 2005) suggests that more than 1 million distinct genomes might exist in the pristine soil, exceeding the previous estimates by two orders of magnitude. Further, it was shown that metal pollution could reduce the genomic diversity of pristine soil by more than 99.9%, revealing the highly toxic effect of metal contamination, especially for rare microbial taxa (Gans et al. 2005).

Interactions amongst soil microbes and between plants and microbes in the rhizosphere are emerging as fascinating areas of molecular biology. Hopefully elucidation of the mechanisms and specific role of the recognized species would lead to better farm management practices in future. These new approaches for investigating soil microbial diversity based on nucleic acids and proteins (soil proteome) are discussed by Nannipieri and Smalla (2006). Besides the species diversity and functional redundancy, there is enormous genetic diversity within the same species which remains largely unexplored, except for the species producing the antibiotics. The new evidences also show that soil bacteria often swap genes among themselves (Chandler 2006).

Using the molecular techniques significant changes in soil microbial communities have been shown in recent years both in the natural and cultivated areas. Microbial activities using rRNA abundance in soil samples showed that the differences between conventionally managed and never cultivated fields were significant but not between the latter and those abandoned for nine years (Buckley and Schmidt 2003). Fields abandoned for more than 45 years showed microbial activities similar to those that were never cultivated. In wooded mountain pastures, cattle grazing which involves repeated mowing, trampling and addition of urine and dung had more profound effects on microbial communities in sunny areas compared to the shaded (Kohler et al. 2005a,b). These studies led the authors to infer that in natural pastures the microbial communities below ground also change similar to the shifting mosaic of the plant species above ground. Plant species composition and richness determined the soil microbial community resistance and resilience to experimentally imposed drying on pasture plant species (Orwin and Wardle 2005). Cluster roots of white lupine (*Lupinus albus* L.) plants that secrete organic acids into soil were used to monitor the community structure in microcosms (Weisskopf et al. 2005). Frequencies of auxin producers were higher in juvenile and mature cluster roots and significantly decreased with their senescence. Proportion of the active population was higher in proximity to the roots. Comparative analyses of prokaryotic genomic sequences suggest the importance of ecology in determining microbial genome size and gene content. The significant variability in genome size and gene content among strains and species of prokaryotes indicate the highly fluid nature of prokaryotic genomes, a result consistent with those from multilocus sequence typing and representational difference analyses. The integration of various levels of ecological analyses coupled to the application and further development of high throughput technologies are accelerating the pace of discovery in microbial ecology (Xu 2006). The evidences based on molecular techniques cited above point to large diversity. However, identification of microbes responsible for the key processes remains an immense challenge (Madsen 2005).

2.3 Evolution of Farming

A brief consideration of the evolution of farming systems is necessary for a correct perception of the change from natural to agro-ecosystems. Some 10–12,000 years back, humans started clearing the natural vegetation, and planting seeds of the crops such as wheat and barley which earlier they had been collecting from natural stands. Cultivation of crops was possible only after clearing the existing natural vegetation, and often burning the same at site, similar to slash and burn agriculture still practiced in many places by tribal populations. Farming practices continue to evolve with developments in other areas of technology. Early farmers soon realized that repeatedly growing cereals in the same land reduced the soil fertility, which could be restored by keeping the plots fallow or through cultivation of leguminous crops. Practices of growing and turning in green manure crops were evolved. With the domestication of animals and the use of farmyard manures, various methods of composting were developed to maintain soil productivity. This type of subsistence agriculture continued in most parts of the world till the development of synthetic chemical fertilizers – N, P and K. The use of chemical fertilizers enhanced productivity several fold, and with the resultant increased harvests the micronutrient deficiencies started showing up. The need to control insect pests, pathogens and weeds led to the development of chemical pesticides which ultimately end up in the soil. As the pesticide residues and their degradation products increased, soil biological activity and crop productivity were affected in contaminated fields. At the same time various physical processes that cause soil degradation such as excessive use of irrigation, mining of ground water and nutrients, loss of top soil, unbalanced use of fertilizers etc. also affect microbial biodiversity resulting in deterioration of soil quality.

Soil health and productivity management have gained immense importance in recent years, especially in the intensively cropped regions in the tropics and sub-tropics as soils in these areas are often poor in SOM. The concepts of Integrated Soil Management (ISM) and Integrated Nutrient Management (INM) have been developed for more sustainable production systems. Practices such as no till farming that cause minimal disturbance to the top soil are increasing (Kirchmann and Thorvaldsson 2000).

A strong lobby against the use of chemical fertilizers and pesticides has emerged, especially in the European Economic Community. The opponents of modern farming practices would like to revert back to organic or ecological (also biodynamic) farming without the use of synthetic fertilizers and pesticides. The ability to feed the present and projected population in year 2050 based on organic farming has been questioned by those supporting modern farming practices. The soils, their SOM content as well the climatic conditions vary in different parts, and therefore the ISM and INM practices cannot be universal. They necessarily need location specific development.

2.3.1 Present and the Future Scenario

Globally more food and other farm products are needed to meet the growing requirements of increasing, economically ascendant world population. Predictions based on the trend of the past 50 years indicate that by 2050, N, P and pesticide use would increase more than 2.5 times the amount used in the year 2000 (Tilman et al. 2001). In general, it is not possible to increase the crop land area in most parts of the world and hence, the increased production must come from the presently cropped area by enhancing land productivity. Further, the additional production must use less of water, chemical fertilizers, pesticides, energy, and manual labor to bring about reduction in the adverse impact on the different components of the environment – soil, water and atmosphere.

Firewood obtained from trees was the main source of energy for cooking and heating homes till the coal, electricity and oil emerged as the alternative and more convenient energy sources. With the realization that the fossil fuels are not unlimited and their increasing cost has brought focus on crops as a renewable source of liquid fuels – ethanol and bio-diesel. This implies that the same land resources should meet the growing demand of food as well as part of the energy. The human civilization developed entirely depending on renewable resources for food and energy but the population was limited at that time. Moreover, virgin land was available for cultivation and grazing of animals. In order to meet the food, and a substantial part of the energy needs of the present 6 billion plus, expected to reach 9 billion by 2050, using only renewable resources does not appear feasible with the existing technologies and knowledge. At the same time, pursuing the path followed in the last century would further exacerbate the environmental problems. The challenge therefore is to increase productivity in an environmentally sustainable manner. This may involve exploitation of soil microbe–microbe and microbe–plant interactions (Morrissey et al. 2004) particularly in low input cropping systems (Johansson et al. 2004). Considering the present level of crop productivity, population growth rates and other factors, countries can be broadly put into four groups:

1. Abundant food production, declining population and stable demand for food, but not major exporters of food items, stabilized soils with high SOM, adequate water resources and forest cover; mainly in the temperate regions.
2. Abundant food production, stabilized or slow population growth, major exporters of food grains and other agri-products, cultivable land set aside, large forest cover, very limited soil degradation. Such regions can easily divert access food grain production for bio-fuels, and or land area to energy crops.
3. Regions with high population growth where food self sufficiency has been achieved in recent years with high inputs of chemical fertilizers and pesticides. Water resources are limited, poor soils with high degradation, low SOM, and limited forest cover.
4. High food insecurity, subsistence farming, very limited use of irrigation, fertilizer and pesticides. High population growth rate; poor soils low in SOM and inadequate water resources.

The developed countries of Europe and North America fall in 1 or 2 of the above, while most of the developing countries with poor economic growth come under 3 and 4.

2.4 Carbon Flow into Agro- and Natural Ecosystems

Cropping systems aim to maximize the fixation of solar energy, a free resource into phytomass within the constraints of temperature, water and plant nutrients through human intervention. Soils provide water and nutrients for enlarging the leaf canopy for intercepting solar radiation. Carbon, hydrogen, oxygen, nitrogen and sulfur, along with the other macro and micronutrients, are incorporated into organic molecules through light dependent reactions. These are utilized for construction, maintenance and turnover of different macromolecules and plant organs. Improved farming practices such as irrigation, fertilizers, pesticides and management amplify the fixation of solar energy, and energy flow into the cropping systems. A part of the resources are used to protect plants from insects and pathogens (Mitra and Bhatia 1982). The above ground plant cover provides C, N and other nutrients to the soil microbes through root exudates, and decomposition of aerial and root phytomass litter (Fig. 2.1). It is decomposed by the soil microbes and partly mineralized; CO_2 and methane are released into the atmosphere. The respiratory activities of plant roots, associative mycorrhizal fungi and free living heterotrophs in soil determine the CO_2 evolution from soil as a component of global carbon cycle. Plant root systems are the main pathway for C inputs into soil. Benefits of plant–microbial interactions also has a cost in terms of C inputs (Morgan et al. 2005). Dynamic simulation models have been developed (Wu et al. 2007). Current photosynthesis in boreal forests in the northern latitudes has been shown to drive soil respiration (Högberg et al. 2001). Girdling of the pine trees through removal of the bark to prevent the supply of current photosynthates to the roots reduced the number of ectomycorhizal fungi from 11 in the control plots to 1. The same was also true for wet tropical forest where phytomass litter inputs were positively correlated to fungal and bacterial biomass, and the latter to soil CO_2 efflux (Li et al. 2005).

Soil is considered as an important net sink for carbon, estimated to contain around 1500×109 tons globally (Copley 2000). This amount is estimated to be 300 times the amount of carbon released currently through burning of fossil fuels. It was assumed that the carbon locked in the soil is inert and stays there. However, recently it has been shown that SOC is more vulnerable to land use and changing climate (Bellamy et al. 2005; Schulze and Freibauer 2005). Soils could therefore also be a source rather than the sink for atmospheric C (Chen et al. 2005; Janzen 2006) largely due to the activities of the soil microbes.

2.4.1 Increase of Greenhouse Gases and Climatic Change

There is increasing evidence that the world's climate is changing and that the rate of change since the onset of the industrial revolution is greater than would be expected from natural variability alone. Clearing of forests for agriculture and burning of fossil fuels are considered the two main cause of elevated CO_2 concentration in the atmosphere (Schimel et al. 2001; Oren et al. 2001). Besides CO_2 increase in methane and nitrous oxide (N_2O) in the atmosphere are linked to agriculture. These gases trap the earth's outgoing infra red part of the solar radiation leading to increase in temperature as in greenhouses, and hence referred as greenhouse gases. They are the cause of global warming and predicted climate changes. CO_2 is the major component of greenhouse gases and the cause of global rise in temperature. It is foreseen that the increase

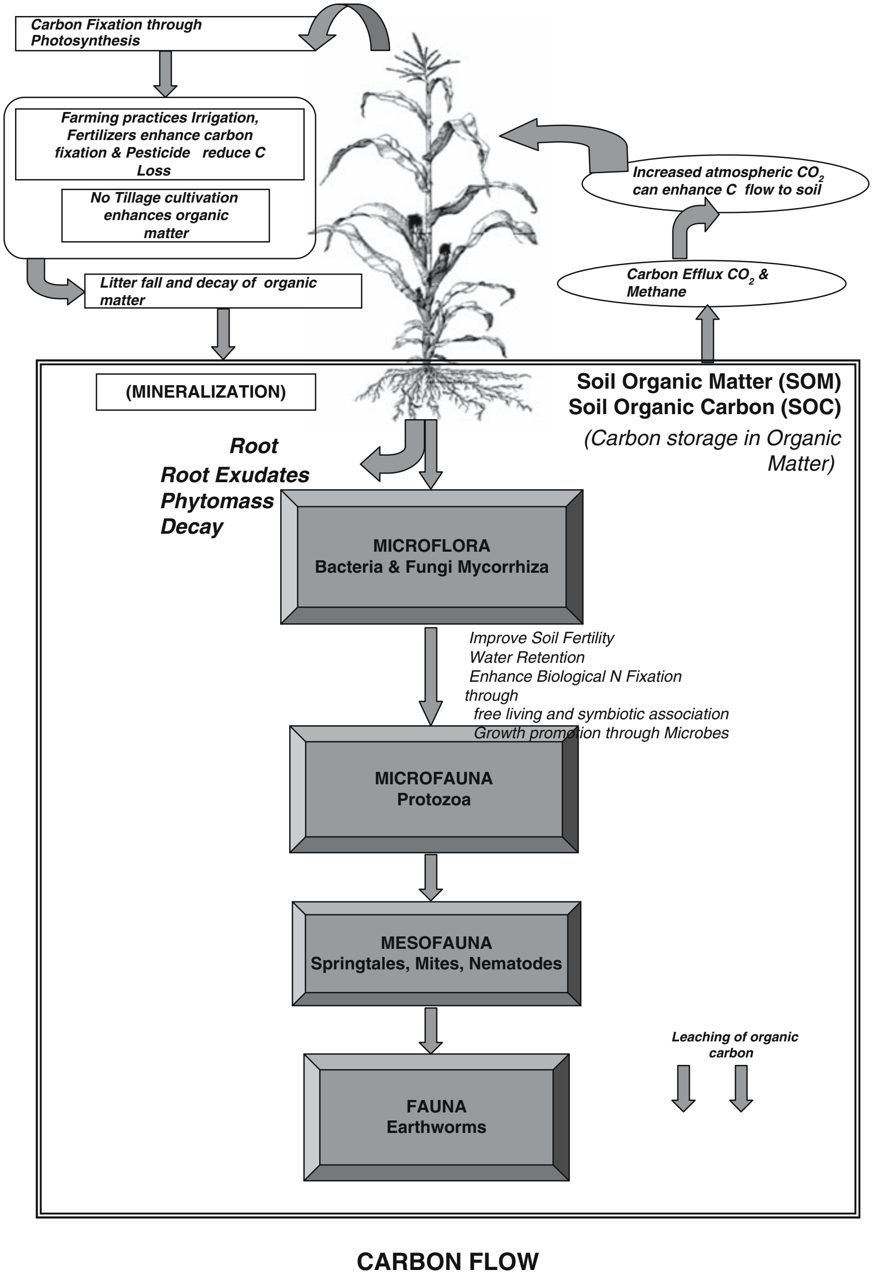

Fig. 2.1 Carbon flow and efflux from soil

in temperature would lead to melting of the snow in the polar regions, raising the sea level causing flooding, and submergence of the low lying coastal areas. All these events would impact plant growth, and alter soil biodiversity that are difficult to model. Climate change associated with greenhouse gas (GHG) emissions was recognized as a global concern in 1979, which led to the adoption of Kyoto Protocol as a first step to achieve stabilization of greenhouse gases. Projected scenarios indicate that increased temperatures and CO_2 concentrations have the potential to enhance herbage growth, but changes in seasonal precipitation would reduce these benefits particularly in areas with low rainfall. Increased frequency of droughts, storms and other extreme events may have further implications for grasslands. Potential farm-scale adaptive responses to climate change have been identified; at the same time grassland agriculture also contributes to GHG emissions, particularly methane and nitrous oxide. The net carbon balance and carbon sequestration depends on the management practices. Hopkins and Prado (2007) have recently reviewed management for mitigating grassland's contribution to GHG emissions which need to be further developed in a holistic way considering other location specific constraints.

2.4.2 Increasing Carbon Sequestration in Soils

The implications of global warming and elevated CO_2 on man made agro- and natural ecosystems with plants as the primary producers are enormous and difficult to predict. Increased atmospheric CO_2 would enhance photosynthesis leading to increased flow of carbon assimilates into soil. Higher temperatures and associated alterations in precipitation would lead to considerable changes in cropping patterns, and may have profound effects on soil biodiversity. However, soil microbes due to their short life cycles, and extensive exchange of genetic material are better endowed to cope with the environmental changes than the plants.

It is advocated that sequestration of CO_2 in SOM could contribute to its reduction in the atmosphere (Schlesinger 1999). Adoption of conservation tillage, including no till cultivation practices, could sequester all CO_2 released from agricultural activities. Application of N fertilizers and CO_2 enrichment are the other alternatives for increased C sequestration into soil. The Royal Society of UK disagrees with the proposition of soil as C sink (Adam 2001) stating that improved management could enhance C sequestration on short term to bring about 25% reduction in atmospheric CO_2 by 2050, but thereafter the potential would be limited. Uncertainties were pointed out that some soils could even release their C. This has been shown by Bellamy et al. (2005) mentioned earlier. Use of N fertilizers to enhance C sequestration could also increase release of other greenhouse gases – methane and NO.

CO_2 enrichment experiments provide data on the possible effects of increased CO_2 concentration in the atmosphere. Annual grass land exposed to enhanced CO_2 levels increased N uptake by the plants resulting in reduction of extractable N in soil (Hu et al. 2001). Microbial biomass C increased while N remained unchanged resulting in a higher C:N ratio, suggesting increased fungal/bacterial ratio. Bacterial (*Pseudomonas* *fluorescence* and *Pantoea agglomerans*) inoculation of maize increased carbon flow into the system, with higher net CO_2 assimilation rate, larger C allocation to the roots with increased amino acid exudation, and higher root/rhizosphere respiration indicating intensive C turnover (Schulze and Pöschel 2005). Other studies also indicate that in the short term CO_2 enrichment enhances C gain of the ecosystem through stimulation of photosynthesis (Diaz et al. 1993; Zak et al. 1993; DeLucia et al. 1999). Microbial processes in the soil gain with the increased C availability, but when N becomes limiting, plants and microbes compete for N (Hu et al. 2001, 2005). Enhanced C input and limitation of N favors fungi over bacteria as fungal biomass has a lower C:N ratio. In northern mid-latitude pine forests N fertilization and C enrichment increased growth by 74% (Oren et al. 2001). Under poor soil fertility C enrichment had no effect. C and N metabolisms are interdependent in plants (Swank et al. 1982) and microbes. The effect of CO_2 enrichment in different soil types and defined microbial population can provide new knowledge to enhance C sequestration and limiting the loss of C and N from farm and forest land (Fontaine and Barot 2005). In temperate and boreal forests in the Northern hemisphere it has been shown that C sequestration is largely driven by N deposition (Magnani et al. 2007). Legume based cropping systems have reduced C and N losses (Drinkwater et al. 1998). The future of the terrestrial carbon sink seems to be uncertain. The elevated global temperatures and changes in precipitation predicted for 2050 could induce a switch from soil as sink to source of carbon since warmer soils release more carbon, and forests suffer increasing periods of drought. Humans have been managing terrestrial ecosystems for their own ends for millennia – from deforestation and clearing of natural vegetation for cultivation of crops to increasing fertilizer and pesticide use. Of the 50 billion tonnes of carbon currently locked up in terrestrial biomass and vulnerable to release in the next 20 years, 40 billion tonnes is put at risk not by changes in climate but by changes in land use. As pointed out by Reay et al. (2007) climate change may be the greatest threat to this huge carbon stock towards the end of the century. In the shorter term, it is chainsaws and ploughs, not drought and extreme temperatures that we must address.

Human interventions could exploit enhanced plant biomass accumulation in elevated atmospheric CO_2 concentration to reduce the future rate of increase in CO_2 levels, and associated global warming. However, N availability constraints could limit CO_2-induced stimulation of plant growth and biomass accumulation. Reich et al. (2006) have recently indicated that variation in both the availability of soil N and deposition of atmospheric N are likely to influence plant biomass accumulation under elevated atmospheric CO_2. Considering that productivity of both natural and managed vegetations are limited by the availability of N, soil N would be a major constraint on global terrestrial responses to elevated CO_2.

2.5 Nitrogen Fixation Through Soil Microbes

The role of soil microbes in fixation of atmospheric N is the best known and agronomically exploited area of soil microbiology (Sprent and Sprent 1990). Free living bacterial species such as *Azospirullum, Azotobacter* and several photosynthetic cyanobacteria fix atmospheric N. However, the biological nitrogen fixation (BNF) by the leguminous crops and tree species is an important component of the nitrogen cycle in agricultural and natural ecosystems. Legumes form an important component in natural pastureland vegetation. They have been traditionally used as a component of cereal–legume rotations or specific legumes as green manure crops to restore soil fertility. Such farming systems were sustained over a long period though cereal productivity was much lower in comparison to those currently harvested using chemical fertilizers. Ley farming and cultivation of green manure crops has become uneconomical in densely populated regions (Ali 1999). Atmospheric N fixation by leguminous plants is a complex process that has been extensively investigated, yet its understanding remains inadequate to realize the full potential. It involves interactions between the legume plant and the *Rhizobium, Bradyrhizobium, Sinorhizobium* and *Azorhizobium* species of soil bacteria collectively referred as rhizobia.

In response to a variety of substances exuded by the host plant roots, specific soil bacteria are attracted to a particular legume species, leading to the formation of specialized root organ - the nodules. The different steps involved in the establishment of successful symbiotic association are processes controlled by the host and bacterial genes in the following sequence: multiplication and colonization at the root surface (*Roc* **ro**ot **c**olonization), adhesion of bacteria to root hair surface (*Roa* **ro**ot **a**dhesion), curling or branching of root **ha**ir (*Hab* and *Hac* root **ha**ir **b**ranching and **c**urling), formation of **inf**ection thread (*Inf*), induction of meristem in the host roots for the nodule initiation and differentiation, **ba**cteroid **r**elease from the infection thread (*Bar*), **ba**cterial **d**ifferentiation (*Bad*), onset of **ni**trogen **f**ixation (*Nif*), **no**dule function **p**ersistence/maintenance (*Nop*), development of **co**mplimentary **f**unctions associated with N fixation (*Cof*) and its transport (Caetano-Anolles and Gresshoff 1991). Nodulation mutants have been isolated in many of the legume crops (Gresshoff 1993) that broadly classified into four classes (Sagan et al. 1994): nod − (no nodule formation), nod +/− (few nodule formation), fix − (ineffective nodules), nod ++ (super or hyper nodulation) and nts (**n**itrate **t**olerant **s**ymbiosis that fix N even in the presence of high nitrate in soil). In field experiments having native microbes a more descriptive classification – non-nodulating with native root nodulating bacteria (RNB); non-nodulating with a specific strain, low nodulating with native RNB; low nodulating at low N; high nodulating at low N but low nodulating at high N and high nodulating at high N have been used (Wani et al. 1995). How the different steps in the nodulation process are affected after inoculation with specific *Rhizobium* strains, in presence of abundant native populations, is not known except for the fact that often the introduced strains lose in competition to the native strains.

Genetic manipulation of the legume host as well as *Rhizobium* species provide enormous opportunities to enhance BNF, and reduce fertilizer N requirement for the following cereal or other non-legume crops (Bhatia et al. 2001). The available non-nodulation and hyper-nodulation mutants can be used for selection of the best host and bacterial combinations. The characterization of soybean as well as the *Rhizobium* genome would further facilitate development of such combinations. At the same time, there is a need to develop user friendly methods for the delivery of the desired strains to minimize competition from the native rhizobia already present in soil.

The possibilities of making cereals, particularly rice, fix nitrogen like the grain legumes have been explored (Dey and Datta 2002). Cereals lack the large number of genes involved in successful symbiotic partnership with rhizobia. Some homologues of nodulin genes have been identified in rice, formation of nodular structures on rice seedling roots were reported (Al-Mallah et al. 1989). Occurrence of early nodulin genes have been reported in rice (Reddy et al. 1999) and two soybean genes related to nodulation have been transferred into rice (Day et al. 2000). However, rice or other cereals having root nodules with nitrogen fixing microbes are still a long way off if at all feasible.

2.6 Effect of GE Crops on Microbial Diversity

A large number of plants expressing alien genes have been developed using recombinant DNA techniques in the last 20 years, and many of them are under commercial cultivation after mandatory clearances from regulatory agencies. The first generation GE plants were aimed to control pest and diseases (Christou et al. 2006), and now more plants with altered metabolic pathways are being developed. Ever since GE crops were developed in the 1980s, supporters and those opposing cultivation of such crops have made conflicting claims on their biosafety and environmental effects. Many questions have been raised regarding the unintended environmental effects of such plants (Bhatia and Mitra 1998; Conner et al. 2003; Ammann 2005). In the present context, the potential short- and long term effects of GE crops on the rhizosphere are a matter of concern as brought out by Bruinsma et al. 2003. Model systems to monitor the effect on non target soil microorganisms have been developed (Turrini et al. 2005). The regulatory aspects related to the soil systems for GE crops, and for the GE microbial pesticides have been discussed by Sayre and Seidler (2005). The possible risks associated with such plants are evaluated in relation to the overall benefits. The results obtained have been reviewed by Lynch et al. (2004) and Liu et al. (2005). Large scale cultivation of GE crops can alter the soil microbial communities in two ways: release of transgene product into the rhizosphere through root exudates or through phytomass degradation, and transfer and integration of plant DNA into resident microbes through horizontal gene transfer. Both these are also possible from the cultivation of crop cultivars developed conventional plant breeding methods. The need for comparing the environmental perturbations caused by GE crops with conventionally bred crop cultivars grown has been advocated (Conner et al. 2003; Ammann 2005). A major limitation in following such effects is the absence of base line data on microbial diversity and the methods to quantify soil microbial diversity. Maize plants expressing *Bacillus thuringiensis* (Bt) *cry1Ab* gene released the toxin into the rhizosphere through root exudates and litter decay (Saxena et al. 1999; Saxena and Stotzky 2001). It remained bound to soil and active for 234 days – the duration of the experiment.

In vitro experiments using the toxin from three different strains of Bt showed no microbiocidal or microstatic activity against selected bacteria, fungi and algae (Koskella 2002). In another study the two maize lines expressing Bt genes, the root exudates of Bt 176 significantly reduced pre-symbiotic hyphal growth of arbuscular mycorrhizal fungi compared to Bt 11 and non-transgenic corn lines. However, no differences were found between control and defensin expressing lines. Bt toxin as well as defensin did not affect the fungal-host recognition mechanism. Bt 176 affected appressoria development; 36% of them failed to produce infection pegs (Turrini et al. 2005). Higher numbers of fungi were recovered from the roots of potato lines expressing a synthetic anti-microbial peptide magainin II, in comparison to the control plants (Callaghan et al. 2005). Rhizosphere bacterial isolates showed different susceptibility to magainin analogues in in vitro experiments. Effect of transgenic potato line, modified for its starch composition characteristics by RNA antisense, on soil and rhizosphere bacterial and fungal diversity were investigated using molecular techniques based on bacterial and fungal rDNA (Milling et al. 2005). No significant differences between the transgenic, unmodified parental line and another cultivar on microbial community structure were observed. When *Pseudomonas* specific primers were used differences in the rhizosphere patterns of transgenic and the parental cultivar were observed. However, similar differences in *Pseudomonas* community were observed in comparisons between two standard, non transgenic, cultivars.

In one of the most comprehensive studies Heuer et al. (2002) investigated the effects of T4-lysozyme release from transgenic potato roots which enhances bactericidal activity against *Bacillus subtilis*. Genetically engineered T4-lysozyme producing, control without T-4 lysozyme gene, and the parental lines were compared at two different field sites for three years. Soil bacterial communities were analyzed using three different complimentary techniques – fatty acid analysis of cultured organisms, Biolog GN microplates, and 16S rRNA gene fragments using DDGE or by cloning and sequencing. No significant effects of the T4-lysozyme expression on rhizosphere communities, over the other environmental variables were observed.

Horizontal transfer of genes from GE crops to the soil bacteria has been reviewed by Lynch et al. (2004) and Mercier et al. (2006). Persistence of plant DNA in soil for a long period has been shown. Soil colloids adsorb biological molecules retarding their microbial degradation. The other variable is the presence of bacteria in competent state to take up the exogenous DNA in the soil. Lastly, the frequency of such events will determine the gene transfer into soil bacteria. The significance of such events should be considered in view of the normal high gene exchange among the different bacteria (Chandler 2006).

Effect of Bt-corn on soil macroflora has been investigated. Finely ground *Bt*-corn leaves, expressing Cry1Ab protein, when added to soil had no deleterious effects on survival, growth, development and reproduction of earthworm population (Vercesi et al. 2006). Juvenile earthworms in pots with *Bt*-corn plants had no effect. A slight negative effect was observed on cocoon hatchability. It is apparent that the GE crops approved for commercial cultivation cause minor changes in microbial community structure and function. However, it is widely accepted now that all transgenic events need to be evaluated on case by case basis for different biosafety concerns before approval for commercial cultivation.

Cultivation of herbicide glyphosate resistant (GR) wheat and canola rotations had little effect on soil microorganisms (Lupwayi et al. 2007). Soil microbial biomass, bacterial functional diversity, community structure and dehydrogenase enzyme activity were monitored in the rhizosphere and bulk soil samples at six sites in Canada. These included fields that followed direct seeding or conventional tillage and different wheat – canola rotations. Out of 22–40 plots significant differences were observed in only in 2–3 plots; however, the observed differences were not consistent. The authors conclude that "Overall, GR crop frequency effects on soil microorganisms were minor and inconsistent over a wide range of growing conditions and crop management."

The above discussion brings out that some genetically engineered crops may affect soil ecosystems, but the long-term significance of any of these changes was not clear. Alterations of soil ecosystems could decrease plant decomposition rates and hence soil C and N levels and soil fertility. Similarly, declining species diversity of soil microorganisms, in some cases, can cause lower community diversity and productivity above ground (van der Heijden et al. 1998; Wolfenbarger and Phifer 2000).

2.6.1 GE Crops Exuding Specific Molecules into Rhizosphere and Possibilities of Enhancing Microbial Cooperation

Plant roots are known to secrete as much as 20% of their stored assimilates into the rhizosphere (Whipps 1990; Uren 2001; Walker et al. 2003). These exudates contain many different compounds including sugars, amino acids, proteins and signal peptides (Uren 2001), and are reported to change the rhizosphere biology and increase the availability of micronutrients (Mraschner and Römheld 2001). Root exudates increase the growth of soil bacteria and their predators enhancing SOM degradation and N mineralization. Model for the same has been developed by Raynaud et al. (2006). Genetic engineering provides unique opportunities for introducing the desired recombinant proteins into the rhizosphere by growing such modified crops. This opens up possibilities for enhancing microbial cooperation in the rhizosphere (Barea et al. 2005) or what Brussard et al. (2007) has referred as "planned microbial diversity".

Tesfaye et al. (2005) reported transfer of fungal endochitinase gene from *Trichoderma harzianum* Riafi into alfalfa (*Medicago sativa* L.) plants. Root exudates of these transgenic plants showed the presence of endochitinase with antifungal activity as demonstrated by inhibition of spore germination of two fungal pathogens. These experiments have shown the potential for introducing the desired proteins into the rhizosphere that could be exploited as a biocontrol method for protecting the plants from soil borne pathogens or for accelerating bio-remediation processes. The recombinant proteins/enzymes can also be used to influence growth promoting microbes, and microbial symbiosis (Austin et al. 1995; Tesfaye et al. 2005).

Transgenic *Arabidopsis thaliana* plants expressing phytase gene from *Aspergillus niger* were first shown to release extra cellular phytase and utilize P supplied as phytate in the medium (Richardson et al. 2001). Later using a root hair specific promoter increased acquisition of P from phytate in the medium in *Arabidopsis* (Mudge et al. 2003) and potato (Zimmerman et al. 2003). Expression of the fungal phytase gene in *Nicotiana tabaccum* improved P nutrition in amended soils (George et al. 2005a). Limitations to the potential of transgenic plants that exude phytase in different soil types were brought out by George et al. (2005b). *Trifolium subterraneum* L. plants constitutively expressing a chimerical phytase gene, and showing a 77-fold increase in exuded phytase activity, were grown in a range of soils differing in organic P content.

Transgenic plants that exuded phytase showed better growth and P nutrition only in a soil containing large concentration of organic P amenable to hydrolysis by plant derived phytase, and also total organic P. In the transgenic line the root growth was shorter than that of the control plants and the longer root system of the latter may have given greater access to soil P. The natural microbial diversity in the soil had no significant influence on P availability. The authors point out the inherent limitations of single trait alterations and infer that “such approaches can be successful under certain edaphic conditions”. With better understanding of the processes involved it should be possible to develop plants with improved P nutrition by incorporating more than one gene. Transfer of nine genes into *Brassica juncea* for metabolic engineering for production of long chain fatty acids have been reported (Wu et al. 2005).

Spaepen et al. (2007) have recently reviewed the role of bacterial IAA in different microorganism–plant interaction and highlight the fact that bacteria use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and circumvention of basal plant defense mechanisms.

2.7 Genetically Engineered Bio-control Agents

As a result of increased concern over the use of chemical pesticides in agriculture, the biological control of pests and disease-causing organisms using antagonistic micro-organisms remains a viable option for sustainable agriculture. The interactions within microbial populations and between microbes and higher organisms are the basis for biocontrol. Use of antagonistic microorganisms is thus emerging as an environment friendly means to control pest and disease causing organisms in integrated pest management (IPM) programs. New and improved strains produced by genetic manipulations provide opportunities for developing more effective biocontrol organisms. Information on the effects of released wild-type or GE organisms on resident communities is important to assess the potential risks associated with the introduction of such organisms into agro ecosystems. Rhizocompetent *Pseudomonas* species have been used for suppression of crop diseases (Nautiyal et al. 2002). They have also been identified as ideal candidate for strain improvement using the rDNA techniques. In view of the biosafety considerations, there is considerable interest in the impact of released GE bio-control agents (GE-BCA) on non target species and microbial diversity (Glandorf et al. 2001; Moenne-Loccoz et al. 2001). Impact of field releases of GE *Pseudomonas fluorescence* on indigenous microbial populations of wheat (De Leij et al. 1995, Glandorf et al. 2001) and sugar beet (Thompson et al. 1995; Moenne-Loccoz et al. 2001) has been reported. Timms-Wilson et al. 2004 improved a *Pseudomonas fluorescens* strain SBW25 by chromosomal insertion of constitutively expressed phzABCDEFG genes for the biosynthesis of antifungal compound phenazine-1-carboxylic acid (PCA). The most effective GE strain 23.10 was used on pea, wheat and sugar beet grown on soil infected with *Phythium ultimum* that causes damping-off in several crops. Colony isolation and different molecular methods were used to assess the impact on microbial diversity. Root-mycorrhiza associations were followed using microscopic examination. The results indicate that plant type, age and disease have a greater effect on the abundance, diversity and succession patterns of rhizosphere bacterial and fungal communities than the inocula of GE-BCA. Further, the presence of inocula reduced the impact of disease on the microbial diversity and function. A transient, small decrease in mycorrhizal association was observed four days after inoculation. The authors infer that no major groups were excluded or enriched as a result of GE-BCA.

Pseudomonas fluorescens CHA0-Rif and its derivative CHA0 Rif/pME3424, which has improved biocontrol activity and enhanced production of the antibiotics 2,4-diacetylphloroglucinol (Phl) and pyoluteorin (Plt), were introduced into soil microcosms and the culturable bacterial community developing on cucumber roots was investigated (Natsch et al. 1998). The introduction of either of the two strains led to a transiently enhanced metabolic activity of the bacterial community on glucose dimers and polymers as measured with BIOLOG GN plates. The introduced strains did not significantly affect the abundance of dominant groups of culturable bacteria discriminated by restriction analysis of amplified 16S rDNA of 2500 individual isolates. About 30–50% of the resident bacteria were very sensitive to Phl and Plt, but neither the wild-type nor CHA0-Rif/pME3424 changed the proportion of sensitive and resistant bacteria in situ. In microcosms with a synthetic bacterial community, both biocontrol strains reduced the population of a strain of *Pseudomonas* but did not affect the abundance of four other bacterial strains including two highly antibiotic-sensitive isolates. The authors concluded that detectable perturbations in the metabolic activity of the resident bacterial community caused by the biocontrol strain CHA0-Rif are (i) transient, (ii) similar for the genetically improved derivative CHA0 Rif/pME3424 and (iii) less pronounced than changes in the community structure during plant growth. From a biological safety assessment point of view, the data therefore suggested that a genetically improved biocontrol strain may have specific interactions with fungal pathogens rather than general effects on bacterial communities. However, the possibility that specific interactions could occur between introduced strains and non-culturable resident bacteria can not be ruled out.

The antibiotic 2,4-diacetylphloroglucinol (Phl) is produced by a range of naturally occurring fluorescent pseudomonads. One isolate, *P. fluorescens* F113, protects pea plants from the pathogenic fungus *Pythium ultimum* by reducing the number of pathogenic lesions on plant roots, but with a concurrent reduction in the emergence of pea plants. The genes from F113 were isolated and a 6.7-kb gene cluster was inserted into the chromosome of the non-Phl-producing *P. fluorescens* strain SBW25 *EeZY6KX* (Bainton et al. 2004). Pea roots inoculated with SBW25 *EeZY6KX* have significantly lower indigenous populations than with F113 and the control. The authors achieved the integration of the Phl antibiotic and competitive exclusion mechanisms into a single strain Pa21. Impact of Pa21 on survival and plant emergence was investigated following inoculation of pea seedlings where it provided protection against *P. ultimum* but did not cause lower seed emergence. Thus, strain Pa21 possesses the necessary qualities to provide effective integrated biocontrol, through maintaining its wt trait of competitive exclusion on the plant roots and expressing the antibiotic genes. Such modified candidate strains may be of some agronomic benefit.

2.8 Conclusions and Outlook for the Future

The recent reports on the soil microbial diversity using the molecular techniques show that the microbial communities are highly diverse, and change with minor perturbations. This may turn out to be a universal phenomenon as more information is gathered from different soils and climatic conditions. As knowledge of the so-called “black box” of the soil microbial diversity increases, an enormous source of knowledge of species and genes would be available. These can be utilized with the application of conventional and new molecular techniques to evolve more productive and sustainable cropping patterns.

There would be increased demands for food, feed, fiber, timber and bio-fuels. The choice of the crops grown for bio-fuels would vary in different regions depending upon the climatic conditions. The legume oil yielding plants are the likely crops of choice for bio-fuels in many areas as they meet their N requirement through fixation, in comparison to sugarcane and sweet sorghum that require application of N fertilizers for increased productivity.

Tremendous increase in the productivity of crops has been brought about with the application of contemporary genetic knowledge and tools since 1900, when Mendel’s Laws of Inheritance were rediscovered. All the improvements made so far have aimed at the alterations in the above ground parts of the plants. The genetic diversity in the “hidden part” below ground, in the root characteristics, the soil microbes in the rhizosphere, and their positive interactions have hardly been exploited. The increasing amount of genomic data will further broaden insight into the role of specific key molecules in the rhizosphere to enhance cooperation among the interacting organisms for improved productivity. These along with the tools and techniques of no till or limited tillage, precision farming using slow release forms of agro-chemicals, built in disease and pest resistance, beneficial microbial inoculants for growth promotion or the control of soil borne pests and pathogens should play a significant role in future. With the genetic tools now available it may be relatively easy to introduce the desired traits in the soil microbes of choice, but to make them successfully compete with the native strains already present, and environmental safety of such modified organisms would be a bigger challenge.

Discussions of the environmental risks and benefits of adopting GE organisms are highly polarized between pro- and anti-biotechnology groups. The current state of available knowledge is frequently overlooked in this debate. A review of existing scientific literature reveals that key experiments on the long term environmental risks are lacking. The complexity of ecological systems presents considerable challenges for experiments to assess the long term risks. The existing studies emphasize that these can vary depending upon the trait and organism modified.

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