Soil Microbes: The Invisible Managers of Soil Fertility

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Abstract

Soil health is represented by its continuous capacity to function as a vital living system. Since soil health is the major driving factor for sustainable agriculture, it has to be preserved. Microorganisms are an essential and integral part of living soil influencing various biogeochemical cycles on major nutrients such as carbon, nitrogen, sulphur, phosphorous and other minerals and play superior role in maintaining soil health than other biological component of soil. They also have the capacity to suppress soil borne pathogens and indirectly help in agricultural productivity. Besides contribution of specific microbes to soil health by participating on nutrient cycles, certain other microbes directly/indirectly promote plant growth through the production of phytohormones, enzymes and by suppressing phytopathogens and insects. The vast functional and genetic diversity of microbial groups including bacteria, fungi and actinomycetes supports in all the above ways for soil health. This book chapter gives an outline of such microbes and their contribution in promoting soil health and its role as soil health indicators.

Keywords

Soil health • Microorganisms • N fixation • Nutrient cycling • Climate change

1.1 Introduction

Soil, a finite and non-renewable resource, supports numerous terrestrial life forms through its critical functions. Soil health is defined as 'the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity,

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promote the quality of air and water environments and maintain plant, animal and human health' (Doran and Safley 1997). In the context of sustainable agriculture, soil health is meant for crop productivity and protection via the functions such as N_2 fixation and phosphorus (P) solubilization, homeostasis of biogeochemical cycles, maintenance of soil structure, detoxification of pollutants and suppression of plant pathogens. In the absence/inefficiency of these functions, the soil is regarded as an inanimate entity with minerals and chemicals. In addition, soil regeneration through chemical and biological process/weathering of underlying rock requires geological time (Huber et al. 2001; Buscot and Varma 2005). So, maintenance of soil health is crucial for sustainable productivity.

The following 14 nutrients are vital for a proper plant growth and development – macronutrients, which are further divided into (1) structural nutrients: C, H, O; (2) primary nutrients: N, P, K and (3) secondary nutrients: S, Ca, Mg; and micronutrients: Fe, B, Cu, Cl, Mn, Mo, Zn, Ni. Besides the structural nutrients (which are obtained from air and water), the remaining 11 nutrients are obtained through soil and absorb only in some specific/available forms as follows: N – NH_4^+ (ammonium) and NO_3^- (nitrate); P – $H_2PO_4^-$ and HPO_4^{-2} (orthophosphate); K – K^+; S – SO_4^{-2} (sulphate); Ca – Ca^{+2}; Mg – Mg^{+2}; Fe – Fe^{+2} (ferrous) and Fe^{+3} (ferric); Zn – Zn^{+2}, Mn – Mn^{+2}; Mo – MoO_4^{-2} (molybdate); Cu – Cu^{+2}, Cl – Cl^-; B – H_3BO_3 (boric acid) and $H_2BO_3^-$ (borate). Though many of the soil flora and fauna are responsible for bringing these nutrients, microorganisms are the drivers behind various biogeochemical cycles and making the organic and inorganic nutrients in their available form to the plants (Lucas and Davis 1961; Mengel and Kirkby 2001).

Microbes are the largest population that exists in soil with a high diversity index. However, the microbial groups vary in their number *vs.* biomass. The number (number/g soil) and biomass (g/m^2) of various microbial groups are, bacteria: 10^8—10^9 *vs.* 40—500; actinomycetes: 10^7—10^8 *vs.* 40—500; fungi: 10^5—10^6 *vs.* 100—1,500; algae: 10^4—10^5 *vs.* 1—50; protozoa: 10^3—10^4 *vs.* varies (Hoorman and Islam 2010). Typical soil samples have about thousands of individual taxa (also known as operational taxonomic units, OTUs) of bacteria, archaea and fungi. It is understood from some estimates that there can be >106 individual species-level OTUs in a single soil sample (Fierer et al. 2007). During the analysis on genome size of microbial community among soil samples by re-association of community DNA, it is known that, the microbial community genome size equals the size of 6,000—10,000 *Escherichia coli* genomes in unperturbed organic soils, and 350—1,500 genomes in arable or heavy metal polluted soils. Still, the rare and unrecovered microorganisms may not be included in the analysis. In contrast, the genomic complexity recovered by culturing methods was less than 40 genomes. This complexity in microbial community genome size denotes the diversity in terms of genetic information present in the soil and also the overall functional variability (Torsvik et al. 1998; Øvreås 2000).

Among the microbial groups, fungus have higher tolerance and surviving capacity against fluctuating soil disturbances, untilled or no-till soils than bacteria and actinomycetes; though the latter groups also have the tolerance (Hoorman and Islam 2010; Meliani et al. 2012). Besides the smaller voluminous nature, soil microbes are the key drivers of biogeochemical cycles on major nutrients such as C, N, S, P and other mineral cycles (Bloem et al. 1997). They also suppress the soil pathogens via various antibiosis compounds and helps in plant disease protection (Haas and Défago 2005). This book chapter deals with role of microbes in improving soil fertility and also the available techniques for indicating soil microbial activity.

1.2 Carbon Cycle

Carbon (C) in the atmosphere is transferred to soil by photosynthetic plants and photo/chemo autotropic microorganisms for the synthesis of organic materials. Hence, the largest carbon pool on the earth's surface (2,157—2,293 Pg) is/becomes soil. The reverse process, i.e.,

decomposition of organic material built in plants and microbes was carried out by organic C utilizing heterotropic microorganisms as a substrate for their metabolism and energy source. The remaining C is liberated as metabolites or CO_2 to the atmosphere (Prentice et al. 2001). The decomposition product termed as soil organic carbon (SOC) is the largest pool within the terrestrial C cycle with an annual turnover of about 60 Gt (Schlesinger 1997). During the SOC formation, the organic materials were either mineralized to CO_2 or humified. Since the SOC affects plant growth by serving as energy source and by influencing nutrient availability through mineralization, it is one of the most important constituents of the soil.

It is understood that microbes transfer the C primarily for their survival. Under oxic conditions, i.e., in surface of soil and oxic layers of wetland systems, aerobic methane-oxidizing bacteria play the role (Chistoserdova et al. 2005; Gupta et al. 2013), whereas under waterlogged anoxic soils, CO_2 is reduced by hydrogenotropic archaea and methoanogenic bacteria (Lu and Conrad 2005; Trumbore 2006). Typically microbial C accounts for a minimum of 100─1,000 μg g^{-1} in arable soils and a maximum of 500─10,000 μg g^{-1} in forest soils with the intermittent values in other ecosystems such as grasslands and semi-arid regions (Kandeler et al. 2005). Besides the considerable variations, microbial biomass C generally accounts for about 0.9─6 % of total organic C with an indirect relationship for increasing soil depth.

Formation of soil organic matter (SOM), a major fraction containing SOC is aided by the decomposition process through various lytic enzymes including, amylase, glucosidase, proteases, cellulase, chitinase and phenol oxidase. These enzymes convert the complex macromolecules into low molecular weight compound for the ready assimilation of microbial components or for their transformation into CO_2 for energy (Burns and Dick 2002). Though the enzymes were released from plants/animals/microorganisms, the latter are major contributors (Tabatabai 1994). Among the microbial groups, fungi are reported to have higher enzyme activity than bacteria (Baldrian et al. 2010). Role of these lytic enzymes in maintaining soil health is previously reviewed by Das and Varma (2011) and hence a brief note on some essential enzymes is described here.

Amylase: Starch hydrolyzing enzyme breaks the complex polysaccharides and releases low molecular weight simple sugars which acts as an energy source for microbes (Rahmansyah and Sudiana 2010) and it is confirmed by the positive correlation between as enzyme activity and SOM (Kujur et al. 2012).

Cellulase: Cellulose in plant debris is degraded by a group of enzymes called cellulases into glucose, cellobiose and high molecular weight oligosaccharides. Soil fungus is the major contributors of this enzyme activity. Report of Arinze and Yubedee (2000) supports this by documenting negative correlation between increasing fungicide concentration in agricultural soils and cellulase activity. Previous studies by Vincent and Sisler (1968) and Atlas et al. (1978) also documented the same effects.

Chitinase: Chitin is a major component of fungal cell wall, exoskeleton of insects and many arthropods. As already quoted, the higher fungal biomass present in soils will be degraded by the chitinases after the cell death with the release of simple organic molecules. Besides contributing for nutrient cycling, it serves majorly for the control of soil borne fungal phytopathogens such as *Sclerotium rolfsii* and *Rhizoctonia solani*. This indirectly helps in increasing plant growth and yield (El-Tarabily et al. 2000; Sindhu and Dadarwal 2001).

Oxidase: In contrast to the hydrolytic enzymes, oxidases were produced for a variety of functions including ontogeny, defence and the acquisition of C and N by microorganisms (Sinsabaugh 2010). Representative of these enzymes include fungal laccases and prokaryotic laccase-like enzymes (Baldrian 2006; Hoegger et al. 2006).

Dehydrogenase: It is related during microbial respiration, where it oxidizes soil organic matter by transferring protons and electrons from substrates to acceptors and the activity

depends on soil type and soil air–water conditions (Wolińska and Stępniewska 2012; Kumar et al. 2013).

Sequential changes in climatic conditions and related ecosystem factors in the current situation affect all of the nutrient cycles. Hence, the research trend has been directed towards (1) effect on climate change including seasonal variations, elevated CO_2 and long-term climate change disturbances (Durán et al. 2014; Haugwitz et al. 2014); (2) effect of fertilizers (Strauss et al. 2014), soil amendments (Anderson et al. 2011) on long-term (Tyree et al. 2006) and short-term scales (Tyree et al. 2009) and (3) effect of SOM (Schmidt et al. 2011) etc. It is understood that, though the importance of soil microorganisms for global C cycling is well known; only few research attempts have been made to evaluate the chemical and microbiological views of C cycling (Kandeler et al. 2005).

1.3 Nitrogen Cycle

Nitrogen (N), an essential element for the synthesis of amino acids and nucleotides is required by all forms of life in large quantities. It is also involved in several respiratory energy metabolisms in which N compounds may serve as either oxidant or reductant. Atmosphere is the largest reservoir of N (78 %) in the form of triple bonded N_2 gas, though it is not freely available to most living organisms. It is accessible only by N_2 fixing bacteria and archaea which pave the way for other organisms to use the fixed N for its incorporation into their biomass. This fixed N constitutes less than 0.1 % of the N_2 pool and is able to limit the primary production in both terrestrial and marine ecosystems. Within the organisms, N exist in most reduced forms and during the cell lysis it is nitrified to nitrate which in turn denitrified to N_2 gas. So, a balanced N cycle requires the dual action of assimilatory (N fixation and incorporation into biomass) and dissimilatory (recycling of fixed nitrogen to N_2) transformations (Vitousek and Howarth 1991; Canfield et al. 2010).

The first step in N cycle, assimilation, i.e., N fixation (also known as biological nitrogen fixation, BNF) is aided by a group of bacteria called diazotrophs including cyanobacteria, green sulphur bacteria, Azotobacteraceae, rhizobia and *Frankia* at various ecosystems in which the former three occurs by/through non-symbiotic process and the latter two through symbiotic process. BNF occurs through a cascade of reactions involving complex enzymes systems and accounts for about 65 % of N currently used in agriculture (Thamdrup 2012; Peoples et al. 1995). Major quantity of N fixed under the control of legume–rhizobia is harvested as grains. The left out N in the soil, roots and shoot residues supports the succeeding crops for N supply. Hence legume–rhizobial symbiosis substantially reduces the N requirement from external sources (Bhattacharyya and Jha 2012). Crops like wheat, rice, sugarcane and woody species also have the capacity to fix atmospheric N using free living or associative diazotrophs. However, the contribution of legume–rhizobia symbiosis (13–360 kg N ha^{-1}) is far greater than the non-symbiotic systems (10–160 kg N ha^{-1}) (Bohlool et al. 1992). Review of Herridge et al. (2008) on global N_2 fixation estimated from FAO databases and other experimental reports also indicates the higher contribution of legume–rhizobia than other systems in N fixation (Table 1.1). However, N fixation efficiency of legumes depends on the host genotype, rhizobial efficiency, soil conditions, and climatic factors (Belnap 2003). Difference in N fixation efficiency of various legumes is shown in Table 1.2.

BNF is an energy demanding process through which atmospheric N is converted to plant usable organic N and plays an important role in the N cycle. This can be understood by the complexity of the enzyme nitrogenase, a major enzyme involved in the nitrogen fixation, which has two components – dinitrogenase reductase, the iron protein and dinitrogenase (metal cofactor). The iron protein provides the electrons with a high reducing power to dinitrogenase which in turn reduces N_2 to NH_3. Based on the availability of metal cofactor, three types of N fixing systems viz. Mo-nitrogenase, V-nitrogenase and

Table 1.1 Comparison of symbiotic and non-symbiotic N fixation in agricultural systems

Agent	Agricultural system	Area (Mha)	Crop N fixed (Tg/year)
Legume–rhizobia	Crop (pulse and oilseed) legumes	186	21
	Pasture and fodder legumes	110	12–25
Azolla-cyanobacteria	Rice	150	5
Endophytic, associative & free-living bacteria	Sugar cane	20	0.5
	Crop lands other than used for legumes and rice	800	<4
	Extensive, tropical savannas primarily used for grazing	1390	<14

Source: Herridge et al. (2008)

Table 1.2 Comparison data for N fixation efficiency of various legumes

Legume	Shoot N (Tg)a	Crop N (Tg)b	%Ndfa	Crop N fixed (Tg)c
Common bean	1.03	1.45	40	0.58
Cowpea	0.27	0.37	63	0.23
Chickpea	0.48	0.96	63	0.60
Pea	0.65	0.90	63	0.57
Lentil	0.24	0.33	63	0.21
Faba bean	0.27	0.38	75	0.29
Groundnut	2.16	3.03	68	2.06
Soybean	16.11	24.17	68	16.44

Source: Herridge et al. (2008). %Ndfa – Percentage of plant N derived from N_2 fixation
[a]Using %N shoots of 3.0 % for soybean, 2.3 % for groundnut, 2.2 % for fababean and 2.0 % for the remainder
[b]Multiplying shoot N by 2.0 (chickpea), 1.5 (soybean) and 1.4 (remainder) to account for below-ground N
[c]Crop N × %Ndfa

Fe-nitrogenase were documented. Complexity of nitrogen fixation can be further understood by participation of multiple gene clusters as follows: (1) nodulation (including nodA-acyltransferase, *nodB*-chitooligosaccharide deacetylase, *nodC*-N-acetylglucosaminyltransferase, *nodD*-transcriptional regulator of common nod genes, *nodPQ*, *nodX*, *nofEF*, *nodIJ*-Nod factors transport, *NOE*-synthesis of Nod factors substituents, *nol* genes-several functions in synthesis of Nod factors substituents and secretion); (2) nitrogen fixation (including *nifA, nifHDK*-nitrogenase, *fixLJ, nifBEN*-biosynthesis of the Fe-Mo cofactor, *fixK*-transcriptional regulator, *fixABCX*-electron transport chain to nitrogenase, *fixGHIS*-copper uptake and metabolism, fdxN-ferredoxin and *fix-NOPQ*-cytochrome oxidase) and (3) other essential elements (including *hup*-hydrogen uptake, *exo*-exopolyssacharide production, *gln*-glutamine synthase, *nfe*-nodulation efficiency and competitiveness, *dct*-dicarboxylate transport, *ndv-β*-1,2 glucan synthesis, *pls*-lipopolysaccharide production) (Laranjo et al. 2014).

It is a well-known fact that rhizobia belong to the families Rhizobiaceae (excluding the *Frankia* sp.), Bradirhizobiaceae and Phyllobacteriaceae. Rhizobia have a unique association with root nodules of leguminous plants and induce plant growth in many ways. They also have capacity to induce plant growth of non-leguminous plants (Mehboob et al. 2012). The number of species reported in Rhizobiaceae family increased considerably from 8 in 1980 to 53 in 2006. This drastic increase was mainly due to dispersion of leguminous plants to new geographical locations. The other possible reasons could be: (1) only 57 % of 650 genera of leguminous plants have been studied for nodulation and nitrogen fixation, and (2) recent advancements in the taxonomic research with the aid of specific molecular tools (Willems 2006). Besides its role in efficient N fixation, they have multiple plant growth promoting traits such as mineral enhancing capacity, phytohormone production and alleviating biotic and abiotic stress (Gopalakrishnan et al. 2014a). All these help in developing formulation of rhizobial inoculants to achieve substantial increases in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels for succeeding crops (GRDC 2013). It is already reported that, inoculation of soybean with rhizobial inoculants showed substantial increases

in nodulation, grain and biomass yield and N fixation (Thuita et al. 2012).

Besides the rhizobia, the associative and free-living nitrogen fixing bacteria were also formulated and commercialized as biofertilizers. The genus *Azospirillum*, an associative N fixing bacteria comprises nearly 15 species: *A. lipoferum, A. brasilense, A. amazonense, A. halopraeferans, A. irakense, A. largimobile, A. dobereinerae, A. oryzae, A. melinis, A. canadense, A. zeae, A. rugosum, A. palatum, A. picis* and *A. thiophilum*. Reis et al. (2011) also reported for its multiple plant growth promoting traits. The next important genus is *Azotobacter*, a free-living nitrogen fixer which comprises of seven species: *A. chroococcum, A. vinelandii, A. beijerinckii, A. paspali, A. armeniacus, A. nigricans* and *A. salinestri* (Jiménez et al. 2011). Besides the N fixing capacity, this genus has the history of more than 35 years in promoting plant growth through multiple phytohormone production, enzymes, enhanced membrane activity, proliferation of the root system, enhanced water and mineral uptake, mobilization of minerals, mitigation of environmental stress factors, and direct and indirect biocontrol against numerous phytopathogens (Bashan and de-Bashan 2010).

The N fixed in the form of ammonium during assimilation process, is further dissimilated by two-step microbial process, i.e., nitrification (the aerobic oxidation of ammonium to nitrite and then to nitrate) and denitrification (the respiratory anaerobic reduction of nitrate via nitrite, nitric oxide, and nitrous oxide to N_2, coupled with the oxidation of organic matter, hydrogen, or reduced iron or sulphur species) (Simon 2002). Nitrification is further carried out by two sets of microbial groups: (1) ammonia oxidizers (nitrosifyers) which convert ammonia to nitrite by the activity of ammonia monooxygenase, e.g. *Nitrosomonas, Nitrosospira* and *Nitrosococcus*; and (2) nitrite oxidizers (the true nitrying bacteria) which convert nitrite to nitrate by the activity of nitrite oxidoreductase, e.g. *Nitrobacter* and *Nitrococcus* (Vaccari et al. 2006).

Though the physiology of nitrogen fixation process is reasonably well characterized, still research studies on the phylogenetic diversity of rhizobial species in the context of common core symbiotic genes (Masson-Boivinemail et al. 2009) and invasive mechanisms behind the symbiotic process (Kiers et al. 2003) are going on. However, the understanding of ecological controls on N fixation is sparse (Vitousek et al. 2002) and it is essential for developing a commercial microbial inoculants. Current research trend is looking over the effect of various environmental factors that limit N fixation, such as soil moisture deficiency, osmotic stress, extremes of temperature, soil salinity, soil acidity, alkalinity, nutrient deficiency, overdoses of fertilizers, pesticides and contaminants (Vance 2001; Galloway et al. 2004; Mohammadi et al. 2012; Peoples et al. 2012).

1.4 Sulphur Cycle

The sulphur (S) present in soil (>95 % of total S) is in the bound form with organic molecules, and it is not directly available to the plants, i.e., inorganic S which constitutes about only 5 %. This minimal part of available S in agricultural soils leads to S deficiency symptoms in plants (Schnug and Haneklaus 1998). Besides the contribution of plant and animal-derived organic S, *in situ* synthesis is also observed, which is mainly mediated by microbial process via immobilization of inorganic S to organic S, interconversion of various organic S forms, mineralization of inorganic sulphur in order to support plant growth. Rhizospheric microbes are the major players in allowing plants to access soil organosulphur (Kertesz 1999). Besides the mineralization and immobilization, oxidation and reduction reactions also influence S cycling. Oxidation of elemental S and inorganic S compounds to sulphate is carried out by chemoautotrophic (*Thiobacillus* sp., *T. ferrooxidans* and *T. thiooxidans*) and photosynthetic (Green and purple bacteria, *Chlorobium* and *Chromatium*.) bacteria. Besides this, heterotrophic bacteria such as *Bacillus, Pseudomonas*, and *Arthrobacter*, fungi such as *Aspergillus* and *Penicillium* and some actinomycetes are also reported to oxidize sulphur compounds. The process of sulphate/sulphuric acid formation has the following advantages: (i) it is the anion of strong mineral acid (H_2SO_4)

which can render alkali soils fit for cultivation by correcting soil pH; and (ii) solubilize inorganic salts containing plant nutrients and thereby increase the level of soluble P, K, Ca, Mg, etc. for plant nutrition. Dissimilatory sulphate reduction also occurs in order to balance the contents, where sulphate-reducing bacteria such as *Desulfovibrio, Desulfatomaculum* and *Desulfomonas* play the key roles through the enzyme activity of desulfurases/bisulphate reductase. Among them, *Desulfovibrio desulfuricans* can reduce sulphates at rapid rate in waterlogged/flooded soils, while *Desulfatomaculum* – a thermophilic obligate anaerobes – can reduce sulphates in dry land soils (Tang et al. 2009). Though many studies have been conducted to evaluate the role of microbes in S cycle, now the research focus has been moved in to deal with enzymes, organisms, pathways, comparative approaches, symbiosis, and environments factors related to the S nutrition (Klotz et al. 2011).

1.5 Phosphorous Cycle

Phosphorous (P) is a key component of nucleic acids, energy molecule ATP and membrane component phospholipids. P accounts for about 0.2—0.8 % of the plant dry weight, but only 0.1 % of this P is available for plants from soil (Zhou et al. 1992). The P content of agricultural soil solutions are typically in the range of 0.01—3.0 mg P L^{-1} representing a small portion of plant requirements. The remaining must be obtained through intervention of biotic and abiotic processes where the phosphate solubilizing activity of the microbes has a role to play (Sharma et al. 2013). Soil microbes help in P release to the plants that absorb only the soluble P like monobasic ($H_2PO_4^-$) and dibasic ($H_2PO_4^{2-}$) forms (Bhattacharyya and Jha 2012). Many bacteria (*Pseudomonas* and *Bacillus*) (Rodriguez and Fraga 1999), fungi (*Aspergillus, Penicillium* and *Trichoderma*) (Mittal et al. 2008) and actinomycetes (*Streptomyces* and *Nocardia)* (Tallapragada and Seshachala 2012) are noticed for P solubilizing capacity and enhancement of plant growth. This is aided by the synthesis of protons and organic acids, the significant contributors for solubilization of metal compounds though the excretion of other metabolites, siderophore also contribute to the solubilization process (Sayer et al. 1995). Low molecular organic acid – 2-ketogluconic acid – with a P-solubilizing ability has been identified in *R. leguminosarum* (Halder et al. 1990) and *R. meliloti* (Halder and Chakrabarty 1993). Mineralization of organic P takes place by several enzymes of microbial origin, such as acid phosphatases (Abd-Alla 1994), phosphohydrolases (Gügi et al. 1991), phytase (Glick 2012), phosphonoacetate hydrolase (McGrath et al. 1998), D-α-glycerophosphatase (Skrary and Cameron 1998) and C-P lyase (Ohtake et al. 1996). Other mineral elements also turn into their available form by any of the above mechanism.

1.6 Suppression of Soil Borne and Other Phytopathogens

Soil health is not only based on abundance and diversity of total soil microbes but also on high population of beneficial organisms. Incidence and severity of root diseases is an indirect assessment of soil health (Abawi and Widmer 2000). Certain rhizospheric microorganisms are known to have antagonistic activities against soil borne and other phytopathogens. This may be achieved by lytic enzymes cellulase, chitinase, protease and β-1, 3-glucanase which either induces direct suppression of plant pathogens or indirectly by enhancing the host plant resistance. Some oligosaccharides derived from fungal cell wall breakdown contribute to indirect mechanism (Pliego et al. 2011; Kilic-Ekici and Yuen 2003). Role of the genus *Pseudomonas* in disease suppression is reviewed by Haas and Défago (2005) in the context of antifungal antibiotic production, induction of systemic resistance in the host plant or interference on fungal pathogenicity factors. Mycorrhizal associations are one among them which are found in all ecological situations including normal cropping systems and in natural ecosystems. Among them arbuscular mycorrhizas (AM) are the most common (Harley and Smith 1983; Gianinazzi and Schüepp 1994), but

the excellency depends on its pre-establishment and extensive development on plant roots before the pathogen attack. Still, AM's broad-spectrum inhibition was noticed against pathogens such as *Aphanomyces, Chalara, Fusarium, Gaeumannomyces, Phytophthora, Pythium, Rhizoctonia, Sclerotium* and *Verticillium* (Azcón-Aguilar and Barea 1996). Another soil fungus *Trichoderma*, a well-known avirulent plant symbiont, characterized as biocontrol agent against broad range of phytopathogens works via competition, mycoparasitism, induced resistance, antibiotic and enzyme production. Beside this, it acts as plant growth promoting agents (Howell 2003; Harman et al. 2004). Others such as *Bacillus, Paenibacillus* and *Streptomyces* were also found to have inhibitory activity against soil borne and other phytopathogens (Cao et al. 2011; Köberl et al. 2013). A list of available commercial formulations of these microbes has been summarized by Junaid et al. (2013).

1.7 Indicators of Soil Health

It is understood from the literature that soil health is the result of continuous conservation and degradation processes in an ecosystem with the unique balance of chemical, physical and biological (including microbial) components. So, evaluation of soil health requires indicators of all these components. Since microbes quickly respond to changes in the soil ecosystem and vice versa, they are the excellent indicators of soil health. Changes in microbial populations or activity can precede detectable changes in soil physical and chemical properties, thereby providing an early sign of either soil improvement or an early warning of soil degradation (NERI 2002). The techniques were improved on the basis of the continuous identification and documentation of microbial processes. Some of the analytical and molecular techniques available are summarized in Table 1.3.

Table 1.3 Biological, physical and chemical indicators used for determining soil health

Indicator	Analytical techniques	Molecular techniques
Microbial biomass	Direct microscopic counts	Fluorescence microscopy
	Chloroform fumigation	Computerized image analysis
	SIR	Soil DNA estimation
	CO_2 production	FISH
	Microbial quotient	
	Fungal estimation	
	PLFA	
Microbial activity	Bacterial DNA synthesis	RNA measurements using RT-PCR
	Bacterial protein synthesis	
	CO_2 production	FISH
Carbon cycling	Soil respiration	SIP
	Metabolic quotient (qCO_2)	FISH
	Decomposition of organic matter	
	Soil enzyme activity	
Nitrogen cycling	N-mineralization	SIP
	Nitrification	FISH
	Denitrification	
	N-fixation	
Biodiversity and microbial resilience	Direct counts	–
	Selective isolation plating	
	Carbon and nitrogen utilization patterns	
	Extracellular enzyme patterns	
	PLFA	

(continued)

Table 1.3 (continued)

Indicator	Analytical techniques	Molecular techniques
Genetic and functional biodiversity	–	DGGE
		TGGE
		T-RFLP
		mRNA diversity using RT-PCR
		BIOLOGTM assay
Microbial resilience	–	Equitability (J) index
Bioavailability of contaminants	Plasmid-containing bacteria	RNA measurements
	Antibiotic-resistant bacteria	Geochemical indicators
Physical and chemical properties	Bulk density	–
	Soil physical observations and estimations	
	pH	
	EC	
	CEC	
	Aggregate stability and soil slaking	
	Water holding capacity	
	Water infiltration rate	
	Macro/micronutrient analysis	
SOM lipid analysis	–	PLFA(GC-MS)
SOM humic substances analysis	–	Non-destructive techniques:
		15 N-NMR, 13C NMR
		UV/Vis and IR spectroscopy
		Destructive techniques:
		Pyrolysis-GC-MS
		Chemolysis-GC-MS

Source: Arias et al. (2005)

1.8 Work at ICRISAT

Microbes play positive roles in plant growth promotion in addition to its direct or indirect participation in the nutrient cycles. These are called plant growth promoting (PGP) microbes which reside in rhizosphere/rhizoplane and promotes plant growth: (1) by using their own metabolism (solubilizing phosphates, producing hormones or fixing nitrogen) or directly affecting the plant metabolism (increasing the uptake of water and minerals), enhancing root development, increasing the enzymatic activity of the plant or helping other beneficial microorganisms to enhance their action on the plants; and (2) by suppressing plant pathogens (Pérez-Montano et al. 2014). Representative genera are *Bacillus, Pseudomonas, Trichoderma, Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium* and *Streptomyces* (Vessey 2003). Many reviews were periodically available on these PGP microbes (Loon 2007; Bloemberg and Lugtenberg 2001; Saharan and Nehra 2011; Bhattacharyya and Jha 2012). So this book chapter just gives a glimpse on those and the related studies conducted by our research group.

ICRISAT has identified over 1,500 microbes including bacteria and actinomycetes, isolated from various composts and rhizospheric soil, in which at least, one out of six has documented either single or multiple agriculturally favourable traits. Our research group has a collection of 59 PGP bacteria and actinomycetes isolated from various herbal vermi-composts and organically cultivated fields with documented PGP traits *in vitro* and also at field conditions (Gopalakrishnan et al. 2014b). PGP bacteria such as *Pseudomonas plecoglossicida, P. monteilii, Brevibacterium*

antiquum, B. altitudinis, Enterobacter ludwigii and *Acinetobacter tandoii* isolated from rhizospheric soil of system of rice intensification (SRI) fields has documented *in vitro* PGP traits and also under field conditions on rice. Enhanced growth performance was observed via increased tiller numbers, panicle numbers, filled grain numbers and weight, stover yield, grain yield, total dry matter, root length, root volume and root dry weight (Gopalakrishnan et al. 2012). Similar type of enhanced growth performance on rice by actinomycetes such as *Streptomyces* sp., *S. caviscabies, S. globisporus subsp. caucasicus, S. griseorubens* is also recorded. In addition, upregulation of PGP genes such as indole acetic acid and siderophore producing genes were documented (Gopalakrishnan et al. 2014c). A PGP bacterium *Pseudomonas geniculata* IC-76 showed its capacity on chickpea under field conditions by enhanced plant growth performance and also agronomic performance via increased nodule number, nodule weight, pod number, pod weight, seed number and seed weight (Gopalakrishnan et al. 2014d).

Besides increasing plant growth, they significantly enhanced rhizospheric total nitrogen (8—82 %), available phosphorous (13—44 %) and organic carbon (17—39 %). Production of lytic enzymes such as cellulase, chitinase, lipase and protease by these microbes (Table 1.4) is an additional evidence for the enhanced soil organic carbon and nitrogen contents (Gopalakrishnan et al. 2014b, 2014c). Analysis of soil health microbial indicators recorded enhanced microbial biomass carbon (23—48 %), microbial biomass nitrogen (7—321 %) and dehydrogenase activity (14—278 %) on experimental plots over the uninoculated control during our field studies on crops such as rice (Gopalakrishnan et al. 2012; 2013; 2014c), chickpea (Gopalakrishnan et al. 2014d) and sorghum (unpublished results). Figures 1.1, 1.2, and 1.3 illustrate the combined results of our published reports on rhizospheric PGP microbes on increasing soil health during the field trials.

Apart from the PGP traits, they also have the capacity to act as biocontrol agents by suppressing soil pathogens, one of the keystone logic for healthy soil. Our PGP bacteria such as *P. plecoglossicida, B. antiquum, B. altitudinis, E. ludwigii, A. tandoii* and *P. monteilii*, and actinomycetes *Streptomyces* sp., *S. tsusimaensis, S. caviscabies, S. setonii* and *S. africanus* were found to have inhibitory activity against soil borne pathogens such as *Fusarium oxysporum* f. sp. *ciceri*, and *Macrophomina phaseolina* under greenhouse conditions. Antagonistic activity of these PGP actinomycetes on *Fusarium* wilt-sick fields has also been demonstrated (Gopalakrishnan et al. 2011a, b). These strains are already reported for lytic enzymes in the context of biocontrol such as chitinase and β-1,3 glucanse (Gopalakrishnan et al. 2014b).

Table 1.4 Extracellular enzyme profile identified for PGP bacteria and actinomycetes

Isolates	Cellulase	Chitinase	Lipase	Protease
PGP bacteria				
SRI-156	+	+	+	+
SRI-158	+	+	+	+
SRI-178	+	+	+	+
SRI-211	+	+	+	+
SRI-229	+	+	+	+
SRI-305	+	+	+	+
SRI-360	+	+	+	+
SBI-23	+	-	-	+
SBI-27	+	-	-	+
PGP actinomycetes				
KAI-26	+	+	+	+
KAI-27	+	+	+	+
KAI-32	+	+	+	+
KAI-90	+	+	+	+
KAI-180	+	+	+	+
SAI-13	+	+	-	+
SAI-25	+	+	+	+
SAI-29	+	+	-	+

Source: Gopalakrishnan et al. (2014b)

1.9 Future Outlook

Microbes have multiple functions and features in influencing soil health and also in promoting plant growth and controlling diseases. Hence maintenance of beneficial microbial load will help in replacing inorganic fertilizer, pesticides and artificial plant growth regulators which have numerous side effects to sustainable agriculture. Beside this,

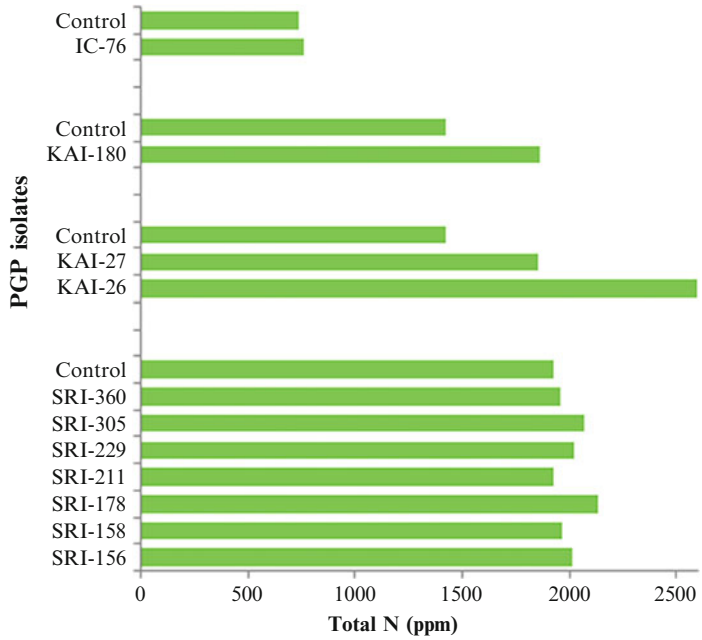

Fig. 1.1 Effect of PGP bacteria and actinomycetes on soil total N under field conditions of chickpea and rice cultivation (Note: Control indicates the treatment groups without any PGP bacterial inoculation, Gopalakrishnan et al. 2012, 2013, 2014c, d)

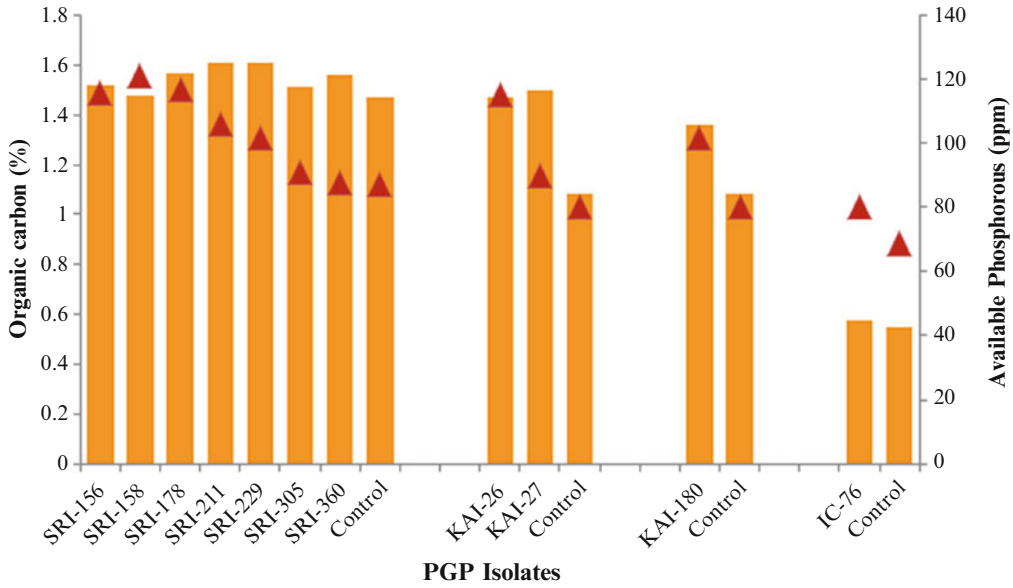

Fig. 1.2 Effect of PGP bacteria and actinomycetes on soil organic carbon and available phosphorous under field conditions of chickpea and rice cultivation
Solid bars (■) are the % organic carbon on the left axis and solid triangles (▲) are the available phosphorous (ppm) on right axis. Control indicates the treatment groups without any PGP bacterial inoculation (Gopalakrishnan et al. 2012, 2013, 2014c, d)

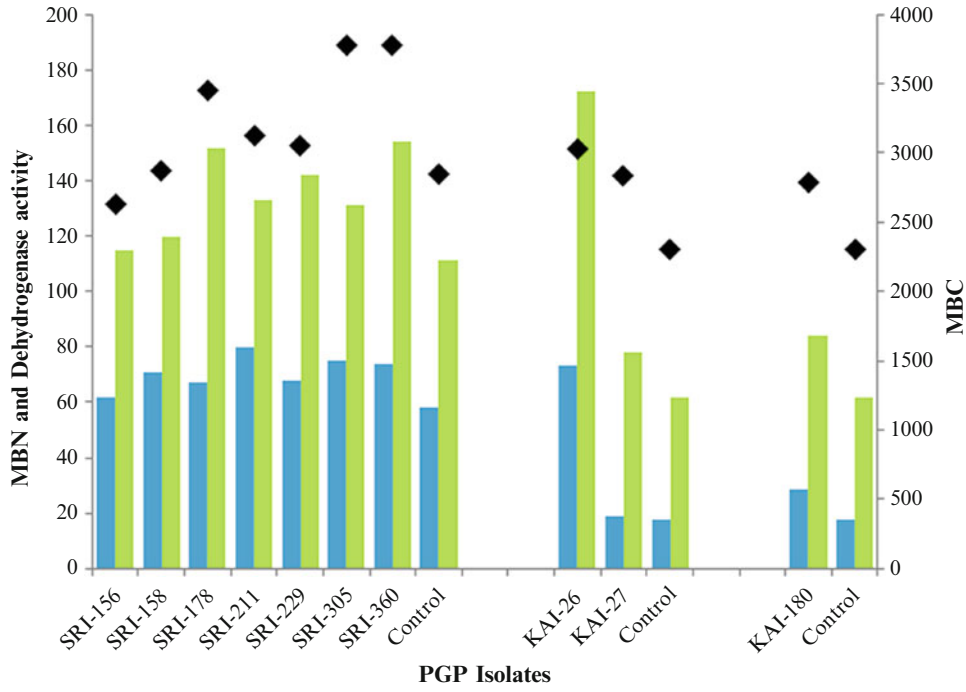

Fig. 1.3 Effect of PGP bacteria and actinomycetes on soil health indicators during field trials of rice cultivation Solid bars (■;■) are the microbial biomass nitrogen (µg g−1 soil) and dehydrogenase activity (µg TPF g−1 soil 24 h−) on left axis and solid diamond (◆) is the microbial biomass carbon (µg g−1 soil) on right axis. Control indicates the treatment groups without any PGP bacterial inoculation (Gopalakrishnan et al. 2012, 2013, 2014c)

understanding the responses of terrestrial ecosystems to global climatic changes and modern agricultural practices remains a major challenge, since soil has a mixed interaction with physical, chemical and biological component along with the influence of water, air/atmosphere, soil amendments etc. So research in each of this context individually and also in combination at various ecosystem levels is necessary.

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