

Chapter 5

Soil Microorganisms and Plant Growth

Hafiz Naeem Asghar, Rizwan Ahmad and Muhammad Javed Akhtar[†]

Abstract

Soil microbiology is concerned with microbes living in the soil, their interactions with each other and with plants in relation to soil management, agricultural productivity and environmental quality. The diversity of microbes in soil is the highest on Earth. These microbes are involved in different vital functions in soil. The most important function in soil is decomposition of organic matter, which is mediated through microbes, which has an enormous influence on soil structure, soil fertility, plant growth and carbon reserves. Soil microbes are involved in reduction of atmospheric gaseous nitrogen (N) to ammonia, which is said to be a second most important biochemical process on Earth after photosynthesis. Thus microbes regulate and maintain organic and inorganic pool of N in soil. Microbes transform different nutrients from unavailable form to plant available form. Microbes do much more than nourishing plants. Soil microbes drive carbon, nitrogen, phosphorus (P) and sulfur (S) cycles in soil, thus play critical roles in controlling composition, chemistry and physics of the atmosphere; therefore, have substantial impact on climate change. Soil microbes attribute in biological processes to degrade, transform, breakdown or remove contaminants from soil and water. Microbial activities are very important for bioremediation of xenobiotic and recalcitrant compounds in soil to improve soil

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health and quality. Diversified and healthy soil microbial ecology is considered as a sensitive indicator of soil health and fertility.

Keywords: Rhizosphere, Microbes, Symbiosis, Decomposition, Remediation and Nutrients.

5.1. Introduction

Soil is a complex habitat for living organisms. These organisms are numerous and diverse. The branch of soil science which is concerned with microorganisms found in soils and their relationship to soil management, agricultural production and environmental quality, is called as soil microbiology. More simply, soil microbiology may be defined as “the study of microorganisms that live in soils, their metabolic activity, and their roles in energy flow and nutrient cycling” (Atlas and Bartha 1993). The Soil Science Society of America (1998) defines soil microbiology as “the branch of soil science concerned with the soil-inhabiting microorganisms, their functions and activities”. Soil microbiology takes into consideration the morphological and physiological characters of soil micro-organisms and their interactions with other organisms, plants, soil and environment, role of microbes in nutrient cycling, geochemical transformations and climate changes. The intense interactions between plants, microbes and soil are translated into plant health, and that is why the soil microbial diversity is said to be an important index of agricultural productivity.

Soil organisms are diverse, ranging from microscopic microorganisms to small mammals. The soil microbial diversity is estimated to range from several thousand to several million different genomes per gram of soil (Whitman et al. 1998; Torsvik and Øvreås 2002). Bacteria are the dominant inhabitants of soil, exceeding their population of 100 million in one gram of soil with approximately 10^4 to 10^6 different species. While population of actinomycetes is 10^6 to 10^7 per gram soil and of fungi 10^4 to 10^6 per gram soil. Collectively, all microorganisms (bacteria, actinomycetes, and fungi) are usually said to be soil microflora, while small microscopic animals are designated as soil microfauna (Sylvia et al. 2005).

The physical, chemical and even biological properties of the soil habitat and their interaction with the resident community of soil microorganisms have a significant impact on growth and activity of microorganisms. Understanding functions and activities of microbial community in soil can enable us to maintain soil health and to achieve the goal of sustainable agriculture.

Few examples of important aspects of soil microbiology are:

- Symbiotic N fixation.
- Decomposition of organic waste to make it a useful product like compost/biofertilizer.
- Nutrient transformations in soil; N, P and S cycles.
- Microbial mediated enzymes production in soil; phosphatases, ureases, cellulases, ACC-deaminases.
- Production of growth regulators; auxins cytokinins and gibberellins.

- Bioremediation of organic and inorganic pollutants.
- Carbon cycling and emission of greenhouse gasses.

Along with soil microorganism, in soil there are many macroorganisms, which are also very important and cannot be ignored as a part of soil biology. Macrofauna includes earthworms, termites, ants, myriapoda, fly larvae and beetles. The soil macrofauna can modify soil chemical, physical and biological properties. They can mix the soil profile by burrowing action. Ants, termites, earthworms and ground beetles can bring soil from deeper layer to the surface, as well as enhance porosity and aeration. They can fragment litter, enhance decomposition of organic matter and formation of humus. They also ingest soil particles/organic matter with their food and thus contribute to aggregate formation and mineralization of organic matter and release of nutrients. Soil macrofauna disseminates bacteria and spores through excrement dispersion in soil or by on body transport (Ruiz 2008). The soil fauna contributes as much as 30% of the total mineralizable N. This contribution becomes more where C:N ration is high. Among macrofauna role of earthworms is well documented. They ingest organic debris, partly digesting and reducing the particle size of the substrates. Such activities enhance microbial ability to further decompose organic matter. In addition, worms excrete thick mucus containing polysaccharides, proteins and other compounds. Bacteria and fungi use these readily available substrates as additional nutrients (Sylvia et al. 2005). Along with these positive effects soil fauna has also some negative effects. Plant parasitic nematodes are best known for their negative effects on crop production, which can potentially reduce the amount of plant detritus deposited in the soil surface, such changes in litter inputs can decrease the number and activities of microbes in soil (Sylvia et al. 2005).

5.2. Historical Aspects of Soil Microbiology

Historically, humans have managed microorganisms consciously or unconsciously since time immemorial. First microbial process occurred over 100,000 years ago when fruit fermented and formed wine (Purser 1977). Also, cheese making and cheese consumption evidence dates back to 6000 years BC (Smith 1995). In 1800s, Edward Jenner inoculated people with cowpox germs as a preventive measure against smallpox (Wilson 1976). History revealed that Romans recognized relationship between legume crops and soil fertility. They knew that alfalfa and clover boost soil fertility but they did not know why? So this could be assumed as the initiation of soil microbiology but the main problem was that people could not actually see the microorganisms responsible for the changes they observed.

During the 17th century Antoni van Leeuwenhoek (1632-1723) made his own microscope and revealed the small creature as bacteria. He did not write stuffy academese, which was yet to be invented but he was the first human to see the new creature, the microbes (Anderson 2014). In the later half of 19th century, Ferdinand Cohn, Louis Pasteur, and Robert Koch were responsible for methodological innovations in aseptic technique and isolation of microorganisms (Madigan and Martinko 2006). Robert Hooke (1635-1703) from Royal Society of England (Coyne 1999) validated Leeuwenhoek's observations and published in his book

Micrographia in 1665, which may be considered as the first textbook of microbiology. Koch's findings are summarized as Koch's postulates giving very important information about the isolation and growth of pure culture. Koch was basically concerned with disease causing organisms, which are mostly culturable, while in case of soil microorganisms it has been observed that some microbes are viable but not culturable. Microbial activities directly related to soil were elaborated by a Russian scientist, Sergei Winogradsky (1856-1953), who is often called the "Father of Soil Microbiology". His investigations are worth to mention regarding the followings:

- Winogradsky column, a self-contained ecosystem for studying the S cycle.
- Nitrification process in which ammonium (NH_4^+) is converted to Nitrate (NO_3^-).
- Microbial oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}).
- Isolation of anaerobic, spore forming, N-fixing *Bacilli*.

Another scientist, Martinus Beijerinck (1851-1931), cultured the first N-fixing bacteria that grew symbiotically in association with legumes and the first aerobic N-fixing bacteria that grew asymbiotically as a free-living soil organism. These were *Rhizobium* and *Azotobacter*, respectively.

Near the end of the 19th century at the University of Delft (the Netherlands), Beijerinck developed enrichment techniques that allowed Beijerinck's crucial discoveries including microbiological transformations of nitrogen and carbon, and also other elements such as manganese (Madigan and Martinko 2006).

Jacob Lipman was the founder of American soil microbiology. He considered soil as a complex and living entity which needed to be understood and studied from the standpoint of soil fertility and crop production. This revolutionary concept stands as a milestone in soil microbiology. Two prominent individuals who shared Lipman's revolutionary concept of soil were Selman Waksman and Robert Starkey. Besides promoting the "Lipman Philosophy," Waksman's book, Principles of Soil Microbiology, and a later work by Waksman and Starkey, The Soil and the Microbe, were considered as the standard soil microbiology texts for most of the period between 1925 and 1950. However, in many circles Waksman is remembered less for his contributions to soil microbiology than for his discovery of the antibiotic streptomycin, for which he was awarded the Nobel prize in physiology and medicine in 1952.

5.3. Microbial Diversity in Soils

Plants, animals, human beings, microbes, and every individual are fairly different from another one, even within the same genera and species. Variation is beauty of nature, its law of nature to maintain sustainability of the fittest in the community. The variation may be linear or cyclic. The variety and variability of organisms and ecosystem is referred to as diversity or biodiversity (Singh et al. 2012). Biodiversity comprises different aspect of ecosystem in addition of being concerned with only number of species present in certain ecosystem. It is actually the degree of variation

of life; this may be linked with genetic variation, species variation or ecosystem variation. Biodiversity can be assessed by focusing on the clusters of soil organisms that play key roles in soil ecosystem (Coleman et al. 2004). Microorganisms in soil play different roles like decomposition of organic matter, formation of soil structure, removal/degradation of pollutants in soil, transformation of nutrient in soil to regulate the consistent supply of nutrient to the plants. Microbes are also responsible for production of growth regulators like auxins, cytokinins, gibberellins, suppressing soil borne diseases and to regulate plant growth in biotic and abiotic stress through production of different enzymes. Soil biodiversity has also very important role in soil and atmospheric changes thorough carbon dioxide flux and carbon sequestration. Soil biodiversity helps to maintain soil quality. Soil quality reflects the ability of soil to support plants, to sustain or improve soil, air and water quality and functioning to support human health. Soil quality is directly linked with soil microbial community, morphologically and phenotypically diverse microbial community is required to mediate different processes carried out in soil (Sylvia et al. 2005). To gain an insight in biodiversity of microbial community composition and its dynamics at both taxonomic and functional levels, it needs to be fully elucidated. Changes in microbial community composition may be monitored by substrate utilization pattern (Biolog), by using fatty acids as markers of microbial diversity and DNA sequencing.

Bacterial diversity has been arranged into different groups (Singh et al. 2012) as under:

- 1) Spirochetes (e.g., *Triponema palidum* and *Borrelia recurrentis*);
- 2) Aerobic/microaerophilic, motile, helical/vibroid Gram negative bacteria (e.g., *Spirillum volutans*, *Aquaspirillum* sp., *Campylobacter* sp., *Helicobacter pylori*, *Bdellovibri obacteriovorus*);
- 3) Non-motile or rarely motile Gram negative curved bacteria (e.g., *Cyclobacterium*, *Ancylobacter* and *Brachyarchus*);
- 4) Gram negative aerobic/microaerophilic rods and cocci (e.g., *Pseudomonas*, acetic acid producing *Acetobacter*, *Gluconobacter* and *Frateuria*; N fixing *Azotobacter chroococcum*, *Agrobacterium tumefaciens* and *Rhizobium*; methylotrophic bacteria in water bodies, *Legionella* and intracellular *Neisseria gonorrhoeae*);
- 5) Facultative anaerobic Gram negative rods (e.g., enteric bacteria *Escherichia coli*, *Salmonella*, *Serratia marcescens*, cholera causing *Vibrio cholera*, luminescent *Photobacterium*);
- 6) Gram negative anaerobic, straight curved and helical bacteria (e.g., *Haloanaerobium* that prefers 13% NaCl, *Halobacterioides* that prefers 8.5 to 14% NaCl, *Thermosipho* that prefer 1-3% and *Thermotoga* with preference of 3-6% NaCl);
- 7) Dissimulatory sulphate reducing and sulphur reducing bacteria (e.g., *Desulfotomaculum acetoxidans*);
- 8) Anaerobic Gram negative cocci (e.g., *Veillonella* found in saliva, tongue, cheek, mucosa and gingival crevices of human oral cavity);
- 9) Rickettsia and Chlamydias;

- 10) Phototrophic bacteria that are of two types: Anoxygenic phototrophic bacteria (e.g., purple *Chromatium*, or green sulphur bacterium *Chlorobium*) and Oxygenic phototrophic bacteria (e.g., *Cyanobacteria*, *Chroococcus*, *Spirulina*, *Lyngbya*, *Nostoc* and *Anabaena*);
- 11) Aerobic chemolithotrophic bacteria, which are:
Hydrogen oxidizing bacteria (e.g., *Alcaligenes eutrophus*), colorless sulphur oxidizing bacteria (e.g., *Achromatium*, *Thiobacterium*, *Thiospira* and *Thiobacillus*), iron oxidized and manganese oxidizing bacteria (e.g., *Gallionella*) magnetotactic bacteria and nitrifying bacteria (e.g., *Nitrococcus* and *Nitrobacter*);
- 12) Budding and appendaged bacteria (e.g., *Caulobacter crescentus*, *Hypomicrobium* and *Planctomyces maris*);
- 13) Sheathed bacteria (e.g., *Leptothrix discophora*);
- 14) Bacteria with gliding mobility, which may be non-photosynthetic non-fruiting gliding bacteria (e.g., *Cytophaga* and *Simonsiella*) fruiting gliding bacteria (e.g., *Myxobacterium*, *Stigmatella*);
- 15) Gram Positive cocci (e.g., *Deinococci*, *Staphylococcus aureus* and *Streptococcus pneumoniae*);
- 16) Endospore forming Gram positive rods and cocci (e.g., *Bacillus sphaericus*, *Bacillus subtilis* and *Clostridium perfringens*);
- 17) Asporogenous Gram positive rods, which are non-spore forming Gram positive rods (e.g., *Lactobacillus*, *Listeria* and *Renibacterium*) irregular non-spore forming Gram positive rods (e.g., *Arthrobacter globiformis*);
- 18) Mycobacteria (e.g., *Mycobacterium tuberculosis*, *M. bovis* and *Arthrobacter globiformis*); and
- 19) Actinomycetes (e.g., *Streptomyces* and *Planomonospora*); mycoplasmas or the cell wall-less bacteria (e.g., *Mycoplasma pneumonia*, *M. hominis* and *Spiroplasma*).

5.3.1. Factors affecting microbial diversity in soils

The microbial diversity has impact on the functional stability of an ecosystem and especially on its resilience towards any disturbance (natural or man-made). The diversity of microbial community has impact on stability of ecosystem functions and resilience to disturbance in soil ecosystem. Microbial community also affects the soil and plant quality and ecosystem sustainability. There are different factors which affect microbial diversity in soil.

- Soil management practices
- Application of pesticides
- Introduction of genetically modified microorganisms
- Physicochemical properties of soil (i.e. pH, cation exchange capacity, aeration, porosity, soil structure, organic matter, soil water and soil temperature).

USDA, Natural Resources Conservation Service (1998) has recommended following management practices to maintain soil biodiversity.

5.3.1.1. Cultivation

Deep and frequent tillage has deleterious effects on microbial diversity while minimum tillage or no till maintains soil physical properties, hence improves biological habitat and diversity of microbes in soil.

5.3.1.2. Compaction

Compaction suppresses pore spaces in soil and reduces the soil aeration for suitable habitat of soil microorganisms. Compaction can create anaerobic conditions in soil and ultimately can affect type and distribution of soil organisms.

5.3.1.3. Pest control

Pesticides, herbicides, fungicides and insecticides can destroy soil microbial diversity. Recommended dose of herbicides and insecticides has comparatively a minor impact on soil organisms, while fungicides and fumigants have more deleterious effect (USDA Natural Resources Conservation Service 1998).

5.3.1.4. Fertility

Availability of limiting factors like balanced nutrients enhances biological diversity. Plenty of carbon (green manures, compost, organic residues) improves biological activity in soil.

5.4. Soil Microbes and Soil Fertility

Microorganisms play a major role in the biogeochemical cycling of different nutrients required for plant growth. Most important nutrients affected by soil microorganism are C, N and P. Some free living or symbiotic microorganisms have the mechanisms to fix the atmospheric N and make it available for plant use. Similarly certain groups of bacteria and fungi have the ability to solubilize fixed nutrients particularly P and refresh the soil fertility status. Heterotrophic microbes are the consumers and their activity plays a key role in cycling the fixed carbon and other nutrients through decomposition of the organic fraction of soil, i.e. plant and animal residues. Initially, easiest compounds are broken down and eventually resistant organic residues along with microbial waste product combine to form soil humus. Plant growth promoting rhizobacteria are the bacteria residing near the plant roots which help the plant in a number of ways, like alteration in soil pH, production of certain enzymes and plant hormones which stimulate growth, physiology, as well as mitigate certain stress factors or protect plant against diseases. Basic understanding about the role of microbes in soil fertility and crop production is discussed in the following section.

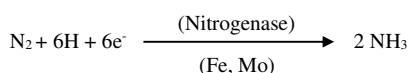
5.4.1. Biological nitrogen fixation

Nitrogen is the foremost nutrient required by the plants in large quantity, and most often its deficiency can limit plant growth. Although elemental N constitute nearly

79% of the atmosphere as molecular N gas (N₂), but it is completely un-available to majority of green plants in this form. The stability of the N≡N triple bond makes N₂ extremely inert and demands high activation energy. There are two most common pathways that make the inert N available to the plants.

- i) Through the formation of industrial fertilizers; and
- ii) N fixation by microbes, i.e., the biological N fixation.

In biological N fixation, N is drawn from atmosphere through N fixation activity by some prokaryotes. Atmospheric N is reduced by prokaryotic bacteria to ammonia, thus, utilization of atmospheric di-N gas (N₂) by certain microbes, through its reduction to ammonia is called biological N fixation.



The reduction of N₂ to ammonia is catalyzed by nitrogenase, a key enzyme of biological N fixation system. The genes that code for the enzyme nitrogenase are collectively called *nif* gene.

5.4.1.1. Modes of nitrogen fixation

There are three principal modes adopted by microbes to fix atmospheric N to ammonia. These are: non-symbiotic, associative symbiotic, and symbiotic.

i) Non symbiotic nitrogen fixation

This type of biological N fixation is processed by the microorganisms which live freely and independently in the soil. A large number of bacteria and some cyanobacteria are capable to fix N₂ non-symbiotically.

ii) Associative symbiotic nitrogen fixation

Associative symbiotic N fixation mostly takes place in association with the roots of grasses and cereal plants. In this type of association no nodules are formed like symbiotic bacteria. The population of bacteria near the roots increases and fixes the atmospheric N. Representative genera involved in associative symbiotic N fixation are *Azospirillum*, *Enterobacter*, *Azotobacter*, *Pseudomonas*, *Klebsiella* and *Bacillus*, etc.

iii) Symbiotic nitrogen fixation

(a) Through nodule formation in legumes

Symbiotic association is the most interesting and important plant-bacteria interaction; the plants being the legumes and the bacteria being *Rhizobium*, *Bradyrhizobium*, *Sinrhizobium*, *Mesorhizobium* and *Azorhizobium*. Infection of the roots of a leguminous plant (e.g. soybean, clover, alfalfa, beans, and peas) with respective rhizobium results in the formation of nodule where atmospheric N is converted to ammonia. Legume-rhizobium N fixation is of considerable agricultural significance as it leads to greater quantitative enhancement of combined N in the soil. Nitrogen fixation rates vary enormously in different legumes, up to 600 kg ha⁻¹ yr⁻¹

have been reported in forage legumes (Coyne 1999), most rates are much less, in general grain legumes fix less N than do the forage legumes. *Rhizobium* strains are highly specific to legume species. A single *rhizobium* strain can generally infect only certain species of legumes and not others. A group of *rhizobium* strains capable of infecting a group of related legumes is referred to as cross-inoculation group (Table 5.1). Before these bacteria can fix N, they must establish themselves in the root cortical cell of the host plant, to form 'root nodules'.

(b) Through nodule formation in non-legumes

Recently it has been observed that certain non-leguminous plants form nodules to fix N. The best known plant, in temperate regions, is alder (*Alnus* spp.); the microbes involved in nodule formation are actinomycete of genus *Frankia* (Wall 2000).

Table 5.1 Cross-inoculation groups of Rhizobia bacteria and associated legumes

Bacteria (Genus)	Species/subgroups	Host legume
<i>Rhizobium</i>	<i>R. leguminosarum</i>	
	<i>bv. Viceae</i>	<i>Vicia</i> (vetch), <i>Pisum</i> (pea), <i>Lens</i> (lentils), <i>Lathyrus</i> (sweet pea)
	<i>bv. Trifolii</i>	<i>Trifolium</i> spp. (most clovers)
	<i>bv. Phaseoli</i>	<i>Phaseolus</i> spp. (dry bean, runner bean, etc.)
	<i>R. Meliloti</i>	<i>Melilotus</i> (sweet clover, etc.), <i>Medicago</i> (alfalfa), <i>Trigonella</i> , (fenugreek)
	<i>R. loti</i>	<i>Lotus</i> (trefoils), <i>Lupinus</i> (lupins), <i>Cicer</i> (chickpea), <i>Anthyllis</i> , <i>Leucaena</i> , and many other tropical trees
Bradyrhizobium	<i>R. Fredii</i>	<i>Glycine</i> spp. (e.g., soybean)
	<i>B. japonicum</i>	<i>Glycine</i> spp. (e.g., soybean)
	<i>B. sp.</i>	<i>Vigna</i> (cowpeas), <i>Arachis</i> (peanut), <i>Cajanus</i> (pigeon pea), <i>Pueraria</i> (kudzu), <i>crotolaria</i> (crotolaria), and many other tropical legumes

Source: Brady and Weil (2007)

5.4.2. Decomposition of organic matter and nutrient release

Soil organic matter includes all materials of plant, animal or microbial origin regardless their degree of decomposition. As soon as plant and animal residues are added to the soil, soil microorganisms immediately act on them to gain food and energy. Organic matter does not decompose at once as a whole. Degree and rate of decomposition depends on many factors, i.e., environmental conditions, nature of the material, nutrient status of soil and micro fauna present in the soil. Simple sugars, amino acids, most proteins and certain polysaccharides decompose very quickly. Large macromolecules such as cellulose are cleaved into oligosaccharides first and then into simple sugars. Lignins decompose very slowly, a less lignified plant residue such as green manure of leguminous plants will decompose faster than more lignified material like wheat straw.

Besides microbial synthesis, during decomposition a heterogenetic mixture of complex nature; 'humus' is also formed and the process is called humification. Humus is a brown to dark brown amorphous and colloidal substance of complex nature rather resistant to further break down. The process of decomposition is carried out through microbial enzymes and greatly influenced by factors like temperature, moisture, pH and C: N, C: P and C: S ratios of the decomposable organic residues. Thus, nutrients released by organic matter decomposition help in maintaining soil productivity and increasing its fertility. During decomposition, plant and animal residues release N, P, S and other essential nutrients for plant growth. Soil microbes play a significant role in converting these elements into ionic species which can be utilized by plants. This conversion is referred as mineralization. The biotransformation of mineral forms of nutrients into organic forms is called immobilization. This inter-conversion of nutrients and humus formation has a great role in slow releasing of plant nutrients which ultimately maintains soil fertility and productivity.

5.4.3. Biotransformation of nutrients in soil

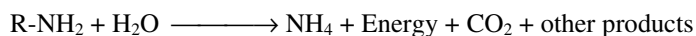
5.4.3.1. Carbon cycle

Carbon dioxide is fixed in different organic forms by photosynthetic organisms in the presence of sunlight. Fixed carbon dioxide is consumed by animals and heterotrophic microorganisms. These organisms release carbon dioxide into the atmosphere during respiration. Respiration processes can be aerobic or anaerobic releasing CO₂ or reduced products such as CH₄.

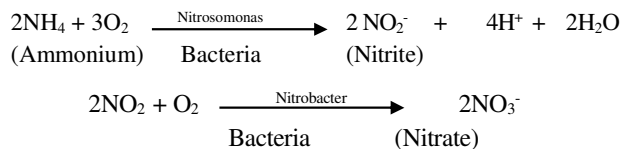
To optimize soil fertility by way of desirable soil physical properties, like structure, and moisture retention to gain slow release of nutrient for plants, it is necessary to optimize carbon pool in the soil. Crop residues, farm yard manure, composts and other carbon sources can be incorporated into the soil to keep the soil micro biota alive and active, improve soil physical properties and attain release of plant nutrients.

5.4.3.2. Nitrogen cycle

The transformations of Nitrogenous substances in soil are largely function of microorganisms. Three fourth of the soil N contents come from biological N fixation. The release of N as ammonium during the process of decomposition is called ammonification.

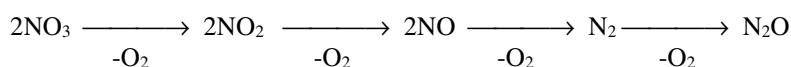


Ammonium in the presence of oxygen is oxidized to nitrate by certain bacteria. The conversion of ammonium to nitrate is called nitrification



The bacteria contributing in the process are called nitrifiers. In first step ammonium is oxidized to nitrite by *Nitrosomonas* and oxygen is used as electron acceptor. Nitrite is toxic substance and quickly oxidized to nitrate by another bacterium called *Nitrobacter*.

Under anaerobic conditions microbes use nitrate as terminal electron acceptor and N is lost into the atmosphere as molecular N, this process is called as denitrification.



The nitrification process is very important as all aerobic plants take up N predominantly in the form of nitrate. Beside microbial immobilization small quantities of N is also lost by leaching to lower layers of the soil, beyond the root zone. So proper soil management practices to maintain healthy microbial community in soil could be a good strategy to maintain soil fertility.

5.4.3.3. Phosphorus cycle

In soil P exists in various forms like phosphates of iron, aluminum, calcium, etc. Due to their low solubility, only a small fraction of total P present in soil is available for plant growth. Several microorganisms cause solubilization of P by producing organic acids that lower the soil pH. Phosphorus Solubilizing Bacteria (BSP), particularly *Pseudomonas* and *Bacillus*, possess the ability to transform insoluble forms of P to plant available forms by secretion of organic acids and phosphatase enzyme (Hussain et al. 2013)

5.4.3.4. Sulphur cycle

Sulphur generally is not a limiting nutrient for plant growth; however, it has a role in producing high crop yield under intensive agricultural system. In soils, it mainly occurs in organic form but small quantity of inorganic sulphur also exists as sulphate. Plants and microorganism consumes sulphur as sulphate and it makes approximately 1% of the dry weight of soil bacteria. Soil bacteria are involved both in oxidation and reduction of S.

5.4.4. Bio-fertilizers

Biofertilizers comprise of living cells of certain types of microorganisms and/or their products which have the ability to transform/mobilize nutritionally important plant elements from non-available forms to available forms through biological processes. Biofertilizers do not directly increase soil fertility but they initiate, stimulate or accelerate the processes which could contribute to improve soil fertility. Microbes in biofertilizers can fix N symbiotically in the roots of leguminous crops or non-symbiotically by free living microorganisms or transform fixed soil nutrients such as P, Fe, S, Cu and Zn, to usable forms or may carry those microbes which are able to produce growth regulators and enzymes, which can help plants to cope with water and salinity stress.

Biofertilizers are cost effective and environment friendly agricultural input which play a significant role in sustainable agriculture. Effective quality control measures

are very important in production of good quality biofertilizer. Efficacy of microbial strains present in commercial biofertilizer must be ensured. Viability of inoculants with desired population of approximately above one hundred million per gram can indicate a good quality biofertilizer. Packing and selection of good carrier material can make biofertilizer really a good supplementary option to fulfill the crop nutritional requirements to improve crop production and food safety.

5.5. Plant-microbe Interactions

It mainly constitutes the association of microorganism with plants either in a positive or negative way. The positive approach is mainly the symbiotic relationships and the negative approach constituents mainly pathogen plant interactions.

5.5.1. The rhizosphere

The rhizosphere is the portion of soil around the vicinity of plant roots that is directly influenced biologically, physically and chemically by plant roots, leading to a favorable conditions/habitat for microorganisms. Typically, rhizosphere contains almost 10^9 microbes per g of soil. Rhizosphere is rich with root exudates and root deposits, which are important sources of substrates for microbial communities present in the rhizosphere. It has been investigated that composition of root exudates and root deposits may vary from species to species, cultivars to cultivars and also depend on plant developmental stage. In response to the impact of root exudates in rhizosphere, the microorganisms in turn influence the plant growth. Environmental and soil conditions have known effects on both plant and microbe. However, it is difficult to tear apart complex interactions between these organisms (Elsas et al. 2007).

5.5.2. Plant's influence on microbes

Plants influence the microbes through secretion of fixed carbon, organic acids, sugar and amino acids in the rhizosphere. These root exudates provide nutrients for proliferation of the microbes in vicinity of roots and indirectly influence on microbial community composition and control over selection or rejection of species that are the most suitable to utilize those nutrients. Plants sense and react to physical stimuli from harmful and beneficial microorganism (Jayaraman et al. 2014). Some exudates can specifically recruit beneficial microbes. Release of malic acid from plant attract favorable microbes while release of proteins and defense chemicals suppress the growth of plant pathogens like *Rhizoctonia solani*, *Pythium aphanidermatum*, *P. ultimum* and *Pseudomonas solanacearum* (Flores et al. 1999; Lugtenberg et al. 1999; Simons et al. 1997).

5.5.3. Microbial influence on plants

In rhizosphere the microbes have both, beneficial and deleterious effects on plants. Some microbes are known plant pathogens, such as *Phytophthora cinnamomi* and some can secrete phytotoxic metabolites, such as *Fusarium moniliforme* suppresses

the seed germination. Some toxic metals become more available to the plants, additionally microbes compete with plants for essential nutrient. Compare to deleterious effects of soil microbes, beneficial effects are more prominent. Many microbes in the vicinity of plant roots are considered as plant growth-promoting rhizobacteria; these bacteria live in rhizosphere and regulate plant growth and development through N fixation, production of growth regulators, enzymes, siderophores and formation of biofilms to exclude toxic metals (Elsas et al. 2007; Glick et al. 1998).

5.6. Environmental Implications of Soil Microbiology

In modern society, daily life starts with the use of different organic chemicals and use of such chemicals is increasing day by day. Among these chemicals some are xenobiotic compounds which are “a stranger to life” because these are not produced naturally in biosphere. The biodegradation of such compounds is very difficult. Chemical approaches to degrade/transform these chemicals to environment safer form are usually costly, demand chemical expertise and sometimes byproducts are more toxic than the parents.

Biological processes may lead towards complete transformation of such organic molecules to inorganic products or comparatively less toxic molecules. The major agents affecting the fate of chemical are naturally occurring microorganisms (e.g., fungi and bacteria). The application of microbes to handle toxic materials is generally termed as bioremediation. Along with microorganisms, certain plants also play important role to remove, contain or transform contaminants; this is called phytoremediation. This approach of remediation is effective for organic as well as inorganic pollutants. Through thermophilic decomposition waste materials can be converted into useful products, called compost. This section explains bioremediation, microbial-assisted phytoremediation and composting.

5.6.1. Bioremediation

Bioremediation is the use of living organisms to clean the environment from hazardous compounds. It involves microorganisms, plants or their enzymes to remove the contaminants for the purpose of cleaning the environment (Hillel 2008). Increase in industrialization, modernization and urbanization caused the production and release of toxic compounds into the soil and environment. These contaminants are mainly divided into two types, organic and inorganic.

Organic contaminants can stay for longer period of time in the environment and bring great threat to soil fauna, flora and to human health. Organic contaminant includes polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPHs) and polychlorinated biphenyls (PCBs), other chlorinated aromatics such as halogenated compounds, polychlorinated terphenyls (PCTs), and pesticides like bentazon and atrazine (Saleh et al. 2004).

Inorganic contaminants are heavy metals which include chromium, lead, cadmium, copper, nickel and mercury etc. Many of the recent studies have reported the success of bioremediation technique to clean polluted soil (Khan et al. 2013).

In order to control or eliminate these contaminants from soils, chemical, physical and biological techniques can be used. Biological methods have advantages over physical and chemical techniques, but comparatively they are slow. Different bacteria/rhizobacteria have been reported to have their important role to combat organic and inorganic pollutants. Among these bacteria some colonize the plant root and have ability to improve plant growth as well.

There are different strategies for bioremediation, like passive bioremediation (by indigenous microorganism), biostimulation (addition of nutrients), bioventing (addition of gaseous stimulants, such as oxygen and methane) and bioaugmentation (inoculation of a contaminated site with microorganisms).

5.6.1.1. Remediation of inorganic pollutants

Rhizosphere bacterial communities withstand high concentration of heavy metals and can remove the heavy metals from contaminated sites through bioreduction, biotransformation, biosorption and bioaccumulation. Microbes improve plant growth in metal contaminated soil thus increase plant biomass, which is basic requirement of plant to act as hyper accumulator. Bacteria can stimulate growth of plant through production of ACC deaminase, indole-3-acetic acid (IAA), siderophores, and antibiotics or through stimulation of several metabolic pathway and enhance metal availability and uptake to the plant through redox changes, acidification or by producing iron chelates.

5.6.1.2. Remediation of organic contaminants

In contrast to the inorganic contaminants, microorganisms play significant role in biodegradation of organic contaminants. Bacteria having capacity to degrade the organic compounds like polychlorinated biphenyls have been obtained from the contaminated sites. Several techniques have been developed to promote the degradation and tolerance of bacteria to soils contaminants. Due to variety of contaminants, microbes and plants separately become less efficient to remediate contaminants. Alternatively, the combined use of plants and degrading microbes was found to be more efficient. Both plants and microbes assist each other and synergistic use of both multiplies the efficiency of bioremediation process.

5.6.2. Phytoremediation

Phytoremediation is a combination of the Greek word *phyto* which means plant and the Latin word *remedium* which means to correct or remove an evil. Plants are typically used when environment is contaminated with heavy metals like lead, chromium, mercury and selenium. This technique encompasses use of plants to remove, detoxify, transform or contain the contaminants. It is a technology that involves the application or use of higher plants to remove or degrade contaminants directly or indirectly. Directly, by releasing the enzymes that degrade the contaminants or indirectly by secreting organic acids that promote the efficiency of

microorganisms to biodegrade the contaminants. It involves the accumulation of contaminants by plants or degradation of contaminants through root colonizing microorganisms (Fig 5.1). This technique is less expensive, natural process and has high public acceptance.

5.6.2.1. Techniques of phytoremediation

Phytoremediation includes phytoextraction, phytodegradation, phytovolatilization, phytostabilization and phytodetoxification (Fig. 5.1) (Sylvia et al. 2005).

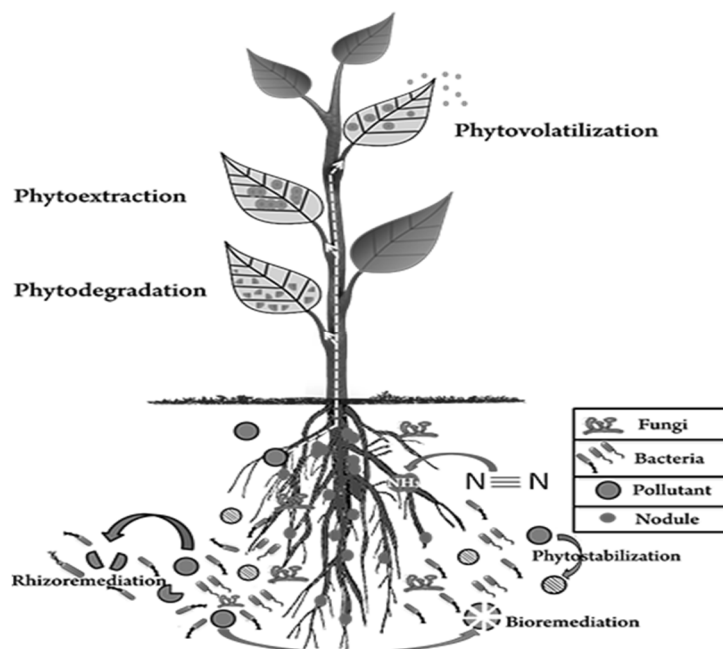


Fig. 5.1 Different approaches of phytoremediation/bioremediation

i) Phytoextraction

Uptake and accumulation of contaminants by plants is known as phytoextraction. In this case hyper accumulator plants are used. Hyper accumulator plants uptake significant amount of contaminants in their bodies. Plants contained heavy metals are harvested or disposed off.

ii) Phytodegradation

It is also called as phytotransformation, it is use of plant secreted organic acid or enzymes to degrade the contaminants around the plants or breakdown of the contaminants which are taken up by the plants and then in the metabolic process plant transform these contaminants.

iii) Phytovolatilization

Volatilization of accumulated contaminants in plants from plant tissues is known as phytovolatilization. Water soluble contaminants taken up by the plants, may become modified and then released in to the environment

iv) Phytostabilization

In this technique of phytoremediation, organic acids secreted by plants make complex with contaminants and stabilize them in soil. Phytostabilization is an *in situ* approach where interactions of metal tolerant plants with different soil amendments reduce the mobility of contaminants to plant, soil, air, water and ground water.

5.6.3. Composting

Composting is a process of converting raw organic waste to a beneficial end product called compost which is a humus-rich soil amendment; this process is carried out by the sequential action of microbes. Composting is different from natural decomposition as it can be accelerated by monitoring, modifying and controlling various factors involved in the process. This process leads to decomposition and stabilization of raw organic waste into a product that can be used as soil conditioner and/or organic fertilizer which is stable having dark-brown or black color with earthy smell.

5.6.3.1. Composting process

A variety of organic materials, like leaves, grass clipping, kitchen waste, animal or poultry waste and municipal waste, can be composted. Composting is a dynamic process in which three groups of microorganisms namely bacteria, actinomycetes and fungi are involved. This mixed microbial population acts in a rapid succession. Most organisms required for composting are aerobic, as they are needed for rapid and complete composting. As the process continues bulky volumes of decomposable organic substances reduces to small volumes (Cambardella et al. 2003).

At the initial stage, mesophilic microorganisms take soluble sugars and amino acids from the plant materials followed by starch. As temperature increases due to oxidation of carbon compounds thermophilic take over. Temperature rises from 45-70 °C that is enough to kill pathogens, weed seeds and phytotoxins. Oxidation of organic compounds releases essential plant nutrients like N, P, S and micronutrients in small amounts. Depletion of readily available food resources leads toward decreased microbial activities. Temperature falls gradually and second mesophilic stage prevails. Finally compost enters the maturation or curing stage, time required may be different in different cases, depending upon the nature of the material and microbes involved. If temperature, moisture, nutrients, aeration and microbial activities are managed properly, the time span for maturation of compost may be reduced. Final product is nutrient rich, free of viable weed seeds, pathogens and easy to apply for potted plants or field crops. Along with providing different nutrients to the plants, compost also acts as good soil conditioner and improves soil physical, chemical and biological properties.

i) Physical properties

Compost is more stable form of organic matter than raw waste and its application in soil improves soil structure, aeration, porosity, soil aggregation, water holding and filtration. Compost stop rapid changes in soil temperature, hence, provide better conditions for growth of root. Compost decreases soil crusting, bulk density, run off, erosion and maintain the soil quality and productivity.

ii) Chemical properties

Application of compost influences chemical properties of soil. Cation exchange capacity (CEC) is increased while pH is slightly decreased with application of compost. Availability of nutrients is increased in composted soil. Compost buffers the soil against rapid changes due to salinity, alkalinity, acidity, pesticides and heavy metals.

iii) Biological properties

Addition of organic matter through compost enhances microbial activity and diversity in soil. Overall improvement in microbial activities in soil enhances decomposition of organic matter, improves nutrient availability and strengthen the symbiotic relationship with plants for N fixation and P accusation, thus improves the soil quality.

5.7. Conclusion

Microbes are the most diverse group of soil organisms, yet very little is known about them. Until recently the research is only focused on those microbes which are cultureable, identification and exploration of many viable but un-cultureable microbes is yet a challenge. New research methods involving molecular techniques are required to understand taxonomic and functional diversity in soil system. Various kinds of organic wastes are being converted into useful soil amendments through recycling by microorganisms, however, in future, various kinds of organic wastes may accumulate in huge quantities due to population outburst, urbanization, economic growth and industrialization. So to keep our environment healthy on sustainable basis, there is dire need to use the microorganisms which are efficient decomposer but can also add beneficial metabolites to final soil amendment to reduce waste burden on the environment and for sustainable agriculture. Use of new organic compounds and heavy metals in industrial and commercial processes is posing serious threat to soil, water and environment. A better understanding of the interactions between bacteria and host plants can play a key role to enhance remediation efficiency; and genetically modified microbes may help in this regard. To address the threat of environmental degradation, the challenge is to have better understating of the carbon sequestration process and different geochemical cycles to control atmospheric gasses and climate change. Biofertilizers are very good supplement of chemical fertilizers but to maintain their quality up to the farmer's field is a challenge to the soil microbiologists. The life span of biofertilizer is short, as they are living products and can be used with full benefits provided the material is not subjected to high temperature or other un-favorable conditions. The

maintenance of the same during transportation and storage is real task. Moreover, the availability of cheap, easily accessible and efficient carrier material is a major hurdle for production at large scale. However, on the part of soil microbiologists the real future challenge is to explore the strains which can tolerate the diversified agro-climatic conditions and selection of the suitable carrier which store and sustain the living strains of biofertilizers for longer time under variable environmental conditions.

No doubt soil fertility is a complex property of soil ecosystem but it could be more understood and managed by studying soil microbial ecology and managing beneficial microbial population in soil. Management of microorganisms in soil helps to ensure soil and environmental health and ultimately more crop growth.

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