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Abstract

Microbes are omnipresent in the biosphere, and their presence invariably affects the environment in which they grow. The effects of microbes on their environment can be beneficial or harmful or inapparent with regard to human measure or observation. The most significant effect of the microbes on earth is their ability to recycle the primary elements that make up all living systems, especially carbon, oxygen, and nitrogen (N). Primary production involves photosynthetic organisms which take up CO₂ from the atmosphere and convert it to organic (cellular) material. The process is also called CO₂ fixation, and it accounts for a very large portion of organic carbon available for synthesis of cell material. Decomposition or biodegradation results in the breakdown of complex organic materials to other forms of carbon that can be used by other organisms. There is no naturally occurring organic compound that cannot be degraded by some microbe, although some synthetic compounds such as Teflon, plastics, insecticides, and pesticides are broken down very slowly or not at all. Through the microbial metabolic processes of fermentation and respiration, organic molecules are eventually broken down to CO₂ which is returned to the atmosphere for continuous process of primary production. Biological nitrogen fixation is a process found only in some bacteria which remove N₂ from the atmosphere and converts it to ammonia (NH₃), for use by the plants and animals. Nitrogen fixation also results in replenishment of soil nitrogen removed by agricultural processes. Thus along with all these benefits, microbes greatly contribute in maintaining sustainability of environment. This chapter mainly focuses on beneficial and harmful impacts of microbes on environment and their role to maintain quality, health, and sustainability of environment.

Keywords

Microbe • Environment • Species interaction • Nutrient cycle • Bioremediation • Pathogen • Disease

3.1 Introduction

The Earth is known as a “closed system” where materials cycle between lithosphere (rocks), atmosphere (air), hydrosphere (water), and biosphere (organism) (Fig. 3.1). Together, they make up all the components of our planet, both living and nonliving. Earth produces everything it needs to ensure the survival and growth of its residents. Environment is defined as the circumstances or conditions that surround an organism or group of organisms. Environment is the complex of social or cultural conditions that affects an individual or community. Since humans inhabit the natural world as well as the built or technological, social, and cultural world, all constitute an important part of our environment.

Environmental studies need to understand the life processes at the microscopic level and ecologist levels from species to ecosystem. Species refer to organisms of the same kind that are genetically similar enough to breed in nature and produce live, fertile offspring. Population consists of all members of the same species living in a given area in the same time. All the populations of organism living and interacting in a particular area make up a biological community. An ecological system or ecosystem is composed of a biological community and its physical environment. The environment includes abiotic factor such as climate, water, minerals, and sunlight as well as biotic factors such as organisms, their products, and effect in a given area.

Photosynthesis is the basis of energy economy of all, but a few specific ecosystem and ecosystem dynamics are based in how organisms share food resources. In

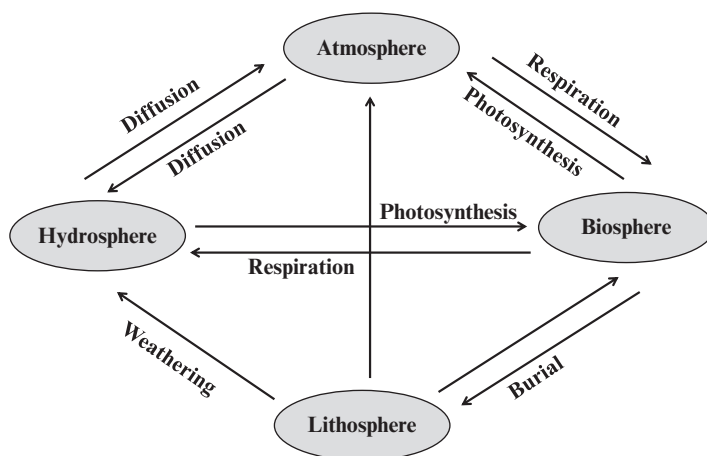


Fig. 3.1 Exchange of materials between different spheres of earth

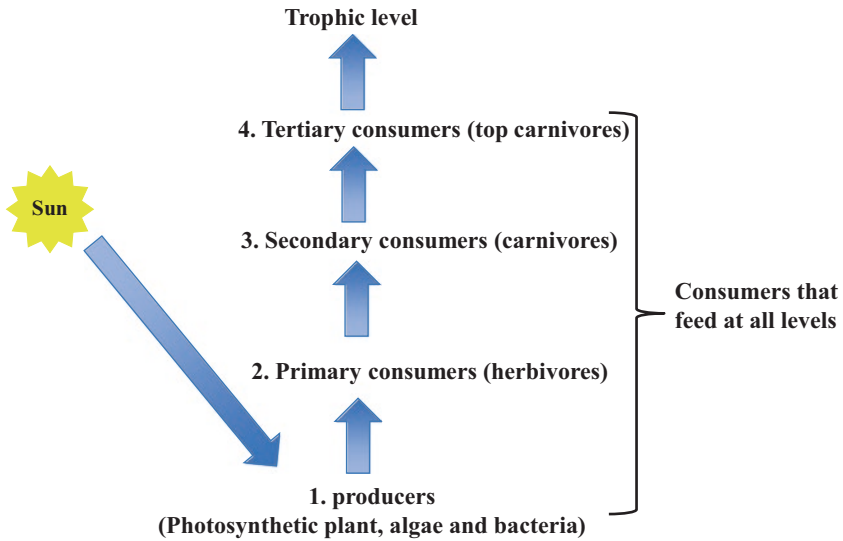


Fig. 3.2 Trophic cascade

fact one of the major properties of an ecosystem is its productivity, the amount of biomass produced in a given area during a given period of time. The rate of production of food creates a linked series of feeding known as food chain, whereas when individual food chains become interconnected, they form food web. An organism's feeding status in an ecosystem can be expressed as trophic level shown in Fig. 3.2.

3.2 Environmental Communities and Their Factors

The groups of similar species create population which results in a community. Microbial community means all microbial populations in a habitat. The activities of complex communities of microbes affect biogeochemical transformations in natural, managed, and engineered ecosystem. Microbial communities are very important for the rigorous progress in the field of agriculture which increases the rate of crop production. Microbial community may be terrestrial or aquatic.

3.2.1 Terrestrial Communities

A community of microbes and their environment that occurs on the landmasses of continents and islands form a terrestrial microenvironment. Terrestrial microenvironment is distinguished from the aquatic microenvironment ecosystems by the lower availability of water and the consequent importance of water as a limiting factor. Rain forests are the most diverse and productive terrestrial microenvironment, but their soil is nutrient deficient due to extensive leaching by rainwater.

3.2.1.1 Soil

Soil formation is a slow process that involves physicochemical weathering and biological processes over millions of years. Microbes play an important role in soil aggregate formation and soil stability that confer fertility and productivity to soil. The soil microbes participate in these processes through many ways, e.g., filamentous microbes assemble clay particles using extensive network of hyphae resulting into soil aggregates. Additionally, some microbes secrete exopolysaccharides or cause compaction of clay particles that promote soil aggregation. The surface soil is always rich in indigenous population of bacteria (including actinomycetes), fungi, algae, and protozoans. Additionally, human and animal activities also introduce specific microbes in the soil by several ways. Human activity directly adds bacteria as biodegradative agents or applying sewage sludge to agricultural fields. Animals introduce microbes through bird dropping or excretion.

3.2.1.2 Air

The atmosphere is an inhospitable climate for microbes because of stress due to dehydration. This results in a limited time frame for microbes to be active; however, some microbes get resistance to these stresses through specific mechanisms promoting loss of their biological activity. Spore-forming bacteria, molds, fungi, and cyst-forming protozoans all have specific mechanisms through which they are protected from these harsh gaseous environments. Therefore, viability is highly dependent on the environment, time they spend in the environment, and type of microbes. However, many other factors also influence the viability of microbes such as humidity, temperature, oxygen content, specific ions, UV radiation, various pollutants, and other air-associated factors (AOFs).

3.2.1.3 Relative Humidity

The relative humidity or relative water content of the air is critical for survival of airborne microbes. Most of the gram-negative bacteria associated with aerosols are able to survive for longer period at low relative humidity, whereas in contrast gram-positive bacteria remain viable longer in association with high relative humidity. The ability of microbes to survive in aerosol is related to the organism's surface biochemistry. One possible explanation of this fact could be a structural change in lipid bilayers of the cell membrane in response to very low humidity. During loss of water, the cell membrane bilayer changes from the typical crystalline structure to a gel phase and affects the surface protein configuration resulting into inactivation of the cell. The viruses with enveloped nucleocapsids (e.g., influenza virus) have longer airborne survival in low relative humidity below 50%, whereas viruses without nucleocapsids (e.g., enteric viruses) are able to survive in high relative humidity above 50%.

3.2.1.4 Temperature

Temperature is a critical factor influencing the activity of microbes. In general, high temperature leads to inactivation due to desiccation and protein denaturation, whereas lower temperature promotes longer survival rates. At very low temperature,

some microbes lose viability because of ice crystal formation on their surface due to freezing.

3.2.1.5 Radiation

Mostly radiation at low wavelengths, e.g., UV radiation and ionic radiation (X-rays), is harmful for microbes causing DNA damage. These radiations target DNA by producing single or double strand breaks and changing the structure of nucleic acid bases. UV radiation causes damage by forming intra-strand thymidine dimers causing inhibition of biological activity such as replication of genome, transcription, and translation. Several mechanisms including association of microbes with large airborne particles, pigments or carotenoids, high relative humidity, cloud cover, etc. protect microbes from these harmful radiations. However, many microbes (e.g., *Deinococcus radiodurans*) have evolved mechanisms to repair DNA damage caused by UV radiation.

3.2.1.6 Oxygen, OAF, and Ions

Oxygen, open-air factors (OAF), and ions combine to inactivate many species of airborne microbes. Some reactive forms of oxygen including superoxide radicals, hydrogen peroxide, and hydroxide radicals are produced due to lighting, UV radiation, or pollution and cause DNA damage by producing mutations. Similarly, OAFs (mixture of ozone and hydrocarbons) also cause inactivation of microbes by damaging nucleic acids and enzymes. In addition to these factors, positive ions cause only physical decay, e.g., inactivation of cell surface proteins, whereas negative ions confer both physical and biological damages such as DNA damage.

3.2.2 Aquatic Communities

Aquatic microenvironments occupy more than 70 % of the earth's surface including mostly ocean but also others such as estuaries, harbors, river, lakes, wetlands, streams, springs, aquifers, etc. The microbiota, living in aquatic environment, are the primary producers (responsible for approximately half of all primary production on earth) and primary consumers as well. A large variety of microbial communities live in aquatic environments such as the planktonic, sediment, microbial mat, bio-film communities, etc. Planktons refer to photoautotrophic microbial community including both eukaryotes (algae) and prokaryotes (cyanobacteria) and heterotrophic community including bacteria (bacterioplankton) and protozoans (zooplankton). Phytoplanktons are the primary producers in the food web using their ability to fix CO₂ into organic matter through photosynthesis. Aquatic microenvironment is further classified into three microenvironments, occupied by microbes living in freshwater, brackish water, and marine water.

3.2.2.1 Freshwater

The study of freshwater microenvironment is known as micro-limnology. There are two types of freshwater environment: standing water or lentic habitats (e.g., lakes,

ponds, bogs) and running water or lotic habitats including springs, rivers, and streams. Lentic habitats are dominated by phytoplankton, forming distinct community gradients based upon the wavelength and the amount of light that penetrates to a depth, e.g., *Chlorobium*. *Chlorobium* can utilize longer wavelength than other phototrophs and survive with little or no oxygen by consuming H_2S instead of H_2O for photosynthesis. In freshwater environment, two types of lakes are present: eutrophic and oligotrophic lakes. Oligotrophic lakes have higher rate (20–120 mg carbon/ m^3 /day) than eutrophic lakes (1–30 mg carbon/ m^3 /day) because eutrophic lakes have much higher levels of organic matter causing turbidity and interfering with light penetration. However, in terms of secondary productivity, eutrophic lakes have much higher rates (190–220 mg carbon/ m^3 /day) as compared to oligotrophic lakes (1–80 mg carbon/ m^3 /day).

3.2.2.2 Brackish Water

Brackish water environment is more saline than freshwater but less saline than marine water environment. An estuary, a part of river that meets with sea, is the best example of brackish water environment. Estuaries are highly variable environments because salinity changes drastically over a relatively short distance. Despite this, estuaries are highly productive environments, e.g., mangrove swamps in the Everglades of Florida, USA. Estuaries are generally turbid due to the large amount of organic matter brought by rivers and the mixing action of tides; therefore, light penetration is poor. Primary producers vary from 10^0 to 10^7 organisms/ml and in relation to depth and proximity to littoral zones. Despite the low primary productivity, substrate availability is not limited, and heterotrophic activity is high ranging from 150 to 230 mg carbon/ m^3 /day.

3.2.2.3 Marine Water

Marine water environments are highly diverse and contain 33–37% salinity. The ocean is divided into two zones on the basis of light availability: photic zone, where light can penetrate, and aphotic zone with lower light. Marine microenvironment is further divided into four habitats: neuston, pelagic, epibiotic, and endobiotic. Habitat at the surface of sea (air-water interface) is termed as neuston. On the basis of the precise depth, pelagic habitat is subdivided into epipelagic and benthopelagic zones. Epipelagic zone is found in upper 100 m of the water column, and a large proportion of organisms living in it are photosynthetic, whereas benthopelagic zone is sea-sediment interface. The third major habitat is the epibiotic habitats referring to surfaces on which attachment of communities occur, while the fourth is the endobiotic habitat with organisms (e.g., *Epulopiscium*) found within the tissues of other larger organisms such as fish.

3.2.3 Extremophilic Communities

The organisms living in physically or geochemically extreme conditions that are detrimental to most life on earth are termed as extremophiles. Most of the

extremophiles are microbes and belong to the domain Archaea. Here are some extreme environmental conditions where extremophiles survive.

3.2.3.1 High Temperature

Environments with high temperature ($>70^{\circ}\text{C}$) including terrestrial and submarine springs with a temperature of 100°C , hydrothermal vents with a temperature more than 300°C are inhospitable for most forms of life except some bacteria and archaeobacteria, e.g., *Thermus*, *Methanobacterium*, *Sulfolobus*, *Pyrodictium*, and *Pyrococcus*. *Pyrodictium* and *Pyrococcus* are capable of surviving at temperature $>100^{\circ}\text{C}$. Another example of such renowned extremophiles is *Thermus aquaticus* having thermotolerant DNA polymerase, which is widely used in the polymerase chain reaction (PCR). These thermophiles have developed such characteristic mechanisms facilitating proteins in folded state even at high temperatures due to increased salt bridges (cations that bridge charges between amino acid residues).

3.2.3.2 High Solute

Some organisms require salt concentrations substantially higher than that found in seawater for their growth, and they are known as halotolerant. *Halobacterium* and *Halanaerobium* are two examples of halotolerant bacteria; however, some algae and fungi also exhibit halotolerance feature. The main mechanism of salt tolerance operates by internal sequestration of high balancing solute (K^+ in bacteria and glycerol in halotolerant eukaryotes) equal to external salt concentration. A second mechanism of salt tolerance involves proteins with acidic and low proportion of nonpolar amino acids. These proteins require high salt concentrations to balance their charge for their optimal activity. Therefore, some obligate halophiles are unable to survive in the environment lacking high salt concentration due to these macromolecular modifications.

3.3 Microbes

There is a wide range of microbes present in our biosphere depending on their physical and other characteristics. Microbes fall into two groups, prokaryotes and eukaryotes, depending upon whether they have nucleus or not. Prokaryotes lack this membrane around their genetic material, and this group includes viruses, bacteria, and related archaea. The other category of microbes includes algae, fungi, protists, and other microscopic animals, having cell nucleus.

3.3.1 Bacteria and Archaea

Bacteria and archaea are the smallest free-living, unicellular organisms present on the earth. Their cell sizes typically range from 0.5 to $1.0\ \mu\text{m}$ in diameter. Both exist in various cell shapes, e.g., cocci, rods, or spirals, and some soil bacteria form branching filaments, e.g., actinomycetes. Their DNA is found free in the cell

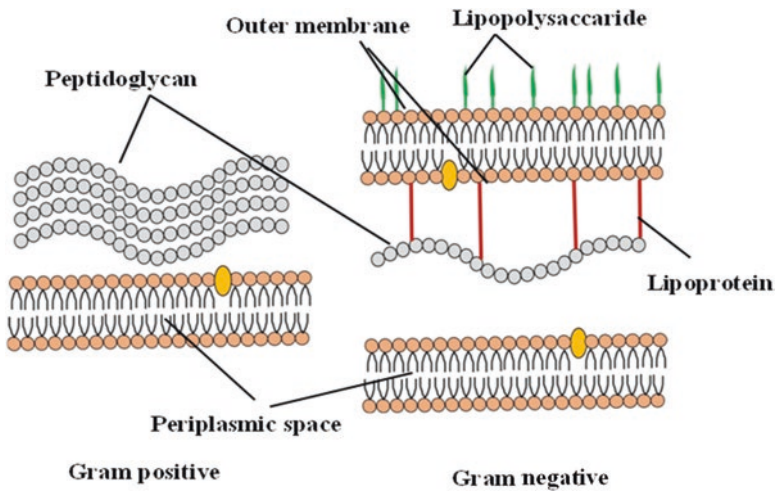


Fig. 3.3 Difference in gram-positive and gram-negative bacteria

cytoplasm and lack a true nuclear envelope, and the genome is mainly composed of single double-stranded DNA molecule with smaller DNA elements known as plasmids. The size of bacterial genome typically ranges from four to six million nucleotides in length and enable to code 3,000–4,000 genes. A bacterial cell envelope is composed of two layers, the inner layer is cell membrane made of phospholipids and the outer layer is cell wall made of proteins, carbohydrates, and lipids, but its composition varies based on the type of organism. Most of the microbes move through flagella (whiplike extensions from the cell) and file filaments, e.g., pili. The pili enable them to attach with each other or to soil particles. Additionally these pili are also involved in transfer of genetic material between bacterial cells, known as conjugation. These microbes usually reproduce asexually, e.g., binary fission, resulting in the formation of two genetically identical bacterial cells. On the basis of gram staining, bacteria are of two types: gram positive and gram negative; both vary in cell structure and physiology (Fig. 3.3). Bacteria and archaea both require carbon as building blocks of their cellular materials and energy to drive the reactions involved in cell biosynthesis and metabolism. Most of the bacteria utilize oxygen, whereas some bacteria and archaea grow anaerobically by using alternative electron acceptors, e.g., nitrate and sulfate.

Basically microbes are classified into autotrophs and heterotrophs. Autotrophs utilize sunlight or inorganic compounds such as Fe^{2+} , nitrate, or nitrite as energy source to fix atmospheric carbon dioxide to produce carbohydrates, fats, and proteins. However, heterotrophic bacteria use organic compounds as a source of carbon and energy. Archaea were originally known to be found in extreme environments and termed as “extremophiles,” but now they are widely distributed and are found in many environments including soil.

It is hard to distinguish both archaea and bacteria on the basis of their morphology. But most recently their classification using molecular phylogenetic tools based

on a comparison of 16S ribosomal rRNA sequences has revealed three separate domains of life: eukaryotes, bacteria, and archaea. Archaea are closely related to eukaryotes (all multicellular organisms) than the bacteria (Woese et al. 1990).

3.3.2 Fungi

Fungi belong to eukaryotes and, therefore, are more closely related to plants and animals than bacteria or archaea. Fungal cell consists of membrane-bound nucleus with chromosomes containing genetic material, e.g., DNA, membrane-bound organelles, e.g., mitochondria, and a cell wall composed of glucans and chitin. Fungi are basically heterotroph organisms meaning thereby that they derive their food from nonliving organic sources, e.g., saprophytic fungi, which feed on dead or decaying organic materials. Few fungi also exist as unicellular organisms, e.g., yeast, which grow through cylindrical threadlike structures (2–10 cm in diameter) known as hyphae. These hyphae may be either septate, e.g., compartmentalized through cross walls, or nonseptate. The hypha is a main part of fungus and constitutes a mycelium. Finely branched mycelium occupies a large surface area in the soil and produces a range of enzymes acting on soil organic matter to produce nutrients and energy required for fungal growth. Fungi can reproduce by both sexually, e.g., through spores, and asexually, e.g., budding or binary fission. Fungi are highly diverse and play a wide range of role in their surrounding environment such as decomposers, mutualists, endophytes of plants, pathogens, and predators. Fungal hyphae are the basic components of soil food webs since they constitute a food source for soil biota, whereas fungal sporocarps provide food for larger animals.

3.3.3 Protists

Protists are unicellular eukaryotes having characteristic organelles but lacking cell wall. Protists may be free living, parasitic, or opportunistic based on environmental changes. The size of protists varies from 2 μm to several centimeters. These are classified into four groups: flagellates (Mastigophora), amoebae (Sarcodina), sporozoans (Sporozoa), and ciliates (Ciliophora). Flagellates are characterized by the presence of flagella as a locomotive tool, multiplication by binary fission, and having both heterotrophic and autotrophic feeding mechanisms. Flagellates are further classified into two major divisions: photosynthesizing (Phytomastigophora) and non-photosynthesizing (Zoomastigophora). Autotrophic flagellates possess chloroplast and synthesize their own food or nutrients by photosynthesis such as *Euglena*, whereas heterotrophic flagellates are parasitic and take their nutrients from their host, for example, *Leishmania* and *Giardia*. Amoebae have pseudopodia as their locomotive tool. Pseudopodia help in ingestion of food materials and provide an extended basis of further classification. The Rhizopoda move by a fluid endoplasm, whereas the Actinopoda have a spikelike pseudopodium for their movement and feeding. These organisms are vacuolated and covered in a shell-like outer layer

known as “test.” These shells may be composed of proteinaceous, siliceous, or calcareous substances and have either a single or multiple chambers. Some testate amoebae are also found in soil, building their shells by excreting substances capable of aggregating soil particles. Some important free-living soil amoebae are grouped in the family *Vahikampfia*. *Entamoeba* is well-known parasitic amoeboid causing dysentery in humans. The ciliates have hairlike structures in an ordered array surrounding the cell known as cilia that divides by transverse fission. Cilia help in their locomotion and feeding. Ciliates are generally free living such as *Paramecium*, but some species are adapted to parasitic life cycles. Sporozoans are mostly parasitic and form spores. Few members are adapted in a symbiotic relationship and have no locomotive organ; therefore, they rely on vectors or direct contact with susceptible host to continue their growth and replication. For example, some species have evolved to enable digestion in the gut of domestic livestock. However, parasitic members are totally dependent on host for their nutrition, for example, *Toxoplasma*, *Isospora*, and *Plasmodium*. Most of parasitic protists are of obvious public health concern causing deadly diseases such as malaria, sleeping sickness, Chagas disease, leishmaniasis, giardiasis, cryptosporidiosis, etc. These parasites have adapted themselves for surviving and reproducing in their hosts by evading host immune responses. Many flagellated protists are capable of forming cysts that are known to survive conventional methods of disinfection and can be transmitted to their host via a water route. *Entamoeba histolytica* is a parasitic amoeba and causes diarrhea and dysentery. *Naegleria* is a free-living amoeba in freshwater, causing infections in nasal passage of humans and capable of invading brain tissues. *Toxoplasma* is an invasive protist that causes blindness and serious illness or death in unborn fetuses. *Cryptosporidium* is responsible for a number of epidemics including the largest US waterborne outbreak in Milwaukee. *Plasmodium* is a main causative agent of mosquito-borne disease, malaria. Domestic animals are also at risk of serious illness and death from parasitic protozoans, for example, *Histomonas*, *Trichomonas*, etc.

3.3.4 Viruses

Viruses are small, obligate, intracellular pathogens that require a host cell for their growth and replication. They can survive outside the host cell but not multiply without a host. Viruses are generally species specific and can infect all types of life forms: bacteria, plants, and animals. Public focus is most often on (1) plant viruses affecting important crops such as tobacco, potatoes, and tomatoes or (2) animal viruses causing deadly diseases: herpes, smallpox, rabies, mumps, measles, meningitis, hepatitis, encephalitis, influenza, diarrhea, yellow and dengue fever, etc. The basic virus structure includes a capsid protein coat and an internal nucleic acid (RNA or DNA), but some viruses may also have protein and lipid envelopes, glycoprotein spikes, or more complex tail and sheath structures. A number of protein capsomers held together by non-covalent bonds form a capsid coat surrounding the nucleic acid molecule. The size of capsids ranges from 18 μm , as in small parvovirus of animals, to several hundred nanometers, as in some filamentous plant viruses.

This outer coat protects and shields the viral nucleic acid and harbors specific receptor sites for attachment on hosts. The viral capsids have two types of symmetrical organization: helical and icosahedral. The helical viruses look like a spiral or a helix with a cylindrical shape, whereas icosahedral viruses adopt a 20-triangular-sided spherical shape when viewed with an electron microscope. Viruses have either RNA or DNA in the double-stranded or single-stranded form. Viral nucleic acid length varies from 1.7 to over 200 kb, and it encodes 4–200 genes.

3.4 Microbial Diversity

Early studies on diverse soil bacteria and fungi are mainly focused on what could be easily cultured from soils, but the fact is that less than 10% of the soil bacteria could be cultured, suggesting the requirement of other approaches. Norman Pace and colleagues in 1980 found that microorganisms could be identified in naturally occurring microbial populations without culturing them (Hugenholtz et al. 1998), but this process requires the PCR amplification of the rRNA genes using rRNA specific primers and RNA extracted directly from the cells present in soil. These specific primers may differentiate among various microbial communities at level of different domains such as Bacteria, Eukarya, and Archaea or phylum (e.g., *Actinobacteria* or *Bacteroidetes*) (Fig. 3.4). However, a range of approaches could be adopted in order to separate and sequence the rRNA genes. The advanced high-throughput DNA sequencing now allows the identification of each individual in thousands of samples within a short duration (Caporaso et al. 2012). Once we compare these rRNA gene sequences from cultivated species using various online databases, e.g., GenBank, allowing identification of evolutionary (phylogenetic) relationships

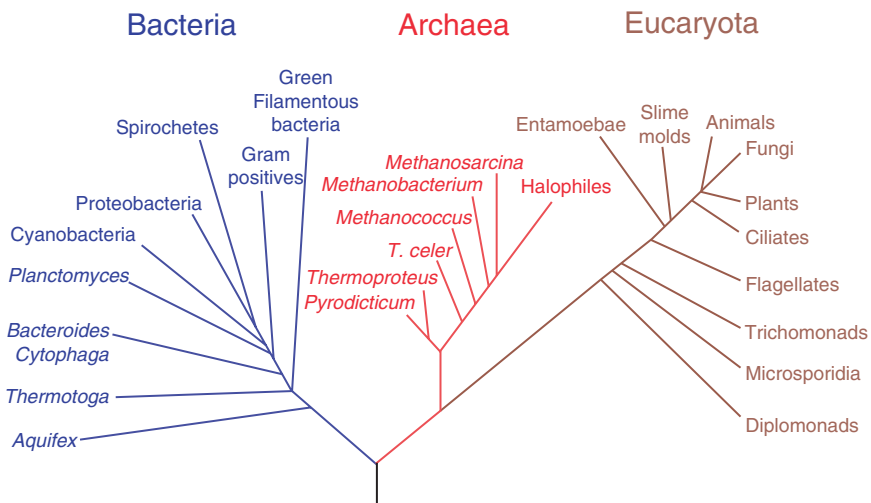


Fig. 3.4 Phylogenetic classification of living world based on 16S and 18S rRNA gene sequences

among various unknown and known organisms, it displays an estimate of the genetic diversity of organisms in a particular community. It is easy to speculate about the organism's characteristics and its closest cultivated relative on the basis of sequencing details. Phylogenetic information sometimes also provides details about the physiology, e.g., all cyanobacteria constitute a monophyletic group in a similar way as sulfate-reducing bacteria, halophiles, and methanogenic archaea do.

3.4.1 Bacterial Phyla

The composition of in situ environmental bacterial communities has been investigated using various molecular tools. The relative abundance of the major phyla varies among diverse soils and environmental conditions such as some members of the phyla, i.e., *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*, are abundant and widely distributed; however, members belonging to *Verrucomicrobia*, *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, *Planctomycetes*, and *Gemmatimonadetes* are comparatively less prevalent.

The *Proteobacteria* is a diverse group of organisms among various subphyla out of which, α -, β -, γ -, and δ -*Proteobacteria* are most commonly found in soil. The members belonging to α -, β -, and γ -subphyla are copiotrophs (an organism able to grow in nutrient-rich environments particularly carbon in contrast to oligotrophs, those found in environments with much lower carbon concentration). The *Proteobacteria* are more prevalent in those area rich in resource availability, e.g., rhizosphere soils.

The α -*Proteobacteria* consist of various metabolically diverse heterotrophic and autotrophic bacteria, e.g., heterotrophic bacteria such as *Sphingomonas*, that are able to degrade various toxic compounds including pentachlorophenol and polycyclic aromatic hydrocarbons and also involved in weathering of minerals. Some heterotrophs that are also able to fix atmospheric nitrogen by forming symbiotic relationships with legumes belong to family Rhizobiaceae such as *Rhizobium*, *Mesorhizobium*, and *Bradyrhizobium*. The autotrophic α -*Proteobacteria* also include soil methane oxidizers, e.g., *Methylobacter* and *Methylophilus*, nitrite oxidizers, e.g., *Nitrospira* and *Nitrobacter*, and phototrophs, e.g., *Rhodospirillum* and *Rhodobacter*.

The β -*Proteobacteria* is also found into three groups: heterotrophs, autotrophs, and methanotrophs. Some of the known heterotrophs found in soil belong to the genera *Burkholderia*, *Alcaligenes*, and *Acidovorax*. Out of these, *Burkholderia* spp. is metabolically diverse and uses simple amino acids, sugars, and recalcitrant aromatic and phenolic compounds as a source of carbon and therefore plays a major role in carbon turnover. *Burkholderia* spp. is also known to promote plant growth by fixing atmospheric nitrogen. Some examples of heterotrophic β -*Proteobacteria* are *Collimonas*, able to degrade live hyphae by producing chitinase, and autotrophic β -*Proteobacteria* are *Nitrosospira* (ammonia oxidizer), *Thiobacillus* (iron oxidizer), *Methylomonas* (methane producer), *Rhodocyclus* (phototroph), etc.

The γ -*Proteobacteria* are also categorized into heterotrophs, lithotrophs, and phototrophs. *Pseudomonas* and *Xanthomonas* are well-known heterotrophs.

Pseudomonas spp. has a remarkable nutritional versatility since most of them are able to grow on more than 50 or 100 different substrates including sugars, amino acids, fatty acids, alcohols, and hydrocarbons. The γ -*Proteobacteria* also include various photolithotrophs, e.g., *Thiocapsa* and *Chromatium*, that utilize sulfide or elemental S as an electron donor and CO₂ as a source of carbon under anaerobic conditions in light.

The δ -*Proteobacteria* consist mainly of SO₄²⁻- and Fe-reducing bacteria. *Desulfovibrio*, a sulfate-reducing bacteria, grow anaerobically by utilizing lactate or ethanol as carbon sources, found in oxygen-depleted soil. This group also includes a parasitic bacteria named as *Bdellovibrio*.

Acidobacteria are widely distributed and found in abundance in the soil with low pH. Since they are poorly present in soil culture collections, a little knowledge is available about their metabolic capabilities. The complete genome sequencing of cultured soil *Acidobacteria*, e.g., *Acidobacterium capsulatum*, implies that they may be oligotrophs and able to metabolize a range of simple and complex carbon sources. These bacteria are also found in low nutrient conditions, tolerant to changes in soil moisture. They also play a role in nitrate and nitrite reduction but not in denitrification or nitrogen fixation.

Verrucomicrobia, also found commonly in soil, are oligotrophic in nature; however, the ecology of Verrucomicrobia is poorly understood. The majority of bacteria of this group are *Chthoniobacter flavus* and *Opitutus terrae*. Genome sequencing of a free-living heterotroph bacteria found in aerobic soil, e.g., *Chthoniobacter flavus*, suggests that it is able to metabolize plant polysaccharides but not amino acids except pyruvate. However, genome sequencing of a verrucomicrobium from rice paddy soil, e.g., *Opitutus terrae*, implies that it is an anaerobic bacterium, capable of producing propionate through fermentation of polysaccharides from plants.

Gram-positive bacteria are abundant in soil culture collections and categorized into two groups: *Actinobacteria* and *Firmicutes*. The *Actinobacteria* are commonly found in soil and further classified into three subphyla: *Actinobacteridae*, *Acidimicrobidae*, and *Rubrobacteridae*. The abundance of *Actinobacteridae* in soil relatively increases with addition of labile carbon sources. The *Actinobacteridae* includes metabolically diverse aerobic heterotrophs, e.g., *Arthrobacter*, *Rhodococcus*, *Streptomyces*, and *Mycobacterium*. *Streptomyces* is a well-known bacterium producing antimicrobial compounds. The *Rubrobacteridae* includes two genera not represent in soil culture collections, e.g., *Rubrobacter* and *Solirubrobacter*. *Acidimicrobium ferrooxidans* is an acid-tolerant ferrous iron oxidizer bacterium and has been detected in soil culture collections among few members of *Acidimicrobidae*.

The *Firmicutes* include bacteria that are able to form endospores such as *Bacillus* and *Clostridium*, and because of endospore production, they are able to survive longer in the soil during dry periods. *Bacillus* is capable of degrading various carbon sources, including polysaccharides from plants, whereas *Clostridium* may ferment sugars, starch, pectin, and cellulose. The addition of recalcitrant C compounds in soil favors the growth of Clostridiales.

Planctomycetes multiply through budding and lack peptidoglycan in their cell walls. These bacteria are also involved in ammonium oxidation (Anammox) in soil

under anaerobic conditions. *Planctomycetes*, Verrucomicrobia, and Chlamydia are important from evolutionary point of view since they share numerous features, e.g., presence of membrane-coat-like proteins and condensed DNA, rarely found in bacteria but more common in Archaea and Eukaryotes. *Sphingobacteria* are known to be involved in aerobic degradation of plant materials present in soil and complex organic molecules, e.g., starch, proteins, cellulose, and chitin. Members belonging to genus *Chitinophaga* are filamentous and chitinolytic and exhibit gliding movement.

3.4.2 Archaeal Phyla

Archaea are known to be widely distributed in the soil on the basis of 16S rRNA gene sequences (Bates et al. 2011), and additionally these gene sequences suggest that members belonging to the phylum Crenarchaeota are found in abundance in the marine environment. All cultured Crenarchaeota are thermophilic or hyperthermophilic organisms: having the ability to survive at a temperature up to 113 °C. Crenarchaeota is sulfur-dependent extremophile; one of the best-characterized members is *Sulfolobus solfataricus*. This organism was originally isolated from geothermally heated sulfuric springs in Italy and grows at 80 °C and pH of 2–4. These organism stains are gram negative and are morphologically diverse having rod-, cocci-, filamentous-, and oddly shaped cells. The *Crenarchaea* play a role in ammonia oxidation in the soil. Mesophilic ammonia-oxidizing archaea (AOA) is abundant in a diverse range of marine environments, including the deep ocean, as revealed by the quantification of the archaeal *amoA* gene encoding the alpha-subunit of the ammonia monooxygenase. *Nitrososphaera viennensis* is a most recent ammonia-oxidizer *Crenarchaea* that was extracted from garden soil, and subsequent phylogenetic analysis confirmed its taxonomic affiliation.

Methanogens are strict soil anaerobes and grow in association with bacteria that participate in the anaerobic food chain and convert complex organic molecules to methane (CH₄) and CO₂. Methanogens generate methane through various pathways. They display various activities such as reduction of carbon dioxide and methanol, cleavage of acetate, and methane production from methylated compounds. Members belonging to genera *Methanosarcina*, *Methanosaeta*, and *Methanocella* are widely distributed in the environment, and both *Methanosarcina* and *Methanosaeta* produce methane through acetate reduction.

3.4.3 Fungal Phyla

Seven fungal phyla are currently recognized: *Chytridiomycota*, *Blastocladiomycota*, *Neocallimastigomycota*, *Glomeromycota*, *Ascomycota*, *Basidiomycota*, and parasitic endobionts, e.g., *Microsporidia*. Among these fungal phyla, the first three have flagellated cells at least during one stage of their life cycle and thereby turned into terrestrial organisms in contrast to higher fungi that lack motile cells. Among these

phyla, *Chytridiomycota* is a basal group and may degrade chitin and keratin. *Batrachochytrium dendrobatidis* is a known pathogenic unicellular chytrid of many amphibian species resulting into decline of worldwide amphibian population. *Blastocladiomycota* differ from the *Chytridiomycota* in reproduction since they exhibit different form of meiosis.

Glomeromycota exhibit some features identical to “lower” fungi, e.g., they have multinucleate aseptate mycelia and most of them have no known sexual stages. They reproduce through large thick-walled asexual spores, commonly found in soils, and may germinate in the presence of a plant root. This phylum includes arbuscular mycorrhizal (AM) fungi that form obligate biotrophic symbioses with mosses, approximately 80% of all land plants, and cyanobacterium *Nostoc* forming cyano-lichens. These higher fungal phyla have a characteristic feature having two compatible nuclei in a hyphal cell also known as dikaryon.

Ascomycota is one of the largest fungal phyla with 64,000 or more number of species. The members of this phylum have a characteristic spore-bearing saclike structure also known as asci, produced in large numbers during sexual reproduction. Ascomycetes mostly reproduce asexually and are rarely found to reproduce through sexual mating. Ascomycetes have a typical haploid mycelium with septate hyphae and cell wall made up of chitin and β -glucans. Few macroscopic ascomycetes exhibit well-known reproductive structures such as morels, truffles, etc. However, many ascomycetes are microscopic unicellular organisms, e.g., yeast or *Saccharomyces*, and filamentous fungi, e.g., *Aspergillus*. Most of the Ascomycetes are saprobes and have various enzymes to degrade complex substrates such as cellulose, keratin, and collagen. Therefore, ascomycetes are known to play a critical role in decomposing and nutrient recycling. Approximately 18,000 species of ascomycetes form lichens through symbiotic relationship with green algae and cyanobacteria. However, other *Ascomycota* constitute ectomycorrhizal and/or ectendomycorrhizal associations through symbiosis with woody plants. Some ascomycetes are known plant parasites and predators. The family *Orbiliaceae* includes carnivorous fungi having specialized hyphae to trap prey including a range of soil mesofauna, protists, nematodes, and arthropods.

Members belonging to *Basidiomycota* are also known as club fungi and produce spores during sexual reproduction on club-like stalks known as basidia. These microscopic basidia are basically clustered on specialized structures also known as sporocarps. Numerous haploid spores are produced after meiosis and released in the environment resulting into a new haploid mycelium after germination.

3.5 Microbial Application to Environment

3.5.1 Contribution of Microbes to Nutrient Cycling

3.5.1.1 Microbes in Carbon Cycle

Microbes play a critical role in carbon cycle on the global scale that is a key constituent of all living organisms (Fig. 3.5). Microorganisms avail carbon for living

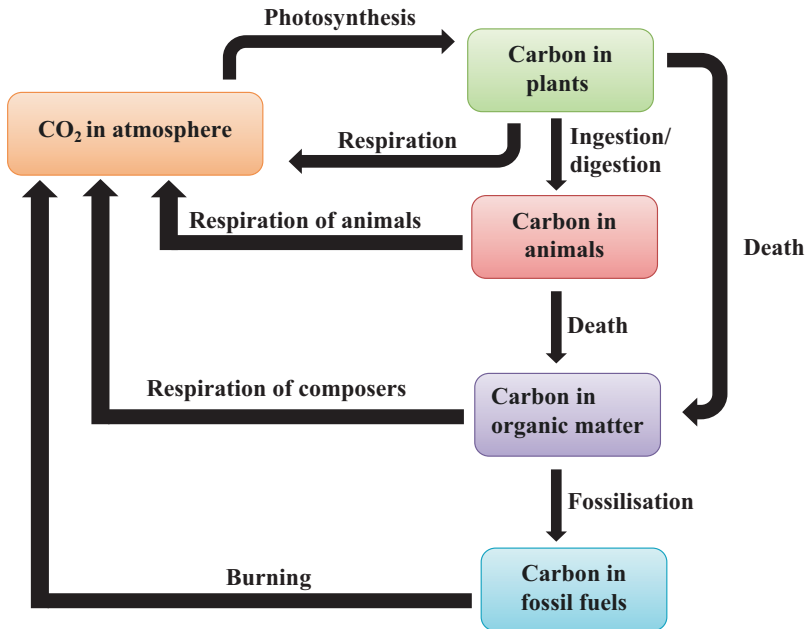


Fig. 3.5 Role of microbes in carbon cycle

organisms and for themselves as well through extracting it from nonliving sources. In aquatic habitats, microbes convert carbon anaerobically, present at oxygen-free zones such as deep mud of lakes and ponds. Carbon dioxide (CO₂) is the most common form of carbon that enters into a carbon cycle. CO₂ is a water-soluble gas present in the atmosphere. Plants and photosynthetic alga use CO₂ during photosynthesis to synthesize carbohydrates. Additionally chemoautotrophs such as archaea and bacteria also utilize CO₂ to synthesize sugars. This carbon, present in the form of sugar, is further processed through a chain of reactions during respiration known as tricarboxylic acid cycle resulting into energy. Microbes may also use carbon under anaerobic conditions to produce energy through a process called as fermentation.

Plants are the primary producers in a terrestrial ecosystem; however, free-living planktons, cyanobacteria, and symbionts such as lichens also contribute in fixing carbon in some ecosystems. Nonliving organic material is recycled by heterotrophic bacteria and fungi, whereas saprobes utilize organic material and produce CO₂ during respiration, thereby contributing to carbon cycle. However, higher animals, e.g., herbivores and carnivores, also digest organic materials to obtain energy using gut microbiota residing in their intestinal tracts; the process is known as decomposition, resulting into inorganic products such as CO₂, ammonia, and water.

Actinobacteria and *Proteobacteria* are capable of degrading soluble organic compounds, e.g., organic acids, amino acids, and sugars (Eilers et al. 2010). Similarly, bacteroidetes are also involved in degradation of more recalcitrant carbon compounds, e.g., cellulose, lignin, and chitin, and utilize higher level of available

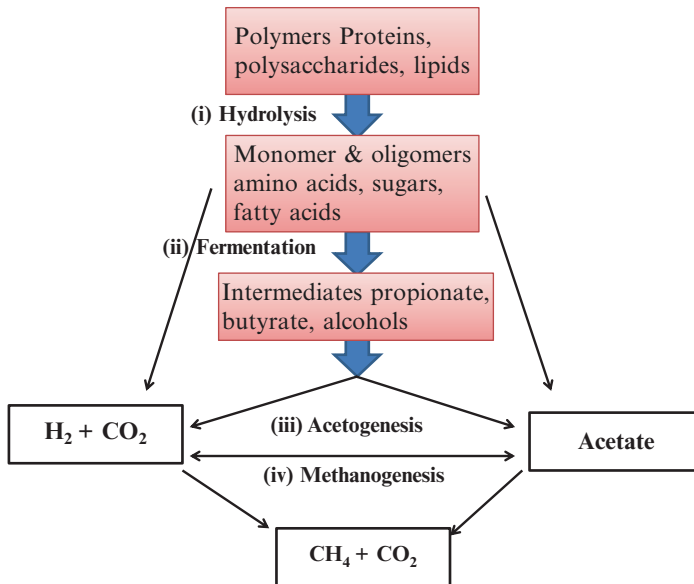


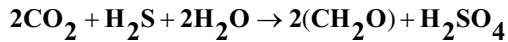
Fig. 3.6 Production of methane by microbes

nitrogen that help in production of extracellular and transport enzymes (Treseder et al. 2011). On the contrary, bacteria living in low-nitrogen environments are more able to metabolize nitrogen-rich organic compounds, e.g., amino acids. The abundance of *α-Proteobacteria* and *Bacteroidetes* favors carbon mineralization, whereas *Acidobacteria* oppose it (Fierer et al. 2007).

3.5.1.2 Microbes in Methane Production

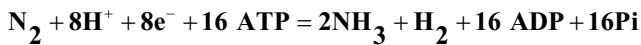
Some microbes execute anaerobic or fermentative degradation of organic compounds into organic acids and some gases, e.g., hydrogen and CO_2 . Methanogens are able to use that hydrogen to reduce CO_2 into methane under strict anaerobic conditions (Fig. 3.6). In order to complete cycle, methane-oxidizing bacteria, e.g., methanotrophs, transform methane to CO_2 , water, and energy under aerobic conditions.

Other microbes such as green and purple sulfur bacteria participate in carbon cycle by degrading hydrogen sulfide (H_2S) into compounds having carbon during energy production (see in reaction). Some bacteria, e.g., *Thiobacillus ferrooxidans*, derive energy from oxidation of ferrous iron to ferric iron and thereby contribute to carbon cycle. Few microbes such as *Bacteroides succinogenes*, *Clostridium butyricum*, and *Syntrophomonas* spp. make a collaborative effort (also known as interspecies hydrogen transfer) for anaerobic degradation of carbon to produce CO_2 and methane in bulk. The following reaction shows anaerobic photoautotrophism in purple sulfur bacteria:



3.5.1.3 Microbes in Nitrogen Cycle

Nitrogen is an essential element present in protein and nucleic acid structure. Microorganisms play a critical role in nitrogen cycle through various processes such as nitrogen fixation, nitrate reduction, nitrification, denitrification, etc. (Fig. 3.7). The microbial processes limit the productivity of an ecosystem because nitrogen availability is a limiting factor for plant biomass production. Ammonification involves decomposition of organic nitrogen into ammonia. Both bacteria and archaea are capable of fixing atmospheric nitrogen through reduction into ammonium (Fig. 3.7). Nitrogenase is an oxygen-sensitive enzyme that catalyzes nitrogen fixation under low oxygen environment. N-fixation requires energy in form of ATP (16 mol) per mole of fixed nitrogen.



The free-living microbes such as *Azotobacter*, *Burkholderia*, and *Clostridium* have an ability to fix nitrogen, and few of them form a symbiotic relationship with the rhizosphere of plants such as *Rhizobium*, *Mesorhizobium*, and *Frankia*. *Sophora* and *Clianthus* are native legumes and form a symbiotic relationship with *Mesorhizobium* or *Rhizobium leguminosarum*. The symbiotic rhizobia are able to fix nitrogen by two or three orders of magnitude higher than free-living soil bacteria.

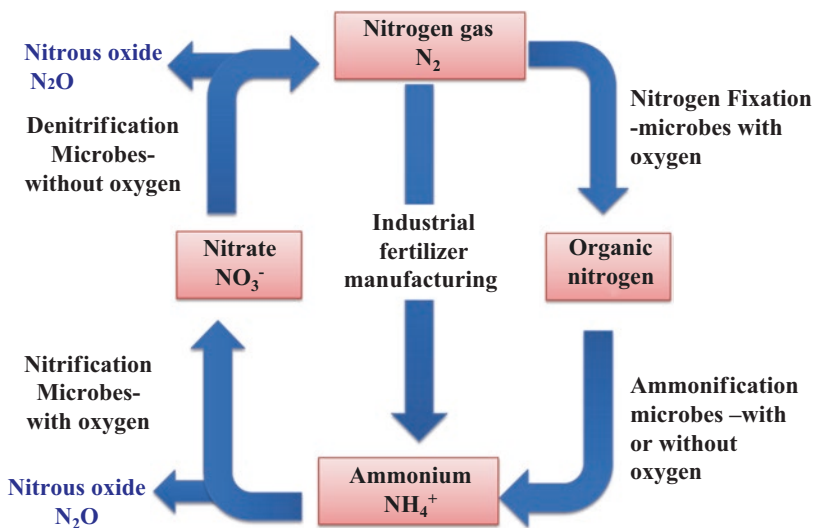


Fig. 3.7 Role of microbes in nitrogen cycle

Nitrification involves two steps: first, ammonia is oxidized to nitrite and then to nitrate. The oxidation of ammonia to nitrite is carried out by few soil bacteria, e.g., *Nitrosospira*, *Nitrosomonas*, *Crenarchaeum*, or *Nitrososphaera*, and thereafter nitrite is oxidized to nitrate by some bacteria, e.g., *Nitrobacter* and *Nitrospira* (Fig. 3.7). Nitrification also changes the ionic state of soil from positive to negative through oxidation of ammonia to nitrite and release of energy, which is used by nitrifying microbes to assimilate CO_2 .

Denitrification involves sequential reduction of nitrate (NO_3^-), nitrite (NO_2^-), and nitric oxide (NO) to the greenhouse gas nitrous oxide (N_2O) or benign nitrogen gas (N_2). Since this process requires limiting oxygen, therefore, it occurs mostly in waterlogged areas that provide anaerobic environment. Nitrogen cycle involves denitrification process through which fixed nitrogen returns back to the atmosphere from soil and water in order to complete the nitrogen cycle. Denitrification involves a range of soil microbiota belonging to *Proteobacteria*, *Actinobacteria*, and *Firmicutes* and other soil eukaryotes. Most of the bacteria lack single or multiple enzymes involved in denitrification and known to be incomplete denitrifier, for example, most of the fungi and bacteria lack nitrous oxide reductase and thereby produce N_2O as a final product. Therefore, incomplete denitrification results into emission of greenhouse gases.

3.5.1.4 Microbes in Sulfur Cycle

Sulfur is an important component of a couple of vitamins and essential metabolites, and it is found in two amino acids, cysteine and methionine. In spite of its paucity in cells, it is an absolutely essential element for living systems. Like nitrogen and carbon, the microbes can transform sulfur from its most oxidized form (sulfate or SO_4) to its most reduced state (sulfide or H_2S) (Fig. 3.8). The sulfur cycle, in

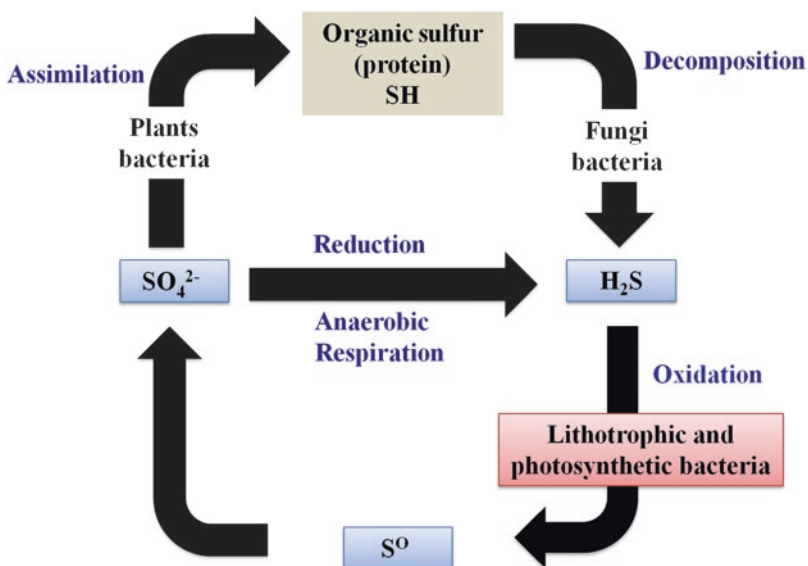


Fig. 3.8 Role of microbes in sulfur cycle

particular, involves some unique groups of prokaryotes. Two unrelated groups of prokaryotes oxidize H_2S to S and S to SO_4 . The first is the anoxygenic photosynthetic purple and green sulfur bacteria that oxidize H_2S as a source of electrons for cyclic photophosphorylation. The second is the “colorless sulfur bacteria” (now a misnomer because the group contains many archaea) which oxidize H_2S and S as sources of energy. In either case, the organisms can usually mediate the complete oxidation of H_2S to SO_4 .



Sulfur-oxidizing prokaryotes are frequently thermophiles found in hot (volcanic) springs and near deep-sea thermal vents that are rich in H_2S . They may be acidophiles as well, because they acidify their own environment by the production of sulfuric acid. Since SO_4 and S may be used as electron acceptors for respiration, sulfate-reducing bacteria produce H_2S during a process of anaerobic respiration analogous to denitrification. The use of SO_4 as an electron acceptor is an obligatory process that takes place only in anaerobic environments. The process results in the distinctive odor of H_2S in anaerobic bogs, soils, and sediments where it occurs. Sulfur is assimilated by bacteria and plants as SO_4 for use and reduction to sulfide. Animals and bacteria can remove the sulfide group from proteins as a source of S during decomposition. These processes complete the sulfur cycle.

3.5.1.5 Microbes in Phosphorus Cycle

Phosphorus is a critical element of various building blocks such as nucleic acids, e.g., DNA and RNA, ADP, ATP, and phospholipids. Phosphorus is a rare element in the environment because of its tendency to precipitate in the presence of divalent and trivalent cations at neutral and alkaline pH.

Microorganisms (bacteria and fungi) mineralize organic phosphate in the form of phosphate esters into inorganic phosphate through a process driven by phosphatase enzymes (Fig. 3.9). Additionally, they also convert insoluble phosphorus into soluble form by a reaction with resulting byproducts such as organic acids. Mycorrhizal fungi help plants to overcome phosphorus limitation through its mobilization from insoluble mineral form by producing oxalate, e.g., various ectomycorrhizal basidiomycetous fungi express phosphate transporters in their extraradical hyphae during phosphorus deficiency in surrounding environments.

3.5.2 Contribution of Microbes in Recycling Wastes and Detoxification

Bacteria and fungi are able to biodegrade or detoxify substances through various ways; thereby, microbial processes are extensively used for bioremediation.

3.5.2.1 Biodegradation

Bioremediation/biotransformation is a waste management tool that involves naturally occurring organisms to remove or neutralize hazardous waste into less toxic or

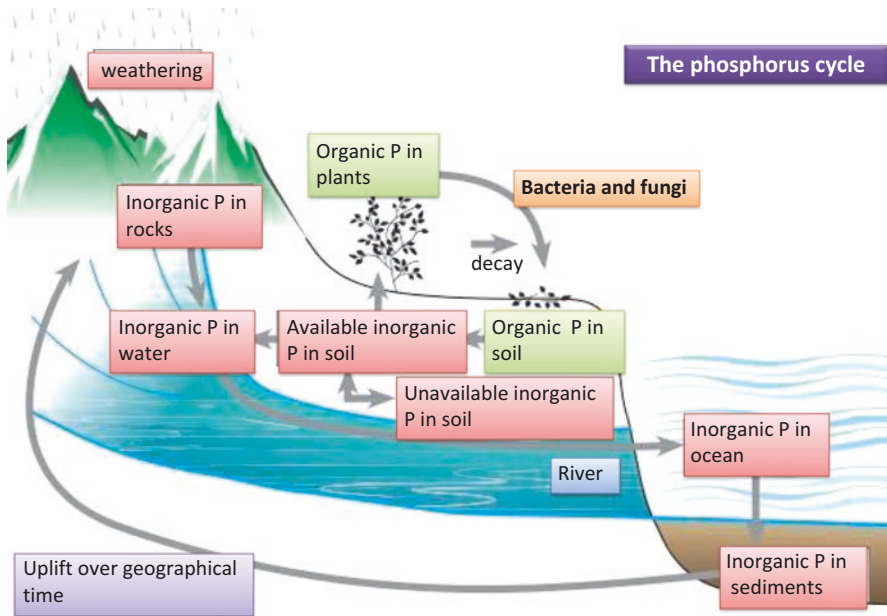


Fig. 3.9 Role of microbes in phosphorus cycle

nontoxic substances. The most commonly used microorganisms are *Flavobacterium*, *Arthrobacterium*, and *Azotobacter*. Bioremediation focuses on different sources and hence is called with different names:

Plant → Phytoremediation

Fungi → Mycoremediation

Biotechnological treatment of waste management involves use of microorganisms to detoxify air, water, and soil pollutants and carried out at lower temperature and pressure; therefore, it requires less energy than the conventional physicochemical treatment method. Depending upon the types of contaminants' site of monitoring and favorable environmental conditions, bioremediation may be carried out either *in situ* or *ex situ* (Table 3.1).

Biostimulation and bioaugmentation processes promote the rate of degradation of organic and inorganic pollutants (Fig. 3.10). These treatment technologies have been found eco-friendly and cost-effective means of pollution control leading to increased public acceptance and compliance with environmental legislation.

Heterotrophic microbes such as *Pseudomonas*, *Sphingomonas*, and *Mycobacterium* are known to be involved in oil degradation. *Pseudomonas* is one of well-studied bacteria capable of degrading alkanes, monoaromatics, naphthalene, and phenanthrene under aerobic conditions. The hydrocarbon-degrading bacteria are dominant in soil contaminated with oil; however, higher concentration of hydrocarbons may deplete available nitrogen and phosphorus in that area since these elements are assimilated during biodegradation.

Table 3.1 Bioremediation and its types

Type	Example	Benefits	Limitations	Factors involved
<i>In situ</i>	Bioventing	Cost efficient, noninvasive, relatively passive, natural attenuation, treats soil and water	Environmental constraints, extended treatment time difficulties	Biodegradation abilities of indigenous microorganism, presence of metals and other inorganics, biodegradability of pollutants, chemical solubility, geological factor and pollutant distribution
	Biosparging			
	Biostimulation			
	Bioaugmentation			
	Rhizofiltration			
<i>Ex situ</i>	Landforming	Cost-effective, less time	Space requirement, extended treatment time, abiotic loss, mass transfer problem, bioavailability limitation	As above, toxicity of amendment, toxic concentration of contaminants
	Composting			
	Biopiles			
Bioreactor	Slurry reactor	Rapid degradation, optimized environmental parameters, enhanced mass transfer, effective use of intoxicant and surfactants	Soil require excavation, relatively high cost	As above, toxicity of amendment, toxic concentration of contaminants
	Aqueous reactor			

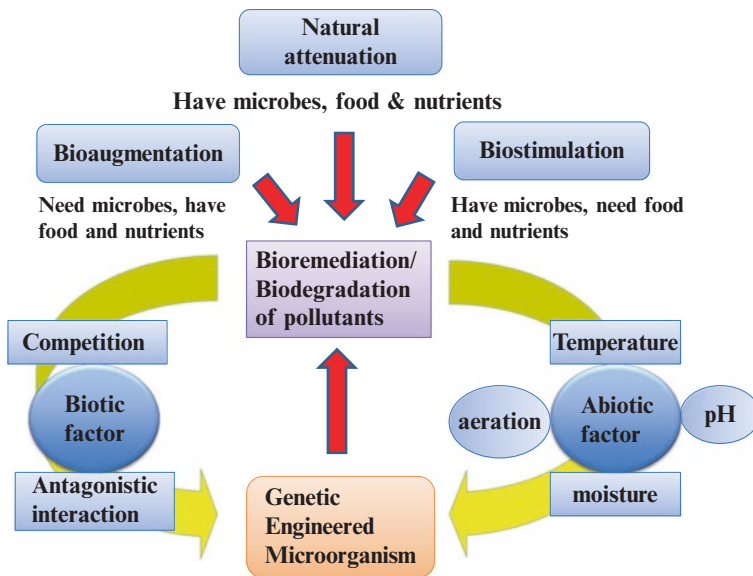


Fig. 3.10 Overview of bioremediation

Microbes (bacteria and fungi) are able to degrade a range of biodegradable pesticides such as atrazine, which is degraded by a bacterium, e.g., *Arthrobacter nicotinovorans*, and related derivatives such as simazine, propazine, and cyanazine (Aislabie et al. 2005). Non-biodegradable pesticides, e.g., DDT (dichlorodiphenyl-trichloroethane), are not readily degraded and still persist in the soil. Some fungi having ability to degrade lignin, such as *Phanerochaete chrysosporium*, are able to degrade various contaminants such as pentachlorophenol and dioxin, and the best example are *Zygomycetes* that degraded various contaminants during wood-treating operation in Whakatane (Thwaites et al. 2006).

Biodegradation of a contaminant depends upon its chemical structure and physical state since various contaminants, e.g., oil are readily degradable, but synthetic contaminants, e.g., DDT and aldrin, are nondegradable and persist in the environment. The ability for degradation also depends upon rare and novel structures and water solubility since less soluble compounds are difficult to degrade. Additionally, poorly water-soluble or hydrophobic contaminants also readily bind to clay particles and, therefore, are easily available to microbes present in soil. These soil microbes utilize these contaminants as energy source, present at higher concentration, and these could be toxic for them, resulting into slow biodegradation. Biodegradation also involves a contact between contaminants and microbes. Some microbes, e.g., chemotactic bacteria and fungi, have an ability to sense and move toward them.

3.5.2.2 Metal Detoxification

The microbial ability to withstand metal toxicity and their physiological adaptation to metal stress has an important significance. Indeed, the expression and activity of proteins involved in metal uptake are crucial for metal resistance, and different bacteria adapt distinct complements of these systems. Bacteria have evolved some regulatory mechanisms to control membrane transporter activity that take up metals, and some of these activities are determined by regulators that bind to metal ions with femtomolar activities. In response to metal exposure, some microorganisms upregulate the expression of extracellular polymers or siderophores containing functional groups that are capable of coordinating metal ions and may be subject to reduced uptake or increased efflux by membrane transporters upon binding to toxic metals. Many microbes have ability to precipitate metals as metal oxides, metal sulfides, metal protein aggregates, or metal crystals forming particulates in close association with cytoplasmic membranes. In addition, some microbes can use cytoplasmic proteins, e.g., bacterioferritin and metallothioneins, to bind, sequester, or store metals (Carrondo 2003). Some microbes use metals in specific redox and covalent reactions that convert toxic metal species into less toxic forms either oxidative or reductive metabolism (Fig. 3.11).

Few microbes have evolved detoxification mechanisms during their exposure to heavy metals, e.g., copper, mercury, lead, zinc, cadmium, etc. One of the known examples is cadmium accumulation in agricultural soils in New Zealand due to extensive use of superphosphate fertilizer (Loganathan et al. 2003). Due to metal toxicity, microbes have evolved few defense mechanisms such as metal

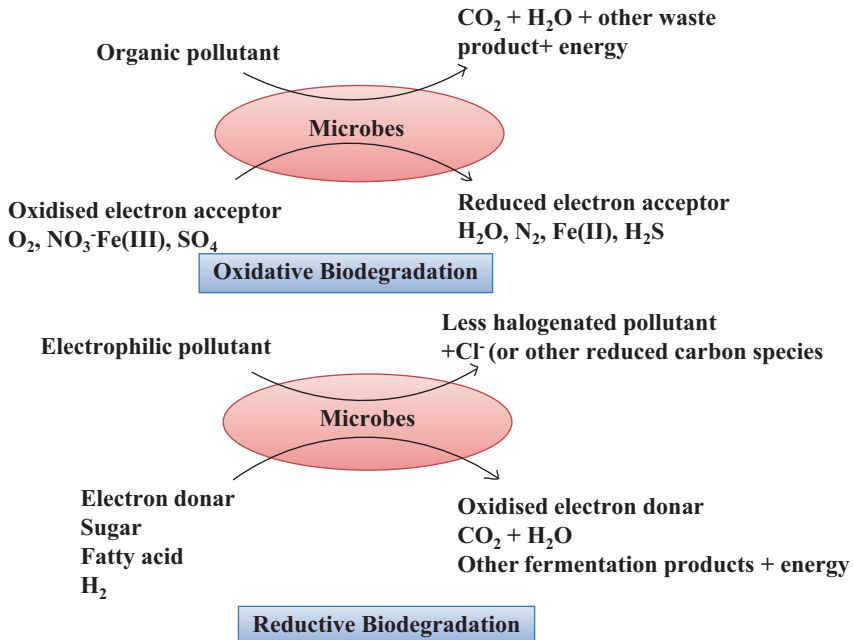


Fig. 3.11 Oxidative and reductive biodegradation of environmental pollutants

sequestration, detoxification, and efflux of ions. Bacteria sequester heavy metals through their binding with cell membrane, cell wall, and extracellular polysaccharides (Harrison et al. 2007). Microbes may also detoxify toxic metals through reduction using various cellular enzymes, e.g., mercury oxidase reduces Hg^{+2} to Hg , which has a low evaporation point and, therefore, diffuses from cell (Nies 1999). Few gram-negative bacteria, e.g., *Alcaligenes eutrophus*, have evolved a mechanism to fight with metal toxicity by expelling them from cytoplasm to external environment through cation/proton antiporter, present at cell membrane (Silver and Phung 1996). Nowadays, the microbial ability to transform heavy metals is being extensively used as a tool for bioremediation.

3.6 Microbial Enzymes in Bioremediation

Microbes are ultimate garbage disposal of nature that clean up or transform contaminants into non-hazardous or less hazardous substances. Various microorganisms such as bacteria and fungi detoxify hazardous substances by secreting various enzymes (Table 3.2), also involved in various industrial applications.

Table 3.2 Industrial applications of microbial enzymes

S. No.	Enzyme	Subclass	Substrate	Reaction(s)	Applications
1.	Oxygenase	Monooxygenase	Alkanes, steroids, fatty acids, and aromatic compounds	Use substrates as a reducing agent by incorporating single oxygen atom, i.e., desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, etc.	Bioremediation, protein engineering, synthetic chemistry, etc.
		Dioxygenase	Aromatic compounds	Incorporating two oxygen atoms to the substrate and resulting into aliphatic products	Bioremediation, pharmaceutical industry, synthetic chemistry, etc.
2.	Laccase	N/A	Ortho- and parapiphenols, aminophenols, polyphenols, polyamines, lignins, and arylamines	Oxidation, decarboxylation, and demethylation of substrates	Bioremediation, food and paper industry, textile industry, cosmetics, synthetic chemistry, etc.
3.	Peroxidase	Lignin peroxidase	Halogenated phenolic, polycyclic, aromatic, and other aromatic compounds	Substrate oxidation using H ₂ O ₂ as a co-substrate and mediator like veratryl alcohol	Bioremediation, food and paper industry, textile industry, pharmaceutical industry, etc.

		Manganese peroxidase	Lignin and other phenolic compounds	Oxidizes Mn^{2+} into Mn^{3+} which results into an Mn^{3+} chelateoxalate that in turn oxidizes the phenolic substrates	Bioremediation, food and paper industry, textile industry, pharmaceutical industry, etc.
		Versatile peroxidase	Methoxybenzenes and phenolic aromatic compounds	Catalyzes the electron transfer from an oxidizable substrate with reduction of complex I and II intermediates	Bioremediation and industrial biocatalyst
4.	Hydrolase	Lipase	Organic pollutants, e.g., oil spill	Hydrolyzes triglycerides to glycerols and free-fatty acids	Detergent production, baking and paper industry, personal products, etc.
		Cellulase	Cellulosic substances	Hydrolyzes substrate to simple carbohydrates	Bioremediation, paper and textile industry, detergent production
		Protease	Proteins	Hydrolyzes peptide bonds	Leather, laundry, biocatalyst, bioremediation, etc.

3.6.1 Oxidoreductases

Microbial oxidoreductases detoxify toxic xenobiotics such as phenolic compounds, produced from the decomposition of lignin through polymerization, copolymerization with other substances, or binding to humic substances. Most of the metal-reducing bacteria reduce the radioactive metals into insoluble forms that appear as a precipitant with the help of an intermediate electron donor (Leung 2004). The paper and pulp industry produces chlorinated phenolic compounds upon partial degradation of lignin during pulp bleaching process. These recalcitrant wastes are removed by the action of various fungal extracellular oxidoreductase enzymes such as laccase, manganese peroxidase, and lignin peroxidase that are released from fungal mycelium into their neighborhood environment. The plants belonging to families such as Fabaceae, Gramineae, and Solanaceae release oxidoreductases, which are recruited in the oxidative degradation of certain soil constituents.

3.6.2 Oxygenases

Oxygenases recruit oxidation of reduced substrates through oxygen transfer from molecular oxygen (O_2) using various co-substrates (FAD, NADH, NADPH). Oxygenases fall into two major categories on the basis of number of oxygen atoms used during oxygenation: monooxygenases and dioxygenases. Oxygenases mediate dehalogenation of halogenated pollutants, methanes, ethanes, and ethylenes through association with multifunctional enzymes (Fetzner and Lingens 1994).

Monooxygenases play an important role in bioremediation process as a biocatalyst due to their high selectivity and stereoselectivity on the wide range of substrates. Most of the monooxygenases are known to have cofactors but few act without them. Monooxygenases incorporate single atom of oxygen molecule into the substrate and are further classified into two subgroups based on the presence of cofactor. P_{450} monooxygenases are heme-containing oxygenases, while flavin-dependent monooxygenases consist of flavin as a prosthetic group and require NADP or NADPH as a coenzyme. Monooxygenases catalyze desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, and biotransformation of various aromatic and aliphatic compounds. Methane monooxygenase is the best-characterized monooxygenase involved in the degradation of various hydrocarbons. Monooxygenases exhibit differential activity in the presence or absence of oxygen. Monooxygenases catalyze oxidative dehalogenation under oxygen-rich conditions, whereas under low oxygen conditions, they catalyze reductive chlorination.

Microbial dioxygenases primarily oxidize aromatic compounds and are involved in bioremediation process. Aromatic hydrocarbon dioxygenases belong to Rieske non-heme iron oxygenase family and are involved in oxygenation of various substrates. An example is naphthalene dioxygenase having Rieske (2Fe-2S) cluster and mononuclear iron molecule in each alpha-subunit (Dua et al. 2002). One of the best nature's strategies for bioremediation is the catechol dioxygenase found in soil

bacteria which is involved in transformation and degradation of aromatic molecules into aliphatic products.

3.6.3 Laccases

Laccases are the members of multicopper oxidase family, produced by certain plants, fungi, insects, and bacteria and catalyze oxidation of a wide range of phenolic and aromatic substrates. Most of the microbes produce intra- and extracellular laccases, catalyzing the oxidation of aminophenols, polyphenols, ortho- or para-diphenols, lignins, aryl diamines, etc. These enzymes act not only by oxidizing phenolic and methoxy-phenolic acids but also through their decarboxylation and demethylation. Laccases are also involved in depolymerization of lignin resulting into various phenols that are utilized by microorganisms.

3.6.4 Peroxidases

Microbial peroxidases catalyze oxidation of lignin and other phenolic compounds in the presence of hydrogen peroxide (H_2O_2) and a mediator. Among all microbial peroxidases, lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), and versatile peroxidase (VP) have shown potent activity to degrade toxic substances.

Lignin peroxidases are heme-containing proteins secreted by white rot fungi during secondary metabolism and play an important role in degradation of lignin from plant cell wall. Manganese-dependent peroxidase is an extracellular heme-containing enzyme secreted by basidiomycete fungi. Mn^{2+} stimulates MnP production and itself oxidizes to Mn^{3+} by MnP. This results into Mn^{3+} chelateoxalate, which in turn oxidizes various phenolic substances. Versatile peroxidases directly oxidize Mn^{+2} , methoxy benzenes, phenolic aromatic substrates similar to MnP and LiP. VP exhibits broad substrate specificity and is able to oxidize substrates even in the absence of manganese as compared to other peroxidases. Hence, bioremediation and biotechnological applications for industrial processing need efficient VP production.

3.6.5 Hydrolases

Microbial hydrolases play an important role in bioremediation process and act by disrupting chemical bonds in toxic compounds and thereby reduce their toxicity up to some extent. These enzymes are readily available and do not need any cofactor for stereoselectivity. Some extracellular hydrolases such as amylases, proteases, lipases, DNases, and xylanases exhibit potential role in various sectors, e.g., food industry, biomedical sciences, and chemical industries. Other hydrolases, e.g., hemicellulases, cellulases, and glycosidases, have shown more importance because they are involved in biomass degradation.

3.6.6 Lipases

Lipases are capable of degrading lipids (e.g., triglycerides) derived from a range of microbes, plants, and animals, into glycerol and free-fatty acids. Recent reports suggest a close association of lipase with organic pollutants present in the soil, and its activity results into reduced hydrocarbon content in the contaminated soil. Microbial lipases are extensively used in industries since these enzymes catalyze various chemical reactions such as hydrolysis, esterification, alcoholysis, aminolysis, etc. Lipase activity is an important indicator or parameter for testing hydrocarbon content present in the soil. Lipases are widely used in pharmaceutical, food, chemical, cosmetic, and paper industries, but the cost of their production limits their potent application in the industries.

3.6.7 Cellulases

Cellulases convert cellulosic waste materials to glucose and have been implicated in intense research for bioremediation processes. Some bacteria and fungi express extracellular cellulases, hemicellulases, and pectinases at very low levels, e.g., *Bacillus* strains produce alkaline cellulases and *Trichoderma* and *Humicola* fungi produce neutral and acidic cellulases. Cellulases are widely used in paper and pulp industry for ink removal during paper recycling, in ethanol production from cellulosic biomass, and in brewing industry to enhance juice release from fruit pulp.

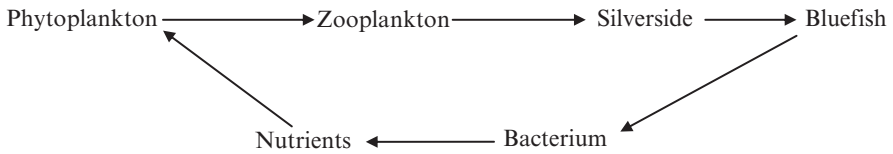
3.6.8 Proteases

Proteases hydrolyze the proteinaceous substances in the atmosphere resulting from animal death, shedding, and molting of appendages, as a by-product of poultry, fishery, and leather industries. Proteases are divided into two groups: exopeptidases and endopeptidases. Exopeptidases are further classified into aminopeptidases and carboxypeptidases depending on their site of cleavage either at N- or C-terminus of a peptide chain. Endopeptidases are also grouped based on the position of active site such as serine endopeptidases, cysteine endopeptidases, aspartic endopeptidases, and metallopeptidases.

Microbial proteases have been employed in cheese and detergent manufacturing industries since many years. Some proteases have been used in production of non-calorific artificial sweetener, e.g., dipeptide aspartame. Alkaline proteases are extensively used in leather industry for removal of hairs and parts on animal skin. Some proteases are also used in combination with broad-spectrum antibiotics in the treatment of wounds, cuts, and burns.

3.7 Contribution of Microbes in Food Web Maintenance

Food chains show the relationships between producers, consumers, and decomposers, showing who eats whom with arrows. The arrows show the movement of energy through the food chain. For example, in the food chain shown below, the small fish (silverside) gets its energy by eating the plankton and the large fish (bluefish) gets its energy by eating the small fish. Finally, the bacterium eats the fish after it dies, getting its energy from the large fish. The bacterium also returns nutrients back to the environment for use by the phytoplankton.



Thus the food chain becomes a complete circle. Animals may eat more than one type of food. They may eat many different types of plants or many different animals. This makes everything more complicated, and the food chain becomes a food web. A food web is made up of interconnected food chains. Most communities include various populations of producer organisms that are eaten by a number of consumer populations (Fig. 3.12). The green crab, for example, is a consumer as well as a

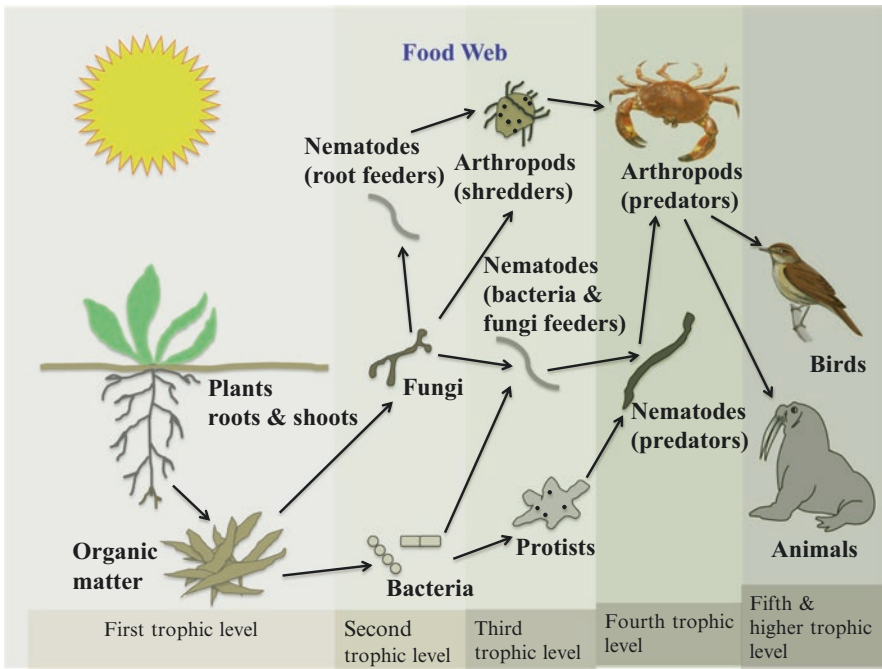


Fig. 3.12 Food web: an interaction between different species in ecosystem

decomposer. The crab will eat dead things or living things if it can catch them. A secondary consumer may also eat a number of primary consumers or producers. This nonlinear set of interactions which shows the complex flow of energy in nature is more easily visualized. In a food web, nutrients are recycled by decomposers in the end. Animals like shrimp and crabs can break the materials down to detritus. Then bacteria reduce the detritus to nutrients. Decomposers work at every level, setting free nutrients that form an essential part of food chain. Large number of primary producers such as bacteria and algae can maintain the base of pyramid to balance the biomass in trophic levels. In a food chain, energy is lost in each step of the chain in two forms, first by the organism producing heat and doing work and second by the food that is not completely digested or absorbed. Therefore, the food web depends on the constant supply of energy from producer and nutrients that are recycled by the decomposition of organism.

As food is passed along the food chain, only about 10% of the energy is transferred to the next level. For example, 10% of the energy phytoplankton received from the sun can be used by zooplankton at the next level. From one level to the next, about 90% of the energy used by the previous level is lost. This means that there has to be a lot more organisms at the lower levels than at the upper levels. The number of organisms at each level makes a pyramid shape and is called a food pyramid. To better understand this energy loss, it is helpful to look at a food pyramid. Thus food web may create the capacity of coexistence which was responsible for species evolution and maintenance of microbial diversity.

3.8 Beneficial and Pathogenic Host-Microbe Interactions

Host-microbe symbiosis exists in almost all animals, and the symbiotic bacteria can be profitable, harmful, or of no effect to the host. For example, the harmless *Escherichia coli* strains commonly found in intestine are a normal part of the gut flora and can advantage their hosts by producing vitamin K and by keeping pathogenic bacteria from colonizing the intestine. The interactions between host and microbe form complicated networks. By contrast, some others like *E. coli* strain O26 can cause disease in its host. Host and microbe interaction can be beneficial or harmful but have an important impact on the environment. When the organism is able to produce disease even in an apparently healthy host, it is referred as primary pathogen, but when it causes disease only when host's defenses are impaired, it is called secondary pathogen. The microbes consistently associated with a host are called flora. These microbes have a full range of symbiotic interactions with their hosts. Some host-microbe interactions are given below (Table 3.3).

Symbiosis A relationship in which two dissimilar organisms (symbionts) live in close association with one another.

Table 3.3 Types of host-microbe interactions

Types of interaction	Response	
	Species A	Species B
Neutralism	0	0
Mutualism	+	+
Commensalism	+	0
Competition	-	-
Amensalism	-	0
Predation	+	-
Parasitism	+	-
Parasitoidism	+	-

0 (No effect), - (harmful effect), + (beneficial effect)

Table 3.4 List of some useful plant-associated bacteria

Taxon	Functions
<i>Agrobacterium radiobacter</i> K84 and K1026	Biological control
<i>Agrobacterium</i> spp. M4	Source for a drug for cholesterol degradation
<i>Agrobacterium radiobacter</i> J14	Biodegradation of atrazine (herbicide)
<i>Agrobacterium tumefaciens</i>	Plasmid vector for plant transformation
<i>Erwinia amylovora</i>	Source of harpin, an elicitor of disease resistance in plants
<i>Xanthomonas campestris</i> pv. <i>campestris</i>	Xanthan gum used in food, cosmetics, etc.
Other plant-associated bacteria	Restriction endonucleases and other enzymes

Commensalism A relationship between two species in which one is benefited and the other is not affected, neither negatively nor positively.

Mutualism Mutually beneficial relationship between two species.

Parasitism A relationship between two species in which one benefits (parasite) from the other (host); it usually involves some detriment to the host.

3.9 Beneficial Plant-Microbial Interactions

Beneficial plant-microbial interactions are categorized into three parts. First, microbes either through direct interaction with the plants or indirectly through influencing biotic or abiotic parameters of soil supply minerals to support plant growth. Second, some microbes inhibit the growth and activity of plant pathogens to promote plant growth. Third, few microbes produce phytohormones that stimulate the growth of plants (Table 3.4). Additionally, some saprophytic microbes establish

neutral interactions with plants without directly benefiting or harming them. These microbes enrich soil nutrient levels by decomposing organic components and thereby influence their productivity and growth. The plant rhizosphere is the major soil ecological environment for plant-microbe interactions. In rhizosphere different microbes colonize around growing roots, which may either result in symbiotic, neutralistic, or parasitic interactions depending upon nutritional status of soil, soil environment, plant defense mechanism, and the type of microbial proliferation in the rhizosphere zone. The microbial community living in the rhizosphere zone benefits plant by promoting their growth and are also known as plant growth-promoting rhizobacteria (PGPR). These PGPRs include various bacteria, e.g., *Azospirillum*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Stenotrophomonas*, and *Streptomyces* and fungi, e.g., *Ampelomyces*, *Coniothyrium*, *Trichoderma*, etc. These PGPRs support plant growth by increasing soil fertility, secreting phytohormones, and protecting them from various diseases by producing antibiotics and inducing plant defense system. *Pseudomonas* and *Bacillus* are well-studied PGPRs and dominating bacteria, present in rhizosphere. PGPR bacteria have following roles to play:

1. PGPR bacteria suppress the growth of pathogenic microbes by lowering iron availability through secretion of low molecular weight siderophores.
2. PGPR can reduce the activity of pathogenic microorganisms by activating the plant to induced systemic resistance (ISR) or systemic acquired resistance (SAR). These plant resistance systems are induced by signaling molecules, e.g., jasmonic acid, ethylene, and salicylic acid.
3. PGPR bacteria enhance the production of phytohormones (e.g., auxin, cytokinin, gibberellins), besides having nitrogen-fixing ability. These phytohormones play a critical role in root initiation, cell division, and cell growth. Auxin is most prominently secreted by *Azospirillum* spp.
4. Several commercial PGPRs support plant growth by several means such as bio-protectants, biostimulants, and biofertilizers.
5. PGPR bacteria, e.g., *Azospirillum*, also provide nutrition to plants by liberating phosphorous from organic compounds and thereby support plant growth.

Root-colonizing microbes are guided by chemical plant signal overlap. For example, plant flavonoids act as chemoattractants for nitrogen-fixing bacteria, mobile zoospores, and symbiotic fungi. During interaction of microbes with plant epidermis, plants secrete signal molecules in the form of flavonoids and flavones in the rhizosphere that drive the differentiation between pathogenic, associative, symbiotic, or neutralistic adaptation of microbes with the plants.

In legume *Rhizobium* symbiosis, the rod-shaped soil bacterium *Rhizobium* induces nitrogen-fixing nodules on the roots of leguminous plants that convert approximately 80% of chemically inert nitrogen present in the atmosphere into ammonia through reduction process using bacterial enzyme nitrogenase in nitrogen-deficient condition (Zahran 1999). During this symbiotic relationship, plant root releases elicitors of *nod gene* expression, bacteria releases Nod factor, and plant root demonstrates ion flux, expresses nodulin proteins, and undergoes nodule

morphogenesis. The plant supports metabolism of bacterial endosymbionts by providing a micro-aerobic environment for effective functioning of the oxygen-sensitive nitrogenase, encoded by bacterial *nif* genes and carbohydrates. In return, bacteria fix atmospheric nitrogen for plants to meet their biological needs. The other diazotrophs such as *Azotobacter*, *Azospirillum*, as well as rhizosphere fungi and bacteria especially *Pseudomonas* and *Bacillus* also interact with *Rhizobium* affecting nodulation and nitrogen fixation and help in creating a beneficiary region where interacting microbes get benefit from additional nutrient resources. Therefore, a mutualistic relationship exists between *Azotobacter* and *Azospirillum* where both interact with *Rhizobium* to improve plant growth, and these beneficiary effects are mainly attributed to improvements in root development, increase in water and mineral uptake by roots, the displacement of fungi and pathogenic bacteria, and, to lesser extent, biological nitrogen fixation (Heath and Tiffin 2009). Nodule formation involves expression of rhizobia specific genes: bacterial genes (*nod* genes) and plant genes (*nodulin* genes). The component, enzymes, and their function are leghemoglobin (protection against oxygen), nitrogenase (N₂ fixation), glutamine synthetase (N-detoxification), and uricase (N-detoxification).

A mycorrhiza is a symbiotic association of a fungus and roots with vesicular plant. In a mycorrhizal association, the fungus colonizes the host plant roots either intracellularly as in arbuscular mycorrhizal fungi (AMF or AM) or extracellularly as in ectomycorrhizal fungi (Sikes 2010). In ectomycorrhiza, a fungus does not enter into plant cell, whereas it colonizes the outer cell layers and forms a Hartig net. Hartig net is a soil network that connects several organisms and protects against pathogenic fungi and soil bacteria. In this association, fungi form a net around the roots (hairs) to extend their access to soil nutrients. Ectomycorrhiza promotes growth of tree seedlings and germination of seeds. This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates such as glucose and sucrose. The carbohydrates are translocated from their source (usually leaves) to root tissue and on to the plant's fungal partners. In return, the plant gains the benefits of the mycelium's higher absorptive capacity for water and mineral nutrients due to the comparatively large surface area of the mycelium/root ratio, thus improving the plant's mineral absorption capabilities. Additionally, mycorrhizal plants are often more resistant to disease caused by soilborne pathogens and metal toxicity. The mycorrhizal fungi, especially the vesicular arbuscular mycorrhizae (VAM) belonging to the *Zygomycetes* class, play an important role in phosphorous mobilization in soils having a relatively low level of available phosphorous for the better growth of cereals as well as legumes. The fundamental characteristics of fungal species that form VAM are:

- They all belong to *Glomales* (*Zygomycetes*).
- Initiation of interaction through germinating spores on plant plasma membrane.
- Hyphae form appressorium (attachment site).
- Formation of an extracellular hyphal system in the apoplast.
- Formation of haustorium: penetration into plant cell (intracellular arbuscules).
- Enlargement of interaction surface.
- Lifetime of arbuscule: a few days.

Extracellular hyphae of the fungal species collect nutrients and transfer them to the fungus. The association of mycorrhizal fungi with legumes has a great impact on root and shoot development and phosphorous uptake resulting in the enhancement of nodulation and nitrogen fixation. Benefited fungi activate the defense genes that encode defensin proteins and may produce the reactive oxygen species through NADH oxidase to protect crops against pathogenic microbes. The yield of crop plants may increase four times higher with mycorrhizal fungi.

3.10 Pathogenic Microbes

Infectious diseases are caused by pathogenic microbes that attack and obtain their nutrition from the host they infect. A pathogen is a microorganism that has the potential to cause disease. An infection is the invasion and multiplication of pathogenic microbes in an individual or population. An infection does not always result in a disease. When an infection causes damage to the individual's vital functions in system, it leads to a disease. Ability to cause disease is pathogenicity, whereas the degree of pathogenicity is known as virulence. To cause disease, a pathogen must gain an access to the host, adhere to host tissues, penetrate or evade host defense system, and damage the host either directly or indirectly by accumulation of microbial wastes.

Numerous fungi, bacteria, viruses, and nematodes are pathogenic in nature and caused many plant and animal diseases (Tables 3.4 and 3.5). Disease triangle is one of the first concepts published by Stevens in 1960 and recognized the interaction among the host, pathogen, and environment (Fig. 3.13).

This triangular relationship is unique to phytopathology in comparison to veterinary and medical sciences because terrestrial plants possess little thermal storage capacity, and their immobility prevents escape from inhospitable environment. The sophisticated immune system found in mammals is absent in plant, and this places an emphasis on the earth's genetic constitution. Finally the predominance in the phytopathology of fungi, which are also highly dependent on environment, may have contributed to the development of this paradigm. Any disease caused by a pathogen is a chain of events involved in the development of pathogen and the effects of disease on the host. All infectious disease-causing agents go through a disease cycle. A generalized disease cycle is illustrated (Fig. 3.14).

3.11 Mechanism of Pathogenesis

Mechanisms of pathogenesis determine the relationship between virulence and components of parasite fitness, such as transmission to new hosts and survival within the host. By making explicit how the biochemical mechanisms of pathogenesis set the relations between parasite fitness and virulence, we expand the

Table 3.5 List of human pathogenic microbes

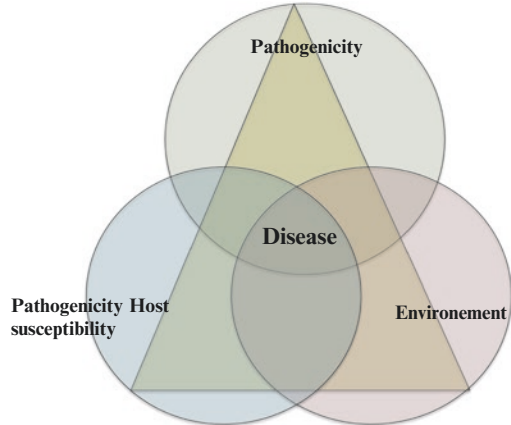
Microbes	Name of disease	Parasite name
Bacteria	Anthrax	<i>Bacillus anthracis</i>
	Abscess	<i>Bacteroides</i> spp.
	Whooping cough	<i>Bordetella pertussis</i>
	Lyme disease	<i>Borrelia burgdorferi</i>
	Campylobacter enteritis	<i>Campylobacter</i> spp.
	Trachoma, conjunctivitis, respiratory infection	<i>Chlamydia</i> spp.
	Botulism, tetanus, gangrene	<i>Clostridium</i> spp.
	Diphtheria	<i>Corynebacterium diphtheriae</i>
	Gastroenteritis, urinary infection, meningitis	<i>Escherichia coli</i>
	Vaginitis, vulvitis	<i>Gardnerella</i> spp.
	Meningitis, pneumonia	<i>Haemophilus influenzae</i>
	Peptic ulcer	<i>Helicobacter pylori</i>
	Pneumonia	<i>Klebsiella pneumoniae</i>
	Pontiac fever	<i>Legionella</i> spp.
	Conjunctivitis	<i>Moraxella lacumata</i>
	Tuberculosis, leprosy	<i>Mycobacterium</i> spp.
	Fatal pneumonia	<i>Mycoplasma pneumoniae</i>
	Gonorrhoea	<i>Neisseria gonorrhoeae</i>
	Pasteurellosis	<i>Pasteurella</i> spp.
	Dermatitis, enteritis	<i>Pseudomonas aeruginosa</i>
	Rocky mountain spotted fever	<i>Rickettsia</i> spp.
	Food poisoning or typhoid fever	<i>Salmonella</i> spp.
	Dysentery	<i>Shigella</i> spp.
Wound infection and food poisoning	<i>Staphylococcus aureus</i>	
Rheumatic fever	<i>Streptococcus pyogenes</i>	
Syphilis	<i>Treponema pallidum</i>	
Cholera	<i>Vibrio cholerae</i>	
Plague	<i>Yersinia pestis</i>	
Fungi	<i>Aspergillosis</i>	<i>Aspergillus niger</i>
	<i>Blastomycosis</i>	<i>Blastomyces dermatitidis</i>
	<i>Candidiasis</i>	<i>Candida albicans</i>
	<i>Coccidioidomycosis</i>	<i>Coccidioides</i> spp.
	<i>C. neoformans infection</i>	<i>Cryptococcus neoformans</i>
	<i>C. gattii infection</i>	<i>Cryptococcus gattii</i>
	<i>Fungal eye infection</i>	<i>Cryptococcus neoformans</i>
	<i>Histoplasmosis</i>	<i>Histoplasma capsulatum</i>
	<i>Mucomycosis</i>	<i>Mucoromycotina</i> spp.
	<i>Pneumocystis pneumonia</i>	<i>Pneumocystis jirovecii</i>
	<i>Ringworm</i>	<i>Trichophyton</i> , <i>Microsporum</i> , and <i>Epidermophyton</i>
	<i>Sporotrichosis</i>	<i>Sporothrix schenckii</i>

(continued)

Table 3.5 (continued)

Microbes	Name of disease	Parasite name
Virus	Influenza (Flu)	Influenza (flu) virus
	Small pox	Variola virus
	Chicken pox	Varicella zoster virus
	Mumps	Paramyxovirus
	Measles	Rubeola virus
	German measles	Rubella virus
	Yellow virus	<i>Flavivirus</i>
	Severe acute respiratory syndrome	<i>Coronavirus</i>
	Swine flu	Triple-reassorted flu virus A (H ₁ N ₁)
	Genital herpes	Herpes simplex virus
	Genital warts	Human papilloma virus
Protozoa	Anemia	<i>Nector americans</i>
	Hay fever	<i>Enterobius vermicularis</i>
	Trichinosis	<i>Trichinella spiralis</i>
	Cysts	<i>Trichuris trichiura</i>
	Onchocerciasis	<i>Wuchereria bancrofti</i>
	Blindness	<i>Onchocerca volvulus</i>
	Ulcer	<i>Dracunculus medinensis</i>
	Ascariasis (intestinal infection)	<i>Ascaris lumbricoides</i>

Fig. 3.13 Disease triangle, illustrating the factors required for disease development



conceptual framework of parasite virulence to encompass many cases that are not addressed by the prior theories of virulence. Human pathogens may enter into the host by different routes such as the mucous membranes, skin, and parental route and cause many diseases (Table 3.4). Most microbes must enter through their preferred portal of entry in order to cause disease, whereas some can cause disease from many routes of entry. The likelihood of disease increases as the number of invading pathogens increases. Infectious dose (ID₅₀) and lethal dose (LD₅₀) are used to determine

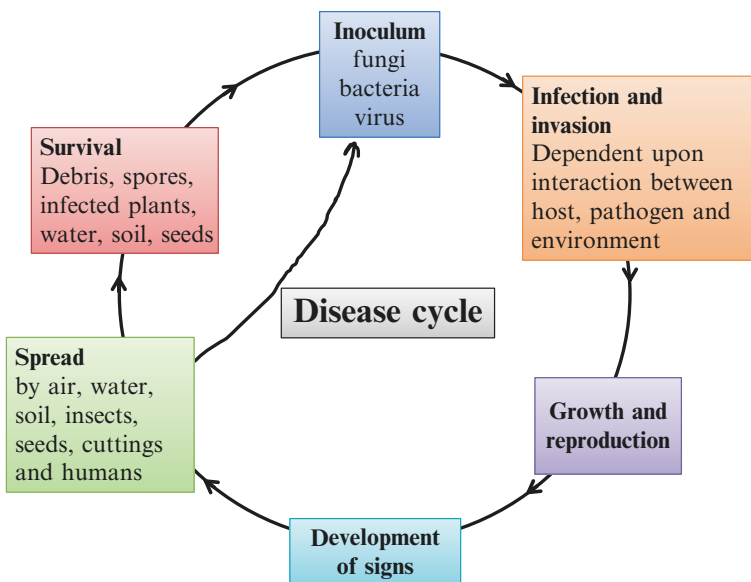


Fig. 3.14 Disease cycle

the number of microbes. ID_{50} is the number of microbes required to produce infection in 50% of the population, whereas LD_{50} is the amount of toxin or pathogen necessary to kill 50% of the population necessary in a particular time frame.

The role of microbes in plant diseases has been recorded as far back 700 BC (Table 3.5). Whereas some bacteria cause hormone-based distortion of leaves and shoots known as fasciations or crown gall, a proliferation of plant cells leading to swelling at the intersection of stem and soil and on roots happen as well. However, symptoms may vary with photoperiod, variety of plants, temperature, humidity, and infective dose. Some plant pathogenic microbes cause severe economically damaging disease such as spots, mosaic patterns, or pustules on leaves and fruits, smelly tuber rots, etc. (Table 3.5). Most of the plant pathogens induce a hypersensitive reaction (HR) in nonhosts or indicator plants, and this HR acts as a plant defense mechanism elicited by the presence of a pathogen in nonhost tissue. Although most plant disease is caused by fungi (85%), only a small fraction of fungi in the environment cause disease. Plant pathogenic fungi have played a powerful role in human and natural history. Fungal pathogens produce toxins causing allergies, e.g., mushrooms produce hallucinogenic mycotoxins (black molds). Protozoa can grow inside host cells causing lysis. They may use host cells, food source, and microbial wastes (Table 3.6).

In sum, it may be said that microbes play a significant role to maintain our environmental sustainability by maintaining biogeochemical and nutrient cycles. Microbes protect our environment from hazardous compounds by using a technique known as bioremediation and keeping our environment healthy. This chapter also provides evidences to explore PGPRs in sustainable agriculture to improve

Table 3.6 List of plant diseases caused by microbes

Microbes	Name of disease	Name of pathogenic microbe
Bacteria	Black rot	<i>Xanthomonas campestris</i> pv. <i>campestris</i>
	Bacterial canker	<i>Clavibacter michiganensis</i> pv. <i>michiganensis</i>
	Bacterial soft rot	<i>Pseudomonas</i> spp., <i>Erwinia</i> spp.
	Bacterial leaf spot	<i>Xanthomonas campestris</i> – various strains
	Bacterial wilt	<i>Ralstonia solanacearum</i>
	Bacterial blight	<i>Pseudomonas syringae</i> – various strains
	Bacterial blight	<i>Pseudomonas syringae</i> pv. <i>lisi</i>
	Bacterial speck	<i>Pseudomonas syringae</i> pv. <i>tomato</i>
	Bacterial brown spot	<i>Pseudomonas syringae</i> pv. <i>syringae</i>
	Crown gall disease	<i>Agrobacterium</i> spp.
	Bacterial speck of tomato plant	<i>Pseudomonas syringae</i>
	Scabs	<i>Burkholderia</i> spp.
Fungi	Late blight	<i>Phytophthora infestans</i>
	Early blight	<i>Alternaria solani</i>
	Gray mold	<i>Botrytis cinerea</i>
	Downy mildew	<i>Plasmopara viticola</i>
	Powdery mildew	<i>Uncinula necator</i>
	Stem rust	<i>Puccinia graminis</i>
	Glume blotch	<i>Staganospora nodorum</i>
Protists	Root knot	<i>Meloidogyne</i> spp
	Root lesions	<i>Pratylenchus coffeae</i> and <i>Helicotylenchus multicinctus</i>
	Cluster of sprouts on tubers	<i>Ditylenchus dipsaci</i>
	Reduced plant growth	<i>Globodera rostochiensis</i>
	Devitalized buds	<i>Aphelenchoides fragariae</i>
	Leaf discoloration	<i>Aphelenchoides besseyi</i>
	Root surface necrosis	<i>Tylenchulus semipenetrans</i>
	Curly tip	<i>Xiphinema</i> spp.
	Root rot	<i>Ditylenchus destructor</i>
Discoloration of foliage	<i>Pratylenchus coffeae</i>	
Virus	Fruit distortion on eggplant fruit	<i>Tomato bushy stunt virus</i>
	Bark scaling	<i>Citrus psorosis virus</i>
	Yellow vein banding	<i>Grapevine fanleaf virus</i>
	Yellow mosaic symptoms on lettuce	<i>Lettuce mosaic virus</i>
	Sugarcane leaf mosaic	<i>Sugarcane mosaic virus</i>
	Black necrotic ring spots in cabbage	<i>Cauliflower mosaic virus</i>
	Maize dwarf syndrome	<i>Maize dwarf mosaic virus</i>
	Peanut dwarfing or stunting	<i>Peanut stunt virus</i>
	Barley yellow dwarf	<i>Barley yellow dwarf virus</i>
	Yellow streaking of leaves	<i>Tobacco mosaic virus</i>
	Alfalfa mosaic	<i>Alfalfa mosaic virus</i>
	Curly top	<i>Beet curly top virus</i>

productivity and other environmental prospects. Therefore, current agricultural practices need to be improved through use of biopesticides and biofertilizers in order to minimize environmental and health problems.

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