

Chapter II: Soil Microbiology

Soil is far more than a simple support for plants; it is a living, complex, and dynamic ecosystem that hosts an immense diversity of microorganisms. Soil microbiology is the branch of microbiology that studies these microscopic forms of terrestrial life, their diversity, their activity, their interactions with one another and with their environment, as well as their ecological roles.

I. Specific features of the soil ecosystem

The soil ecosystem, or pedosphere, is one of the most complex and dynamic environments in the biosphere. It is characterized by strong physico-chemical and biological heterogeneity and harbors exceptional microbial biodiversity. This specificity relies on several fundamental aspects:

1. Complex physical structure

Soil is a complex, heterogeneous system composed of four fundamental, constantly interacting components: mineral matter, organic matter, soil solution, and soil atmosphere. Mineral matter represents the inorganic fraction of the soil and derives from the parent rock. It is made up of primary components such as quartz (silica) and feldspars (sources of aluminum, potassium, calcium, etc.), and secondary components such as clay minerals and iron and manganese oxides, which result from the chemical transformation of primary minerals through weathering processes. These elements play a crucial role in soil structure and in the retention of nutrients.

Organic matter is formed by the decomposition of plant and animal residues. Humus, its stable form, is an essential component that improves soil structure, water-holding capacity, and chemical fertility, while serving as an energy substrate for a wide diversity of microorganisms.

The soil solution corresponds to the liquid phase, consisting of water and dissolved mineral and organic substances. This water is held in the soil by capillary forces within aggregates and moves according to a water potential gradient. It contains a variety of available nutrients (monosaccharides, amino acids, ammonium ions, phosphates, potassium, etc.) that are indispensable for plant growth and microbial activity. The chemical composition of the soil solution is dynamic and varies with temperature, evaporation, water inputs (rainfall or irrigation), and biological activity. It plays a key role in the transport of nutrients, energy, and even chemical signals within the soil.

Finally, the soil atmosphere occupies the pores that are not saturated with water. It differs from atmospheric air by its higher carbon dioxide content (up to ten times more) and high humidity, as

a consequence of the respiratory activity of microorganisms and roots. It also contains nitrogen (N_2), oxygen (O_2), carbon dioxide (CO_2), and transient gases such as methane (CH_4), hydrogen sulfide (H_2S), ammonia (NH_3), and other volatile organic compounds (alcohols, esters, fatty acids). When the oxygen concentration falls below 1%, conditions become anaerobic, leading to specific microbial processes such as denitrification, sulfate reduction, or methanogenesis, which profoundly modify soil chemistry.

2. Role of organic matter

Organic matter, derived from the decomposition of plant and animal residues, is the main source of carbon and energy for the soil microflora. It also contributes to soil structure by promoting the aggregation of particles, which influences porosity, water retention, and air circulation.

3. Microbial diversity and density

Soil is one of the richest environments in microorganisms: bacteria, actinobacteria, fungi, microscopic algae, protozoa, and viruses coexist within it. It is estimated that one gram of fertile soil can contain up to 10^9 bacteria, several kilometers of fungal hyphae, and thousands of different species. This diversity allows the performance of multiple ecological functions.

4. Multiple biotic interactions

Soil microorganisms interact not only with each other (competition, symbiosis, antibiosis) but also with plants (rhizosphere) and animals (earthworms, insects, etc.). These interactions modulate nutrient availability, influence plant growth, and play a key role in ecosystem health.

5. Essential ecological functions

Soil microorganisms perform major functions:

- Decomposition of organic matter: recycling of nutrients.
- Biogeochemical cycles: nitrogen, carbon, sulfur, phosphorus.
- Stabilization of soil structure.
- Bioremediation: degradation of organic and inorganic pollutants.
- Beneficial symbioses: e.g. mycorrhizae and nitrogen-fixing bacteria.

6. Influence of abiotic factors

Temperature, pH, moisture, soil texture, and nutrient content directly influence microbial composition and activity. Each soil type (clayey, sandy, loamy) hosts a particular microbial community adapted to local conditions.

II. Soil properties

Soil properties determine its fertility, its capacity to host microbial life, and its ability to support plant growth. They are generally divided into three major categories: physical, chemical, and biological properties, all interdependent and influenced by soil composition and structure.

1. Physical properties

The physical properties of soil concern its texture, structure, porosity, bulk density, water-holding capacity, and temperature.

- Texture: It depends on the relative proportions of mineral particles (sand, silt, and clay). It directly influences aeration, permeability, water retention, and nutrient availability. Clay soils, for example, retain water better but are less well aerated than sandy soils.
- Structure: It describes the arrangement of soil particles into aggregates. Good structure promotes the movement of water, air, and roots while providing varied habitats for microorganisms.
- Porosity: Corresponds to the total volume of voids in the soil. It governs water retention, infiltration, aeration, and therefore microbial activity. It is influenced by compaction and organic-matter content.
- Water-holding capacity: This is the amount of water that the soil can retain after drainage. It depends on texture, structure, and organic-matter content. Capillary water held in micropores is particularly important for microbial life.
- Soil temperature: It affects the rate of biochemical reactions, seed germination, and the activity of enzymes and microorganisms. Extreme temperatures (cold or excessive heat) reduce microbial activity.

2. Chemical properties

The chemical properties of soil control nutrient availability and ionic equilibria.

- Soil pH: It measures soil acidity or alkalinity. Most soil microorganisms are active at near-neutral pH (between 6 and 7). A pH that is too acidic or too alkaline can limit the bioavailability of certain nutrients and restrict biological activity.
- Cation exchange capacity (CEC): This is the soil's capacity to retain and exchange cations (Ca^{2+} , Mg^{2+} , K^+ , NH_4^+ , etc.). It depends mainly on the amounts of clay and organic matter (humus) and determines the chemical fertility of the soil.
- Nutrient content: Soil contains macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur) and trace elements (iron, zinc, copper, manganese, etc.) that are essential for the development of plants and microorganisms. Their availability depends on the soil solution, pH, and interactions with colloids.
- Presence of pollutants: Heavy metals, pesticides, hydrocarbons, and other toxic substances can disrupt the chemical balance of the soil, reduce its fertility, and disturb the microbial ecosystem.

3. Biological properties

The biological properties of soil are related to the diversity, biomass, and activity of the living organisms it contains.

- Microbial biomass: This represents the mass of living microorganisms (bacteria, fungi, actinomycetes, etc.) that actively participate in element cycling, organic-matter decomposition, and humus formation.
- Enzymatic activity: Enzymes produced by microorganisms (e.g. dehydrogenases, phosphatases, cellulases) are sensitive indicators of the biological fertility of soil.
- Soil respiration: Measured by CO_2 production, it reflects the intensity of microbial and root activity. It is influenced by temperature, moisture, and organic-matter inputs.
- Soil biodiversity: Includes not only microorganisms but also mesofauna and macrofauna (nematodes, springtails, earthworms, insects). This biodiversity regulates ecosystem functions and stabilizes the soil against disturbances.

III. Soil microflora

This microflora is extremely diverse and includes several biological groups: bacteria, fungi, actinobacteria, archaea, protozoa, microscopic algae, and viruses.

1. Soil bacteria

Bacteria represent the most abundant and diverse group in soil. They can reach up to 10^9 cells per gram of soil and belong to dozens of different phyla. The most dominant in soils are:

- Proteobacteria: include many rhizospheric bacteria, such as nitrogen-fixing species (*Rhizobium*, *Azospirillum*) and plant growth-promoting rhizobacteria (PGPR).
- Firmicutes: a resistant group capable of forming spores (e.g. *Bacillus*), often active in decomposition under stressful or nutrient-poor conditions.
- Bacteroidetes: specialized in the degradation of complex polymers.

Bacteria perform key functions such as decomposition of organic matter, nutrient mineralization, biological nitrogen fixation, nitrification, and phosphorus solubilization. Some bacteria form biofilms or symbiotic associations with plants, playing a crucial role in plant health and soil structuring.

2. Soil fungi

Fungi account for 60–90% of the microbial biomass in many soils, with mycelial networks that can extend over several meters in just a few grams of soil. They include:

- **Saprophytic fungi** (*Aspergillus*, *Penicillium*, *Trichoderma*): decompose complex organic materials such as lignin, cellulose, and chitin.
- **Mycorrhizal fungi:**
 - Arbuscular endomycorrhizae (Glomeromycota): live in the roots of more than 80% of terrestrial plants and facilitate the uptake of water and phosphorus.
 - Ectomycorrhizae: present in forest trees (oak, pine, etc.), they create underground networks for nutrient sharing.
- **Pathogenic fungi:** *Fusarium*, *Pythium*, *Rhizoctonia*, causing root diseases.

They stabilize soil aggregates through the production of glomalin, improve soil structure and water retention, and play a major ecological and agronomic role.

3. Actinobacteria

These are filamentous bacteria midway between bacteria and fungi. They form a mycelial network, resist desiccation, and degrade highly complex substrates. Some, such as *Streptomyces*, produce natural antibiotics, powerful extracellular enzymes (cellulases, xylanases), and signaling molecules that influence the germination of other fungal spores. They are indicators of the biological maturity of soils.

4. Archaea

Long underestimated, archaea are now recognized as important members of soil microflora. They play a crucial role in:

- Nitrification (ammonium → nitrite), particularly Crenarchaeota (genus *Nitrososphaera*).
- Methanogenesis in anaerobic zones (water-saturated, organic-rich environments).
- Tolerance to extreme conditions (acidity, salinity, drought).

They are often present in deep, poor, or harsh habitats, which gives them significant ecological importance in marginal environments.

5. Protozoa and nematodes

Protozoa (amoebae, ciliates, flagellates) are predatory micro-eukaryotes that feed on bacteria. They regulate bacterial populations, stimulate N mineralization (by releasing ammonium after digestion), and enhance microbial dynamics. Bacterivorous nematodes have similar functions, but some species can also be plant pathogens

6. Microscopic algae

Mainly present at the surface, especially in moist or light-exposed soils, green algae (*Chlorophyceae*), diatoms, and cyanobacteria can form biological soil crusts in arid ecosystems. They carry out photosynthesis, enrich the soil with organic carbon, and contribute to soil stabilization against erosion.

7. Soil viruses

Bacteriophages and fungal viruses are ubiquitous in soils but still poorly studied. They modulate microbial communities through cell lysis, participate in nutrient cycles by releasing intracellular contents, and contribute to the evolutionary dynamics of microorganisms through horizontal gene transfer.

IV. Spatial distribution and ecological niches

The distribution of microorganisms in soil is not homogeneous. It varies with depth, the availability of organic substrates, the structure of aggregates, and the proximity of roots (the so-called rhizosphere). The rhizosphere is particularly rich in microorganisms because of the presence of root exudates (amino acids, sugars, organic acids) that stimulate microbial activity.

Outside the rhizosphere, several other zones can be distinguished:

- The mycorrhizosphere, the zone of interaction between roots, mycorrhizal fungi, and associated bacteria.
- The drilosphere, the soil zone influenced by the activity of earthworms.
- The porosphere, the space inside soil pores.

Ecological functions of the microflora

Soil microflora performs fundamental ecosystem functions:

- Decomposition of organic matter: transformation of organic residues into humus and release of nutrients that can be assimilated by plants.
- Nutrient cycling: direct participation in the cycles of nitrogen (biological fixation, nitrification, denitrification), carbon, sulfur, and phosphorus.
- Formation and stability of soil structure: microbial biofilms and extracellular polysaccharides promote aggregation of soil particles.
- Interactions with plants: stimulation of growth by PGPR (plant growth-promoting rhizobacteria), protection against pathogens, induction of systemic resistance.
- Bioremediation: ability to degrade or immobilize organic pollutants (hydrocarbons, pesticides) or inorganic pollutants (heavy metals), thereby reducing their toxicity.

V. Interactions with fauna, water, and plants

Interactions between microflora, fauna, water, and plants are not independent. They integrate into a dynamic ecological network called the soil–plant holobiont, in which each partner influences the others. These interactions can be mutualistic, competitive, trophic, or chemical, and they determine biological functions, community structure, and the dynamics of terrestrial ecosystems.

V.1. Interactions between microorganisms and plants

Beneficial bacteria: PGPR

- Plant growth-promoting rhizobacteria (PGPR), such as *Pseudomonas*, *Azospirillum*, *Rhizobium*, and *Bacillus*, enhance plant growth by:
- Fixing atmospheric nitrogen
- Solubilizing phosphorus and potassium
- Producing auxins, cytokinins, and gibberellins
- Inducing systemic resistance (ISR) against pathogens

Mycorrhizal fungi

- Glomeromycota form arbuscular mycorrhizae, which facilitate the uptake of phosphorus, zinc, and water.
- They exchange nutrients for plant-produced sugars via an underground network (extramatrical mycelium), acting as biogeochemical mediators.

Endophytic microbiome

- Some microorganisms live inside plant tissues (roots, stems, leaves) without causing disease.
- They improve stress tolerance (drought, salinity), modulate plant gene expression, and strengthen plant defenses.

V.2. Interactions between microorganisms and soil fauna

a. Microbial predators (protozoa and nematodes)

Protozoa and bacterivorous nematodes feed on soil bacteria and fungi. This predation stimulates nitrogen mineralization as ammonium (NH_4^+), releasing nutrients that had been immobilized in bacterial biomass.

b. Earthworms and springtails

Earthworms fragment organic matter and create zones of high microbial activity (drilosphere). Bacteria and fungi multiply rapidly in these zones. Springtails, which feed on fungi, influence the composition of fungal communities and reduce the proliferation of pathogenic fungi.

c. Indirect mutualism

Microorganisms improve the digestibility of organic matter for soil animals, while fauna produces substrates (mucus, excreta) that favor microbial growth.

V.3. Interactions with soil water

Water plays a fundamental role as a driver of microbial activity in soil. It constitutes the main living medium for soil microorganisms, ensuring several functions essential to their survival and metabolism. On the one hand, it facilitates the transport of nutrients such as nitrogen (N), phosphorus (P), and sulfur (S), which are indispensable for microbial growth. On the other hand, it allows the diffusion of chemical signals, particularly those involved in intercellular communication (quorum sensing) and in the production of antimicrobial or competitive compounds. In addition, water enables passive movement of microorganisms through soil pores, allowing them to colonize new microhabitats.

Soil moisture strongly influences microbial activity. Under optimal moisture conditions, enzymatic reactions and microbial processes reach their maximum efficiency. Conversely, excess water leads to a decrease in available oxygen, favoring the development of strict anaerobes such as *Clostridium* or *Methanobacterium*. Under drought conditions, microflora adopts survival strategies: some species form spores or cysts, or take refuge in protective biofilms capable of withstanding desiccation.

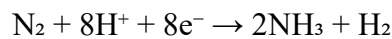
These biofilms, formed on the surface of soil particles, are collective structures that create microenvironments protected from abiotic stresses (heat, toxicity, water deficit). They facilitate the exchange of nutrients and signals between cells, while playing an active role in organic-matter degradation and in the retention of mineral elements. Thus, soil water is not only a dispersal medium but also a vital interface that shapes the distribution, diversity, and activity of soil microorganisms.

VI. Nitrogen cycle in soil

Nitrogen occurs in soil in different forms: N_2 (gas), NH_4^+ (ammonium), NO_2^- (nitrite), and NO_3^- (nitrate). The transformation of nitrogen from one form to another is carried out mainly by microorganisms, forming the nitrogen cycle.

VI.1. Biological nitrogen fixation

Atmospheric nitrogen (N_2), which makes up 78% of the air, is the most abundant but the least directly usable form for living organisms. Only certain bacteria and archaea can fix it thanks to an enzyme called nitrogenase (an oxygen-sensitive enzymatic complex composed of dinitrogenase and dinitrogenase reductase). The reaction produces ammonia:



Nitrogen-fixing microorganisms can be:

- Free-living in the soil: Azotobacter, Clostridium, cyanobacteria (e.g. *Anabaena*).
- Symbiotic with legumes: Rhizobium, Bradyrhizobium, which induce the formation of root nodules where fixation takes place.

VI.2. Ammonification (mineralisation)

Ammonification is the process by which organic nitrogen (from the degradation of amino acids, nucleic acids, urea, etc.) is converted into ammonia (NH_3) by heterotrophic bacteria. Depending on soil moisture:

- In dry soil, NH_3 escapes as a gas.
- In moist soil, it is converted to ammonium (NH_4^+):



VI.3. Nitrification

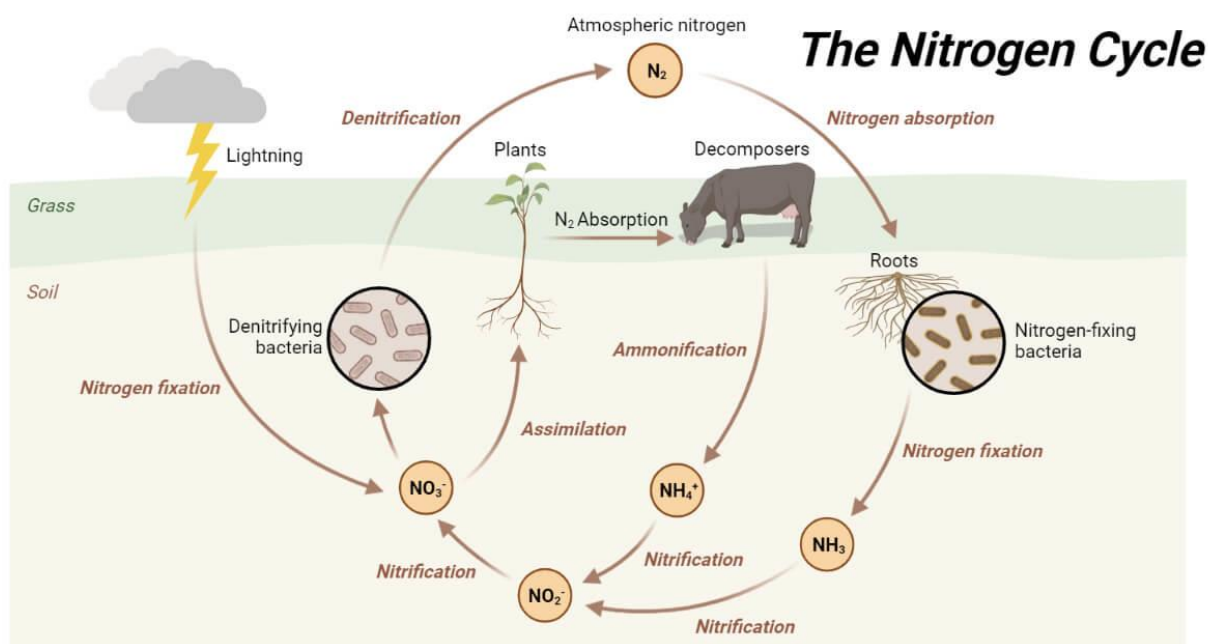
Nitrification is an aerobic microbial oxidation in two steps:

1. $NH_4^+ \rightarrow NO_2^-$ by Nitrosomonas
2. $NO_2^- \rightarrow NO_3^-$ by Nitrobacter

Under anaerobic conditions, anammox bacteria (e.g. *Brocadia*,) can convert directly $\text{NH}_3 + \text{NO}_2^- \rightarrow \text{N}_2$, a process mainly found in sediments, wastewater, and oxygen-poor environments.

VI.4. Denitrification

Denitrification is the reduction of nitrate (NO_3^-) to gaseous nitrogen compounds (NO , N_2O , N_2), which returns nitrogen to the atmosphere. This is a facultative anaerobic process carried out by heterotrophic microorganisms when oxygen is absent, particularly in water-saturated soils. Microorganisms involved include *Pseudomonas*, *Alcaligenes*, *Bacillus*, *Agrobacterium*, and *Flavobacterium*.



Example of symbiosis: Rhizobium–legume

The symbiosis between bacteria of the genus *Rhizobium* and legume plants (peas, beans, clover, alfalfa, soybean, etc.) is a classic example of symbiotic fixation of atmospheric nitrogen in soil. This mutualistic process allows the plant to obtain an assimilable nitrogen source while providing the bacterium with carbon compounds derived from photosynthesis.

1. Molecular recognition and signaling

The plant secretes root flavonoids into the soil, which are perceived by *Rhizobium*. In response, the bacterium synthesizes Nod (nodulation) factors, specific chemical signals that trigger:

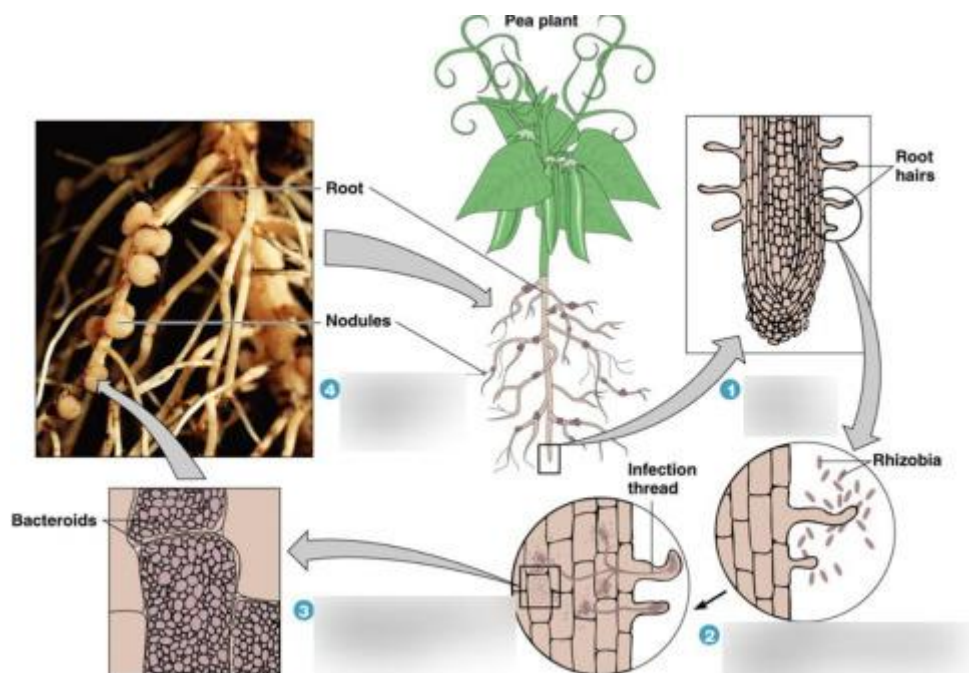
- Deformation and curling of root hairs,
- Formation of an infection thread through which the bacterium enters the root.

2. *Formation of root nodules*

Once inside the plant tissues, *Rhizobium* colonizes cortical cells, inducing the formation of nodules specialised structures in which the bacteria differentiate into bacteroids, physiologically active forms that fix nitrogen. Nodule development is regulated by nodulation (*nod*) genes in *Rhizobium* and symbiotic (*sym*) genes in the plant.

3. *Nitrogen fixation*

Within the nodule, the nitrogenase enzyme catalyses the reduction of molecular nitrogen (N_2) to ammonia (NH_3). Because nitrogenase is inhibited by oxygen, nodules contain a plant protein called leghaemoglobin, which binds and regulates oxygen, maintaining a microaerobic environment favourable to fixation.



(1) Rhizobia attach to root hair. (2) An infection thread is formed, through which bacteria enter root cells. (3) Bacteria change into bacteroides; packed root cells enlarge. (4) Enlarged root cells form a root nodule. (<https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/nod-factor>)