SOIL BIOLOGY AND MICROBIOLOGY

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Contents

Summary

Although soil organisms only constitute a tiny fraction of the total soil, their presence and activity are essential in forming and modifying the soils physical and chemical characteristics. The numbers and diversity of soil organisms is enormous compared to other habitats on earth, and the characterization of the populations and their dynamics is still in its infancy.

Soil biota can be grouped according to size in microbiota, mesobiota and macrobiota. Microbiota consist of bacteria, actinomycetes, fungi, algae and protozoa. They are the dominating group, both according to numbers and total biomass. Mesobiota consist of

nematodes, enchytraeids, collembola or springtails, mites, rotifers and small insects. Macrobiota consist of earthworms, mollusks and larger arthropods. These larger groups are particularly important in modifying soil structure and regulating other soil populations by predation.

Many soil processes, particularly those related to nutrient turnover and mineralization of organic matter are controlled by the soil biota. These processes are essential for the soils capacity to function and carry out vital ecosystem services as buffering and filtering of nutrients and water, and providing a basis for plant production.

Soil quality and the biota living within it are closely interlinked. Soil physical properties as structure, infiltration rate and water holding capacity are influenced by the activity of mainly macrobiota. Conversely, the activity of soil organisms is influenced by the living conditions in the soil. Several attempts to use soil biota as indicators for soil quality have been tried, but so far no standard indicators have been identified.

Although many biological processes in soils are resistant and resilient enough to withstand significant stress, key processes can be reduced or even cease if exposed to adverse conditions. Experience shows that soil functions may be lost, without being noticed, even while the soil appears to be functioning.

1. Introduction

Soil consists of mineral particles, air, water, soil organic matter, plant roots and living soil organisms. In terms of mass and volume, the living organisms constitute by far the smallest part of the soil. Typically, 50% of a soil volume will be mineral particles, 25% air filled pores and 25% water filled pores, although this will vary. The soil organic matter constitutes 0.5-5% of the solid fraction by weight, except in peat soils where it is much higher.

Soil organisms constitute 1-5 % of the organic fraction, hence only 0.04 % of the soil mass. However, soil organisms are involved in most soil processes, and soils cannot function naturally without the presence of life. Soil organisms are centrally involved in soil structure formation, litter turnover, nutrient availability, and buffering and detoxifying properties of the soil. Organisms affect soil physical properties such as water retention, water infiltration and aeration, are crucial in decomposition of organic matter and nutrient cycling, and have potential for cleaning and detoxifying polluted soils or spills. In order to understand soil processes, it is therefore necessary to understand the function of organisms living within it.

The diversity of soil biota is greater in soil than in any other habitat on earth. Millions of species, most of which not yet described, inhabit the soil, which has been referred to as the *poor mans rainforest*. Molecular techniques enable to investigate the species composition in the soil, but there is still a long way to go before the function and metabolism of most species are understood.

2. Soil Biota

Soil biota can be grouped according to size in micro-, meso- and macrobiota. Microbiota are less than 0.2mm and consist of bacteria, actinomycetes, fungi, algae and protozoa. Mesobiota range from 0.2 to 10mm in size and consist of nematodes, enchytraeids, collembola or springtails, mites, rotifers and small insects (arthropods). Macrobiota are organisms larger than 10mm and consist of earthworms, mollusks and larger arthropods.

2.1. Microbiota

2.1.1. Bacteria

Bacteria are the most diverse group in the soil, and the most abundant cell type. Bacteria are prokaryotes, i.e. lacking a nuclear membrane. Bacterial cells are usually in the range of 0.4-2 μm, and can be spherical, rod-shaped or S shaped. Some bacteria have flagella making them mobile. However, in the soil they are most often attached to surfaces by ion exchange.

Bacteria fall in two main groups, the archaebacteria and the eubacteria. The archaebacteria – literally "ancient/first bacteria" – are believed to be the most ancient group of bacteria, and include organisms adapted to extreme environments (halophiles, termophiles etc.) They differ from the eubacteria in their membrane lipid composition and possession of introns in their genome. Eubacteria – literally "true bacteria" is a somewhat larger group of bacteria of various shape, motility and biology. These two groups are as diverse and different from each other as they are from the third large group of organisms – the eukaryotes which include all protozoa, fungi, plants and animals. All three groups are believed to have one common ancestor.

Actinomycetes are bacteria, but due to their mycelial morphology and production of spore bodies, they have traditionally been considered as an intermediate group between bacteria and fungi. *Actinomycetes* in soil are particularly specialized towards decomposition of organic matter, including more complex substrates as chitin and hemicellulose, particularly under adverse conditions as high pH, temperatures and water stress.

Determining the taxonomy of bacteria is challenged by a number of factors. First, bacteria mainly reproduce by binary fission, more rarely by bud formation. Hence, in the absence of sexual reproduction the traditional species definition does not hold. Second, the majority of bacterial strains cannot be cultured on laboratory media, making studies difficult. Third, unlike higher organisms, bacteria lack convenient morphological properties that are well suited for classification purposes. Finally, development of molecular techniques, particularly the use of 16S rRNA for producing phylogenetic trees have revealed considerable discrepancies between the classical taxonomy based on morphology and metabolism and the phylogeny of bacteria.

The reason for this is that microorganisms living in the same environment may develop similar morphological and biochemical characteristics – i.e. develop analogy, although they are not taxonomically related. Horizontal gene transfer between taxonomically unrelated organisms accentuates this problem, as certain genes, and thereby traits can

move between populations by this mechanism. On the other hand, taxonomically related species may develop different traits if occupying different niches, i.e. in aerobic or anaerobic environments.

Nucleotide sequences of DNA and RNA can change because of evolutionary pressure, or due to random substitutions if that particular sequence is not coding for a gene under evolutionary pressure (termed random drift). For these non-coding sequences, sequence differences between species are believed to be correlated with taxonomic distance, or rather distance to nearest common ancestor. The nucleotide sequence of the rRNA has these characteristics; it is very ancient – i.e. present in all organisms – and not coding for genes/traits under evolutionary pressure. Hence, relatively similar rRNA will indicate that the species are closely related, whereas taxonomically more distant species will have undergone more changes in the nucleotide sequence.

2.1.2. Fungi

Unlike the bacteria, fungi belong to the eukaryotes, i.e. organisms with a true cell nucleus. Most fungi are aerobic heterotrophs; some anaerobes occur in guts and in wastewater. Although there are far more species of bacteria than fungi - the ratio between both being dependent on the environment - the biomass of these two major groups in soil is comparable.

The fungi are divided into five main groups (phylla), all of which occur in soil as saprophytes or plant and insect pathogens. The five groups are the *Chitridiomycota, Zygomycota, Glomeromycota, Ascomycota* and *Basidimycota*. One additional group, the *Oomycota* are not true fungi but belong to a separate kingdom. However, due to their similarity with fungi, they are sometimes classified with the true fungi.

The majority of soil fungi consist of a filamentous mycelia made up of hyphen, alternatively as individual cells (e.g. yeast). Sexual reproduction takes place via fruiting bodies produced from the mycelium. These fruiting bodies exhibit a large variation in size and shape, and the large above-ground fruiting bodies from ascomycota and basidomycota are well known as mushrooms. The fruiting bodies also serve as a means of identification and classification of the fungi.

Fungi have several important specialized functions in the soil, as plant pathogens, decomposers and as mycorrhizal symbionts with plants. The soil hosts numerous fungi that are plant pathogens, primarily attacking the roots. Some are very host specific and can be suppressed by avoiding susceptible crops for a number of years.

Certain decomposition processes of highly complex and recalcitrant structures such as lignin are almost exclusively carried out by a limited group of fungi. The white rot fungi (*Basidomycetes*) are capable of complete oxidation of lignin by producing a strongly oxidizing environment by groups of enzymes as laccases and lignin and manganese peroxidases. The resistance of lignin to decomposition results from their random structure; therefore a strongly oxidizing environment, rather than stereospecific bonding by enzymes to the substrate is required for its decomposition.

Unlike bacteria that basically require a substrate to be brought to them (e.g. by diffusion), fungi are capable of moving to their substrate by hyphen growth (and in some cases by motile zoospores). Hence, colonization and decomposition of litters on soil surfaces is mainly carried out by fungi, which colonize the litter layer much faster than bacteria. Fungal decomposers are also capable of acquiring nutrients from one part of their hyphen and carbon substrates from other parts, thereby enabling them to grow on physically separated nutrient and carbon substrates, i.e. low nutrient containing litters on the soil surface.

The same mechanism is characteristic for a certain group of fungi forming symbiotic relationships with plants, by attaching themselves to the roots, either inter-cellularly (ectomycorrhiza) or by penetrating root cells (endomycorrhiza, most commonly as vesicular-arabuscular mycorrhiza). The plants supply the fungi with carbohydrates, and the fungi hyphen act as an extension of the plant roots, enhancing uptake of soil nutrients, primarily phosphorus. The mycorrhizal-plant symbiosis is much more unspecific and common than other interactions between micro-organisms, e.g. N fixation by rhizobium. It is estimated that 80% of all plants can associate with mycorrhiza.

2.1.3. Cyanobacteria and Algae

Taxonomically, cyanobacteria and algae belong to two different domains: cyanobacteria are prokaryotic eubacteria, algae are eukaryotes. However, due to their functional and morphological similarities, the cyanobacteria were previously grouped together with the algae under the name: *Blue-green algae*. Both cyanobacteria and algae are photosynthetic organisms; cyanobacteria are also commonly capable of fixing nitrogen. Cyanobacteria are believed to be one of the oldest life forms on earth, dating back more than 3.5 billion years, and were probably the primary agents in oxygenating the atmosphere and earth.

Both algae and cyanobacteria are found in all soils. Although they need light to grow, they can be found even in deeper soil layers. Whether this is due to alternate metabolic capabilities or simply passive leaching through the soil, is not clear. Algae and cyanobacteria colonize and grow on soil surfaces in very dry or otherwise bare soils (see also *Soils of Arid and Semiarid Areas*), and they can contribute significantly to nutrient flows of the system. Though both groups depend on moisture for growth, they can withstand prolonged dry periods in dormant stages.

2.1.4. Protozoa

There are three main groups of soil protozoa (literally: first animals) in soil; namely the flagellates, amoeba and ciliates. *Protozoa* are heterotrophic eukaryotes, feeding mainly on other micro-organisms as bacteria or dissolved organic substrates. The larger groups, particularly the ciliates also feed on other protozoa. Being much larger than bacteria - flagellates are usually 5 μm in diameter, amoeba 10 μm and ciliates 20 μm - they are restricted to larger pores.

Amoeba are mobile by extending their flexible cell walls (pseudopods) and sliding along

surfaces, flagellates have one to several flagella attached that allow movement in water filled pores, whereas ciliates are more or less densely covered with shorter cilia.

Although restricted to water filled pores for movement and feeding, protozoa are capable of forming encapsulations or cysts, enabling them to survive for extended periods under unfavorable conditions.

2.1.5. Metabolism of Micro-organisms

Microorganisms can be grouped according to their metabolism source as follows:

- Source of energy (chemotrophs and phototrophs).
- Source of electrons (Electron donor) (chemotrophs consisting of either organotrophs or lithotrophs).
- Source of carbon (heterotrophs or autotrophs).

Most microorganisms get their energy by assimilating and oxidizing energy-rich organic compounds; others obtain their energy from the transformation of inorganic compounds, e.g. *Nitrosomonas* that oxidize ammonium to nitrite. Both types of energy acquisition are termed chemotrophic. Phototrophs capture energy from light as plants do, converting it to chemical energy.

Chemotrophs can be subdivided according to their source of reducing power, i.e. electrons. If relying on organic compounds, they are organotrophs, if deriving their reducing power from inorganic compounds they are lithotrophs (literally: living on a rock).

The terms heterotrophy and autotrophy do not refer to the energy supply of the organisms, but to the carbon source. Autotrophs are capable of assimilating inorganic carbon, i.e. CO_2 or carbonates, either via photosynthesis (photo-autotrophs) or coupled to oxidation of inorganic compounds (litho-autotrophs) or organic compounds (organo-autotroph).

Heterotrophs obtain the carbon needed for their anabolism from reduced organic compounds (organic carbon). This group includes all fungi and protozoa, as well as many bacteria. Heterotrophs are most commonly organotrophs and, hence, the term heterotrophy is often used for generalizing chemo-organo-heterotrophs. Likewise, lithotrophs are generally, though not always, autotrophs as are phototrophs. The groups are commonly referred to as litho-autotrophs and photo-autotrophs.

2.2. Mesobiota

Mesobiota range from 0.2 to 10 mm in size. Although much less abundant both in numbers and biomass than bacteria and fungi, mesobiota play an important role in soil functioning. Mesobiota are all eukaryotes, and almost exclusively heterotrophic aerobes. They differ fundamentally from micro-organisms in their size, metabolism and ability to move actively.

Nematodes vary widely in their feeding habits, and their primary food source can usually be identified from the morphology of their mouth. Nematodes may feed on bacteria, fungi, detritus, roots or predate on other micro-fauna in the soil. Hence, the nematodes cover a range of different trophic levels in the soil, and cannot be placed as one group in food webs (see below). The root or plant feeding nematodes are often referred to as parasitic nematodes, as these can significantly affect crop yields when occurring in large numbers.

Collembola dominate the meso-fauna in terms of numbers. *Collembola* as a group are believed to be omnivorous, feeding on detritus, bacteria, fungi or even other components of the mesofauna. However, there exist also more narrow feeding preferences within individual species.

The mites can be grouped in two trophic strata based on their feeding preferences. The oribatid mites primarily feed on detritus and fungi in the soil, whereas the predatory mites feed on nematodes and other groups of the mesofauna.

The mesobiota play an important role in nutrient cycling in the soil. This is less because of the magnitude of their own metabolism, as due to their influence in the bacteria and fungi they predate. Exclusion of mesobiota from the decomposition process may increase speed of decay, if they are significantly suppressing decomposer microorganisms. However, more commonly they have been shown to increase C and nutrient mineralization, by predating on growing microbial populations. For low quality substrates, this also increases nutrient availability by mineralization, leading to a positive feedback on decomposition of the remaining substrate (Bonkowski 2004;Clarholm 2005). In some cases, the grazing of microorganisms has even been shown to increase microbial growth rates by this mechanism of recycling nutrients.

As for microorganisms and macrobiota, the mesobiota tend to be most abundant in the top soil layers, primarily because the main substrate input to the soil occurs via detritus at the surface. The exception from this is found in the rhizosphere, i.e. the soil layer surrounding the roots, where biological activity is several fold increased (see below). Most species are susceptible to soil disturbance and cultivation, and numbers decline after tillage, either because of physical disruption, disturbance of pores and pathways in the soil or exposure to adverse conditions as drying on the soil surface. Loss of micro-site habitats due to soil homogenization may also lead to loss in diversity and numbers.

2.3. Macrobiota

Macrobiota are organisms larger than 10mm. In terms of biomass and abundance, they make up a much smaller fraction of the soil biota than microorganisms and mesofauna. They play, however, an important role in soil function, primarily because they affect physical soil weathering processes, and interact with other groups of organisms, in particular on the transfer of carbon and nutrients in the form of detritus within the soil.

Earthworms are the most important functional group in that they facilitate litter turnover by drawing it into the soil, dividing it into smaller parts, thereby increasing surface area, and by mixing litter with soil and soil organisms in their gut. Other species feed on

humus rich soil, obtaining energy from partial breakdown of soil organic matter, and soil microorganisms. The large impact of earthworms on the soil environment has given them a function as "ecosystem engineers".

Earthworms have also been shown to dramatically increase dispersal of microorganisms in soil through their movement. Together with plant roots, earthworms are the main creators of soil macropores, which may remain functional for years or even decades in clayey, undisturbed soil layers. Macropores radically alter the movement of water and dissolved substances in the soil, and can act as "highways" that increase the speed of percolation, and bypass biological or chemical processes, resulting in leaching of otherwise immobile (phosphate, pesticides) or labile (dissolved organic carbon or nitrogen) compounds from the soil profile. They also increase the speed of transport and, hence, the leaching of mobile nutrients as nitrate. Macropores also increase aeration of the soil, and water infiltration at the surface.

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Biographical Sketch

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