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## The microbiology of soil and of nutrient cycling

Soil is a dynamic habitat for an enormous variety of life-forms. It gives a mechanical support to plants from which they extract nutrients. It shelters many animal types, from invertebrates such as worms and insects up to mammals like rabbits, moles, foxes and badgers. It also provides habitats colonised by a staggering variety of microorganisms. All these forms of life interact with one another and with the soil to create continually changing conditions. This allows an on-going evolution of soil habitats.

The activity of living organisms in soil helps to control its quality, depth, structure and properties. The climate, slope, locale and bedrock also contribute to the nature of soil in different locations. The interactions between these multiple factors are responsible for the variation of soil types. Consequently, the same fundamental soil structure in different locations may be found to support very different biological communities. These complex communities contribute significantly to the continuous cycling of nutrients across the globe.

### 1.1 What habitats are provided by soil?

Soil forms by the breakdown of bedrock material. Erosion of rocks may be the result of chemical, physical or biological activity, or combinations of the three factors. Dissolved carbon dioxide and other gases cause rain water to become slightly to moderately acid. This pH effect may cause the breakdown of rocks such as limestone. Physical or mechanical erosion can result from the action of wind or water, including ice erosion. The growth of plant roots and

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the digging or burrowing activities of animals contribute to the mechanical breakdown of soil. Microbial activity by **thermoacidophilic** bacteria, such as those found in coal slag heaps, results in an extremely acid environment. Leaching of acid from slag heaps may cause chemical changes in bedrock.

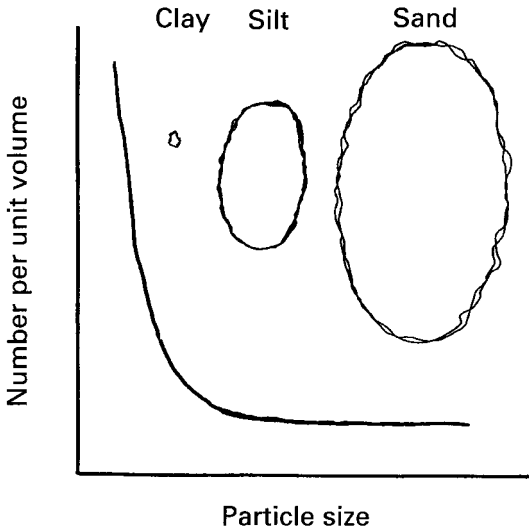
Naked rocks provide a very inhospitable habitat. Even these may, however, be colonised. There is evidence for colonisation all around us. Next time you visit a graveyard, look for lichens on the headstones. Lichens are microbial colonisers of rocks. This is true even if the rock is not in its original environment. Gravestones are conveniently dated. By comparing the age of different headstones and the degree of colonisation you can get some idea of the time it takes to colonise native rocks.

Among the first rock colonisers are cyanobacteria. Parent rocks do not provide nitrogen in a form that is readily available for biological systems. Bacteria are unique among life-forms in that they can fix atmospheric nitrogen so that it can be used by other organisms. Cyanobacteria are ideally placed to colonise rock surfaces because they are nitrogen-fixing **photolithotrophs**. They require only light and inorganic nutrients to grow. Cyanobacteria can provide both fixed nitrogen and carbon compounds that can be used as nutrients by other organisms. They are responsible for the initial deposition of organic matter on exposed rocks. This initiates the biological processes that lead to soil formation and to nutrient cycling. The colonisation of rocks by cyanobacteria is the first step in the transformation of naked rock into soil suitable for the support of plant and animal life. The microbes present in the soil are responsible for re-cycling organic and inorganic material and play an important part in the dynamic regeneration of soil.

As soils develop and evolve, the smallest particles are found nearest the surface of the ground and particle size increases steadily down to the bedrock. Soil particles may be classified by size (Fig. 1.1). Sand particles are typically between 50 micrometres and 2 millimetres. Silt particles are smaller than sand particles, being between 2 micrometres and 50 micrometres. Clay particles are smaller than 2 micrometres. The sizes of the particles present in soil profoundly affect its nature. One cubic metre of sand may contain approximately  $10^8$  particles and has a surface area of about 6000 square metres. The same volume of clay may contain  $10^{17}$  particles with a surface area of about 6 million square metres. As the size of particle decreases, the number of particles present in a unit volume of soil increases exponentially, as does the surface area of the soil. This has important consequences for water retention and hence for other properties of the soil.

Sandy soils, with their relatively small surface area, cannot retain water very well and drain very quickly. This may lead to the formation of arid soils. At

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**Fig. 1.1.** The relative sizes of soil particles. The clay particle is small, the silt particle is of average size and the sand particle is large.

the other extreme, clays have a very large surface area and retain water very easily. Clays also tend not to be porous. As a result of water retention, they also tend to form anaerobic environments. Neither extreme provides an ideal habitat, other than for specialised life-forms. The most fertile soils are loams. These contain a mixture of sand, silt and clay particles and provide a diversity of microhabitats capable of supporting a wide range of organisms. These organisms interact to modify the atmosphere between particles of soil. Consequently, the atmosphere within the soil differs from that above ground. Microbial and other metabolisms use some of the available oxygen present in the soil and so there is less oxygen beneath the ground than there is in the air above the soil surface. Similarly, carbon dioxide is generated as a by-product of microbial metabolism and there is a higher concentration of carbon dioxide within soil than above ground.

Soils may also be grouped by their organic content. At one extreme are mineral soils that have little or no organic content. Such soils are typical of desert environments. At the other extreme are bogs. There is a gradation of soil types between that found in deserts and that in bogs, with an ever-increasing organic content.

Plants are the major producers of organic material to be found in soil, and plant matter accumulates as litter. Animal faeces and the decomposing bodies of dead animals complement this organic supply. Artificially added fertilisers,

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herbicides and pesticides all affect the biological component and hence the organic content of soils. Horse dung and chicken manure are beloved of gardeners. Microbes play a central role in re-cycling such material. Besides recycling of naturally occurring organic compounds, soil microbes are responsible for the chemical degradation of pesticides. Not all pesticides are easily broken down, however. Those compounds that resist microbial decomposition and that consequently accumulate in the environment are known as recalcitrant pesticides.

During the evolution of a soil habitat, its organic content may eventually become predominant. The ultimate organic soil is found in a bog. Bogs are waterlogged and consequently form an anaerobic environment. Any dissolved oxygen is quickly used up by **facultative organisms**. This provides a very inhospitable environment for fungi and **aerobic bacteria**. Since these organisms tend to be responsible for the decomposition of organic structures, bogs provide excellent sites for the preservation of organic matter.

A striking example of the preservative effect of bogs is afforded by the existence of intact human bodies conserved for thousands of years. 'Pete Marsh' was one such specimen. His body was found in a bog in Cheshire. He was in such a good state of preservation that a forensic *post mortem* examination was possible on this archaeological find, showing that the man had died after being garrotted. 'Lindow Man', as he is also known, is now on view in a special atmospherically controlled chamber in the British Museum.

Winchester Cathedral was built on a peat bog. To support this magnificent structure, the medieval architects and masons raised the building on a huge raft made from beech trees. This raft provided a floating foundation for the Cathedral. The wood survived intact for hundreds of years, preserved by the anaerobic, waterlogged environment provided by the marshy ground upon which it rested. It was only during the early twentieth century that a crisis arose. The surrounding water-meadows were drained to conform to the agricultural practices then in fashion. The water table around the Cathedral started to fluctuate and the beech raft was exposed to the air for the first time in centuries. It was also exposed to the microorganisms responsible for wood decay. It was only owing to the engineering expertise of a single diver, William Walker, that the whole structure was saved from disaster. He spent years working alone under the cathedral underpinning its structure. A similar drop in the water table in the Black Bay area of Boston has caused considerable problems of subsidence in some of the older buildings in the area. Again, this is caused by oxygen-dependent fungi rotting the previously soaked timber piles on which the buildings were erected.

Soils contain many aerobic and facultative organisms and, because of the

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microbial manipulation of microenvironments, soils may harbour a large number of **obligate anaerobes**. Bacteria are the largest group of soil microbes, both in total number and in diversity. Indeed the presence of bacteria gives freshly dug soil its characteristic 'earthy' smell. The odour is that of **geosmin**, a secondary metabolite produced by streptomycete bacteria.

Microscopic examination of soil reveals vast numbers of bacteria are present. Typically there are about  $10^8$  to  $10^9$  per gram dry weight of soil. Only a tiny fraction of these can be cultivated upon laboratory culture media. Scientists have yet to provide appropriate culture conditions for the vast majority of soil microbes. Many live in complex communities in which individuals cross-feed one another in a manner that cannot be replicated when the microbes are placed in artificial culture. The microbial activity of soil is severely underestimated using artificial culture. An estimate of the microbial activity of soil is further influenced by the fact that many soil bacteria and fungi are present as dormant spores. These may germinate when brought into contact with a rich artificial growth medium. Spores may also flourish when introduced into cuts and grazes. Gardeners are particularly prone to tetanus, when spores of *Clostridium tetani* are introduced into minor trauma sites.

For many years, the study of soil microbiology was severely limited because of our inability to cultivate the vast majority of soil microbes in artificial culture. Today, great advances are being made by the application of molecular biological techniques to this problem. Sensitive isotope studies are yielding information on the metabolism of soil microbes and **polymerase chain reaction (PCR)** technology is being used to study the taxonomy of non-cultivable bacteria, particularly exploiting 16S ribosomal RNA (rRNA) structure. The structure of the 16S rRNA is conserved within members of a species whereas different species show divergent 16S rRNA structures. Therefore this provides a very useful target in taxonomic studies.

Both bacteria and fungi provide an abundant source of food for soil protozoa. The most commonly encountered soil protozoa include flagellates and amoebas. The abundance of such creatures depends upon the quantity and type of organic matter present in the soil sample. Protozoa play a key role in the regulation and maintenance of the equilibrium of soil microbes. Whereas many microbes obtain their nutrients from solution, protozoa are frequently found to be of a scavenging nature, obtaining their nutrients by devouring other microbes.

The distribution of microbes throughout the soil is not even. Microorganisms tend to cluster around the roots of higher plants. This phenomenon is referred to as the **rhizosphere effect** (*rhiza*: Greek for root; hence the rhizosphere is the region surrounding the roots of a plant). The majority

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of microorganisms found in the rhizosphere are bacteria, but fungi and protozoa also congregate in this region. Microorganisms are thought to gain nutrients from plants, and **auxotrophic** mutants requiring various amino acids have been isolated from the rhizosphere. Plants may also derive benefit from this arrangement. Bacteria may fix nitrogen in a form that can be taken up and used by plants. In certain circumstances, the association between microorganisms and higher plants can become very intimate. **Mycorrhizas** are formed when roots become intimately associated with fungi. Root nodules provide another important example of the close association between leguminous plants and nitrogen-fixing bacteria. In this instance bacteria rather than fungi are involved in the association with plants.

### 1.2 How are microbes involved in nutrient cycling?

Life on Earth is based on carbon. Water and simple organic compounds such as carbon dioxide become elaborated into complex, carbon-based organic structures. These compounds include other elements besides carbon, oxygen and hydrogen. Nitrogen is found in nucleic acids, amino acids and proteins. Phosphorous is a component of nucleic acids, lipids, energy storage compounds and other organic phosphates. Sulphur is found principally in certain amino acids and proteins. All of these elements are continuously cycled through the ecosystem. Many natural biological cycling processes require elements to be in different chemical states in different stages of the cycle. Phosphorous is an exception. It is always taken up as inorganic phosphates. Once absorbed into living organisms, biochemical processes transform phosphorous into more complex forms.

Inorganic phosphates are very widely distributed in nature but are frequently present as insoluble salts. So, despite an apparently plentiful supply of phosphorous, phosphates often represent a **limiting nutrient** in natural ecosystems. This means that as supplies of phosphates run out, uncontrolled growth of organisms is prevented. Insoluble phosphates can be converted into soluble phosphates. This may be achieved by the activity of the acid products of bacterial **fermentations**. These may then be taken up into bacteria. Soluble phosphates may also be added to the land artificially, either as plant fertilisers or as organophosphate pesticides. Phosphates are also used in the manufacture of many detergents. These chemicals can end up in rivers and lakes, artificially increasing the concentration of biologically accessible phosphates. This permits the overgrowth of algae in affected waters, resulting in **algal blooms**. These can deprive other plants of light, thus killing them and

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destroying the natural ecology of the affected waters. Some algal blooms may also be toxic to animals.

Besides the cycling of non-metal elements, microorganisms have a role in the biochemical transformation of metal ions. Bacteria such as *Thiobacillus ferrooxidans* and iron bacteria of the genus *Gallionella* are capable of oxidising ferrous ( $\text{Fe}^{2+}$ ) iron into ferric ( $\text{Fe}^{3+}$ ) iron. Many bacteria can reduce small quantities of ferric iron to its ferrous state. There is also a group of iron-respiring bacteria that obtain their energy by **respiration**. They use ferric iron as an electron acceptor in place of oxygen. Magnetotactic bacteria, exemplified by *Aquaspirillum magnetotacticum*, can transform iron into its magnetic salt magnetite. These bacteria act as biological magnets. Bacteria are also important in the transformation of manganese ions, where similar reactions to those seen with iron are observed.

Without the cycling of elements, the continuation of life on Earth would be impossible, since essential nutrients would rapidly be taken up by organisms and locked in a form that cannot be used by others. The reactions involved in elemental cycling are often chemical in nature, but biochemical reactions also play an important part in the cycling of elements. Microbes are of prime importance in this process.

In a complete ecosystem, **photolithotrophs** or **chemolithotrophs** are found in association with **chemoorganotrophs** or **photoorganotrophs**, and nutrients continually cycle between these different types of organism. Lithotrophs gain energy from the metabolism of inorganic compounds such as carbon dioxide whereas organotrophs need a supply of complex organic molecules from which they derive energy. Phototrophs require light as a source of energy but chemotrophs can grow in the dark, obtaining their energy from chemical compounds. The rate of cycling of inorganic compounds has been estimated and different compounds cycle at very different rates. It is thought to take 2 million years for every molecule of water on the planet to be split as a result of photosynthesis and then to be regenerated by other life-forms. Photosynthesis may be mediated either by plants or photosynthetic microbes. The process of photosynthesis releases atmospheric oxygen. It is probable that all atmospheric oxygen is of biological origin and its cycling is thought to take about 2000 years. Photosynthesis is also responsible for the uptake of carbon dioxide into organic compounds. Carbon dioxide is released from these during respiration and some fermentations. It only takes about 300 years to cycle the atmospheric carbon dioxide.

Because of our familiarity with green plants, life without photosynthesis is perhaps difficult to imagine. This is, after all, the reaction that provides us with the oxygen that we need to survive. It should be remembered, however,

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that photosynthesis is responsible for the production of molecular oxygen. This element is highly toxic to many life-forms. Life on Earth evolved at a time when there was little or no oxygen in the atmosphere. Aerobic organisms can only survive because they have evolved elaborate protection mechanisms to limit the toxicity of oxygen. Equally, not all life depends on sunlight. In the dark depths of both the Atlantic and Pacific oceans are thermal vents in the Earth's crust. These provide a source of heat and chemical energy that chemolithotrophic bacteria can use. In turn, these bacteria provide a food source for a range of invertebrates. These rich and diverse communities spend their entire lives in pitch darkness around the 'black smokers'.

### 1.2.1 How is carbon cycled?

Most people are familiar with the aerobic carbon cycle. During photosynthesis, organic compounds are generated as a result of the fixation of carbon dioxide. Photosynthetic plants and microbes are the primary producers of organic carbon compounds and these provide nutrients for other organisms. These organisms act as consumers of organic carbon and break down organic material in the processes of fermentation and respiration. Chemoorganotrophic microbes break down organic carbon compounds to release carbon dioxide. Chemolithotrophic bacteria can assimilate inorganic carbon into organic matter in the dark. Certain bacteria are also capable of anaerobic carbon cycling. Fermentation reactions, common in bacteria that are found in water and anaerobic soils, are responsible for the breakdown of organic chemicals into carbon dioxide or methane. Hydrogen gas may be released as a product of some fermentations. Methane can itself act as a carbon and energy source for methane-oxidising bacteria. These bacteria can generate sugars and amino acids from methane found in their environments, again helping with the cycling of carbon compounds.

### 1.2.2 How is nitrogen cycled?

One of the crucial steps in the advancement of human civilisation was the development of agriculture. This involves the artificial manipulation of the natural environment to maximise the yield of food crops and livestock. With the development of agriculture came the need to maximise the fertility of soils. The availability of fixed nitrogen in a form that can be used by crop



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plants is of prime importance in determining the fertility of soil. As a consequence, the biological nitrogen cycle (see Fig. 1.4 below) is of fundamental importance, both to agriculture and to natural ecology.

Inorganic nitrogen compounds such as nitrates, nitrites and ammonia are converted into organic nitrogen compounds such as proteins and nucleic acids in the process of **nitrogen assimilation**. Many bacteria reduce nitrates to nitrites and some bacteria further reduce nitrites to ammonia. Ammonium salts may then be incorporated into organic polymers in the process of **assimilatory nitrate reduction**. Ammonia is primarily fixed into organic matter by way of amino acids such as glutamate and glutamine. Other nitrogen compounds can be made from these.

For the continued cycling of nitrogen, organic nitrogen compounds must be broken down to release ammonia. **Putrefactive metabolism** yields considerable quantities of ammonia from **biopolymers** that contain nitrogen. Bacteria may also produce **urease**, an enzyme that breaks down urea to liberate carbon dioxide, water and ammonia. The quantity of ammonia released by the urease of *Helicobacter pylori* is sufficient to protect this bacterium from the acid pH of the human stomach.

Bacteria are also involved in the inorganic cycling of nitrogen compounds. **Nitrifying bacteria** are responsible for the biological oxidation of ammonia. These bacteria are chemolithotrophs, obtaining chemical energy from the oxidation process. This energy is used to elaborate organic compounds from carbon dioxide. Nitrifying bacteria such as those of the genus *Nitrosomonas* produce nitrite ions from the oxidation of ammonia. Bacteria of the genus *Nitrobacter* and a few other genera can oxidise nitrites to nitrates.

As well as their role in the nitrogen cycle, nitrifying bacteria may have a more sinister activity, as illustrated by their effects on buildings such as the cathedrals at Cologne and Regensburg. They have been shown to colonise the sandstone used to build these churches. Water carries the bacteria from the surface and into the matrix of the stone to a depth of up to five millimetres. Here they produce quantities of nitrous and nitric acid sufficient to cause erosion of the stone. Consequently, the decay of great public buildings may not be exclusively caused by acid rain generated by industrial pollution.

Nitrates may be used by some bacteria instead of oxygen for a type of respiration referred to as **dissimilatory nitrate reduction**. During this process, nitrate is reduced to nitrite and thence to ammonia. This may then be assimilated into organic compounds as described above. Not all bacteria follow this pathway, however. Bacteria of the genus *Pseudomonas*, micrococci and *Thiobacillus* species can reduce nitrates to liberate nitrogen gas into the environment. Bacteria that can generate nitrogen gas from the reduction of

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nitrates are commonly found in organically rich soils, compost heaps and in sewage treatment plants.

To complete the inorganic nitrogen cycle, nitrogen gas must be fixed in a form that can be used by living organisms. If this were not the case, life on Earth would only have continued until all the available nitrogen compounds had been converted into nitrogen gas. Nitrogen is converted into ammonia in the process of **nitrogen fixation**. Bacteria are the only life-forms capable of the biological fixation of nitrogen. They are of vital importance if life is to continue on this planet. Green plants are the main producers of organic matter in the biosphere and they require a supply of fixed nitrogen for this process. Fixed nitrogen may be obtained through the death and lysis of free-living nitrogen-fixing bacteria. Nitrogen-fixing bacteria, however, frequently form close associations with plants. In some cases, the relationship becomes so intimate that bacteria live as **endosymbionts** within plant tissues. Bacteria supply the plant with all of its fixed nitrogen demands. In return, they receive a supply of organic carbon compounds (Fig. 1.2).

Not all nitrogen fixation occurs as a result of biological processes. Nitrogenous fertilisers are produced in vast quantities by the agrochemical industry. Oxides of nitrogen are also produced by natural and artificial phenomena in the environment. Ultraviolet irradiation and lightning facilitate the oxidation of nitrogen, particularly in the upper atmosphere. At ground level, these reactions are augmented by electrical discharges and in particular by the activity of car engines.

Biological nitrogen fixation is catalysed by **nitrogenase**. The activity of this enzyme is rapidly lost in the presence of oxygen. Nitrogen-fixing bacteria have evolved a number of strategies for protecting nitrogenase from the harmful effects of oxygen. The simplest solution is to grow under anaerobic conditions. Nitrogen fixers such as *Clostridium pasteurianum* and *Desulfovibrio desulfuricans* are obligate anaerobes. In consequence, they can only grow under conditions that protect the activity of nitrogenase.

Many bacteria are facultative anaerobes. Nitrogen-fixing facultative bacteria are generally only capable of fixing nitrogen when they are growing in anaerobic environments. Examples of such bacteria include some of the Enterobacteriaceae, such as *Enterobacter* species, as well as facultative members of the genus *Bacillus*.

The Gram-negative bacterium *Klebsiella pneumoniae* is capable of nitrogen fixation in a microaerophilic atmosphere as well as in anaerobic conditions. This is because in a microaerophilic atmosphere bacterial respiration can effectively reduce the local oxygen tension to zero. This permits nitrogenase to function. This provides an example of respiration fulfilling two demands