SOIL MICROBES AND THEIR CONTRIBUTION TO SOIL SERVICES

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ABSTRACT: We discuss the roles of microbes in the ecosystem services provided by soils to humans. The diversity of microbes in soil is enormous and they drive many soil services. We examine the functional, metabolic, and phylogenetic diversity of soil bacteria, archaea, and fungi. The roles of these soil microbes are highlighted in the cycling of major biological elements (C, N, P), in the recycling of wastes, and the detoxification of environmental pollutants. Microbes play a pivotal role in the cycling of nitrogen; they exclusively mediate nitrogen fixation, denitrification, and nitrification. We also discuss recent theoretical advances in understanding of ecosystem processes that were made possible through explicit consideration of the roles of soil microbes. Global knowledge of soil microbial diversity and functioning is increasing rapidly, but knowledge of New Zealand's soil microbial resources is sparse, despite their importance in the provisioning and regulating services provided by soil ecosystems.

Key words: archaea, bacteria, detoxification, fungi, nutrient cycling.

INTRODUCTION

Soils are the naturally occurring physical covering of the earth's surface, and represent the interface of three material states: solids (geological and dead biological materials), liquids (water), and gases (air in soil pores). Each soil is a unique product of the combination of geological parent material, glacial and geomorphological history, the presence and activity of biota, and the history of land use and disturbance regimes. Soils are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids, and other invertebrates as well as plants and algae. These soil dwellers provide food or nutrients that support organisms that live above and below ground. Soils also play critical roles in buffering and filtering freshwater ecosystems. Consequently, soils are extremely important to human societies. We depend on soils for the basis on which we and our buildings stand, and for the production of food, building materials, and other resources; indeed, soils influence most ecosystem services on which we depend (Dominati et al. 2010).

Soil microbes, bacteria, archaea, and fungi play diverse and often critical roles in these ecosystem services. The vast metabolic diversity of soil microbes means their activities drive or contribute to the cycling of all major elements (e.g. C, N, P), and this cycling affects the structure and the functions of soil ecosystems as well as the ability of soils to provide services to people. Table 1 provides an overview of roles of soil microbes in these provisioning and regulating ecosystem services.

In this chapter we describe soil microbes, including their diversity, abundance and distribution, and in particular their role in two soil regulating services: nutrient cycling and recycling of wastes and detoxification. Where possible, we refer to studies on the microbiota of New Zealand's natural and managed soils.

What are bacteria, archaea and fungi?

Bacteria and archaea are the smallest independently living, single-celled organisms on earth. Typical cells range from 0.5 to 1.0 μm in diameter. Bacteria and archaea may occur as cocci, rods, or spirals, and some bacteria common in soils, such as the Actinomycetales, can form branching filaments (Figure 1). Most lack a true membrane-bound nucleus, so their DNA lies free in the cell cytoplasm. Their genome typically consists of a single circular molecule of double-stranded DNA, though cells may also harbour smaller DNA elements called plasmids. The size of the genome varies, depending on the lifestyle and complexity of the organism, but typically ranges from 4 to 6 million nucleotides in length and codes for between 3000 and 4000 genes. A cell membrane made of phospholipids surrounds the cell. Outside this is the cell wall, which varies in composition depending on the organism but is usually made of proteins, carbohydrates and lipids. Many microbes can move, using flagella (whip-like extensions from the cell). They can also form fine filaments called pili that can attach the cells to each other or to soil surfaces. Some use special pili to attach to other microbes and transfer DNA in a process known as conjugation. Usually they undergo asexual reproduction, typically by dividing in half; some cells can divide every 12–20 minutes, while others take much longer.

As with all organisms, bacteria and archaea require carbon to provide the building blocks for cell materials. They also require energy to drive the reactions involved in cell synthesis and metabolism. To grow, some bacteria require oxygen while other bacteria and most archaea use alternative electron acceptors including nitrate and sulphate (i.e. they respire nitrate and sulphate). For these anaerobic organisms oxygen may be toxic (refer to Box 1). Broadly, microbes are classed as autotrophs or heterotrophs. Autotrophs use energy from sunlight or inorganic compounds (e.g. Fe^{2+}, nitrate or nitrite) to fix atmospheric carbon dioxide to produce carbohydrates, fats and proteins, whereas heterotrophs use organic carbon compounds as a source of carbon and energy.

Archaea were originally thought to exist only in harsh environments and were often described as 'extremophiles', but we now know they are widely distributed and are found alongside bacteria in many environments including soil. Archaea and bacteria are difficult to distinguish on the basis of their morphology. However, molecular phylogenetic tools based on a comparison of 16S ribosomal rRNA sequences have revealed that all life can be divided into three domains, with Archaea being more closely related to Eukarya (all multicellular organisms) than the Bacteria (Woese et al. 1990).

Fungi are eukarya and hence more closely related to plants and animals than to bacteria or archaea. Like all eukarya, including humans, fungal cells contain membrane-bound nuclei with chromosomes that contain DNA. They also have membrane-bound organelles such as mitochondria. Fungi have a cell wall composed of glucans and chitin (Figure 2). Fungi are heterotrophic organisms, and their 'default' nutritional strategy is to be a saprobe, that is, to feed on decaying matter. While some fungi

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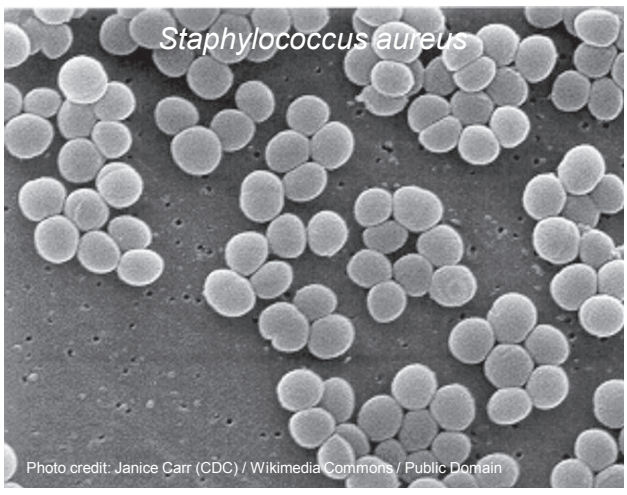
TABLE 1 Role of soil microbes in provisioning and regulating services provided by soil ecosystems (adapted from Dominati et al. 2010)

Soil service	Descriptor	Role of soil microbes
Provisioning services – products obtained from ecosystems		
Physical support	Soils form the surface of the earth and represent the physical base on which animals, humans and infrastructures stand. Soils also provide support to animal species that benefit humans (e.g. livestock).	Microbes contribute to soil formation through nutrient cycling and organic matter production. Microbial products are critical to soil aggregation, improved soil structure making soil more habitable for plants.
Raw materials	Soils can be a source of raw materials (e.g. peat for fuel and clay for potting).	Soil microbes produce antimicrobial agents and enzymes used for biotechnological purposes.
Growth medium for plants	Humans use plants for food, building, energy, fibre, medicines and more. By enabling plants to grow, soils provide a service to humans. Soils physically support plants and supply them with nutrients and water.	Soil microbes mobilise nutrients from insoluble minerals to support plant growth.
Regulating services – enable humans to live in a stable, healthy and resilient environment		
Buffering water flows	Soils have the capacity to store and retain quantities of water and therefore can mitigate and lessen the impacts of extreme climatic events (e.g. limit flooding). Soil macroporosity and hydrological processes like infiltration and drainage impact on this service.	Soil macropores are formed by plant roots, earthworms and other soil biota, which may depend on soil microbes as food or for nutrients.
Nutrient cycling	Soil is the site of the decomposition of organic materials and the mobilisation of nutrients in bedrock and soil aggregates. Soil is also the site of the oxidation and reduction of nutrient elements, symbiotic N-fixation and photoautotrophic activity.	The activities of soil bacteria, archaea and fungi drive nutrient cycling in soils and are involved in weathering minerals.
Recycling of wastes and detoxification	Soils absorb, detoxify, and recycle applied wastes (e.g. effluent disposal), agrochemicals, and spills of fuels and oils, reducing potential harm to humans and to organisms useful to humans.	Microbial processes like mineralisation and immobilisation are responsible for this service. Detoxifying microbes may be limited by the availability of soil nutrients (e.g. N or P), which in turn depends on soil microbial activities.
Filtering of contaminants	If pollutants (e.g. excess nutrients, exotic microbes, metals, organic compounds) are leached from soils, they can contaminate aquatic ecosystems and threaten human health. Soils absorb and retain solutes and pollutants, avoiding their release into water.	In concert with the clay and organic matter content of soils, microbial products contribute to both the hydrophobicity and wettability of soils, impacting on the ability of soils to filter contaminants.
Habitat for biodiversity	A very large component of global biodiversity occurs in soils. Some organisms have above-ground life stages or are food resources for above-ground species. Soils are a reservoir for resting phases of organisms (e.g. seeds, fungal spores) and thus are critical for the rejuvenation of communities.	Soil bacteria, archaea, and fungi comprise the vast majority of the biological diversity on earth. Further, they are the foundation of soil food webs thereby underpinning the diversity of higher trophic levels. Interactions among soil microbes and plants often determine plant biodiversity.
Biological control of pests, weeds and pathogens	Soils provide habitat to beneficial species that regulate the composition of communities and thus prevent proliferation of herbivores and pathogens. This service depends on soil properties and the biological processes driving inter- and intra-specific interactions (symbiosis, competition, host–prey associations).	Beneficial species include bacteria, archaea, and fungi that support plant growth through increasing nutrient availability and by outcompeting invading pathogens.
Carbon storage and regulation of greenhouse gas emissions	Soils play an important role in regulating many atmospheric constituents, impacting on air quality, and on regional and global climate. Soils store carbon as stable organic matter offsetting CO_2 emissions and are home to microbes that release nitrous oxide (N_2O) and methane (CH_4).	By mineralising soil carbon and nutrients, microbes are major determinants of the carbon storage capacity of soils. Denitrifying bacteria and fungi and methane producing and consuming bacteria regulate nitrous oxide (N_2O) and methane (CH_4) emissions from soils.



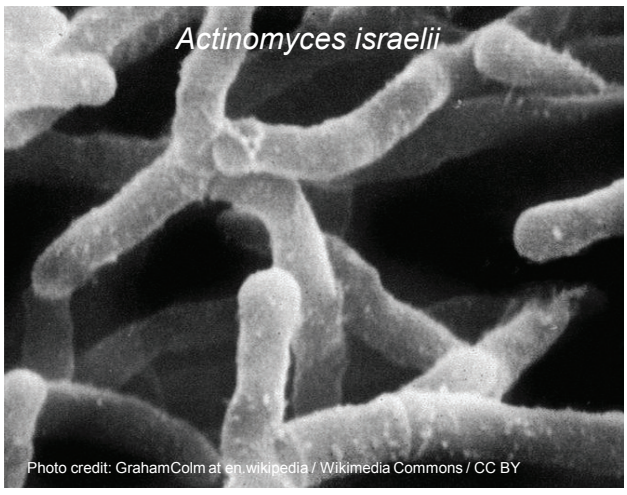


FIGURE 1 Examples of the structure of bacteria and/or fungi.

BOX 1 Metabolic diversity of bacteria

Bacteria are extremely metabolically diverse and can be divided into four groups, based on their source of carbon and their source of energy:

Photoautotrophs like cyanobacteria photosynthesise, obtaining energy from sunlight and carbon by fixing carbon dioxide. Cyanobacteria in soil include *Nostoc*, which is also a nitrogen fixer.

Photoheterotrophs derive energy from photosynthesis if provided with an electron donor (hydrogen or an organic compound) for reductive assimilation of carbon dioxide. Some, such as *Rhodopseudomonas*, will grow on organic substrates if oxygen is provided.

Chemoautotrophs use reduced inorganic substrates to fix carbon dioxide and as a source of energy. The major energy sources for these organisms are hydrogen, ammonia, nitrite, hydrogen sulphide, and the ferrous ion (Fe^{2+}). In soil, this group includes the bacteria involved in nitrification, such as *Nitrosomonas* and *Nitrobacter*, and *Thiobacillus,* which plays a role in formation of acid mine drainage.

Chemoheterotrophs require pre-formed organic molecules as their sources of both carbon and energy. Some bacteria use simple carbon sources like glucose or succinate, whereas others degrade more complex substrates like proteins and carbohydrates. Although some bacteria, like *Pseudomonas*, may utilise up to 100 different carbon sources for growth, most grow on fewer.

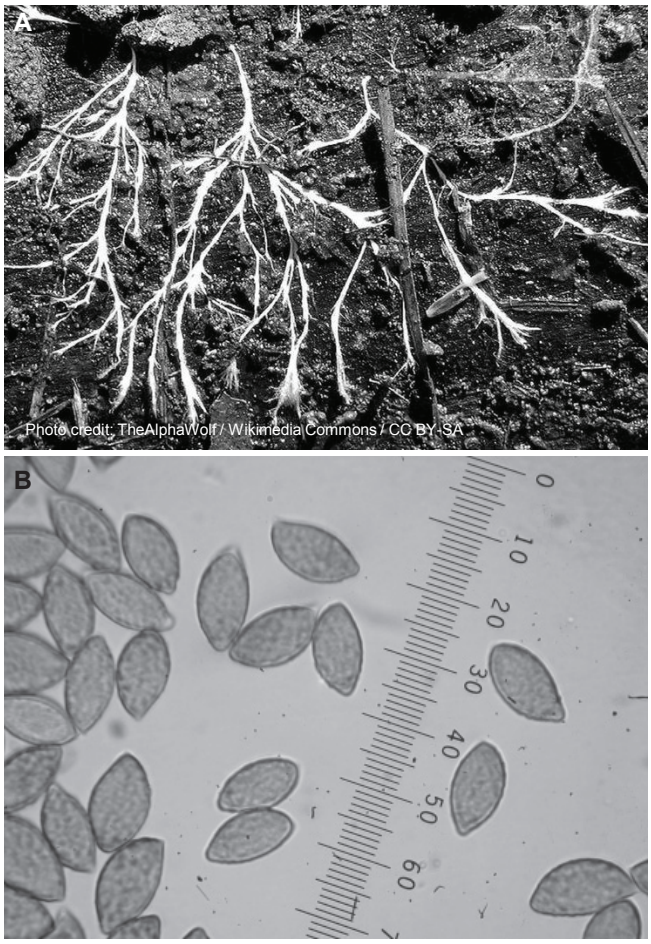


FIGURE 2 Example of A) fungal hyphae in soil, B) fungal spores. Photo credit for 2B: Ronpast / Wikimedia Commons / CC-BY-SA-3.0.

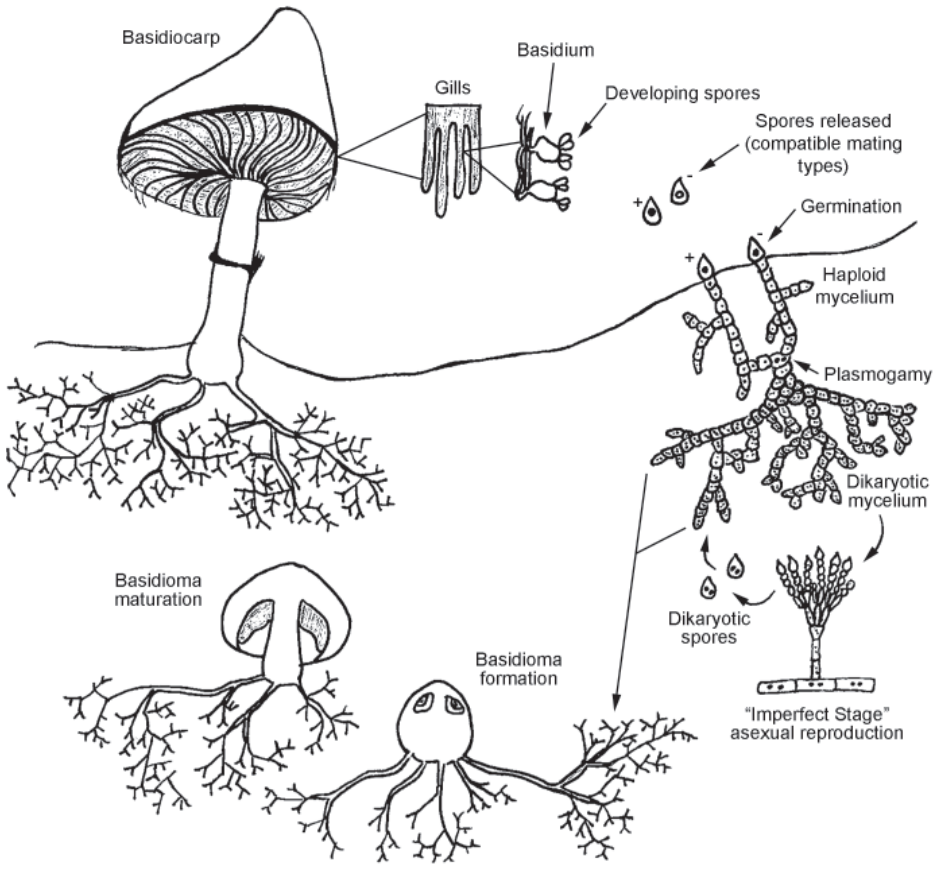


FIGURE 3 A typical fungal life cycle, including sexual reproduction with the mating of compatible spores, and the imperfect stage where asexual reproduction leads to production of spores and budding.

occur as single-celled organisms, generally referred to as yeasts, many grow as hyphae, which are cylindrical thread-like structures, 2–10 μm in diameter. The hyphae may be either septate – divided into compartments separated by cross walls – or non-septate. Fungi grow from the tips of the hyphae. Many intertwined hyphae constitute a mycelium, the main body of the fungus. Finely and complexly branched, the mycelium occupies a large volume of soil and produces a wide variety of enzymes that act on soil organic matter and mineral compounds to release the nutrients and energy the fungus needs for growth.

Fungi reproduce by both sexual and asexual means. Both processes produce spores: a general term for resistant resting structures. Yeasts reproduce by budding or binary fission. A typical fungal life cycle comprises sexual reproduction with the mating of compatible spores, and the 'imperfect stage' where asexual reproduction leads to the production of spores through budding (Figure 3).

Like bacteria and archaea, fungi are extremely diverse and their unique life-history strategies allow them to serve a wide variety of ecological roles, for example decomposers, mutualists, endophytes of plants, pathogens, and even predators. Fungal hyphae are foundational components of soil food webs because they are forage for grazing soil biota. Fungal sporocarps are also important foods for larger animals. Box 2 outlines some of the most prominent roles of fungi in soil ecosystems.

SOILS AS A MICROBIAL HABITAT

Soils harbour enormous microbial diversity. The total fresh weight mass of organisms below temperate grassland can exceed 45 tonnes per hectare, equalling or exceeding above-ground biomass (Ritz et al. 2003). Bacteria are present in greatest numbers, with archaea 10-fold less. Estimates of the number of species of bacteria per gram of soil range from 2000 to 18 000. Fungi, however, often contribute the largest part of the total microbial biomass in soils.

The soil environment is very complex and provides diverse microbial habitats. Soils vary greatly depending on climate, organisms, land form, and parent material. Over time these factors interact so that soils develop characteristic horizons (Figure 4). The profile of a soil reflects the decomposition and incorporation of organic materials into the mineral matrix, the formation of

BOX 2 Major functional roles of fungi in soil

While fungi perform a vast diversity of functions, three functional groups of fungi have particular importance in soil ecosystems: the saprotrophs, the mycorrhizas, and the lichens.

Saprotrophic fungi produce a wide range of enzymes, including amylases, proteases, lipases, and phosphatases. These enzymes are produced by hyphae at the front of the mycelium as it grows through its substrate. From a single germinated spore, the mycelium will often grow radially outwards creating a ring of metabolic activity. The sugars, peptides, amino acids and lipids liberated by the fungal enzymes may not necessarily be acquired by this fungus, but are competed for intensely by bacteria, plants, and other soil biota including other fungi. Thus, by making substrates available to other soil organisms, saprotrophic fungi increase the biomass and diversity of soils and play a critical role in decomposition. This is particularly evident in groups of saprotrophic fungi that specialise in degrading recalcitrant plant and animal compounds such as chitin (other fungi and insect exoskeletons), keratin (animal hair and feathers), cellulose (within plant fibres), and lignin (in plants). For example, 'white-rot' fungi are unique because they can degrade lignin into less recalcitrant molecules, which can be acted upon by enzymes from a wider variety of organisms. Saprotrophic fungi play a critical role in the global carbon cycle.

Mycorrhizal fungi form mutually beneficial symbiotic associations with living plant roots. The symbiosis is based on the exchange of resources: the plant receives soil nutrients from the fungus and the plant provides sugars as a source of carbon to the fungus. The vast majority of all land plants form mycorrhizal associations and these allow plants to occupy a much broader range of soil environments than would otherwise be possible.

Arbuscular (AM) and ectomycorrhizal (EM) fungi form symbioses with the broadest range of host plants. AM fungi colonise approximately 80% of all plant species, and are prevalent among herbaceous species including many important crop plants. In these, the site of nutrient exchange is the arbuscule: a finely branched, tree-like hypha that actually penetrates the plant root cell. Mycelia of AM fungi tend to be small compared with those of EM fungi, but they are particularly important for plant access to inorganic soil phosphorus.
In temperate regions, most dominant trees and woody plants, including commercially important pine, spruce, fir, oak, beech, poplar and willow, form associations with EM fungi. In EM associations, the fungus remains predominantly on the surface of the root and penetrates only between root cells, but may produce an extensive extra-radical mycelium. Like saprotrophic fungi, EM fungi are critical decomposers of organic materials in soils. Because they are fuelled by carbon from the plant, EM fungi may have the energy to produce energetically more expensive enzymes than typical saprotrophs. Saprotrophic fungi often dominate the surface layers of the soil profile, where they decompose recently shed plant litter, while EM fungi dominate lower in the profile, where they mobilise nitrogen for use by their host plants (Lindahl et al. 2007).

The extensive mycelium of EM fungi enables their vegetative hyphae to fuse to one another (anastomose); this, and the tendency for EM fungi to be non-specific to host plants, means EM fungi often form extensive, complex underground connections known as mycorrhizal networks. Mycorrhizal networks (MNs) occur in all major terrestrial ecosystems and allow materials – including carbon, nutrients, water, defence signals and allelochemicals – to be transferred between plants. Virtually all seeds that germinate in soil do so within an existing mycorrhizal network, allowing the young plant to quickly tap into this pathway of below-ground resource transfer (Teste et al. 2009). Thus, MNs have important effects on plant establishment, survival, and growth, as well as implications for plant community diversity and stability in response to environmental stress. MNs are considered fundamental to ecosystems as complex adaptive systems, because they provide avenues for feedbacks and cross-scale interactions that lead to self-organisation and emergent properties (Simard et al. 2012).

Lichens are symbiotic mutualistic associations between a fungus and a green alga (bipartite symbiosis), and sometimes also with cyanobacteria (tripartite symbiosis). The fungus contributes the 'body' of the symbiosis, protecting the photobionts from radiation and dehydration, and secreting organic acids that mobilise insoluble minerals from the substrate. The alga photosynthesises to produce carbon, and the cyanobacteria, if present, fix atmospheric nitrogen into ammonium, a usable form of N. As a symbiosis, lichens are nutritionally independent and are remarkably tolerant to extremes in temperatures and humidity, being particularly adapted to desiccation. This allows them to persist in many habitats inaccessible to plants, including the High Arctic, the Antarctic, and alpine and desert environments. On these barren substrates lichens commonly take on one of three growth forms: crustose (forming a crust), foliose (leafy), or fruticose (lacy). The organic acids they secrete help to break down primary substrates, thereby helping a soil profile to develop and facilitating primary succession of plants onto these new soils.

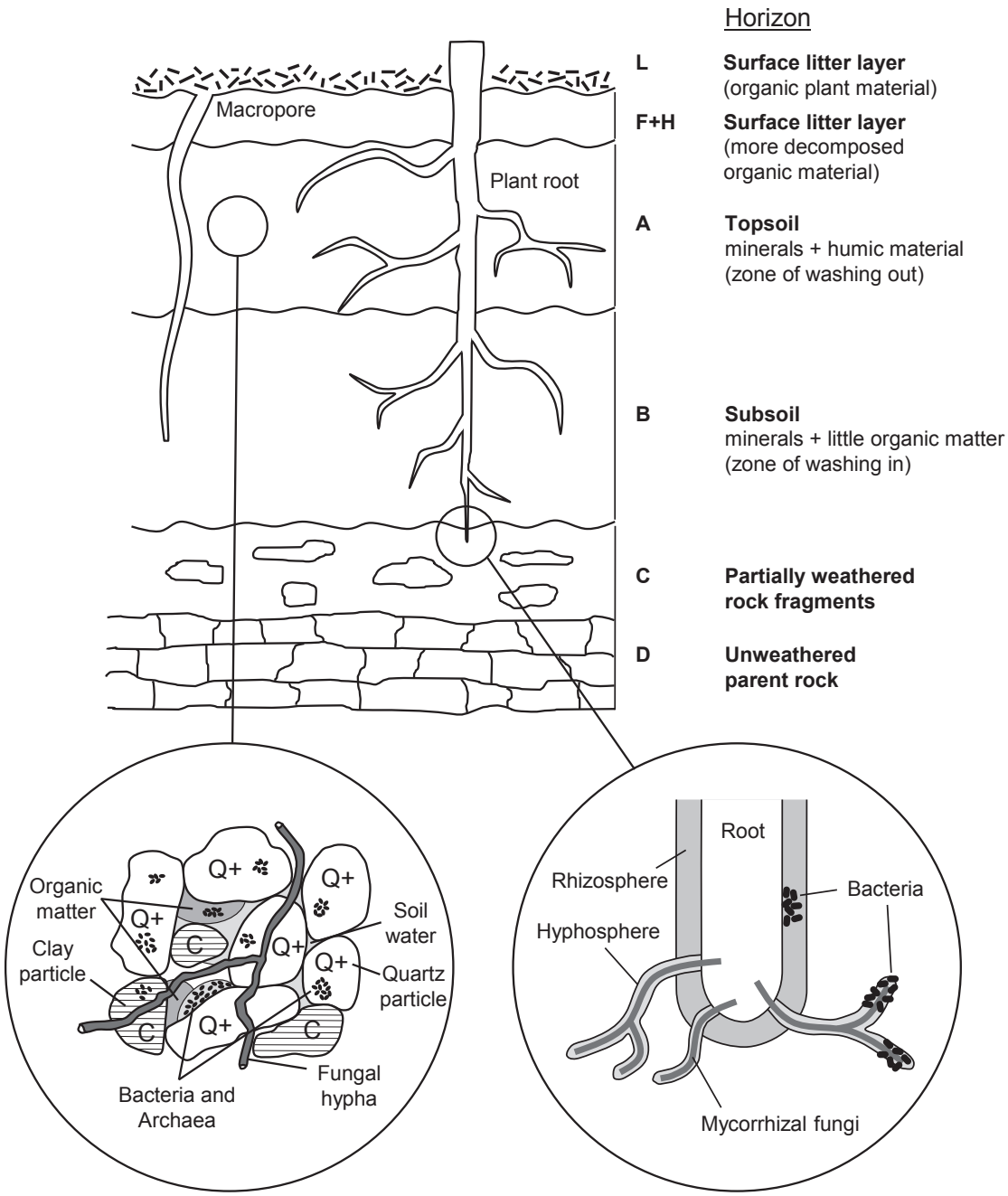


FIGURE 4 A typical soil profile showing horizons and microbial habitats (adapted from Stolp 1988).

humus, and the processes of mineral weathering. Decomposition and weathering are mediated by soil microbes. Typical horizons that develop in soils include the L, F+H, A, E, and B horizons. The L and F+H horizons occur in forest soils, whereas in agricultural soils the top layer is the A horizon. The L horizon is the layer of dead organic materials, including plant leaf litter, windfall, and animal wastes that accumulate on the surface of a soil. Organic materials in the L horizon are relatively undecomposed, with those in the F+H horizons being progressively more so. Immediately below this layer of organic accumulation lies the first mineral soil layer, the A horizon. The A horizon is typically dark in colour because of its high organic matter content. It has the highest density of soil microbes and plant roots and is the site of considerable organic matter decomposition and humification in soil. As water falls onto the soil surface and infiltrates the A horizon, organic compounds and minerals like iron (Fe), aluminium (Al), clays, and other ions are leached. Where this process is pronounced, an E horizon develops; this is lighter in colour than the A-horizon from which it formed. The E horizon retains non-mobile constituents and thus tends to be enriched in some minerals such as quartz. The deeper B horizon, often called the 'subsoil', is a zone of accumulation; it contains leached materials such as Fe, Al and silicate minerals as well as humified organic compounds and clay. Below the B horizon is the least altered parent material. A layer of broken or partially weathered stones often forms a C horizon above solid, unweathered bedrock.

Soil structure refers to the naturally occurring arrangement of soil particles into aggregates. Soil aggregates are initiated by the chemical and physical interaction of microbial and plant derived organic matter (such as polysaccharides and humic acids) with soil clay particles. Over time, physical forces such as drying and rewetting, and the movements of soil biota, shape these organo-mineral complexes into progressively larger aggregates. These aggregates are fundamental to all soil biological processes because they determine the pore size for water and air movement, which in turn controls microbial activity and soil organic matter

turnover. Microbial activity in soil aggregates can influence oxygen distribution within soils, creating habitats for anaerobic microbes that catalyse a variety of soil processes such as methane production and denitrification. Within the soil aggregates most microbes adhere to the surface of soil particles, where they form microcolonies (Figure 4). However, they are unevenly distributed and colonise only a small part of the available surface area. Organic matter and clay content of the soil are particularly important for determining the sorption of microbes to soil.

Microbes exist throughout the soil profile; however, they are most abundant in surface soils, the rhizosphere of plants, and around macropores (Bundt et al. 2001; Fierer et al. 2007). Macropores are channels formed by plant roots, earthworms, and other soil biota and are often lined with organic matter. Both numbers and diversity of microbes are correlated with organic matter. Hence, soil microbial abundance and diversity are highest in the top 10 cm and decline with depth. Interestingly, Eilers et al. (2012) noted that bacterial composition was most variable in the surface horizons whereas lower down the communities were relatively similar. The taxonomic and functional diversity of soil microbes is influenced by the growth of plant roots, which locally modify the chemistry of soil in the rhizosphere by exuding carbon and excreting and adsorbing nutrients. In the rhizosphere plants allocate 1–22% of photosynthetic assimilate to their ectomycorrhizal fungus partner (Hobbie 2006), the mycelium of which represents a major route by which carbon flows between the plant and the soil microbial community. Carbon is released from the hyphae of the EM fungi as exudates like trehalose, mannitol or oxalic acid, and when hyphae senesce. Mycorrhizal root tips and the vegetative mycelium (the hyposphere) also provide a habitat for bacteria (Figure 4).

SOIL MICROBIAL DIVERSITY

Early studies of soil bacterial and fungal diversity focused on what could be readily cultured from soils, but the realisation that less than 10% of the soil bacterial community could be readily cultured meant other approaches were required. In the 1980s Norman Pace and colleagues realised organisms could be identified in naturally occurring microbial populations without first culturing them (Hugenholtz et al. 1998). These techniques typically require the extraction and isolation of ribosomal RNA (rRNA) genes directly from cells in soil. Following isolation, the rRNA genes are amplified from total community DNA using the polymerase chain reaction (PCR) with rRNA-specific primers. These primers can select different microbial groups at level of the domain (Bacteria, Eukarya, and Archaea), or phylum (e.g. Actinobacteria or Bacteroidetes). Different approaches can be taken to separate and sequence the rRNA genes. Advances in high-throughput DNA sequencing now allow thousands of individuals to be identified in each of thousands of samples in a week (Caporaso et al. 2012). Comparison of these sequences with rRNA genes from cultivated species and with sequences in databases such as GenBank allows evolutionary (phylogenetic) relationships between unknown and known organisms to be determined and provides an estimate of the genetic diversity of organisms in the community. Sequence information also allows speculation about the organism's characteristics, given what is known of its closest cultivated relative. Sometimes, phylogenetic information can also be used to infer physiology; for example, all cyanobacteria form a monophyletic group, as do many sulphate-reducing bacteria, halophiles, and methanogenic archaea.

Soil bacterial phyla

Molecular tools have been used to investigate in situ soil bacterial community composition. These investigations have revealed that although bacteria have been subdivided into more than 100 phyla, fewer than 10 are abundant in soil (Table 2) (Janssen 2006). The estimated relative abundance of the major phyla varies between different soils (or samples); members of the phyla Proteobacteria, Acidobacteria, and Actinobacteria are widespread and often abundant, whereas members of the Verrucomicrobia, Bacteroidetes, Firmicutes, Chloroflexi, Planctomycetes, and Gemmatimonadetes are generally less prevalent. While the number of phyla in soil is low it appears the species diversity is

TABLE 2 Dominant bacterial phyla in soil (adapted from Janssen 2006)

Phyla/Subphyla	Mean contribution (%)	Range (%)	Examples
α-Proteobacteria	19	2–43	*Sphingomonas, Rhizobium*, *Mesorhizobium, Bradyrhizobium*, *Methylobacter, Methylophilus, Nitrospira, Nitrobacter, Rhodobacter*
β-Proteobacteria	10	2–31	*Burkholderia, Alcaligenes*, *Acidovorax*, *Collimonas*, *Nitrosospira*, *Thiobacillus*, *Rhodocyclus, Methylomonas*
γ-Proteobacteria	8	1–34	*Pseudomonas, Xanthamonas, Azotobacter*, *Thiocapsa, Chromatium*
δ-Proteobacteria	2	0–10	*Desulfovibrio, Bdellovibrio*
ε-Proteobacteria	<1	0–1	*Helicobacter, Campylobacter*
Acidobacteria	20	0–35	*Acidobacterium*
Actinobacteria	13	0–25	*Arthrobacter*, *Rhodococcus*, *Streptomyces, Mycobacterium*, *Rubrobacter, Terrabacter*, *Acidimicrobium*
Verrucomicrobia	7	0–21	*Chthoniobacter*, *Opitutus*
Bacteroidetes	5	0–16	*Chitinophaga*
Firmicutes	2	0–7	*Clostridium*, *Bacillus, Lactobacillus*
Chloroflexi	3	0–16	
Planctomycetes	2	0–8	
Gemmatimonadetes	2	0–4	*Gemmatimonas*
Other groups	5	2–10	
Unknown	2	0–13	

high compared with other environments (Nemergut et al. 2011). However, more than 10% of the sequences in a soil sample may not be able to be assigned to known phyla (Janssen 2006; Nacke et al. 2011).

The Proteobacteria are a metabolically diverse group of organisms in several subphyla, four of which, α-, β-, γ-, and δ-Proteobacteria, are commonly reported in soil. Members of the α, β, and γ subphyla are considered to be copiotrophs: they are more prevalent where resource availability is high such as in rhizosphere soils (Fierer et al. 2007). Adding low-molecular-weight carbon to soil increased the relative abundances of β- and γ-Proteobacteria (Eilers et al. 2010; Goldfarb et al. 2011), while spiking soils with recalcitrant carbon (cellulose, lignin, or tannin-protein) increased the relative abundance of α-, β-, and δ-Proteobacteria (Goldfarb et al. 2011). Most notably, numbers of bacteria in the class Burkholderiales within the β-Proteobacteria increased in response to both labile and chemically recalcitrant substances (Goldfarb et al. 2011).

The α-Proteobacteria contain metabolically diverse heterotrophic and autotrophic bacteria. Among the heterotrophs are *Sphingomonas*, which degrade a wide range of toxic compounds including pentachlorophenol and polyaromatic hydrocarbons. They have also been implicated in weathering of minerals. The heterotrophs include the nitrogen fixers belonging to the Rhizobiaceae, for example *Rhizobium*, *Mesorhizobium* and *Bradyrhizobium*, all of which form symbiotic relationships with legumes. Soil methane-oxidisers such as *Methylobacter* and *Methylophilus* also belong to the α-Proteobacteria. Among the autotrophs are nitrite oxidisers in the genera *Nitrospira* and *Nitrobacter,* and phototrophs in *Rhodospirillum* and *Rhodobacter*.

The β-Proteobacteria include heterotrophs, autotrophs, and methanotrophs. The best known heterotrophs in soil belong to the genera *Burkholderia, Alcaligenes*, and *Acidovorax*. *Burkholderia* species probably play a major role in carbon turnover: they are metabolically diverse, using simple amino acids and sugars and recalcitrant aromatic and phenolic compounds as carbon substrates. Members of *Burkholderia* are also reported to fix nitrogen and promote plant growth. Among the heterotrophs is *Collimonas*, which produces chitinase and may degrade live hyphae (de Boer et al. 2004). Both *Burkholderia* and *Collimonas* species weather minerals (Uroz et al. 2007). Autotrophs include the ammonia oxidiser *Nitrosospira*, the iron oxidiser *Thiobacillus* and the phototroph *Rhodocyclus*. An example of a methanotroph belonging to the β-Proteobacteria is *Methylomonas*.

The γ-Proteobacteria in soil include heterotrophs, lithotrophs, and phototrophs. Among the best known heterotrophs are *Pseudomonas* and *Xanthamonas*. *Pseudomonas* species exhibit remarkable nutritional versatility. Most grow on more than 50 different substrates, some on more than 100. These substrates include sugars, amino acids, fatty acids, alcohols, and hydrocarbons. The γ-Proteobacteria also include the photolithotrophs *Thiocapsa* and *Chromatium*; under anaerobic conditions in light, these use sulphide or elemental sulphur as an electron donor and carbon dioxide as a carbon source.

The δ-Proteobacteria contain mainly sulphate- and iron-reducing bacteria. In soil the sulphate reducer *Desulfovibrio* grows anaerobically with carbon sources such as lactate or ethanol, which occur in soils where oxygen is depleted due to organic matter decomposition. *Bdellovibrio,* a bacterial parasite, also belongs to this group.

The ε-Proteobacteria comprise few known genera. Among those detected in soil are the curved to spirilloid *Helicobacter* and *Campylobacter*. Both species inhabit the digestive tract of animals and could enter soil following the deposition of faeces.

Proteobacteria commonly detected in the rhizosphere include *Burkholderia, Collimonas*, and relatives of the *Rhizobiaceae.*

Acidobacteria are widespread in soils and increase in relative abundance as soil pH declines (Lauber et al. 2009). Analysis of 16S rRNA gene sequences indicates this phylum is highly diverse. More than 20 different subgroups occur in soils but members of subgroups 1, 2, 3, 4, and 6 are reported to be most abundant in soil (Jones et al. 2009). Very little is known of their metabolic capabilities as they are poorly represented in soil culture collections. However, increasingly they are being isolated by using oligotrophic media and prolonged incubation (Davis et al. 2011). Genome sequencing of three cultured soil Acidobacteria (*Acidobacterium capsulatum* and Ellin 345 from subgroup 1 and Ellin6076 from subgroup 3) suggests that bacteria belonging to this phyla may be oligotrophs that metabolise a wide range of simple and complex carbon sources (Ward et al. 2009). They also appear well suited to low nutrient conditions, tolerate fluctuations in soil moisture, and are capable of nitrate and nitrite reduction, but not denitrification or nitrogen fixation. Bacteria closely related to the genera *Acidobacterium* are reported to be among the most abundant in soil.

Like the Acidobacteria, the Verrucomicrobia appear to be ubiquitous in soil, and may be oligotrophs, which might explain why they are under-represented in culture collections (Janssen 2006). The ecology of Verrucomicrobia remains poorly understood. The major group of Verrucomicrobia found in soil is the class Spartobacteria, of subdivision 2, which is reported to dominate Verrucomicrobia in grasslands and subsurface soil horizons at 10–50 cm depth (Bergmann et al. 2011). This class contains free-living taxa and endosymbionts associated with nematodes of the genus *Xiphinema*. Most phylotypes in soil have been found to be most closely related to *Chthoniobacter flavus,* a free-living aerobic soil heterotroph (Bergmann et al. 2011). Genome sequencing of *C. flavus* Ellin428 has revealed it can metabolise polysaccharides of plant origin but not amino acids or organic acids except for pyruvate (Kant et al. 2011). In contrast, genome sequencing of *Optiutus terrae*, a verrucomicrobium from rice paddy soil, revealed it is a fermentative anaerobe that produces propionate from the fermentation of plant polysaccharides (van Passel et al. 2011).

Microbes with gram-positive cell membranes tend to be abundant in soil culture collections. Gram-positive bacteria fall into two phylogenetic groups, Actinobacteria and Firmicutes. The Actinobacteria in soil are commonly assigned to the subphyla Actinobacteridae, Acidimicrobidae, and Rubrobacteridae (Janssen 2006). The relative abundance of Actinobacteridae in soil increases following addition of labile carbon sources (Goldfarb et al. 2011). Actinobacteria belonging to the subclass Actinobacteridae and isolates from soil include *Arthrobacter*, *Rhodococcus*, *Streptomyces*, and *Mycobacterium*. They are metabolically diverse aerobic heterotrophs. *Streptomyces* are known for their ability to produce antimicrobial compounds. The Rubrobacteridae include the genera *Rubrobacter* and *Solirubrobacter.* Both genera are not common in soil culture collections. *Rubrobacter* are especially prevalent in desert soils and may resist ionizing radiation (Holmes et al. 2000). Among the few cultured members of Acidimicrobidae that have been detected in soil is the acid-tolerant ferrous iron oxidiser

Acidimicrobium ferroxidans.

Members of the Firmicutes include the endospore-forming and the lactic acid bacteria. Among the best known genera of endospore formers in soil are the aerobic to facultatively anaerobic genus *Bacillus* and the anaerobic genus *Clostridium*. *Bacillus* degrades many different carbon sources, including plant polysaccharides. Some are fermentative while others fix nitrogen or denitrify. *Clostridium* is metabolically diverse, and may ferment sugars, starch, pectin, and cellulose. The relative abundance of Clostridiales in soil increases following addition of recalcitrant C compounds (Goldfarb et al. 2011). Production of endospores has been linked to long-term survival in soil during dry periods. Lactic acid bacteria (e.g. *Lactobacillus*) are aerotolerant anaerobes often isolated from decaying plant material.

Bacteria assigned to the Bacteroidetes that are frequently isolated from soil often belong to the Sphingobacteria. They are involved in aerobic degradation of complex organic molecules such as starch, proteins, cellulose, and chitin. In soil they may be important for degrading plant material. Among the Sphingobacteria, close relatives of the genus *Chitinophaga* are reported to be abundant in soil. Members of this genus are filamentous, chitinolytic and can move by gliding. In soil they may use fungal hyphae and insects as sources of carbon. It has been suggested that Bacteroidetes are copiotrophs, because their relative abundance in soil may increase following carbon-addition (Fierer et al. 2007; Eilers et al. 2010). The relative abundances of Bacteroidetes and Actinobacteria tend to increase with increasing soil pH (Lauber et al. 2009).

Very little is known about the physiology, genetics, and ecology of soil bacteria belonging to the phyla Gemmatimonadetes, Chloroflexi, and Planctomycetes because few representatives of these phyla have been cultivated. A few soil isolates of Gemmatimonadetes have been obtained; they belong to subphyla 1 and are aerobic heterotrophs. DeBruyn et al. (2011) suggested they are adapted to low soil moisture conditions. Members of the genus *Gemmatimonas* are reported to be abundant in soil. Aerobic heterotrophs that belong to Chloroflexi and grow on oligotrophic media have been isolated (Davis et al. 2011); there is also evidence that soil Chloroflexi respire organohalide compounds (Krzmarzick et al. 2011). Planctomycetes are organisms that divide by budding and lack peptidoglycan in their cell walls. Members of these phyla have been implicated in anaerobic ammonium oxidation (anammox) in soil (Humbert et al. 2010). Bacteria belonging to the superphylum Planctomycetes-Verrucomicrobia-Chlamydia are notable from an evolutionary standpoint because they have a range of characters rare in Bacteria but common in Archaea and Eukarya (Devos and Reynaud 2010). These include the presence of membrane-coat-like proteins and condensed DNA.

Soil archaeal phyla

The distribution of archaea within soil has been the subject of numerous 16S rRNA gene surveys (Bates et al. 2011). These have revealed the widespread presence of archaea, primarily members of the phylum Crenarchaeota, in soil. They are most abundant below the topsoil. Though crenarchaea are relatively diverse, those abundant in soils tend to be restricted to one specific lineage, namely group 1.1b. There is evidence of soil crenarchaea contributing to ammonia oxidation in soil. Soil metagenomic studies have revealed that crenarchaea affiliated with lineage group 1.1b contain and express *amoA* genes (Treusch et al. 2005). More recently, an ammonium-oxidising crenarchaea, identified as *Nitrososphaera viennensis*, was isolated from garden soil (Tourna et al. 2011), and subsequent phylogenetic analysis confirmed its taxonomic affiliation with group 1.1.b.

Euryarchaeota, specifically methanogens, are present in soil but active only in anoxic conditions, for example when the soils are waterlogged (Angel et al. 2012). They are strict anaerobes and grow in association with bacteria where they participate in the anaerobic food chain, converting complex organic molecules to methane and carbon dioxide. The pathways methanogens use to generate methane vary. They include reduction of carbon dioxide and methanol, cleavage of acetate, and production of methane from methylated compounds. In soil, methanogens belonging to the genera *Methanosarcina, Methanosaeta*, and *Methanocella* are widespread. Both *Methanosarcina* and *Methanosaeta* reduce acetate to produce methane.

Soil fungal phyla

Fungi are ancient. Fungal-like organisms appeared in the fossil record at least 1400 million years ago and all modern classes of fungi had appeared by the Late Carboniferous, approximately 300 million years ago. Fungi are thought to have colonised land during the Cambrian period, well in advance of plants. Not surprisingly, given their ancient origins, fungi have evolved to occupy nearly every ecological niche on earth. It is estimated that there are 1.5 million to 5 million species of fungi. Like plants, fungi were historically classified on the basis of reproductive structures. With the advent of next-generation sequencing technologies and the analysis of multiple genetic marker datasets, fungal taxonomy has changed substantially in recent years. Seven fungal phyla are currently recognised (Hibbett et al. 2007): Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Ascomycota, Basidiomycota, and the relatively recently evolved lineage of parasitic endobionts, the Microsporidia, which are sometimes considered to be a sister-group of the fungi (Liu et al. 2006). Figure 5 depicts the evolutionary relationships among extant groups of fungi.

The first three of these fungal phyla have in common the presence of flagellated cells during at least one stage of their life history. From an evolutionary perspective, these groups differ from the 'higher' fungi, which lack motile cells and have thus become truly terrestrial organisms. Of these three phyla, the widely distributed Chytridiomycota are considered the basal group. They are saprobes and many can degrade chitin and keratin. While some species are unicellular, others form coenocytic thalli. The unicellular chitrid *Batrachochytrium dendrobatidis* is a deadly pathogen of many amphibian species that plays a major role in

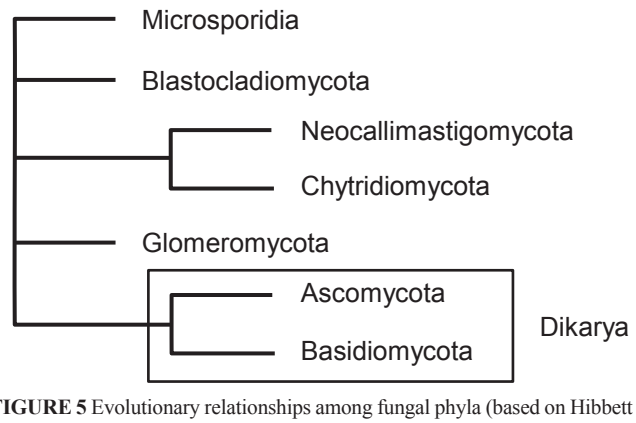


FIGURE 5 Evolutionary relationships among fungal phyla (based on Hibbett et al. 2007).

the decline of amphibian populations worldwide. However, the New Zealand endemic and critically endangered Archey's frog (*Leiopelma archeyi*) appears to naturally eliminate *B. dendrobatidis* when infected (Bishop et al. 2009), and the cause of its decline is still unknown. Also important in moist soil habitats are the Blastocladiomycota which differ from the Chytridiomycota most strikingly during reproduction when they undergo a different form of meiosis. Like the Chytridiomycota, many are saprobes of dead organic matter. Others are pathogens of soil organisms including tardigrades, algae, nematodes, insects, and plants. The phylum Neocallimastigomycota contains fungi that live in the rumens of ungulate animals, where they are vital in digesting fibre.

While completely terrestrial during their life history, members of the Glomeromycota retain other features of the 'lower' fungi. Their mycelia are formed of multinucleate cells that lack cross walls, and hyphal fusion is rare, occurring uniquely through conjugation of specialised hyphae (gametangia) during sexual reproduction. Many Glomeromycota have no known sexual stage. They produce very large (80–500 μm), thick-walled asexual spores, which are common in many soils and germinate in response to the presence of a plant root. While the phylum Glomeromycota contains few species, it has enormous ecological and economic importance. The phylum contains the arbuscular mycorrhizal (AM) fungi, which form obligate biotrophic symbioses with approximately 80% of all land plants (Smith and Read 1997). The fossil record of this group is ancient, extending approximately 460 million years before present (Simon et al. 1993), and clearly showing that Glomeromycota were critical for allowing plants to colonise land in the early Devonian period. In addition to symbioses with higher plants, Glomeromycota form obligate biotrophic symbioses with mosses, and with the cyanobacteria *Nostoc* to form cyano-lichens.

An important derived trait of the 'higher' fungal phyla is the presence of the dikaryon, where a hyphal cell maintains two compatible nuclei. This arises when compatible hyphae fuse to combine cytoplasm but not nuclei. Daughter cells of the dikaryon maintain this binucleate state. The trait, which is thought to have arisen in the last common ancestor of the Ascomycota and Basidiomycota (Tehler 1988), is so important that it has secured these two extant groups their own subkingdom among fungi – the Dikarya.

The Ascomycota are by far the largest fungal phylum with more than 64 000 named species. The definitive feature of this group is the presence of asci: sac-like spore-bearing structures that are clustered together and produced in large numbers during sexual reproduction. Sexual mating, however, is relatively rare among the ascomycetes, and many have only an asexual stage. Consequently, the dominant stage of the life cycle for many members of the Ascomycota is the haploid mycelium, and the formation of a dikaryon may be rare and short-lived. The typical haploid ascomycete mycelium comprises septate hyphae with cell walls containing chitin and β-glucans. This phylum constitutes a huge range of fungi with nearly every imaginable life-history strategy. Some macroscopic ascomycetes produce well-known reproductive structures like morels, truffles, and cup and bird's-nest fungi. Conversely, many members of the Ascomycota are microscopic and exist as single-celled yeasts (e.g. *Saccharomyces*) or as filamentous fungi (e.g. *Aspergillus*). Dimorphic fungi can switch between yeast and hyphal phases in response to environmental conditions. Most Ascomycota are saprotrophic and these have evolved a huge range of enzymes to degrade complex substrates including cellulose, keratin, and collagen; consequently, ascomycetes are critical in soils as decomposers and nutrient recyclers (see Box 2).

Many Ascomycota live symbiotically with other organisms. Approximately 18 000 species of ascomycetous fungi live in symbiosis with green algae, and sometimes cyanobacteria, to form lichens. These ascomycetes form the thalli of 98% (Honegger 1996) of lichen species and include all major lichen growth forms. Lichenisation is believed to have evolved and been lost among the Ascomycota many times (Lutzoni et al. 2001). Other ascomycetes form ectomycorrhizal and/or ectendomycorrhizal associations with woody plants. Many of these fungi are inconspicuous because they fruit below ground; nonetheless, they tend to be widespread because they have broad host ranges (Smith and Read 1997). The Ascomycota are also important parasites of plants. For example, the pathogenic Ascomycete *Cyttaria* infects *Nothofagus* in New Zealand producing 'beech strawberries' during sexual reproduction by the fungus (Figure 6). However, perhaps the most remarkable lifestyle of a member of the Ascomycota in soil is that of predator. Members of the family Orbiliaceae are carnivorous fungi with hyphae that are specialized to trap prey. Some species' hyphae are spring-loaded, ring-shaped traps that respond to the movement of prey, which include a variety of soil mesofauna including protists, nematodes, tardigrades, and arthropods.

Members of the Basidiomycota, commonly known as the 'club fungi', produce spores on club-like stalks called basidia during sexual reproduction. While basidia are microscopic, they are often produced en masse on specialised structures (sporocarps) that we recognise as mushrooms, toadstools, wood-corals,

FIGURE 6 Reproductive structures of the parasitic ascomycete *Cyttaria* sp., commonly known as beech strawberries. Here they are depicted on black beech (*Nothofagus solandri*) in Nelson Lakes National Park, New Zealand.

FIGURE 7 Examples of the unique lifestyles of Ascomycota (top panel) and Basidiomycota (bottom panel) in soils.

shelf fungi, and puffballs. As in the Ascomycota, haploid hyphae fuse to form the dikaryon, but in Basidiomycota the dikaryotic mycelium often becomes the dominant stage of the life cycle, outcompeting the monokaryotic mycelium in soil, and lasting many months or even centuries. In response to an environmental cue like autumn rain, compatible nuclei fuse within the dikaryotic mycelium to produce a diploid sporocarp. Here, meiosis takes place within basidia and spores containing haploid nuclei are released, often forcefully, to the environment, where each will germinate and form a new haploid mycelium (Figure 3).

The Basidiomycota comprise nearly 32 000 species of fungi (Kirk et al. 2008) and three major subphyla (Hibbett et al. 2007): the Pucciniomycotina, Ustilaginomycotina, and the Agaricomycotina. The Pucciniomycotina and Ustilaginomycotina, which include the rust and smut fungi, are pathogens of many economically important plants including oats, wheat, maize, beans, coffee, apple, and sugarcane. The subphylum Agaricomycotina contains many charismatic fungi with important ecological roles in soils. The three classes of Agaricomycotina are delineated on the basis of typical reproductive structures: the Agaricomycetes (mushrooms and toadstools), the Dacrymycetes (puffballs), and the Tremellomycetes (jelly fungi). Members of the Agaricomycotina are particularly important in temperate forests and woodlands where they form the majority of ectomycorrhizas (as well as prized edible mushrooms). Others are critical decomposers. The 'soft', 'brown' and 'white' rot fungi produce hydrogen peroxide and enzymes to degrade complex plant compounds including cellulose and lignin. A few species in the Agaricomycotina are lichenised fungi (e.g. *Omphalina*). Figure 7 illustrates examples of the unique lifestyles of soil Ascomycetes and Basidiomycetes.

CONTRIBUTION OF MICROBES TO NUTRIENT CYCLING

In soils, microbes play a pivotal role in cycling nutrients essential for life. For example, soil microbes play major roles in

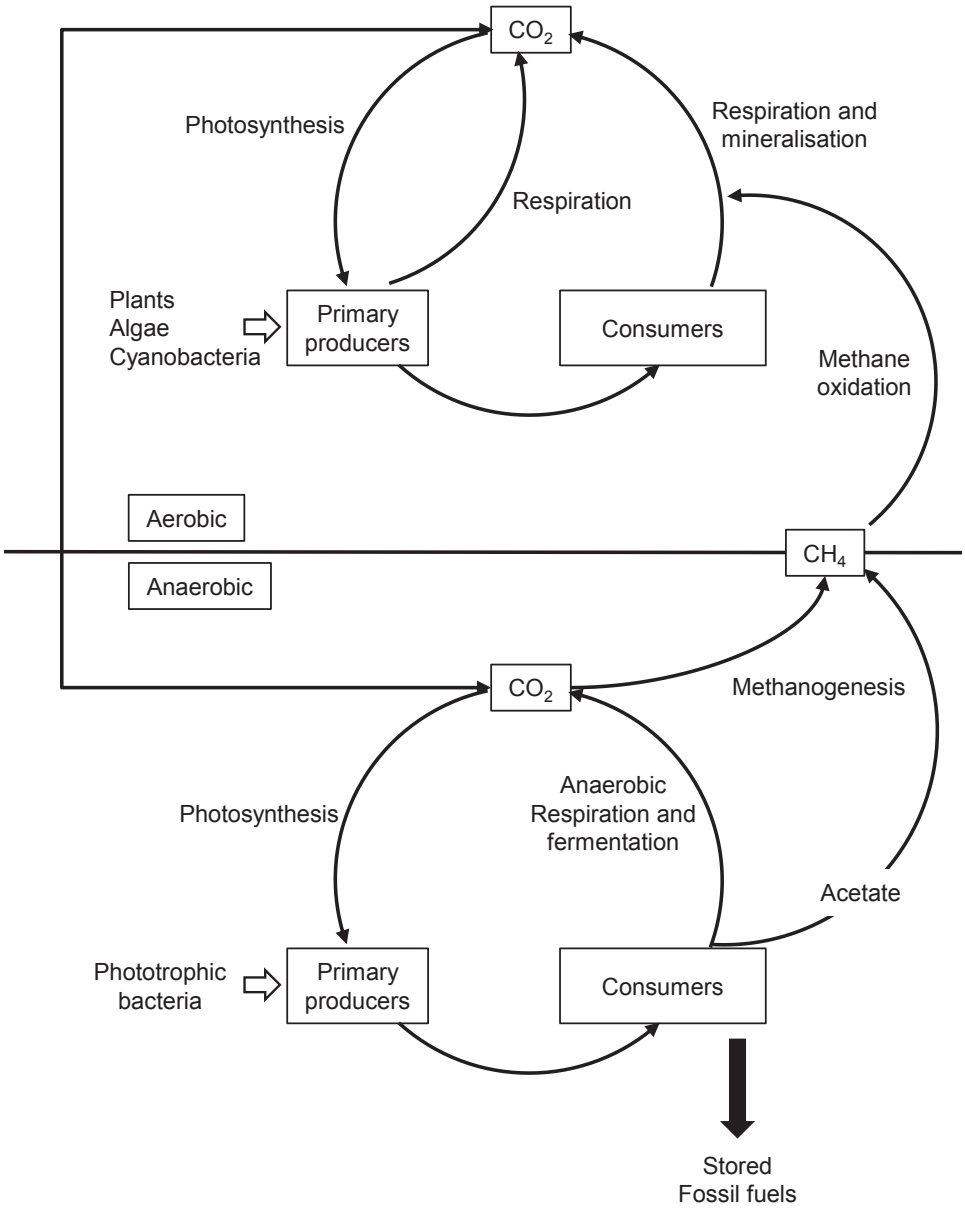


FIGURE 8 Microbial role in the global carbon cycle (adapted from Stolp 1988).

cycling carbon, nitrogen (N) and phosphorus, which are essential for producing biomolecules such as amino acids, proteins, DNA and RNA – the fundamental compounds of life. Many plant nutrients are ultimately derived from weathering of minerals. Silicate minerals such as feldspar, mica and hornblende provide calcium, magnesium and potassium, whereas apatite is the primary mineral source of phosphorus. Mineral weathering by soil bacteria and fungi plays a significant role in ion cycling and plant nutrition.

Carbon cycling

Microbes play major roles in the cycling of carbon – the key constituent of all living organisms (Figure 8). Primary producers fix carbon dioxide and convert it to organic material. In terrestrial ecosystems the primary producers of organic material are plants, although surface-dwelling algae and cyanobacteria, both free-living and symbiotic as lichens, can contribute significantly to carbon fixation in some ecosystems. Within soil, autotrophic microbes can also fix carbon dioxide (Box 1).

Organic materials resulting from primary production reside in living organisms and the non-living organic materials derived from them. Heterotrophic bacteria and fungi are the ultimate recyclers of non-living organic material. These soil saprotrophs complete the carbon cycle, converting organic material formed by primary producers back to carbon dioxide during respiration. They are sometimes aided in this process by higher animals (herbivores and carnivores) that digest particulate organic material with the help of microbes residing in their intestinal tracts. This process is known as decomposition and involves the degradation of non-living organic material to obtain energy for growth. Mineralisation of the organic compound occurs when it is degraded completely into inorganic products such as carbon dioxide, ammonia, and water.

In soil ecosystems, the major agents of organic matter decomposition are fungi, which constitute the majority of soil biomass (Box 2). However, both bacteria and fungi degrade complex organic molecules that higher organisms cannot break down. A wide variety of bacteria, especially those belonging to Actinobacteria and Proteobacteria, degrade soluble organic molecules such as organic acids, amino acids, and sugars (Eilers et al. 2010). Likewise, some bacteria, such as Bacteroidetes, help degrade more recalcitrant carbon compounds such as cellulose, lignin and chitin. Bacteria that target these recalcitrant carbon compounds may require relatively high levels of available N to support the production of extracellular and transport enzymes (Treseder et al. 2011). In contrast, bacteria adapted to low N environments are more adept at metabolising organic N compounds such as amino acids. Net carbon mineralisation in soils was reported to be positively correlated with β-Proteobacteria and Bacteroidetes abundance and negatively correlated with Acidobacteria (Fierer et al. 2007).

Microbes are unique in their capacity to carry out anaerobic (fermentative) degradation of organic matter, which results in the fermentation of organic compounds to organic acids, and generates gases such as hydrogen and carbon dioxide. Under strictly anaerobic conditions the hydrogen may be used by methanogens to reduce carbon dioxide to produce methane gas. Some methanogens can metabolise methanol, acetate or methylamine to methane and carbon dioxide. The oxidation of methane by soil bacteria is described in Box 3.

Nitrogen cycling

All organisms require nitrogen, because it is an essential element in protein and nucleic acids. Animals derive nitrogen from organic sources while plants require inorganic nitrogen sources such as ammonium and nitrate, or relatively depolymerised nitrogen sources such as single amino acids (e.g. glycine)

BOX 3 Methane oxidation

Lower in the profile of some soils, where anaerobic conditions predominate in micropores, (especially in bogs, fens and landfills) fermentative metabolism by methanogens may lead to the production of methane gas. As methane filters upwards in the soil profile through soil pores it may be oxidised by methanotrophs before it escapes to the atmosphere. Methanotrophs are bacteria and some fungi that oxidise methane to carbon dioxide. They are unique in being able to use single carbon compounds as their sole carbon source, and thus are said to have C1 metabolism. Figure 9 illustrates C1 metabolism, wherein microbial enzymes convert methane to methanol and formaldehyde for the production of biomass.

Among bacterial methanotrophs, two separate metabolic pathways have evolved to assimilate methane-C. Among the γ-Proteobacteria the ribulose monophosphate (RuMP) pathway is used and these bacteria are said to be Type I methanotrophs. Type II methanotrophs belong to the α-Proteobacteria and use the serine pathway for carbon assimilation. In New Zealand soils, Type II methanotrophs are the most dominant and active methane oxidisers in pine and shrub soils, while Type I methanotrophs (related to *Methylococcus capsulatus*) dominate activity and populations in pasture soil (Singh et al. 2007).

In many parts of New Zealand, soils are of volcanic origin. These soils tend to be fine textured and highly porous, and these characteristics enhance methane oxidation. Furthermore, in geothermally active areas natural methane seeps may occur, promoting the growth of methanotroph communities. Microbes in New Zealand volcanic soils may be useful for the development of methane mitigation technologies such as biofilters for dairy wastes (Pratt et al. in press).

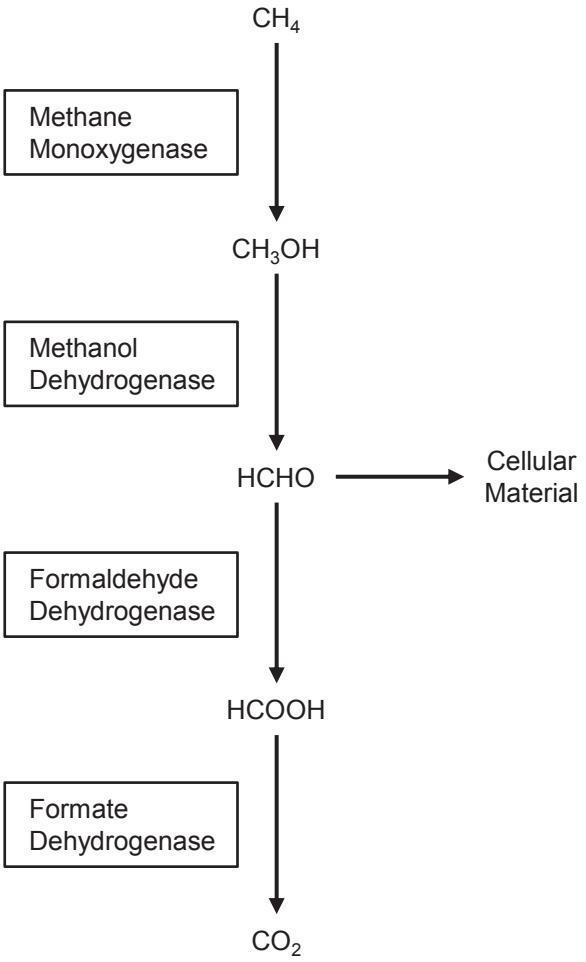


FIGURE 9 Illustration of single carbon compound (C1) metabolism used by methanotrophs for biomass production.

(Schimel and Bennett 2004). Most microbes can use ammonium or nitrate for growth.

Microbes play an important role in the nitrogen cycle (Figure 10). They carry out several processes not carried out by other organisms, namely nitrogen fixation, dissimilatory nitrate reduction to ammonia (DNRA), nitrification, anammox, and denitrification. Because nitrogen is often the major limiting nutrient for plant biomass production in terrestrial habitats, the rates of these microbial processes often limit ecosystem productivity. Some steps in the nitrogen cycle are mediated by few microbial groups (e.g. nitrogen fixation or nitrification) and are referred to as narrow processes, whereas others are mediated by many groups (e.g. DRNA) and are considered broad processes. The release of ammonium from soil organic matter during decomposition is known as ammonification.

Only bacteria and archaea carry out biological nitrogen fixation (N-fixation), the reduction of atmospheric nitrogen gas to ammonium. N-fixation is the only natural process through which new N enters the biosphere, so it is critically important for ecosystem function. N-fixation is catalysed by the enzyme nitrogenase. This enzyme is extremely sensitive to oxygen, requiring a low oxygen environment for activity. N-fixation is energetically expensive, consuming 16 moles of ATP per mole of N fixed. The ammonium produced through N-fixation is assimilated into amino acids and subsequently polymerised into proteins. Under nitrogen-limiting conditions, N-fixing microbes have an advantage. N-fixation is carried out by free-living microbes (e.g. *Azotobacter*, *Burkholderia*, *Clostridium* and some methanogens), some of which may be associated with the rhizosphere of plants, and bacteria that form symbiotic relationships with plants (e.g. *Rhizobium, Mesorhizobium, Frankia*). Exudates from plants may supply some of the energy required for N-fixation. In agricultural soils in New Zealand, rhizobia that form root nodules in symbiotic relationships with introduced legumes such as clover, lucerne or lotus are a significant source of N. Similarly, native legumes (e.g. *Sophora* and *Clianthus*) form symbiotic relationships with *Mesorhizobium* or *Rhizobium leguminosarum* (Weir et al. 2004). Notably, the strains of rhizobia on native legumes differed from those on weed legumes like gorse. The rates of N-fixation by symbiotic rhizobia are often two or three orders of magnitude higher than by free-living bacteria in soil.

During nitrification, ammonia or ammonium ions are oxidised to nitrite and then to nitrate. In soil, nitrification is aerobic and appears to be restricted to a few autotrophic bacteria and Crenarchaea. The two steps in nitrification – the formation of nitrite, then nitrate – are carried out by different microbial groups. In soils, oxidation of ammonia to nitrite is mediated by bacteria like *Nitrosospira* and *Nitrosomonas* or the crenarchaeum *Nitrososphaera*, whereas the oxidation of nitrite to nitrate is mediated by bacteria such as *Nitrobacter* and *Nitrospira*. Nitrifying microbes utilise the energy derived from nitrification to assimilate carbon dioxide. Nitrification is especially important in soils, because the oxidation of ammonium to nitrite and nitrate ions changes their charge from positive to negative. This leads to nitrate leaching, because the positively charged ammonium ions (NH_4^+) tend to be bound by negatively charged clay particles but the negatively charged nitrate ions (NO_3^-) can be readily

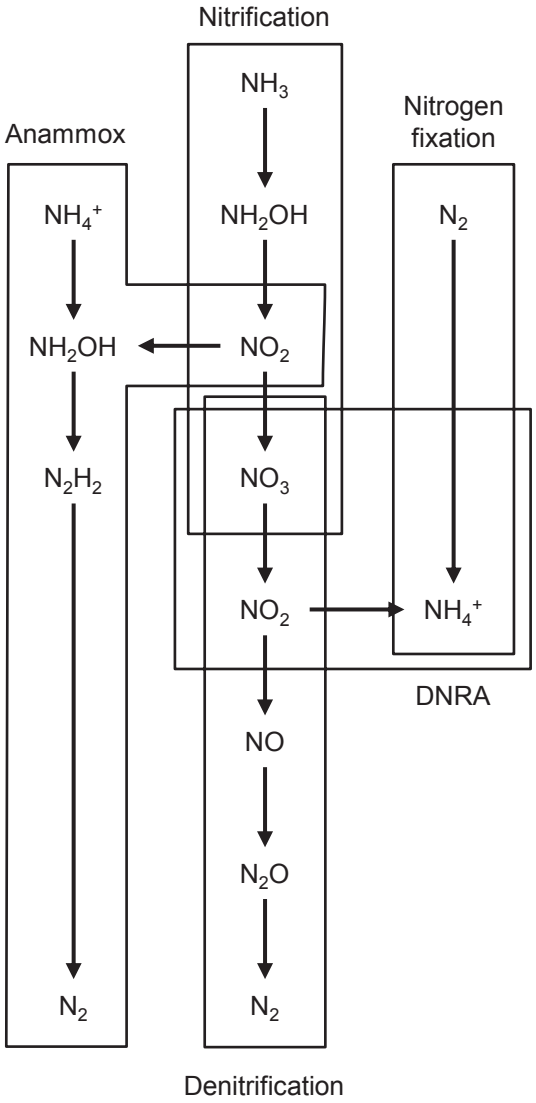


FIGURE 10 Microbial role in the nitrogen cycle (adapted from Philippot et al. 2007).

leached into groundwaters. To minimise this nitrate leaching, nitrification inhibitors have been applied to New Zealand soils. In New Zealand grassland soils, ammonia-oxidising bacteria (AOB) were more abundant in topsoils, whereas ammonia-oxidising archaea (AOA) were more abundant in one of the subsoils (Di et al. 2010). Apparently, AOB and AOA may prefer different soil N concentrations to grow: AOB dominate under high ammonia substrate conditions, AOA dominate under low ammonia substrate concentrations.

Denitrification is a microbial respiratory process during which soluble nitrogen oxides are used as an alternative electron acceptor when oxygen is limiting. It consists of the 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). It occurs predominantly in waterlogged areas that have become anaerobic. Complete denitrification (to N_2) is the major biological mechanism by which fixed N returns to the atmosphere from soil and water, completing the nitrogen cycle. It results in considerable loss of fixed N from soil, and has important consequences because nitrogen is the limiting nutrient for crop production. The ability to denitrify has been identified in a diverse range of phylogenetically unrelated soil bacteria including members of the Proteobacteria, Actinobacteria, and Firmicutes, as well as in fungi and other soil eukaryotes. However, many denitrifiers lack one or more of the enzymes involved in denitrification, and are thus often said to be 'incomplete'; for example, most fungi (Kobayashi et al. 1996) and approximately one-third of sequenced bacterial denitrifiers (Philippot et al. 2011) lack N_2O reductase, so their final denitrification product is N_2O. Incomplete denitrification is a major source of greenhouse gas emissions from pastoral agriculture in New Zealand (Saggar et al. 2012).

In an alternative process called dissimilatory reduction of nitrate, a variety of facultative anaerobic bacteria including *Alcaligenes* or *Escherichia* reduce nitrate to nitrite under anaerobic conditions. The nitrite produced by these species is excreted or, under appropriate conditions, some microbes reduce nitrite via hydroxylamine to ammonia. These organisms do not produce gaseous nitrogen products: that is, they do not denitrify.

Anammox bacteria anaerobically oxidise ammonium to nitrogen gas (N_2) (Humbert et al. 2010). The anammox reaction depends on the concomitant presence of both oxidised and reduced inorganic nitrogen compounds under anaerobic conditions. In soil, anammox bacteria have been detected in permafrost and agricultural soil, and from bulk soils and soil associated with nitrogen-fixing plants. The bacteria that carry out this reaction form a deep-branching, monophyletic group within the Planctomycetes. Phylogenetic analysis has revealed that 16S rRNA gene sequences cloned from these bacteria in soils were closely related to sequences from the candidate genera 'Kuenenia' and 'Brocadia'.

Some bacteria can participate in multiple steps in the nitrogen cycle. For example, *Rhizobium*, *Bradyrhizobium* and *Azospirillum* have members that both fix nitrogen and denitrify. Moreover nitrifying bacteria such as *Nitrosomonas* can carry out denitrification: this process is called nitrifier denitrification.

Phosphorus cycling

Phosphorus (P) is not an abundant element in the environment, and its availability is further restricted by a tendency to precipitate in the presence of divalent and trivalent cations at neutral and alkaline pH. Microbes transform phosphorus in two main ways. In one, they mineralise organic P (occurring mainly as phosphate esters) to form inorganic phosphate in a process catalysed by phosphatase enzymes, which are produced by many bacteria and fungi. In the other, they transform insoluble, immobilised P to soluble or mobile P in a process normally mediated by the production of organic acids. Microbes release sufficient P for their own use and that of plants and other soil organisms.

Mycorrhizal fungi produce oxalate to release phosphate from insoluble mineral P. This mobilisation of P by fungal symbionts is a major strategy that allows plants to overcome P-limitation. For example, several ectomycorrhizal basidiomycetous fungi have high-affinity phosphate transporters that are expressed in extraradical hyphae in response to P deficiency in their host (Plassard and Dell 2010). In New Zealand pasture soils, P-solubilising bacteria have been found in the Proteobacteria (in particular *Pseudomonas*), Actinobacteria, Firmicutes, and Bacteroidetes (Mander et al. 2012), but their numbers and diversity are affected by farm management strategies, with highest numbers in soils low in P. There is evidence that long-term application of P-rich fertiliser can alter the diversity of Actinobacteria and arbuscular mycorrhizal fungi in pasture soils (Wakelin et al. 2012).

MICROBES AS AGENTS FOR RECYCLING WASTES AND DETOXIFICATION

Naturally occurring microorganisms – particularly bacteria and fungi – have evolved an impressive array of mechanisms to

biodegrade or detoxify substances hazardous to human health or the environment. These microbial processes are being harnessed for bioremediation.

Biodegradation

Many years of laboratory studies have provided a wealth of information about how microbes biodegrade or detoxify organic contaminants. These studies describe the establishment of enrichment cultures for detection of biotransformation of contaminants under a range of environmental conditions: for example, pH, or nutrient or oxygen availability. The source of microbes for the enrichment cultures are typically soils contaminated with the compound of interest. Where possible, pure cultures that can degrade the contaminant are obtained and have been used for biochemical and molecular characterisation of the degradation pathways.

Heterotrophic bacteria in soil – for example *Pseudomonas*, *Sphingomonas* and *Mycobacterium* – have often been implicated in oil degradation. *Pseudomonas*, for example, has been well studied and the genes and enzymes responsible for degrading alkanes, monoaromatics, naphthalene, and phenanthrene as a sole carbon source under aerobic conditions are well understood. Knowledge of the mechanisms that microbes use to degrade oil has been applied in situ. For example, enhancing oil degradation in soil typically involves addition of nutrients (N and P) and sometimes oxygen and water. There is usually no need to add hydrocarbon-degrading bacteria to oil-contaminated sites because they are ubiquitous in soil and when oil is spilled they increase in numbers. However, high concentrations of hydrocarbons can deplete available nitrogen and phosphorus because these elements are assimilated during biodegradation; consequently, activity of the hydrocarbon degraders may become limited by these nutrients.

Bacteria and fungi also degrade pesticides. For example, the bacterium *Arthrobacter nicotinovorans* HIM, isolated from a New Zealand agricultural soil, degraded atrazine as a sole source of carbon and nitrogen. In addition to atrazine the bacterium also degraded the related triazine compounds simazine, terbuylazine, propazine, and cyanazine (Aislabie et al. 2005). Pesticides broken down rapidly in soil may not effectively control pests. Others like DDT, which was used extensively in New Zealand for the control of grass grub, are not readily degradable and persist in soil. Under aerobic conditions DDT is converted to DDE, which was considered a dead-end metabolite. However, *Terrabacter* sp. Strain DDE-1, isolated from soil from Winchmore Research Station, metabolised DDE when grown on biphenyl (i.e. when biphenyl was provided as an alternative for growth) (Aislabie et al. 1999).

Ligninolytic fungi such as the white rot fungus *Phanaerochaete chrysosporium* can degrade a diverse range of environmental contaminants such as pentachlorophenol and dioxin under co-metabolic conditions (i.e. with alternatives for growth such as sawdust, straw or corn cobs). This impressive ability has been attributed to the mechanisms these fungi have evolved to degrade lignin (Barr and Aust 1994). New Zealand strains of white rot fungi and also Zygomycetes degraded pentachlorophenol and selected dioxin and furan cogeners in soil samples from a former dip tank wood-treating operation in Whakatane (Thwaites et al. 2006).

Biodegradation in situ is a function of three independent but interrelated factors: the contaminant, the microbes, and the environment.

Both the chemical structure and the physical state of an organic contaminant affect the rate at which it is biodegraded. In general, microbes can degrade naturally occurring organic contaminants such as those associated with oil, whereas some synthetic molecules like DDT and aldrin persist in the environment and are not readily degraded. Synthetic molecules often contain novel arrangements rarely found in nature, which increases persistence. Resistance to degradation is linked with a decrease in water solubility, so larger molecules tend to be less soluble and harder to degrade. Many organic contaminants are hydrophobic or poorly soluble in water, and they bind to soil organic matter or clay surfaces. This may reduce their toxicity but it also reduces their biodegradability. Degradation may also be impeded when the contaminant concentration is too high because these organic contaminants, while serving as carbon and energy sources for microbes, may be toxic at high concentrations.

For biodegradation to proceed, microbes with the appropriate biodegradative ability must be present in sufficient numbers. This will depend in part on how long they have been exposed to the contaminant. As some pollutants contain a mixture of compounds (e.g. oil) a mixture of microbes is required because no single microbe has the metabolic potential to degrade all contaminants. It is essential that the microbes and the contaminant are in contact for biodegradation to occur. Some bacteria are mobile and chemotactic, sensing the contaminant and moving towards it; other microbes such as fungi grow as filaments towards the contaminant.

The presence of the required microbial population is not enough: environmental conditions in situ must permit microbial growth or activity. Microbial growth and activity are sensitive to pH, temperature, moisture, nutrient availability and oxygen concentrations, with most microbes growing optimally over a narrow range of these conditions. Hydrocarbons are readily degraded aerobically whereas reactions involving dechlorination (e.g. degradation of trichloroethylene) often require anaerobic conditions. Other compounds, such as the alkylated benzenes, are degradable under aerobic and anaerobic conditions. Nutrients that may limit biodegradation in situ include nitrogen, phosphorus, potassium, and iron.

Detoxification of heavy metals

Environmental exposure of microbes to heavy metals has led to the evolution of detoxification mechanisms. In soils, heavy metal contaminants include copper, mercury (Hg), zinc (Zn), lead, cobalt (Co) and cadmium (Cd); in New Zealand agricultural soils, cadmium accumulation is linked to the use of superphosphate fertiliser (Loganathan et al. 2003). Metals may be toxic to soil microbes due to their chemical affinity for thiol groups on biomolecules such as proteins. To avoid cellular damage caused by these metals, bacteria have evolved three general mechanisms for metal tolerance. The first is sequestration of the metals by binding to cell constituents, which reduces the concentration of free ions in the cytoplasm. The metals can be adsorbed by cell membranes, cell walls, and extracellular polymeric substances (EPS) such as polysaccharides. Several metals can be sequestered in EPS, including copper and lead (Harrison et al. 2007). The second mechanism involves detoxification through reduction of intracellular ions. For example, Hg^{+2} may be reduced to Hg^0 by mercury reductase (encoded by the *merA* gene), and the Hg^0 then diffuses from the cell because of its low evaporation point (Nies 1999). The third mechanism involves extrusion of ions from the cell by efflux systems. The cation/proton antiporter *Czc*, known for example in *Alcaligenes eutrophus,* mediates resistance

to Cd^{2+}, Zn^{2+} and Co^{2+} by expelling metals from the cytoplasm through the cell membrane to the environment (Silver and Phung 1996). These microbial transformations of heavy metals are being harnessed for bioremediation of wastes containing heavy metals.

RECENT TRENDS IN SOIL MICROBIAL DIVERSITY RESEARCH

Despite the recognition of the importance of microbes in sustaining soil ecosystem services there is still 'a lack of understanding of fundamental processes that drive, maintain and affect microbial diversity in soil and of the role of diversity in essential soil processes' (Stein and Nicol 2011). Although we are beginning to understand the scale of microbial diversity, we remain largely ignorant of the role and importance of this vast diversity from an ecological perspective. A comprehensive understanding of the relationship between soil microbial diversity and ecosystem functions is essential for determining whether factors that affect the diversity, activity, and physiology of microbes will alter the functioning of terrestrial ecosystems.

Insights from molecular technologies

In recent years, our knowledge of the structure of soil microbial communities has been greatly advanced with the development of molecular tools. We can now report what is present in soil and have begun to unravel key issues in soil microbiology including:

- Spatial distribution of soil microbes at local, regional and continental scales
- Drivers of soil microbial community structure
- Co-occurrence patterns among soil bacteria and between soil bacteria, fungi and plants
- The influence of changing land use and climate change on soil microbial community structure.

Molecular tools have facilitated the investigation of soil bacterial communities at local (Acosta-Martínez et al. 2008), regional (Dequiedt et al. 2009; Griffiths et al. 2011), and global scales (Lauber et al. 2009). These studies have revealed that bacterial communities exhibit biogeographic patterns and that they decline in similarity with geographic distance (Martiny et al. 2011). Determining the underlying causes of this 'distance-decay' pattern is an area of intense research because such studies of beta diversity (variation in community composition) yield insights about how diversity is maintained. Beta diversity could be driven by differences in environmental conditions. The traditional view is that soil microbes occur everywhere (i.e. no dispersal limitations) and the environment determines which organisms are abundant. This view suggests the structure of a soil microbial community is influenced by both biotic and abiotic factors, including soil type, mineral composition and texture, nutrient availability (C, N and P), moisture and oxygen status, and associated plant communities. Recent investigations, however, indicate that the major driver of soil bacterial communities appears to be soil pH (Lauber et al. 2009). Among bacteria the relative abundance of Bacteroidetes increases with pH, whereas that of Acidobacteria Gp3 declines (Nacke et al. 2011). In contrast to bacteria, the fungal community composition is less strongly affected by pH (Rousk et al. 2010) but soil nutrient status may be an important driver (Lauber et al. 2008).

In contrast to the traditional view, another factor that may explain patterns in beta diversity is limitations to dispersal. Some evidence suggests organisms that are abundant in soil bacterial communities are more likely to be widely distributed (Nemergut et al. 2011). For example, 10 of the most abundant bacteria in four soils from distinctly different sites in North and South America were found in two or more of those soils (Fulthorpe et al. 2008). Among the 10 most abundant bacteria were members of the genera *Chitinophaga*, *Acidobacterium* and *Acidovorax*.

The structure of the soil microbial community is influenced by land use. This is to be expected, as changing land use will modify soil properties. More bacterial phyla were found in grassland soils than in forest soils (Nacke et al. 2011). Decomposer species of the Actinobacteria were more prevalent in non-disturbed grassland systems compared with agricultural soils, whereas the reverse trend was reported for Bacteroidetes (Acosta-Martínez et al. 2008). Adding nitrogen to soils resulted in an increase in relative abundance of bacterial copiotrophic taxa (e.g. members of Proteobacteria or Actinobacteria) with oligotrophic taxa (e.g. Acidobacteria) showing the opposite pattern (Fierer et al. 2012; Ramirez et al. 2012). Similarly, for fungi nitrophilic mycorrhizal fungi (e.g. *Laccaria bicolor*) increased following nitrogen addition to soil whereas Cortinarius spp. declined (Deslippe et al 2011).

Climate change is also predicted to affect soil microbial community structure through the direct impacts of higher soil temperatures and indirect effects such as shifts in the plant community or soil properties. For example, long-term warming simultaneously reduced the evenness (a measure of diversity) of bacterial communities and increased the evenness of fungal communities. Thus, warming increased the most dominant group of Actinobacteria but reduced the rarer Gemmatimonadetes and the Proteobacteria, while the greater evenness of the fungal community was associated with significant increases in the ectomycorrhizal fungi, *Russula* spp., *Cortinarius* spp., and members of the Helotiales, suggesting an important role for the plant community in driving this change (Deslippe et al. 2012).

Understanding the vast diversity

Determining the reason for the vast diversity of soil microbial communities still represents a major conceptual challenge in soil microbial ecology. One theory suggests this enormous biodiversity is driven by several factors: the spatial isolation of microbes within soil, which reduces direct competitive interactions; the amount and heterogeneity of food and energy resources; and time – the fact that today's soil microbial communities are the result of more than 3.5 billion years of evolution (Tiedje et al 2001). However, empirical tests of these hypotheses are rare.

The low phyletic and high species diversity observed in soil bacterial communities may relate to the extreme spatial heterogeneity that exists in soils (Ritz et al 2003). Sampling difficulties, however, limit tests of this mechanism. Current methods to investigate soil microbial communities involve analysing genes, transcripts or genome fragments recovered from nucleic acids extracted from soil samples much larger than the scale at which microbial populations might form discernible patterns. These samples comprise several grams of soil, so when they are processed any physical association and relative spatial distribution is destroyed. Consequently, while these methods provide information on the extant microbial population, they make it difficult to understand microbial interactions. For example, physical fractionation of soil has revealed that macroaggregates (>250 μm) had a relatively high abundance of Actinobacteria and α-Proteobacteria, whereas the silt-clay fractions (<53 μm) were distinguished by the abundance of Gemmatimonadetes (Davinic et al. 2012), so any process that does not differentiate these particle sizes will not recognise this pattern of microbial

distribution. Sampling at an appopriate scale is also an issue when investigating how soil resources control the structure and functioning of soil microbial communities. Some methods are being developed to overcome these scale-related problems – for example, Shi et al. (2012) describe the use of the rhizotron, which allowed in situ sampling of tree root exudates and associated rhizosphere microbial communities – but further effort is required to examine microbial communities and soil resources at the microbe scale. Only when this is achieved will we be able to fully appreciate microbially mediated processes and understand the high diversity of microbes in soil.

Linking the structure of microbial communities with their function

A key challenge for soil microbiology is to link the structure of microbial communities unambiguously with their function (Stein and Nicol 2011). This is difficult, given the vast diversity of soil microbes, few of which are represented in culture collections. This is particularly so for Acidobacteria, Verrucomicrobia, Chloroflexi, Planctomycetes and Gemmatimonadetes, although even some subphyla of the Actinobacteria remain unknown. Among the archaea, Crenarchaeota are rare in culture; so too are mycorrhizal fungi. Continued effort is required to isolate representative strains, because molecular data are meaningless without a context for gene function (Stein and Nicol 2011). The function of at least 30% of genomic content is unknown (Galperin and Koonin 2010), yet this could be resolved in part by studying the functioning of model organisms in the laboratory. Studies like these provide insights into biochemical and structural properties, metabolic pathways, gene regulation, and evolutionary history. Inferences from genomic data may also identify strategies for cultivating currently uncultivated organisms. Among the best known examples of this is the iron-oxidising bacterium *Leptospirillum ferrodiazotrophum* from acid mine drainage (Tyson et al. 2005). From environmental genome data it was predicted that this bacterium was solely responsible for nitrogen fixation in the in situ bacterial community, so a sample containing *L. ferrodiazotrophum* was inoculated into nitrogen-free media, where it grew. More innovative culturing methods (and patience) are required.

A second issue is that microbes can function in diverse ways in soil, and for some processes there is a high degree of functional redundancy. The bacterium *Rhizobium* for example, may fix nitrogen when in a symbiotic relationship, denitrify when free-living, decompose organic matter, and enhance soil aggregation through production of extra-cellular polysaccharides. Functional redundancy is the principle that the more organisms there are to carry out a particular process, the more likely it is that the process will be unaffected should some of these organisms be incapacitated or removed (Andrén et al. 1999). Functional redundancy can hence obscure linkages between bacterial taxonomy and functional traits (Schimel 1995), particularly when examining more broadly defined processes (e.g. carbon cycling of root exudates in the rhizosphere) where many taxa may be responsible for the same biogeochemical function. Not surprisingly, then, most progress has been made in understanding the link between bacterial taxonomy and functional traits for narrow processes involved in nitrogen cycling such as nitrification and denitrification (Bottomley et al. 2012). Recently Schimel and Schaeffer (2012) discussed how microbial community structure may influence carbon cycling. They argued that while microbial community structure may be important in the breakdown of organic matter in the rhizosphere and in leaf litter, it is not likely to be important in mineral soil, where the rate-limiting step for decomposition is physical access to organic matter.

Environmental factors influence the diversity, activity and physiology of soil microbial populations; consequently, determining how these factors affect the functioning of terrestrial ecosystems will require a better understanding of how populations of soil microbes function.

Role of ecological theory and modelling

Ecological theory generates predictions that can be of practical value to people. Traditional ecological theory has focused on communities of plants and animals, yet the vast abundance, biomass, and diversity of microbes, and the importance of microbial activities, suggest the established theory is of limited value if it does not apply to microbial communities (Prosser et al. 2007). Recent efforts have been made to view the accumulating knowledge of the diversity, structure, and function of soil microbial communities through the lens of ecological theory (Prosser et al. 2007), and also to test ecological theories using microbial models (Wittebolle et al. 2009). For example, a trait-based approach has been used to predict nitrifier diversity, ammonia oxidation rates, and nitrous oxide production across pH, temperature, and substrate gradients (Bouskill et al. 2012).

Ecosystem process models are critical for generating predictions of how factors such as land use and climate change will affect the services humans derive from ecosystems. However, these models have historically omitted microbial structures and functions, which may determine the rates of important ecosystem functions (McGuire and Treseder 2010). A recent trend in soil microbial ecology has been to consider microbes explicitly in ecosystem models, and this has led to considerable insights. For example, including organic nutrient uptake by mycorrhizal fungi in an ecosystem carbon model recently revealed that mycorrhizal fungi with this trait (mainly ecto and ericoid mycorrhizae) enhance ecosystem carbon storage (Orwin et al. 2011). This has important implications for land use change and its effects on soil carbon stocks and greenhouse gas emissions in New Zealand and elsewhere.

CURRENT GAPS IN KNOWLEDGE OF NEW ZEALAND'S SOIL MICROBIAL DIVERSITY AND FUNCTIONING

Although global knowledge of soil microbial diversity and functioning is increasing rapidly, knowledge of New Zealand's soil microbes is sparse. First, there is little information about the microbial phylogenetic diversity of New Zealand soils of natural or managed ecosystems. We do not know how the structure and function of soil microbial communities vary within the New Zealand landscape, in different soils, and under different land uses. Most probably, the microbial composition of our soils at the phylum level resembles those reported worldwide, but variations at the species level will reflect local environmental conditions, the communities of plants and animals, and land use. Second, we know little about how land use and climate change will affect the long-term maintenance of our microbial resources.

Investigations of soil microbial diversity and functioning in New Zealand have so far largely focused on microbes important to agriculture, including bacteria that fix nitrogen or mobilise phosphorus and microbes that oxidise ammonium. Some studies have also described microbes with potential applications for bioremediation, but the bacteria commonly isolated from soil in these studies are fast-growing heterotrophs and their ability to perform the desired functions in situ is debatable. Relatively little

emphasis has been placed on soil microbes in native ecosystems. For example, while they are probably the best studied microbes in native soils, only about one third of New Zealand's estimated 24000 fungal species have been described (Landcare Research Fungal Guide, 2013).

As the value of soil services is realised it will become increasingly important to understand the role of microbial diversity in soils and their functioning. Of particular importance is management of soil carbon and nitrogen dynamics. In New Zealand, soil carbon stocks on flat land grazed by dairy cows have declined (Schipper et al. 2010). The role of soil microbes in this decline and how we might manipulate soil conditions to halt it have yet to be resolved. Similarly, we do not know how the increasing inputs of nutrients (C, N and P) into soil from manure and fertiliser will affect microbial diversity and functioning. New Zealand soils are naturally low in phosphorus, yet we know little about how phosphate fertilisation affects native organisms, particularly those involved in phosphorus mobilisation. Understanding impacts of land use and climate change on soil microbial community structure and function is important if we wish to maintain, value, and conserve our microbial resources.

Currently there is little demand for knowledge of New Zealand's soil microbial diversity, although one exception is demand from those who wish to import microbes from overseas and need to know if a particular organism is present in New Zealand. However, companies involved in developing biotechnology may also require knowledge of soil microbial diversity and functioning. Moreover, as land managers and regional councils continue to grapple with maintenance of soil quality and weed invasions, we anticipate an increasing need for indicators of soil microbial diversity and functioning. Particularly useful would be the development of diagnostic tools as indicators of soil health (Kibblewhite et al. 2008). Currently, the only biological soil indicator used in New Zealand is anaerobic mineralisable nitrogen (Sparling and Schipper 2004). Given the reduction in the cost of DNA sequencing, molecular tools may soon be available for routine assessment and monitoring of microbial diversity and function in soils. Unlike plants and animals, microbes are the focus of no conservation efforts, because it is assumed that conservation of ecosystems will ensure conservation of the soil microbial community. This assumption, however, has yet to be tested.

Currently terrestrial ecosystems face increased pressures due to human population growth and associated increases in urbanization, resource extraction, fossil fuel combustion and anthropogenic climate change. These pressures threaten to erode the stability and functions of the ecosystems upon which human civilizations depend, and consequently present major challenges to humanity. In order to overcome these challenges and to preserve essential ecosystem services, we require knowledge of the microbial pillars upon which these systems are founded so that we can avoid the risk of eroding what is most essential. Further, broadening our fundamental knowledge of the diversity and function of soil microbial communities is also likely to illuminate new microbial processes, mechanisms, adaptations and product that present hitherto unknown solutions to many practical problems that we face.

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REFERENCES

Acosta-Martínez V, Dowd S, Sun Y, Allen V 2008. Tag-pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biology & Biochemistry 40: 2762–2770.

Aislabie J, Davison AD, Boul HL, Franzmann PD, Jardine DR, Karuso P 1999. Isolation of *Terrabacter* sp. strain DDE-1 which metabolises DDE when induced with biphenyl. Applied and Environmental Microbiology 65: 5607–5611.

Aislabie J, Bej AK, Ryburn J, Lloyd N, Wilkins A 2005. Characterization of *Arthrobacter nicotinovorans* HIM, an atrazine-degrading bacterium, from agricultural soil New Zealand. FEMs Microbiology, Ecology 52: 279–286.

Andrén O, Brussaard L, Clarholm M 1999. Soil organism influence on ecosystem-level processes-bypassing the ecological hierarchy? Applied Soil Ecology 11: 177–188.

Angel R, Claus P, Conrad R 2012. Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. The ISME Journal 6: 847–862.

Barr DP, Aust SD 1994. Mechanisms white rot fungi use to degrade pollutants. Environmental Science and Technology 28: 79A–87A.

Bates ST, Berg-Lyons DB, Caporosa JG, Walters WA, Knight R, Fierer N 2011. Examining the global distribution of dominant archaeal populations in soils. The ISME Journal 5: 908–917.

Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA, et al. 2011. The under-recognised dominance of Verrucomicrobia in soil bacterial communities. Soil Biology & Biochemistry 43: 1450–1455.

Bishop PJ, Speare R, Poulter R, Butler M, et al. 2009. Elimination of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* by Archey's frog *Leiopelma archeyi*. Diseases of Aquatic Organisms 84: 9-15.

Bottomley PJ, Taylor AE, Myrold DD 2012. A consideration of the relative contributions of different microbial subpopulations to the soil N cycle. Frontiers in Microbiology 3: Article 373.

Bouskill NJ, Tang J, Riley WJ, Brodie EL 2012.Trait-based representation of biological nitrification: model development, testing, and predicted community composition. Frontiers in Microbiology 3: Article 364.

Bundt M, Widmer F, Pesaro M, Zeyer J, Blaser P 2001. Preferential flow paths: biological 'hot spots' in soils. Soil Biology & Biochemistry 33: 729–738.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. 2012. Ultra-high throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6: 1621–1624.

Davinic M, Fultz LM, Acosta-Martinez V, Calderón FJ, Cox SB, Dowd SE, et al. 2012. Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. Soil Biology & Biochemistry 46: 63–72.

Davis KE, Sangwan P, Janssen PH 2011. Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow-growing and mini-colony-forming soil bacteria. Environmental Microbiology 13: 798–805.

De Boer W, Leveau JHJ, Kowalchuk GA, Klein Gunnewiek PJA, Abeln ECA, Figgge MJ, et al. 2004. *Collimonas fungivorans* gen. nov., sp. nov., a chitinolytic soil bacterium with the ability to grow on living fungal hyphae. International Journal of Systematic and Evolutionary Microbiology 54: 857–864.

De Bruyn JM, Nixon LT, Fawaz MN, Johnson AM, Radosevich M 2011. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. Applied and Environmental Microbiology 77: 6295–6300.

Dequiedt S, Thioulouse J, Jolivet C, Saby PPA, Lelievre M, Maron P-A, et al. 2009. Biogeographical patterns of soil bacterial communities. Environmental Microbiology Reports 1: 251–255.

Deslippe JR, Hartmann M, Mohn BW, Simard SW 2011. Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. Global Change Biology 17: 1625-1636.

Deslippe JR, Hartmann M, Mohn BW, Simard SW 2012. Long term warming alters arctic soil microbial communities. FEMS Microbiology, Ecology 81: 594–607.

Devos DP, Reynaud EG 2010. Evolution. Intermediate steps. Science 330: 1187–1188.

Di H, Cameron KC, Shen J-P, Winefield CS, O'Callaghan M, Bowatte S, et al. 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiology Ecology 72: 386–394.

Dominati E, Patterson M, MacKay A 2010. A framework for classifying and quantifying natural capital and ecosystem services of soils. Ecological

Economics 69: 1858–1868.
Eilers KG, Lauber CL, Knight R, Fierer N 2010. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. Soil Biology & Biochemistry 42: 896–903.
Eilers KG, Debenport S, Anderson S, Fierer N 2012. Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology & Biochemistry 50: 58–65.
Fierer N, Bradford MA, Jackson RB 2007. Towards an ecological classification of soil bacteria. Ecology 88: 1354–1364.
Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6: 1007–1017.
Fulthorpe RR, Roesch LFW, Riva A, Triplett EW 2008. Distantly sampled soils carry few species in common. The ISME Journal 2: 901–910.
Galperin MY, Koonin EV 2010. From complete genome sequence to 'complete' understanding. Trends in Biotechnology 28: 398–406.
Goldfarb KC, Karaoz U, Hanson CA, Santee CA, Bradford MA, Treseder KK, et al. 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Frontiers in Microbiology 2: Article 94.
Griffiths RI, Thomson BC, James P, Bell T, Bailey M, Whiteley AS 2011. The bacterial biogeography of British soils. Environmental Microbiology 13: 1642–1654.
Harrison JJ, Ceri H, Turner R 2007. Mulitresistance and tolerance in microbial biofilms. Nature Reviews in Microbiology 5: 928–938.
Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, et al. 2007. A higher-level phylogenetic classification of the Fungi. Mycology Research 111: 509–547.
Hobbie EA 2006. Carbon allocation to ectomycorrhizal fungi correlates with total below ground allocation in culture studies. Ecology 87: 563–569.
Holmes AJ, Bowyer J, Holley MP, O'Donoghue M, Montgomery M, Gillings MR 2000. Diverse, yet-to-be-cultured members of the *Rubrobacter* subdivision of the *Actinobacteria* are widespread in Australian arid soils. FEMS Microbiology Ecology 33: 111-120.
Honegger, R. 1996. Mycosymbionts. In: Nash TH ed. Lichen biology. Cambridge University Press. Pp. 24–36.
Hugenholtz P, Goebel BM, Pace NR 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. Journal of Bacteriology 180: 4765–4774.
Humbert S, Tarnawski S, Fromin N, Mallet M-P, Aragno M, Zopfi J 2010. Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. The ISME Journal 4: 450–454.
Janssen PH 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology 72: 1719–1728.
Jones RT, Robeson MS, Lauber CL, Hamady M, Knight R, Fierer N 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. The ISME Journal 3: 442–453.
Kant R, van Passel MWJ, Palva A, Lucas S, Lapidus A, del Rio T, et al. 2011. Genome sequence of *Chthoniobacter flavus* Ellin428, an aerobic heterotrophic soil bacterium. Journal of Bacteriology 193: 2902–2903.
Kibblewhite MG, Ritz K, Swift MJ 2008. Soil health in agricultural systems. Philosophical Transactions of the Royal Society B 363: 685–701.
Kirk PM, Cannon PF, Minter DW, Stalpers JA 2008. Dictionary of the Fungi. 10th edn. Wallingford, CABI. P. 61.
Kobayashi M, Matsuo Y, Takimoto A, Suzuki S, Maruo F, Shoun H 1996. Denitrification, a novel type of respiratory metabolism in fungal mitochondrion. Journal of Biological Chemistry 271: 16263–16267.
Krzmarzick MJ, Crary BB, Harding JJ, Oyerinde OO, Leri AC, Myeni SCB, et al. 2011. Natural niche of organohalide-respiring Chloroflexi. Applied and Environmental Microbiology 78: 393–401.
Landcare Research Fungal Guide 2013. http://fungalguide.landcareresearch.co.nz/WebForms/FG_About.aspx
Lauber CL, Strickland MS, Bradford MA, Fierer N 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. 40: 2407–2415.
Lauber CL, Hamady M, Knight R, Fierer N 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75: 5111–5120.
Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, et al. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173: 611–620.
Liu YJ, Hodson MC, Hall BD 2006. Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of kingdom Fungi inferred from RNA polymerase II subunit genes. BMC Evolutionary Biology 6: 74–87.
Loganathan P, Hedley MJ, Grace ND, Lee J, Cronin SJ, Bolan NS, et al. 2003. Fertiliser contaminants in New Zealand grazed pasture with special reference to cadmium and fluorine: a review. Australian Journal of Soil Research 41: 501–532.
Lutzoni F, Pagel M, Reeb V 2001. Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411: 937–940.
Mander C, Wakelin S, Young S, Condron L, O'Callaghan M 2012. Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. Soil Biology & Biochemistry 44: 93–101.
Martiny JBH, Eisen JA, Penn K, Allison SD, Horner-Devine MC 2011. Drivers of β-diversity depend on spatial scale. Proceedings of the National Academy of Sciences (USA) 108: 7850–7854.
McGuire KL, Treseder KK. 2010. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biology & Biochemistry 42: 529–535.
Nacke H, Thürmer A, Wollherr A, Will C, Hodac L, Herold N, et al. 2011. Pyrosequencing-based assessment of bacterial community structure along different management types in german Forest and grassland soils. PLOS one 6: Issue 2, e17000.
Nemergut DR, Costello EK, Hamady M, Lozupone C, Jiang L, Schmidt S, et al. 2011. Global pattern in the biogeography of bacterial taxa. Environmental Microbiology 13: 135–144.
Nies DH 1999. Microbial heavy metal resistance. Applied Microbiology and Biotechnology 51: 730–750.
Orwin KH, Kirschbaum MUF, St John MG, Dickie IA 2011. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. Ecology Letters 14: 493–502.
Philippot L, Hallin S, Schloter M 2007. Ecology of denitrifying bacteria in agricultural soil. Advances in Agronomy 96: 249–305.
Philippot L, Andert J, Jones CM, Bru D, Hallin S 2011. Importance of denitrifiers lacking the gene encoding the nitrous oxide reductase for N2O emissions from soil. Global Change Biology 17: 1497–504.
Plassard C, Dell B 2010. Phosphorus nutrition of mycorrhizal trees. Tree Physiology 30: 1129–1139.
Pratt C, Deslippe JR, Tate KR In press. Assessment of a biofilter cover design to mitigate dairy effluent pond methane emissions. Environmental Science and Technology dx.doi.org/10.1021/es303225h
Prosser JI, Bohannan BJM, Curtis TP, Ellis RJ, Firestone MK, Freckleton RP, et al. 2007. The role of ecological theory in microbial ecology. Nature Reviews in Microbiology 5: 383–392.
Ramirez KS, Craine JM, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18: 1918–1927.
Ritz K, McHugh M, Harris J 2003. Biological diversity and function in soils: contemporary perspectives and implications in relation to the formulation of effective indicators. OECD Expert Meeting on Soil Erosion and Soil Biodiversity Indicators, Rome, March 2003. Pp. 1–11.
Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, et al. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4: 1340–1351.
Saggar S, Jha N, Deslippe J, Bolan NS, Luo J, Giltrap DL, et al. 2012. Denitrification and N_2O:N_2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. Science of the Total Environment: http://dx.doi.org/10.1016/j.scitotenv.2012.11.050
Schimel JP 1995. Ecosystem consequences of microbial diversity and community. In: Chaplin FS, Korner C eds Arctic and Alpine biodiversity: patterns, causes, and Alpine consequences. New York, Springer-Verlag. Pp. 239–254.
Schimel JP, Bennett J 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85: 591–602.
Schimel JP, Schaeffer SM 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3: Article 348.
Schipper LA, Parfitt RL, Ross C, Baisden WT, Claydon JJ, Fraser S 2010. Gains and losses in C and N stocks of New Zealand pasture soils depend on land use. Agriculture Ecosystem and Environment 139: 611–617.
Shi S, O'Callaghan M, Jones EE, Richardson AE, Walter C, Stewart A, et al. 2012. Investigation of organic anions in tree root exudates and rhizosphere microbial communities using in situ and destructive sampling techniques. Plant and Soil 359: 149–163.

Silver S, Phung LT 1996. Bacterial heavy metal resistance: new surprises. Annual Reviews in Microbiology 50: 753–789.

Simard SW, Beiler KJ, Bingham M, Deslippe JR, Philip L, Teste F 2012. Hyphal networks: mechanisms, ecology and modelling. Fungal Biology Reviews 26: 39–60.

Simon L, Bousquet J, Levesque C, Lalonde M, 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363: 67–69.

Singh BK, Tate KR, Kolipaka G, Hedley CB, Macdonald CA, Millard P, et al. 2007. Effect of afforestation of pastures on the activity and population dynamics of methanotrophic bacteria. Applied and Environmental Microbiology 73: 5153–5161.

Smith SE, Read DJ 1997. Mycorrhizal symbiosis. 2nd edn. London, Academic Press. 605 p.

Sparling G, Schipper L 2004. Soil quality monitoring in New Zealand: trends and issues arising from a broad-scale survey. Agriculture, Ecosystems and Environment 104: 545–552.

Stein LY, Nicol GW 2011. Grand challenges in terrestrial microbiology. Frontiers in Microbiology 2: Article 6.

Stolp H 1988. Microbial ecology: organisms, habitats, activities. Cambridge University Press. 308 p.

Tehler A 1988. A cladistic outline of the Eumycota. Cladistics 4: 227–277.

Teste FP, Simard SW, Durall DM, Guy RD, Jones MD, Schoonmaker AL 2009. Access to mycorrhizal networks and tree roots: importance for seedling survival and resource transfer. Ecology 90: 2808–2822.

Thwaites JM, Farrell RL, Duncan SD, Lamar RT, White RB 2006. Fungal-based remediation: treatment of PCP contaminated soil in New Zealand. In: Singh SN ed. Environmental bioremediation technologies. New York, Springer . Pp. 465–479.

Tiedje JM, Cho JC, Murray A, Treves D, Xia B, Zhou J 2001. Soil teeming with life: New frontiers for soil science. In: Rees RM, Ball BC, Campbell DC, Watson CA eds Sustainable management of soil organic matter. Wallingford, CAB International. Pp. 393–412.

Tourna M, Stieglmeier M, Spang A, Konneke M, Schintimeister A, Urich A, et al. 2011. *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. Proceedings of the National Academy of Sciences 108: 8420–8425.

Treseder KK, Kivlin SN, Hawkes CV 2011. Evolutionary trade-offs among decomposers determine responses to nitrogen enrichment. Ecology Letters 14: 933–938.

Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk H-P, Schleper C 2005. Novel genes for nitrite reductase and *Amo*-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environmental Microbiology 7: 1985–1995.

Tyson GW, Lo I, Baker BJ, Allen EE, Hugenholtz P, Banfield JF 2005. Genome-directed isolation of the key nitrogen fixer *Leptospirillum ferrodiazotrophum* sp. nov. from an acidophilic microbial community. Applied and Environmental Microbiology 71: 6319–6324.

Uroz S, Calvaruso C, Turpault MP, Pierrat JC, Mustin C, Frey-Klett P 2007. Effect of the mycorrhizospere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. Applied and Environmental Microbiology 73: 3019–3027.

Van Passel, Kant R, Palva A, Copeland A, Lucas S, Copeland A, et al. 2011. Genome sequence of the Verrucomicrobium *Opitutus terrae* PB90-1, an abundant inhabitant of rice paddy soil ecosystems. Journal of Bacteriology 193: 2367–2368.

Wakelin S, Mander C, Gerard E, Jansa J, Erb A, Young S, et al. 2012. Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures. Applied Soil Ecology 61: 40–48.

Ward NL, Challacombe JF, Janssen PH, Henrissat B, Coutinho MW, Wu M, et al. 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Applied and Environmental Microbiology 75: 2046–2056.

Weir BS, Turner SJ, Silvester WB, Park D-C, Young JM 2004. Unexpectedly diverse *Mesorhizobium* strains and *Rhizobium leguminosarum* nodulate native legume genera of New Zealand, while introduced weeds are nodulated by *Bradyrhizobium* species. Applied and Environmental Microbiology 70: 5980–5987.

Wittebolle L, Marzorati M, Clement L, Balloi A, Daffonchio D, Heylen K, et al. 2009. Initial community evenness favours functionality under selective stress. Nature 458: 623–626.

Woese C, Kandler O, Wheelis M 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences (USA) 87: 4576–4579.