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# Managing soil health for sustainable agriculture

## Volume 1: Fundamentals

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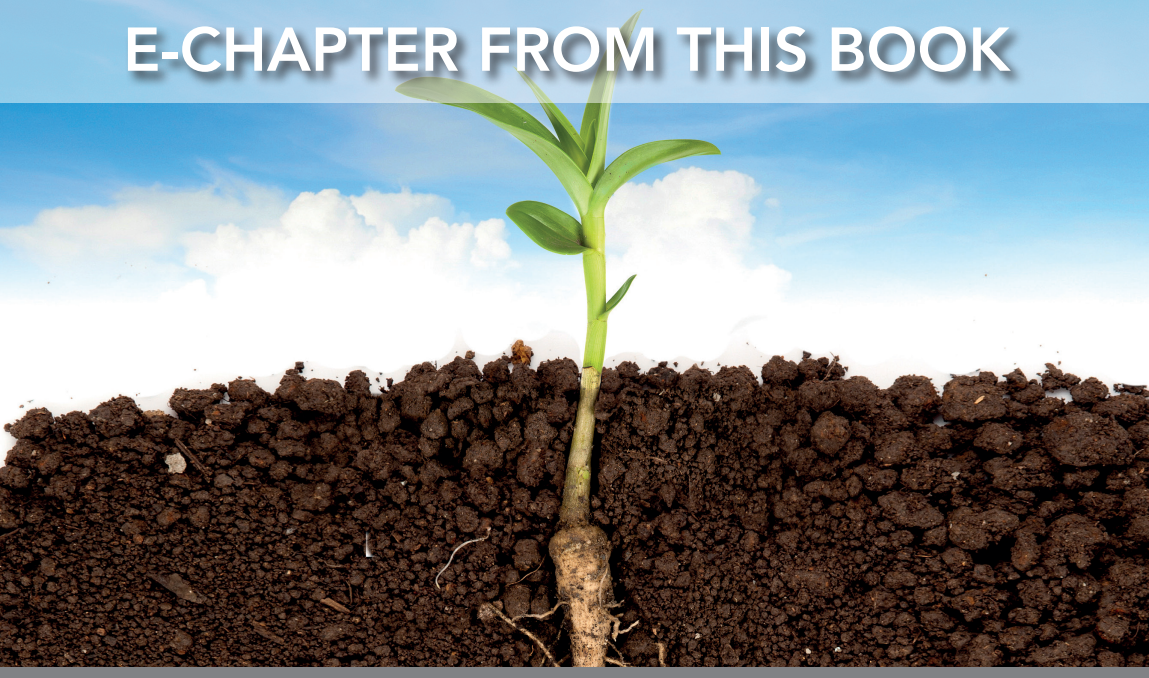
**E-CHAPTER FROM THIS BOOK**



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# Soil microorganisms: role in soil health

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## 1 Introduction

Without life, soil would remain an assemblage of mineral particles produced by weathering of rocks. Soil is itself essential for supporting terrestrial life, providing a substrate for plants and hosting the complex food web of soil biota. This includes microorganisms and micro- and mesofauna, most of which are reliant ultimately on nutrients supplied directly or indirectly by plants. The microfauna range in size from the smallest single-celled protozoans (protists) of around 5 μm to small nematodes, unsegmented worms and tardigrades (eight-legged arthropods) up to 1 mm but not readily visible by eye; mesofauna are larger soil invertebrates which move and shred plant residues or predate on other members of the soil biota (Nannipieri et al., 2003). However, the focus of this chapter is on microorganisms: prokaryotic bacteria and archaea (smaller than 5 μm), and eukaryotic fungi with hyphae up to 50 μm diameter but potentially many metres long. Residues from plant, animal and microbial activity provide organic components, making soils fertile and binding together mineral particles into aggregates that, with the associated pore spaces, confer structure. Soil microorganisms are pivotal for nutrient provision in soil systems through mineralization, where simple and complex organic materials are oxidized to labile inorganic compounds, a large proportion of which is recycled via plants.

Soil microbial communities are both extraordinarily large and diverse with an estimated $10^9$ bacterial and archaeal cells $g^{-1}$ comprising $10^4$ to $10^6$ species $g^{-1}$ in temperate soils (Bent and Forney, 2008). Species are defined in bacteria and archaea as strains that

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show at least 70% DNA homology over the whole genome and at least 97% in the 16S ribosomal RNA (rRNA) gene (Cohan, 2002). Equivalent information on soil fungi and microfauna is not yet available as there is much less sequence information available in the DNA databases. To date (2016), there are >230 000 bacterial and >2000 archaeal genomes available on the US Joint Genome Institute website https://gold.jgi.doe.gov/index (Mukherjee et al., 2017); the fungal portal http://jgi.doe.gov/fungi is aiming to provide 1000 fungal genomes (Grigoriev et al., 2014). The micro-scale structural and physico-chemical variability of soil creates multiple microenvironments providing the basis of the biodiversity, a measure which includes the number of different operational taxonomic units (OTUs) or species (richness) and the relative frequency with which they occur (evenness). The spatial separation of micro-sites enables co-evolution of multiple lineages in any particular soil, over time. This endows the soil with resilience, the ability to continue to function when challenged by stress or perturbation (Allison and Martiny, 2008; Girvan et al., 2005), which is related to functional redundancy, that is, due to the number of different species of different ecotypes that can perform a given function (Konopka, 2009). These properties are essential to maintain microbial activity in the face of periodic or catastrophic changes.

The earth has finite resources, with the elements essential for life partitioned in different reservoirs: terrestrial, aquatic, atmospheric and biotic. Ultimately, solar and geothermal energy drives the geochemical cycles, converting elements between different forms; microorganisms have an essential role in circulating them between abiotic and biotic pools. This includes fixation of carbon and nitrogen, the solubilization and uptake of minerals, and decomposition of organic residues with the associated release of mineral nutrients. Other essential elements including P, K, S, Mg, Ca, Fe and a range of trace element micronutrients are derived initially from minerals and may be released by volcanism or solubilized by weathering and enzymes secreted by microorganisms and plant roots (Falkowski et al., 2008).

Agricultural practice has continually influenced soil properties from its origin as small family settlements cultivating crops and domesticated animals selected for desirable traits, to modern intensive tillage agriculture, over the past 10 000 years (Montgomery, 2007a,b). The impact of human activity on soil microbiology is apparent in ancient sites: Amazonian terra preta, dark coloured soils rich in charred organic material and phosphate, some thousands of years old and thought to be derived from ancient human habitation, have distinct microbial communities with more species than pristine forest (Grossman et al., 2010). Similarly, woodland sites of Roman farms in France are reported to contain mycorrhizal fungi that differ from adjoining areas (Diedhiou et al., 2010). Changes are not necessarily detrimental, however, problems arising from agricultural mismanagement are a recurrent theme in social history, linked to the downfall of civilizations from Europe and Asia to Mesoamerica (Tainter, 1990). A combination of deforestation and intensification to provide food for increasing urban populations and stochastic natural disasters can lead to loss of soil structure and fertility resulting in soil erosion. Problems associated with soil erosion and degradation persist and may be exacerbated by climate change (Quinton et al., 2010; Banwart, 2011). Other activities such as metal mining and refining released toxic compounds creating polluted, infertile soils on a small scale until a massive expansion following the industrial revolution.

Modern agricultural tillage practices including mechanization, chemical fertilizers, pesticides and modern high-yielding crop varieties have increased productivity. Despite its success in sustaining the global human population (the FAO reports that over the

past decade cereal production has risen in line with demand), modern agricultural practice raises many environmental concerns. These include annexing of previously 'natural' environments, soil compaction and erosion due to agricultural vehicle traffic, over-cropping, overgrazing and a reduction in soil organic matter. There may also be depletion of surface and groundwater supplies diverted to crop production, pollution due to run-off of excess applied nutrients and pesticide residues, and generation of greenhouse gasses (GHG). Loss of natural habitats to agriculture, from <10 % of land globally in 1700 to ~40% in 2008 (FAOSTAT), has been detrimental to many wild plant and animal species, raising concerns about the less visible life below-ground. The global human population increased 10-fold between 1700 and 2000 to exceed 6 billion and reached 7 billion in 2011. The UN predicts that this increase in population will rise to ~9 billion by 2050. Pressure on land for food production and aggravated climate change will also increase in the future.

Whilst feedback mechanisms have been identified and mitigation options in soil have been proposed (Singh et al., 2010), there will undoubtedly be stress from broader extremes of temperature and precipitation associated with anthropogenic climate change. To ensure new practices do not compromise food security, it is essential to establish the likely environmental impacts on soil microbial communities and the geochemical cycle functions they perform, especially those where national decisions on land management have global significance.

A practical definition of soil health for any soil, whether nature reserve or intensively managed cropland, is 'the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal, and human health' (Pankhurst et al., 1997). This chapter reviews the contribution of soil microorganisms to sustainable agriculture and considers their role in both crop plant productivity and in major geochemical cycles, how this may have varied over time and how it may be influenced by changes in land use now and in the future. In this context, recent advances in the understanding of microbial community function arising from the application of high-throughput molecular methods for microbial ecology are discussed.

## 2 Methods for investigating microorganisms in soil

Historically, measurement of soil properties such as C and N content, bulk density, pH, cation exchange capacity (CEC) and microbial biomass and functions including aerobic respiration, nitrification and denitrification rates indicated the consequences of changes in land use. However, such 'black-box' approaches cannot describe soil microbial community structure, nor indicate reduced biodiversity and functional redundancy, early indicators of loss of resilience. Prior to development of methods based on the direct extraction of DNA and RNA from soil, termed metagenomics and metatranscriptomics, respectively, results were biased towards a potentially unrepresentative minority of microorganisms: currently, around 99% cannot be grown in the laboratory using standard methods (Hirsch et al., 2010). This may improve following development of the isolation chip (iChip), where soil organisms are incubated in discrete diffusion chambers incubated *in situ* in soil, reported to increase culture success to 50% (Nichols et al., 2010).

Metagenomic approaches that analyse community DNA extracted directly from environments provide data on the diversity and relative abundance of functional and

phylogenetic (mostly based on rRNA) gene sequences. This is a major advance despite limitations on the depth of sequencing that is practical, albeit that costs are falling and efficiency is increasing. Potential bias arises from DNA extraction methods (Delmont et al., 2011) and poor gene assignment due to incomplete knowledge of functional genes (Konopka, 2009). Currently, all methods assessing relative numbers and diversity of genes, whether based on PCR amplification, direct sequencing or hybridization to microarrays, are subject to technical and practical constraints limiting representation of less abundant groups (Bent and Forney, 2008). Other limitations include the fact that DNA can originate from inactive and lysed organisms, so it provides information on the potential rather than the actual activity of the community, and non-functional pseudogenes could skew estimates of redundancy. As the microorganisms represented in the DNA sequence database increase (see above), identification of metagenomic reads is constrained by the bioinformatics methods available (Thomas et al., 2012). The analysis of metagenomic DNA from soil for bacteria and archaea using universal prokaryotic primers to amplify 16S rRNA gene, sometimes referred to as 'barcoding', has provided invaluable information: many studies compare the relative abundance of 16S amplicons in different soils. The equivalent analysis for fungi is usually based on the spacer region that separates ribosomal genes, and for other eukaryotes the 18S rRNA gene or a mitochondrial gene is used but identification of these is limited by a paucity of sequence data from environmental isolates (Pawlowski et al., 2012).

However, information provided by soil metagenomes has proved invaluable for revealing community structure and sequence data to facilitate the design of molecular tools for more detailed analysis of particular genes including probes for microarrays and primers for quantitative PCR (qPCR) and targeted sequencing (Hirsch et al., 2010, 2013). Metatranscriptomics identifies active functional genes if messenger RNA (mRNA) is converted to DNA using reverse transcriptase and subjected to qPCR and methods based on *in situ* labelling of DNA denote actively growing organisms (Hirsch et al., 2010, 2013). Following metagenomics/transcriptomics, soil proteomics is beginning to identify the gene products present at the time of sampling (Keiblinger et al., 2016). A combination of these methods is anticipated to provide increasingly detailed insight of the soil system, illuminating the black box. As the cost of sequencing is reduced, and the length of each sequence is increased, we get closer to the ultimate aim of obtaining full genomes of all the microorganisms present in a soil sample and demonstrating which genes are being expressed (Thomas et al., 2012).

A frequent question in soil ecology concerns the relative abundance and activity of eukaryotic and prokaryotic microorganisms. Metagenomic data is now providing answers to the first part: on average, bacterial DNA comprises 97%, archaeal DNA 1%, fungal DNA 0.3% and other eukaryotic DNA 1.2% (the remainder is assigned to viruses or unknown groups) in eight publically available soil metagenomes, shown in Table 1. If the proportion of fungal DNA is lower than expected from estimates based on other biomarkers, it may be because the ratio of genetic material to total fungal biomass is lower than for the prokaryotes (Strickland and Rousk, 2010). Also, the hyphal and spore walls of many fungi are more difficult to lyse than those of prokaryotic cells and may be under-represented (Mauchline et al., 2002). Modern agricultural tillage disrupts fungal hyphae in soil but evidence that it reduces the proportion of fungal biomass compared to that of bacteria is contradictory (Helgason et al., 2009; Strickland and Rousk, 2010). Our results (unpublished) show that the proportion of fungal DNA is 0.05% of the total extracted in regularly tilled arable soil but 0.7% in undisturbed grassland. This is in marked

**Table 1** A range of soil metagenomes publically available on MG-RAST (http://metagenomics.anl.gov/) unless indicated otherwise, analysed for the percentage of DNA sequences assigned to the Bacteria, Archaea, Fungi or other Eukaryotes

| Biome | Sequencing platform | Bacteria % DNA | Archaea% DNA | Fungi % DNA | % DNA other eukaryotes | Location (reference) |
|---|---|---|---|---|---|---|
| Grassland | 454 | 97.23 | 0.72 | 0.16 | 1.43 | Park Grass, Rothamsted Research, Harpenden, Hertfordshire, UK (Delmont et al., 2012) |
| Grassland | 454 | 97.56 | 1.17 | 0.51 | 0.71 | Kellogg Biological Station, Hickory Corners, MI (Ramirez et al., 2010) |
| Grassland | 454 | 97.01 | 1.17 | 0.18 | 1.60 | Cedar Creek Ecosystem Science Reserve, Bethel, MN (Ramirez et al., 2010) |
| Tropical rainforest | 454 | 96.99 | 1.22 | 0.52 | 1.05 | Luquillo, Puerto Rico (Deangelis et al., 2010) |
| Tallgrass prairie | Illumina | 98.07 | 0.38 | 0.27 | 0.35 | Fricke Cemetery, NE (Fierer et al., 2013) |
| Arid soil | Illumina | 95.75 | 2.48 | 0.27 | 0.21 | Uluru, Northern Territories, Australia (www.bioplatforms.com/soil-biodiversity) |
| Rice paddy soil | Illumina | 97.35 | 1.27 | 0.22 | 0.01 | Typical paddy at unspecified location, South China (http://trace.ddbj.nig.ac.jp/DRASearch/study?acc=SRP039858) |
| Rain-fed bog | Illumina | 97.35 | 1.27 | 0.22 | 0.01 | Marcell Experimental Forest, MN (Lin et al., 2014) |
| **Mean (+/− se)** | | **97.20** (0.43) | **1.07** (0.21) | **0.34** (0.34) | **1.20** (0.21) | |

contrast to a previous study on the same grassland soil using direct measurement of biovolume and the reduction in soil respiration in response to fungal inhibitors, which estimated a fungal:bacterial ratio of 4:1 (Lin and Brookes, 1999). This demonstrates that measurement of DNA (genetic potential), RNA (gene expression), biomarkers (PLFA or ergosterol), biovolume and activity all evaluate different aspects and cannot alone provide a measure of the functional importance of fungi in soil (Strickland and Rousk, 2010). Based on the proportion of metagenomic DNA, fungi were found to be more abundant in the rhizosphere than in bulk soil, 2.54% and 0.39%, respectively, in a recent study (Guo et al., 2016). Currently, there are few metatranscriptomic studies on soil but estimates of fungal activity, according to the proportion of fungal mRNA, are much higher: up to 13% reported

in garden and agricultural soils (Mcgrath et al., 2008); up to 30% in forest soil where soil is not tilled (Baldrian et al., 2012).

# 3 The soil environment

The soil food web recycles nutrients; respiration returns C to the atmosphere as $CO_2$, and other gasses and solutes are also lost from soil due to microbial activity. Nutrients can be relocated by large-scale removal of plant material (roaming herds of herbivores, agricultural practices) and released from plant residues in soil by grazing megafauna above ground and the activity of mesofauna and microorganisms in soil. The major sources, sinks and outflows for C and N in soils, including GHG emissions, are shown in Fig. 1 and in recent reviews (Singh et al., 2010; Gruber and Galloway, 2008).

## 3.1 Sources of natural variation in soil

Most soils developed over millennia and contain a mixture of minerals classified as sand, silt and clay according to their particle size. The relative proportion of these and their parent minerals, together with soil organic matter, determines the physico-chemical properties including pH and CEC. Clays and organic matter contribute to soil CEC and water-holding capacity and are considered beneficial in agricultural soils. Soils are subject to seasonal climatic changes, as well as natural catastrophic events such as freezing, glaciation, flooding, drought, fire and volcanic activity. Since soil is a porous solid matrix,

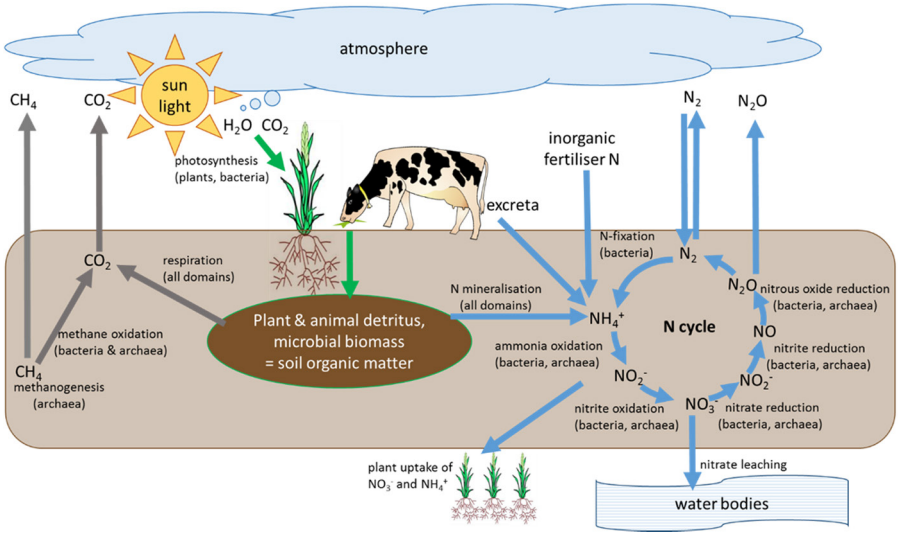


**Figure 1** The major sources, sinks and outflows for C and N in soils, including GHG emissions: carbon dioxide ($CO_2$), methane ($CH_4$) and nitrous oxide ($N_2O$). Plants provide a major sink for inorganic N in soil; in agricultural systems this is removed during harvest or grazing. For more detail and references see Sections 4.1 and 4.2.

the microbiota is relatively immobile, although wind, water flow, root growth and faunal activity all contribute to the dispersion of microbial communities in soil particles. The soil environment varies at the landscape scale according to climatic zone and altitude, underlying geology and the plant and animal communities present. Soil pH, a key factor in mineral nutrient availability, has been shown to exert a major influence on microbial community structure (Fierer and Jackson, 2006). At the field scale, local variation influences plant, animal and hence soil communities (Garbeva et al., 2004); individual plants can have a direct influence due to quantitative and qualitative differences in root exudates and residues; and indirect effects through the herbivores, pathogens and symbionts they support (Bais et al., 2006; Haichar et al., 2008). On a micro-scale, there is extreme heterogeneity due to the variable mineral and organic matter components of soil, pore structure, the presence or absence of particular sources of nutrients (roots and plant or animal detritus) and associated differences in pH (Bronick and Lal, 2005). This is overlaid by gradients of oxygen, $CO_2$ and water, influenced by the frequency of perturbation by animals, freeze-thawing and agricultural cultivation.

## 3.2 Microbial diversity

Bacteria are the dominant microorganisms in temperate soils, archaea comprising <2% of cells (Buckley et al., 1998; Bates et al., 2011). There is strong circumstantial evidence that many archaea are ammonia oxidizers as the archaeal ammonia monooxygenase gene is relatively abundant compared to the bacterial equivalent (Leininger et al., 2006). In anaerobic soils that generate methane (bogs, paddy fields), methanogenic archaea are responsible (Conrad et al., 2006); they are present in most soils but active only in anoxic conditions (Angel et al., 2012). Soil metagenomic sequence data from different biomes shows that the well-known bacterial phyla Proteobacteria, Firmicutes and Actinomycetes are abundant in soil. Some members of these groups are associated with specific functions including nitrogen fixation, nitrification and denitrification. Other more recently described bacterial phyla including the Acidobacteria, Bacteroidetes, Gemmatimonadetes and Verrucomicrobia are also ubiquitous in soil but their role is uncertain other than a heterotrophic contribution to carbon cycling and enhancing the overall resilience of the soil (Girvan et al., 2005).

A comparison of the relative abundance of phyla using 16S rRNA gene amplicon analysis in plots with different long-term treatments on the Rothamsted farm (grassland, arable and a permanent bare fallow without plants) showed that Verrucomicrobia were significantly more abundant in the grassland, Gemmatimonadetes in the bare fallow and Nitrospirae in the arable soil (Hirsch et al., 2016). When all the prokaryotic phyla are displayed on a ternary plot (Fig. 2), it appears that most cluster in the centre indicating no overall preference for one of the treatments but there are some notable exceptions; phyla with known functions. The Thaumarchaeota, an archaeal phylum that can oxidize ammonia, is relatively more abundant in the arable plots that receive ammonium nitrate fertilizer. The photosynthetic cyanobacteria are relatively more abundant in the bare fallow where they are not shaded by plants, and N levels are low. The majority of phyla, those most abundant overall, will provide very broad functions such as $CO_2$ emission. However, when the 16S rRNA gene amplicons are re-analysed at the highest possible resolution (to genus for some, others can only be placed at order or family level), the distribution appears to become more biased to one or another of the treatments (Fig. 3). For example, one Verrucomicrobia family, one α-proteobacteria genus and several groups of Actinobacteria and Acidobacteria are

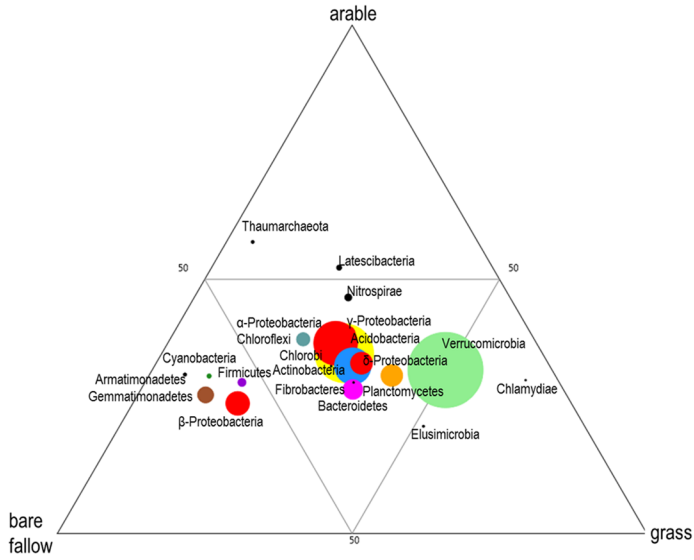


**Figure 2** Ternary plot showing the overall relative abundance indicated by the size of the bubbles for prokaryotic phyla (subphyla for Proteobacteria) in three differently managed soils. The Thaumarchaeota are Archaea; other phyla are Bacteria. The position of each group on the plot shows the relative distribution between treatments. Data from Hirsch et al., 2016.

Each phylum is colour coded, in order of overall abundance in all plots: Verrucomicrobia – light green; Proteobacteria subphyla – red; Gemmatimonadetes – brown; Actinobacteria – blue; Acidobacteria – yellow; Firmicutes – violet; Chloroflexi – greenblue; Bacteroidetes – pink; Planctomycetes – orange; Nitrospirae – dark grey; Cyanobacteria – green; Latescibacteria, Thaumarchaeota, Armatimonadetes, Elusimicrobia, Fibrobacteres, Chlorobi, Chlamydiae – black.

more abundant in bare fallow plots; some Gemmatimonadetes groups favour grassland, in contrast to the overall affinities of their respective phyla shown in Fig. 2.

Thus, the relative abundance of phyla and species varies between biomes, although the implications of this are unclear with the exception of cases where phyla are associated with specific functions. Differences may not be apparent unless extreme stress is applied, such as in highly polluted soil. However, for processes performed by a restricted range of soil species, the stability and efficiency of the function is predicted to be limited when species richness is low, rising as species numbers increase, demonstrated in the case of methane oxidation (Levine et al., 2011).

## 3.3 Microbial interactions with plants

Microorganisms are an essential component of soil: without them, there would be no recycling of nutrients including N, no biological fixation of $N_2$ and no protection for crop plants against adventitious pathogens and pests. The role of specific groups is detailed below, but it is assumed that many different groups mineralize organic N and C. The rhizosphere (area of soil influenced by the root) has numerous active bacteria than bulk soil, but fewer different OTU (Dennis et al., 2010). Many bacteria and fungi possess functions beneficial to plant

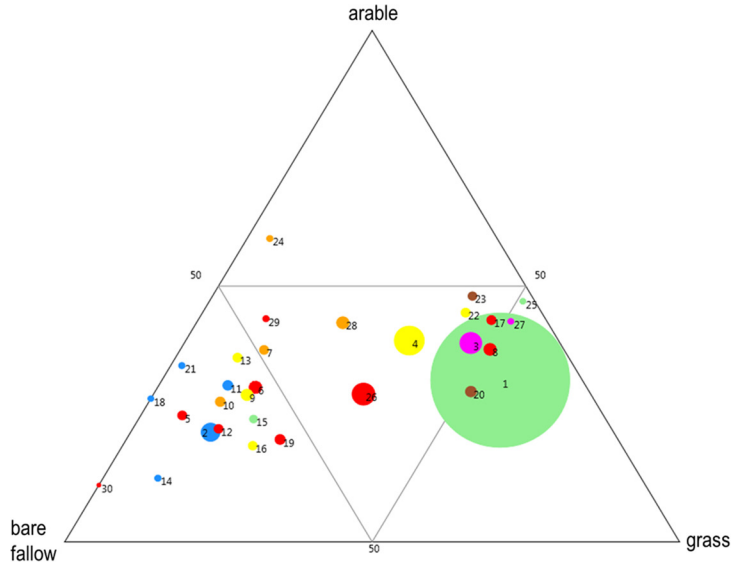


**Figure 3** Ternary plot showing the relative abundance indicated by the size of the bubbles and the distribution between treatments for prokaryotic OTU defined by >97% DNA sequence similarity in the 16S rRNA gene sequence. The taxonomic identification to genus is not possible in many cases: the best match is given. Only the 30 OTU contributing more than 0.8% of the overall differences between plots are shown. Data from Hirsch et al., 2016. Colour coding for the phyla to which OTU belong is similar to the one used in Fig. 2 (Verrucomicrobia – light green; Proteobacteria – red; Gemmatimonadetes – brown; Actinobacteria – blue; Acidobacteria – yellow; Bacteroidetes – pink; Planctomycetes – orange). Key below:

1 Verrucomicrobia; genus DA101
2 Actinobacteria; family Gaiellaceae
3 Bacteroidetes; family Chitinophagaceae
4 Acidobacteria; order iii1–15
5 β-Proteobacteria; family EB1003
6 β-Proteobacteria; order SC-I-84
7 Planctomycetes; genus *Gemmata*
8 δ-Proteobacteria; family Syntrophobacteraceae
9 Acidobacteria; family Koribacteraceae
10 Planctomycetes; family Gemmataceae
11 Actinobacteria; order 0319-7L14
12 β-Proteobacteria; genus *Methylibium*
13 Acidobacteria; order Solibacterales
14 Actinobacteria; family Micrococcaceae
15 Verrucomicrobia; family Chthoniobacteraceae
16 Acidobacteria; order RB41
17 γ-Proteobacteria; family Sinobacteraceae
18 Actinobacteria; genus *Mycobacterium*
19 α-Proteobacteria; genus *Bradyrhizobium*
20 Gemmatimonadetes; class Gemm-1
21 Actinobacteria; order Acidimicrobiales
22 Acidobacteria; genus *Candidatus Koribacter*
23 Gemmatimonadetes; order Ellin 5301
24 Planctomycetes; family Pirellulaceae
25 Verrucomicrobia; family auto67_4W
26 α-Proteobacteria; genus *Rhodoplanes*
27 Bacteroidetes; order Sphingobacteriales
28 Planctomycetes; order WD2101
29 α-Proteobacteria; family Hyphomicrobiaceae
30 β-Proteobacteria; family Oxalobacteraceae

growth in addition to increasing nutrient availability, including phytohormone production and the ability to protect against pasts and diseases. There are specific associations that are mutually beneficial, including leguminous plants with rhizobia and mycorrhizal fungi with their host plants. This topic is too wide to cover in detail in this chapter, but there exist many reviews on the rhizosphere microbiome (Lakshmanan et al., 2014, Philippot et al., 2013, Mcnear Jr., 2013). Exploitation of the rhizosphere microbiome for sustainable agriculture

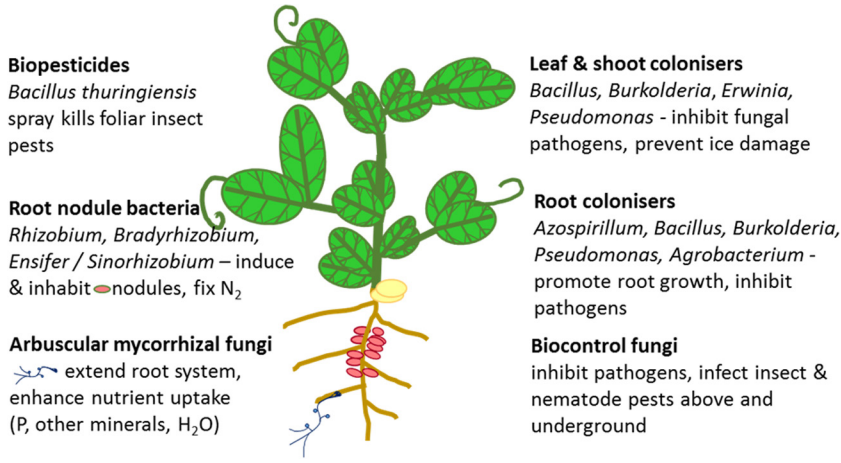


**Figure 4** Examples of beneficial bacteria and fungi that may be exploited to improve plant health and crop yields. For more details and references see Sections 3.3 and 5.4.

has a long history: rhizobial inoculation of legumes on a commercial scale began in the early twentieth century (Hirsch, 2004). Some of the beneficial interactions are summarized in Fig. 4 and there is further discussion in Section 5.4.

# 4 Microbial inputs to geochemical cycles

## 4.1 Carbon cycling

Microorganisms are a reservoir of organic carbon, in addition to breaking down plant and animal residues (Fig. 1). Many different groups of microorganisms contribute to the turnover of the large reservoir of carbon-rich organic matter in soil (Nannipieri et al., 2003), some of which is lost as $CO_2$ and some of which is degraded and condenses over time to form humus (Martin and Haider, 1971). Inversion tillage (ploughing) makes organic matter more available for degradation; in undisturbed soils where anoxia can develop, it tends to accumulate (Bronick and Lal, 2005, Martin and Haider, 1971). Methanogenic archaea generate $CH_4$ in wet, organic C-rich soils (see above). Organic matter incorporation into the soil can be managed and $CH_4$ emission rates can be reduced when rice paddies are drained, but global warming and thawing permafrost are likely to increase methanogenesis in the future (Davidson and Janssens, 2006).

## 4.2 Nitrogen cycling

Soil microorganisms maintain supplies of bioavailable N for plants, whether inputs are from N fixation, manure, compost or N fertilizers based on urea or ammonia. In contrast, nitrate in fertilizers is soluble, mobile in soil and readily available for plants. Excess N, whether from animal excreta or fertilizer application, can be lost from soil by physico-chemical routes (volatilization, leaching) or microbially mediated nitrification and denitrification (Fig. 1).

Nitrogen fixation is an energy-dependent process, often performed by bacteria associated with plants, notably symbiotic endophytic bacteria (Dixon and Kahn, 2004). There are two major functional groups: rhizobia – Proteobacteria that nodulate leguminous plants; and *Frankia* – Actinobacteria that form nodules on some shrubs and trees (Franche et al., 2009). Specific rhizobial symbionts multiply when applied as inoculants (e.g. *Bradyrhizobium* for soya beans, typically applied to large tracts of land) or where host plants are nodulated by indigenous strains such as when clover is cultivated (Hirsch, 1996). Together, free-living and endophytic bacteria are estimated to contribute a similar amount of N to soils as N fertilizers, leguminous crops adding an additional 30%.

Fig. 1 shows how denitrification returns most of the N deposited on, applied to or fixed in soil to the atmosphere and is a major cause of fertilizer N losses from soil and GHG emissions. The many groups of bacteria and archaea capable of denitrification use nitrite and nitrous oxides as respiratory terminal electron acceptors, ultimately producing $N_2$ to complete the N cycle (Zumft, 1997).

Many different soil bacteria and archaea hydrolyse urea from fertilizers or animal excreta to produce ammonia, the substrate for nitrification (Lu et al., 2012, Lu and Jia, 2013). Relatively few groups of these oxidize ammonia to nitrite via hydroxylamine (Poth and Focht, 1985); further oxidation of nitrite to nitrate is performed by a narrow group of Proteobacteria and a deep-branching phylum, Nitrospirae (Spieck et al., 2006). These groups are thought to provide most of the nitrifying activity observed in soil. The nitrite oxidizers do not seem to be rate-limiting, as nitrite is not observed to accumulate in ammonia-fertilized soils unless specific inhibitors are added (Belser and Mays, 1980). Recently, some Nitrospirae have been shown to perform complete ammonia oxidation (comammox) with nitrite as a product (Van Kessel et al., 2015, Daims et al., 2015). These bacteria are associated with aquatic systems and there is evidence for their presence in soil (Pinto et al., 2016), although any role in nitrification in soil is unknown at present. Anaerobic ammonia oxidation, anammox, is important in aquatic systems and sludges. Anammox bacteria from a deep-branching, monophyletic group within the Planctomycetes that oxidize ammonium to dinitrogen gas anaerobically, with nitrite as an electron acceptor (Kuenen, 2008). However, these bacteria are not common in soil and it is unlikely that they are major contributors to soil nitrification (Humbert et al., 2009).

The interaction between different factors favouring ammonia-oxidizing archaea and bacteria (AOA and AOB) is complex and we do not yet have a full understanding of soil nitrifiers. The AOB are autotrophs, obtaining energy from the oxidation of inorganic electron donors in their environments rather than reduced organic carbon compounds whilst some AOA can use organic C (Tourna et al., 2011). AOA are more abundant than AOB in most soils (Leininger et al., 2006). However, there is conflicting evidence concerning the relative contribution of each to soil nitrification and it is uncertain if they are functionally interchangeable (Jia and Conrad, 2009; Xia et al., 2011). AOA require less energy and ammonia to survive than AOB and are likely to be more successful in low-input unfertilized and forest soils (Valentine, 2007; Martens-Habbena et al., 2009) despite having lower cellular rates of ammonium oxidation. The equilibrium between ammonia ($NH_3$) and ammonium ($NH_4^+$) shifts to $NH_4^+$ at low pH, limiting substrate availability and growth of AOB even when N inputs are relatively high; this might favour AOA in low pH soils (Zhang et al., 2012). The first AOA isolated from soil, *Nitrososphaera viennensis,* has mixotrophic growth and can use pyruvate as a carbon source (Tourna et al., 2011). In contrast, *Candidatus Nitrosotalea devanaterra* is an acidophilic chemoautotroph that grows in very low ammonia concentrations (Lehtovirta-Morley et al., 2011). There is further discussion on nitrification and denitrification in Section 6.1.

## 4.3 Other minerals

Whilst most essential elements are recycled in the soil food web, key nutrients may be depleted over time, in particular N and P (Wardle et al., 2004), depending on the underlying soil mineralogy, climatic factors and previous plant cover. The importance of these elements is emphasized in agricultural systems where plant growth is regularly removed and nutrient limitation becomes apparent. Fertilizers containing N, P, K, Mg, Ca, S (macronutrients) and other elements required in smaller amounts (micronutrients) are usually required to maintain yields. Unlike N which is readily recycled, phosphates released from organic debris or mineral fertilizers become firmly bound to soil minerals (Al and Fe oxides) in acidic soils and precipitated as Ca-, Al- or Fe-phosphate in alkaline soils and are not available to plants (Gyaneshwar et al., 2002). This sequestered P is only very slowly released by microbial, root exudate and chemical activity. Nevertheless, pollution from excess soluble phosphates in animal wastes and fertilizers, and soil particles carrying sequestered P, can cause severe eutrophication in receiving water (Tilman, 2001). Although soil microorganisms take up (immobilize) phosphate from mineral and organic sources, they may also make it available to plants. Plants require phosphate rather than organic forms of P and recycling organic residues is important in natural ecosystems as well as for sustainable agriculture. Plants and microorganisms produce organic anions and phosphatases that solubilize mineral phosphates (Jones, 1998; Richardson et al., 2009). Microorganisms can also secrete nucleases, releasing phosphate from nucleic acids, and phytases that degrade phytates, relatively stable plant-derived inositol phosphates (Jones, 1998). Inoculation of plants and amendment of soil with phosphate-solubilizing bacteria and fungi has been proposed since the 1930s, but the extent to which their application has any substantial effects on P nutrition of plants in field conditions is controversial (Gyaneshwar et al., 2002). There is also considerable disagreement over the contribution of microorganisms to plant P nutrition in natural ecosystems, with reports in the literature of estimates ranging from 0 to 90% (Van Der Heijden et al., 2008). By contrast, the importance of mycorrhizal fungi to plant health and P nutrition is widely accepted: they form symbiotic associations with roots in >80% of angiosperm genera, their hyphae extending into soil, effectively increasing the root surface available for nutrient and water uptake, in return obtaining plant sugars (Brundrett, 2002; Finlay, 2008). In natural ecosystems, most plants are found to be mycorrhizal but agricultural cultivation reduces infection levels, in part because tillage breaks up mycelial networks in soil, and plants replete in N and P may be less receptive to colonization by arbuscular mycorrhizal fungi (AMF), although there is considerable variation between species (Treseder and Allen, 2002).

Sulphur is a component of many minerals and it enters the soil S cycle where microorganisms release sulphates that plants can absorb; there are also natural sources of atmospheric S from volcanic eruptions, $H_2S$, dimethyl sulphide in sea spray and oxidation of elemental S by certain bacteria and archaea (Falkowski et al., 2008). Crop growth was not limited by S in most industrialized countries until the end of the twentieth century due to $SO_2$ released from fossil fuels, and serendipitous applications as part of fertilizer formulations (Zhao et al., 1999) but S fertilizer is now recommended for most crops. Indeed, changing $SO_2$ emissions over the past 160 y have been linked to changes in the dominant fungal pathogens causing wheat leaf blotch, detected in DNA extracted from material collected and archived (Bearchell et al., 2005).

# 5 Anthropogenic impacts on soil: land management and crop selection

Traditional agriculture incorporated crop rotation to reduce buildup of pests and diseases and fallow periods to prevent nutrient exhaustion, with ploughing to bury weeds and improve structure in heavy clay soils. N was supplied as human waste, animal manures or by exploiting symbiotic $N_2$-fixing associations of leguminous plants. Annual river flooding provided nutrient-rich sediments in some areas and irrigation systems were constructed to water crops in dry periods. Some developing countries still rely on these methods, but elsewhere they have been replaced by modern agricultural practices including chemical fertilizers, pesticides and modern high-yielding crop varieties driven by improved mechanization.

## 5.1 Deforestation and afforestation

There are distinct differences in microbial assemblages following inter-conversion between forest, pasture and arable land: besides differences in plant cover, forest soils tend to sequester more soil organic carbon (SOC) than pastures, arable land sequesters even less (Cookson et al., 2006). The higher G+C content observed in microbial community DNA isolated from pasture compared to forest soil may be attributed to a predominance of α- and β-proteobacteria in the former, although the reasons are as yet unknown (Nusslein and Tiedje, 1999). Functional gene microarray comparison of ancient forest, spruce plantation and cropped land identified the spruce plantation soil as having the greatest number of carbon-cycling genes, cropped land the fewest, and that genetic diversity seemed to increase with total SOC (Zhang et al., 2007). Total metagenomic analysis of forest soils indicated differences between the organic and mineral layers with relatively more carbon-cycling genes in the former, and long-term residual effects following harvesting of trees (Cardenas et al., 2015).

## 5.2 Tillage

Tilling or ploughing reduces weed growth, improves soil workability and mixes in manures, fertilizers and crop residues. Some soils are vulnerable to damage and erosion caused by excessive tillage. A long-term comparison of conventional, reduced- and no-till in an arable crop rotation in Switzerland indicated that the number of AMF spores was greater in untilled soil with a trend to increased species diversity (determined by ribosomal gene sequencing), although this was not statistically significant (Jansa et al., 2002). Comparison of 16S rRNA gene sequences in long-term corn and soya bean rotations in Kansas showed the overall abundance of Proteobacteria in all treatments. Tillage altered the Acidobacteria and Gemmatimonadetes community structure compared to no-till, and there was significantly higher diversity in the untilled sites in one of two years tested (Yin et al., 2010). Similarly, increased species richness was associated with reduced tillage in a study comparing 16S rRNA genes in a long-term arable rotation in Mexico (Ceja-Navarro et al., 2010). The functional significance of these changes is unclear, with ambiguity over which of several factors are implicated: soil type, climate, crop and management practice all affect microbial communities and their activity (Yin et al., 2010). However, a recent

meta-analysis of 62 studies on the effects of tillage from around the world showed a statistically significant overall reduction in soil microbial biomass and enzyme activities and an increase in $CO_2$ evolution (Zuber and Villamil, 2016).

## 5.3 Flooding

Flooding in rice-growing areas helps control weeds and soil-borne pests. Flooded soils rapidly become anaerobic and soil microorganisms adopt alternative electron acceptors to oxygen (nitrate, Mn (IV) and Fe (III) ions, sulphate, acetate and $CO_2$), resulting in changes in soil chemistry and the generation of nitrous oxides, $H_2S$ and $CH_4$ (Liesack et al., 2000). In a study comparing soil microcosms sampled at time and depth intervals, the active soil microbial community changed substantially within two days of flooding. Species diversity was decreased, with β-proteobacteria dominating the upper oxic zone and Bacteroidetes dominating the lower anoxic zone (Noll et al., 2005).

## 5.4 Crops

Although compositional differences in root exudates influence rhizosphere microorganisms colonizing different plant species, soil properties and cultivation practices associated with particular crops may have more impact on soil microbial communities (Berg and Smalla, 2009; Dennis et al., 2010; Bais et al., 2006). However, pathogens and symbionts build up in the presence of compatible plant hosts (Berg and Smalla, 2009) and crop rotation was developed in part to deplete crop-specific soil-borne pathogens, although this may take several years. Similarly, symbiotic rhizobia have been shown to increase in number during cultivation of their host legume crop and persist for many years afterwards (Hirsch, 1996). There are many reports of microbial community structure (i.e. which species are present or dominant) and its activity reflecting different crop types (Berg and Smalla, 2009), although in recent years the use of culture-independent community studies indicates that most are dominated by Proteobacteria, followed by Firmicutes and Actinobacteria (Deangelis et al., 2009; Mendes et al., 2011; Teixeira et al., 2010; Weinert et al., 2011). Composition of rhizosphere communities may change during plant growth (Deangelis et al., 2009) or vary in different cultivars of a single crop species (Weinert et al., 2011). Apart from pathogens and their specific antagonists (Garbeva et al., 2004; Deangelis et al., 2009; Mendes et al., 2011; Sanguin et al., 2009) the functional implications of differences in rhizosphere community structure are unclear. Some bacteria that promote plant growth have been shown to produce phytohormones which may improve root growth and thus nutrient uptake, drought tolerance and crop yields but those tested by necessity are from well-known groups (Proteobacteria, Firmicutes) and can be grown in lab culture (Ngumbi and Kloepper, 2016). Despite the large numbers of different bacterial species and types present on all plant roots, stable isotope probing shows that particular groups are active on certain plants' roots, for example, *Variovorax* and oilseed rape, Sphingomonadaceae and cereals (Haichar et al., 2008).

Bare fallow soil from which weeds have been removed regularly over a 50-year period seemed to have maintained a similar level of microbial diversity compared to plots under continuous wheat or grass cultivation for the same period, despite having a substantially less abundant soil community (Hirsch et al., 2016). The permanent grassland from which both treatments were derived has a mixed plant community

and similar microbial diversity to the bare fallow, albeit more numerous. However, community structure was different in all three plots (Hirsch et al., 2009, 2016). A comparison using 16S rRNA gene pyrosequencing indicated higher species diversity in a wheat-cotton-corn rotation than pasture, with substantially lower diversity in cotton monoculture (Acosta-Martinez et al., 2008). Similarly, functional gene microarray analysis indicated less diversity in functional genes in soils under monoculture than those with mixed plant species (He et al., 2010).

# 6 Anthropogenic impacts on soil: fertilizers, agrochemicals, soil pH and pollution

## 6.1 Fertilizers

Fertilizers are used to improve plant growth and increase C inputs to soil, but can have indirect effects on the microbial community, in addition to specific impacts of N cycling organisms. Indirect effects arise from soil acidification by ammonium-based fertilizers – this includes urea, which is hydrolysed to ammonia by microbial activity (Bremner, 1995). Organic manures often contain substantial plant residues which complicate direct comparison with mineral fertilizers, particularly when the former are part of 'organic' agriculture regimes that avoid using 'artificial' manufactured fertilizers and pesticides. Like humus, farmyard manure (FYM) and compost improve soil structure, water and nutrient retention, whether part of a traditional, modern intensive or 'organic' agricultural systems. Animal manures in particular carry a distinctive microbial load, some of which will survive in soil and amend the microbiome of the host soil. However, both soils receiving regular FYM or green manures appear to maintain more diverse bacterial and fungal communities based on ribosomal gene diversity (Sun et al., 2004; Sugiyama et al., 2010). Functional diversity is also greater than that in soils receiving only mineral fertilizers, indicated by both enzyme activity and microarray analysis (Reeve et al., 2010; Mader et al., 2002). Nevertheless, fully 'organic' management generally results in lower crop yields than modern farming systems (Mader et al., 2002).

Fertilizers containing N might be anticipated to influence microorganisms involved in N cycling, removing selective pressure for biological $N_2$ fixation. There was no discernible effect on the diversity of the nitrogenase gene *nifH* attributable to N fertilizer inputs in the Broadbalk experiment at Rothamsted (Ogilvie et al., 2008). However, a negative association between *nifH* abundance and SOC was reported at a long-term ecological research site in the United States (Morales et al., 2010). In a comparison of multiple soil types and management regimes in southeast Australia, land use had no significant influence and the amount of soil microbial biomass C was the primary factor influencing *nifH* abundance (Hayden et al., 2010).

Impacts on denitrification are more complex, in part because denitrifiers are more numerous than any other functional group in the N cycle, comprising up to 5% of all soil bacteria (Philippot et al., 2007). Factors influencing denitrification such as anoxia and nitrate concentration are well established. Also, manures and organic fertilizers tend to increase denitrification activity relative to mineral fertilizers (Philippot et al., 2007; Hallin et al., 2009), although changes in denitrifier communities are less clear. The cytochrome $cd_1$ variant of nitrite reductase encoded by *nirS* is reported to be more common than

Cu-dependent *nirK* in cultured environmental bacterial isolates (Coyne et al., 1989). The preponderance of as-yet-uncultured soil bacterial genotypes renders the actual relative abundance uncertain. Environmental factors influencing the prevalence of alternate nitrite reductases *nirK* and *nirS* remain unclear and there is little consensus in the published literature: *nirK* is reportedly more abundant in arable systems (Coyne et al., 1989) and with increasing N fertilizer inputs (Philippot et al., 2007) whilst *nirS* is more abundant in intensively grazed plots with high soil ammonia and nitrate relative to less intensively grazed plots with lower N inputs (Dandie et al., 2011). In a Swedish long-term arable rotation comparing different fertilizer inputs, microbial functions were measured and the abundance of different genes assessed using qPCR. Denitrification activity correlated with the overall bacterial community size, crop yield and *nosZ* abundance whilst pH was the major soil property driving microbial community structure (Hallin et al., 2009). The distribution of *nirK* and *nirS* indicated that the forms are functional analogues (Hallin et al., 2009) and the overall abundance of denitrifiers together with available N and soil conditions determines the denitrification rate.

Continuing the discussion of the AOA and bacteria from Section 4.2, although overall AOA are more abundant than AOB in soil, in arable soils at neutral pH AOB proliferate in response to N fertilizer application and seem responsible for increased nitrification rates in agricultural systems (Wessen et al., 2011; Mendum et al., 1999; Jia and Conrad, 2009; Bates et al., 2011). Similarly, AOB, but not AOA, increased with N in grazed grasslands (Di et al., 2009) whilst in two arable soils, AOA declined with increasing N (Wessen et al., 2011; Bates et al., 2011). However, in acid soils, AOA seem to respond to N application (Gubry-Rangin et al., 2015). This reflects availability of the substrate $NH_3$ and the different pH optima for the two groups: AOB nitrification rate decreases below pH 7; AOA are more abundant in soils below pH 6.0 and nitrification activity decreases as soil pH increases (Nicol et al., 2008). Furthermore, community structure is influenced by soil pH: distinctly different groups of both AOA and AOB (determined by DGGE analysis of PCR products) were found in arable plots maintained from pH 4.5 to 7.5 (Nicol et al., 2008). This may explain why significant farm-scale heterogeneity in AOA and AOB abundance, community structure and function seems to be related to soil properties rather than land management by organic or conventional regimes (Wessen et al., 2011). To summarize, nitrification occurs in most soils: AOA may be responsible for most activity in nutrient-poor acidic conditions and AOB contribute more in fertilized soils at neutral pH. In consequence, there may be a delay in nitrification in response to intensive animal grazing, fertilizer or lime application on previously nutrient-poor soils, until AOB populations increase. There may also be differential responses to nitrification inhibitors: 3,4-dimethylpyrazole phosphate reduces abundance of AOB but not AOA (Kleineidam et al., 2011). Similarly, AOB were inhibited to a much greater degree than AOA by sulphadiazine residues in pig manure (Schauss et al., 2009).

## 6.2 Agrochemicals

As well as fertilizers, other agrochemicals are applied to most food and feed crops in the developed world to increase yields by improving growth, reducing damage from pests and pathogens, and decreasing competition with weeds, thus indirectly affecting diversity. For example, in the United Kingdom, approximately 25% of land receives agrochemical applications (Defra statistics 2009). Some agrochemicals have been reported to show transient effects either because of selective toxicity or because they are selective substrates

metabolized by certain microbial groups (Hussain et al., 2009). For example, glyphosate is considered to be a relatively safe herbicide with limited non-target effects (Duke and Powles, 2008). At high concentrations the formulations used in agriculture are toxic for many microorganisms in laboratory culture, but when applied directly to soil glyphosate they provide a growth substrate, stimulating both microbial activity and functional diversity (Mijangos et al., 2009). When applied to plants it has inconsistent effects possibly due to herbicide-induced changes in root exudation (Mijangos et al., 2009) and had no significant effect on the diversity of bacteria in the maize rhizosphere, assessed by 16S rRNA gene sequencing (Barriuso et al., 2010). Similarly, no effects on microbial processes were detected in soil where spring barley received applications of five pesticides, including glyphosate, over a 20-year period (Bromilow et al., 1996).

## 6.3 Soil pH

The pH of soil is relatively easy to manage, influences nutrient availability and is reported to have the largest overall influence on microbial communities, greater than the effect of soil mineralogy or of plants (Fierer and Jackson, 2006). Natural soils tend to become acid: plant roots respire $CO_2$ forming the weak carbonic acid and also exude protons and organic acids (Jones, 1998). Soil microorganisms contribute by respiration and degradation of plant residues (Catt, 1985; Goulding and Annis, 1998); also rain is naturally acid, historically exacerbated by 'acid rain' arising from industrial processes (Goulding and Poulton, 1985). In agricultural systems acidification occurs, resulting in $Ca^{2+}$ losses (Gasser, 1985) exacerbated by the use of elemental sulphur and ammonia-based fertilizers (Goulding and Poulton, 1985). The Romans used 'lime' ($CaCO_3$ in calcite or chalk, $CaMg(CO_3)_2$ in dolomite) to 'sweeten' soil if a soil suspension tasted acidic (Barber, 1967). The practice was widespread around 100 BCE according to the Roman agronomist Varro. The Romans probably introduced liming to Britain and marl (chalk) pits have been common since Norman times (1066–1154 CE). Generally, lime was applied every five years, but since the nineteenth century, improved understanding and monitoring of soil chemical processes has enabled maintenance of soil pH at levels optimal for particular crops (Goulding and Annis, 1998). Soil pH substantially altered the trajectory of microbial community structure development in a grassland over time, assessed by 16S rRNA gene microarray and qPCR study (Kuramae et al., 2011), and microbial diversity in a pH gradient in arable soil assessed by ribosomal gene sequencing (Rousk et al., 2010). In the latter study, the number of bacterial species doubled between pH 4 and 8, although effects on fungal diversity were not significant. In a landscape-scale study in Burgundy, France, soil pH was the main driver of differences in the diversity and abundance of microbial communities, including those involved in N cycling (Bru et al., 2011).

## 6.4 Pollution

Pollution from natural or anthropogenic sources can have major effects on soil microbial functioning. Soils polluted heavily with toxic metals or organic chemicals from industrial or natural sources may support only a limited number of dominant specialist organisms. However, these organisms may alleviate some of the most harmful effects of acute contamination. For example, they may reduce metal ions to less toxic (Cr(VI) to Cr(III)) or mobile (U(VI) to U(IV)) forms in anaerobic sediments, generating energy for their growth in the process; or by catabolizing polyaromatic hydrocarbons. The number and diversity

of functional genes detected using a functional gene microarray decreased along an oil field contamination gradient, although the abundance of specific organic contaminant degradation genes increased (Liang et al., 2009). Plants are frequently early casualties of contamination; their absence will have a secondary effect on the soil food web. Planting soils with pollution-resistant plants is often a first step in reclamation of mine spoil heaps, providing substrates that stimulate bacterial growth.

Contamination of land with pesticides or animal wastes containing antibiotics frequently has transient effects on soil microorganisms. Metals used in fungicide preparations can be more problematical as they are not degraded and accumulate in soil. Chronic metal pollution can reduce plant productivity, making crops unfit for consumption. In productive soils, the commonest sources of pollution are through the use of copper-based fungicides (commonly applied to vineyards), atmospheric deposition and addition of sewage-sludge or municipal waste to arable soils (Nicholson et al., 2006). Sludges are rich sources of organic matter, N and P but typically contain potentially harmful metal pollutants such as Zn, Cu and Cd. Numerous studies describe the general response (species diversity, population respiration, enzyme activity) of soil microbial populations to metal pollutants in soils, for example, reporting reductions in diversity based on 16S rRNA gene analysis (Moffett et al., 2003; Ranjard et al., 2006; Macdonald et al., 2011b) or abundance of rhizobia based on nodulation gene frequency (Macdonald et al., 2011a). Chronic exposure to metals results in the soil community acquiring tolerance to the metal in a phenomenon termed pollution-induced community tolerance (Davis et al., 2004). Specific changes in functional diversity have been reported in soil microcosms, for example, Cu associated with fungicide application resulted in altered nitrification gene diversity and increased nitrification potential (Demanou et al., 2006). Ag addition also changed denitrification gene diversity, resulting in reduced activity (Throback et al., 2007) and changes in microbial communities in response to Cu addition were associated with an increase in the mineralization of soil organic matter (Bernard et al., 2009). However, short-term studies can be misleading, interactions between metal ions, soil organic matter and minerals, and microorganisms are complex, and few studies compare different soil types contaminated with the same material. Comparison of microbial communities using T-RFLP in five arable and two grassland soils treated 11 years previously with the same sludge containing Cu and Zn (at levels close to the EU maximum) and the untreated soils indicated that the geographic site had an overwhelming influence on soil community structure but some community shifts could be attributed to metals (Macdonald et al., 2011b).

## 7 Future perspectives

Whether for agriculture, forestry, industry or amenity, land use is driven by social, economic and political factors. Consequently, the most sustainable systems do not always prevail and there is a balance between using less land for more intensive food production, or lower input agriculture over a much wider area: 'land sparing' versus 'land sharing'. However, since land is a finite resource, calls for greater intensification are irresistible (Godfray et al., 2010). Eschewing synthetic chemicals cannot replace farming practices in industrialized countries and achieve continually increasing yields (FAO; http://www.fao.org/organicag/oa-faq/oa-faq7/en/). The next 30 years will see inevitable pressure to improve the efficiency of use of both water and fertilizers, whether synthetic, derived from organic wastes or minerals (Tilman et al., 2002). Continued development of chemicals

for crop protection will be essential to provide maximum efficacy against pests with minimal impact on non-target organisms, including consumers (Beddington, 2010). An increase was predicted in the exploitation of higher-yielding or more pest resistant crop varieties with a broader genetic base, including plants modified using GM technology to incorporate novel traits (Godfray et al., 2010) and with targeted mutagenesis using CRISPR-Cas methods (Doudna and Charpentier, 2014). Designing plants to select for the most beneficial microbial colonists, or to modify the soil microbiome to optimize nutrient availability whilst minimizing losses is one potential strategy (Hirsch and Mauchline, 2012; Lareen et al., 2016).

Managing land for environmental improvement is subjective: natural mixed vegetation and their associated microbiota may thrive in soils too acidic and low in nutrients for optimal crop growth; incorporation of organic matter benefits arable soils, sequestering carbon and improving soil structure – but the same treatment may encourage denitrifying and methane-generating bacteria that increase nitrous oxide and methane emissions. Understanding the response of soil microorganisms to natural or anthropogenic perturbation including differences in land use management should enable optimal soil management, to maximize crop production, improve structure or regenerate 'natural' ecosystems.

Recent technological advances are opening the soil 'black box' and providing insight into how communities respond to changing land use (Hirsch et al, 2010). Although the rarest species may remain undiscovered, and scant information gained on less abundant phyla, high-throughput sequencing is providing increasingly deep sequencing, revealing improved details of complexity and greater knowledge of community structure and dynamics. This is complemented by new culture methods such as the iChip (Nichols et al., 2010), discussed above (Section 2), that enable isolation of previously obscure soil microorganisms.

High species diversity and substantial functional redundancy both confer resilience to soil communities, making it unlikely that essential functions will be lost permanently even if the environment is not conducive to survival of organisms and expression of genes. Mankind has manipulated soils deliberately to improve crop yields since the emergence of agriculture, often producing concomitant changes in microbial communities. Sometimes this has led to inadvertent loss of soil structure and chemistry, occasionally causing major environmental problems, but mitigation methods have developed alongside agriculture, in particular, management of soil nutrients and pH. Crop varieties and agrochemicals seem less likely to influence soil microorganisms than associated major changes in land management that affect the physico-chemical structure of soil (Griffiths and Philippot, 2013) and it is these, together with climate change, that could potentially destabilize biogeochemical cycles.

## 8 Where to look for further information

Additional information can be found online, in books and research papers. A comprehensive collection of methods for rhizosphere research is provided by *Molecular Microbial Ecology of the Rhizosphere* (de Bruijn, 2013). Molecular methods evolve rapidly and journal articles provide recent advances in technology, but the US Department of Energy Argonne National Laboratory hosts the Earth Microbiome Project website which provides current

methods for DNA extraction and preparation of samples for amplicon sequencing to measure microbial diversity (http://www.earthmicrobiome.org/protocols-and-standards/).

A useful online overview of soil biology with links to related information on soil health is provided by the US Department of Agriculture Soil Biology Primer (https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/biology/). Further information on soil biology, with a large number of illustrations including soil mesofauna is available from the Global Soil Biodiversity Initiative which has produced an online atlas (https://globalsoilbiodiversity.org/). The FAO Soils Portal provides overviews of soil biodiversity and its importance for nutrient cycling and other aspects of soil health (http://www.fao.org/soils-portal/soil-biodiversity/en/).

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