MICROBIOME

Embracing the unknown: disentangling the complexities of the soil microbiome

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Abstract | Soil microorganisms are clearly a key component of both natural and managed ecosystems. Despite the challenges of surviving in soil, a gram of soil can contain thousands of individual microbial taxa, including viruses and members of all three domains of life. Recent advances in marker gene, genomic and metagenomic analyses have greatly expanded our ability to characterize the soil microbiome and identify the factors that shape soil microbial communities across space and time. However, although most soil microorganisms remain undescribed, we can begin to categorize soil microorganisms on the basis of their ecological strategies. This is an approach that should prove fruitful for leveraging genomic information to predict the functional attributes of individual taxa. The field is now poised to identify how we can manipulate and manage the soil microbiome to increase soil fertility, improve crop production and improve our understanding of how terrestrial ecosystems will respond to environmental change.

Soil microbiome
A general term describing all microorganisms that can be found in soil, including archaea, bacteria, viruses, fungi, protists and other microbial eukaryotes.

How can I stand on the ground every day and not feel its power? How can I live my life stepping on this stuff and not wonder at it? — W. B. Logan[1]

Soil microbial biomass rivals the aboveground biomass of plants or animals, with soil often containing >1,000 kg of microbial biomass carbon per hectare[2,3]. These soil microorganisms have crucial roles in nutrient cycling, the maintenance of soil fertility and soil carbon sequestration, and the soil microbiome has both direct and indirect effects on the health of plants and animals in terrestrial ecosystems. The importance of the soil microbiome has been recognized for more than a century[4], and there is a long history of research that describes the microorganisms that inhabit soil, their metabolic capabilities and their influence on soil fertility. In fact, many of the important discoveries made by microbiologists — including the discovery of antibiotics and unique microbial metabolic pathways (for example, nitrogen gas fixation and ammonia oxidation) — were largely derived from work with soil microorganisms.

Recent methodological advances have enabled researchers to chart the full extent of soil microbial diversity and to build a more comprehensive understanding of specific microbial controls on soil processes. In particular, DNA-based and RNA-based analyses of the soil microbiome are now relatively common and have greatly expanded what is known about the phylogenetic and taxonomic structure of soil microbial communities. It is now recognized that typical culture-based approaches substantially underestimate soil microbial diversity and that soils contain a broad diversity of microbial taxa from all three domains of life, the majority of which remain uncharacterized[5–8].

In this Review, I summarize what has been learned from recent work on the soil microbiome, discussing what microorganisms live in soil, what factors affect the composition of the soil microbiome across space and time, and why it remains difficult to link specific soil microbial taxa to many soil processes. I then focus on recent advances in our conceptual understanding of soil microbial communities and their metabolic capabilities, and emphasize how we can continue to leverage genomic, metagenomic and marker gene data to infer the ecological attributes of undescribed soil microbial taxa. Next, I discuss the challenges and opportunities associated with efforts that aim to manage soil microbial communities to maximize agricultural productivity and sustainability. Last, this Review highlights key research directions that could shape the future of basic and applied research into the soil microbiome.

Structure of the soil microbiome

Soil is not a single environment; rather, it encompasses a wide range of environments that can contain distinct microbial communities (FIG. 1). Distinct soil environments may be only micrometres to millimetres apart, but they can differ considerably in their abiotic characteristics, microbial abundances, rates of microbial activity and microbial community composition.

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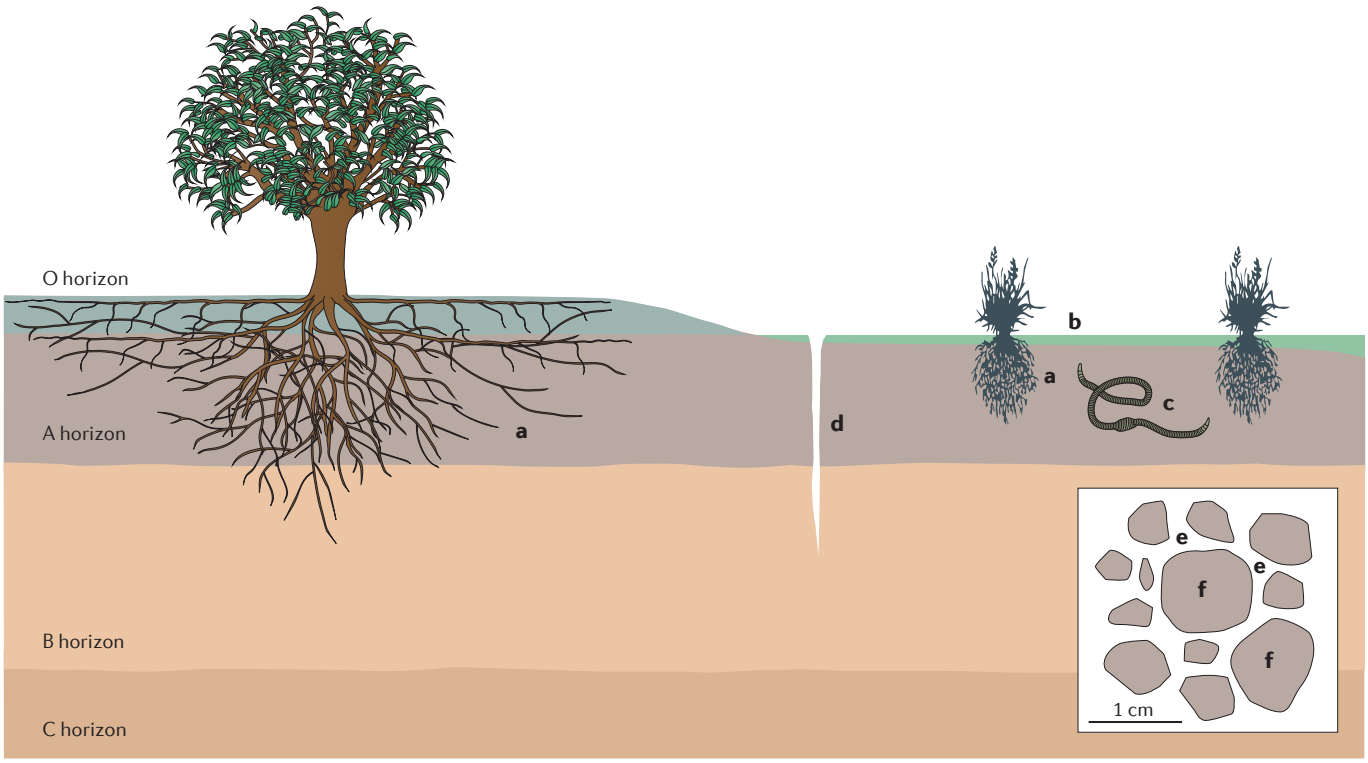


Figure 1 | **The macroenvironments and microenvironments of soil.** Soil is not a single environment; instead, soil encompasses a broad range of different microbial habitats. These include the rhizosphere (soil in close proximity to plant roots; part **a**), surface layers that are exposed to light (part **b**; the photic zone), soil associated with earthworm burrows (the drilosphere; part **c**), and soil found in preferential water flow paths, including cracks in the soil (part **d**). Moreover, there are microenvironments associated with soil aggregates; the conditions found on aggregate surfaces or on the water films between aggregates (part **e**) are distinct from the conditions found inside aggregates (part **f**). Finally, there are marked shifts in microbial communities and abiotic conditions with soil depth. Although most studies have focused exclusively on the microorganisms found in surface soil horizons (layers), communities found in the litter layer (or O-horizon) are often distinct from those found in underlying mineral soil horizons (A and B horizons) and deeper saprolite (C horizons)[123]. Key soil properties — such as pH, organic carbon concentration, salinity, texture and available nitrogen concentration — can vary substantially across these distinct soil environments.

Characteristics of the soil environment. At the global scale, soil environmental conditions are highly variable. Decades of research has shown that the properties of surface soils — including pH, organic carbon concentration, salinity, texture and available nitrogen concentration — exhibit an enormous range. This variation is a product of the main factors that affect soil formation: namely, climate, organisms (including both macroorganisms and microorganisms), relief, parent material and time (as reviewed in REF. 9). Even in a given soil profile, environmental conditions can vary considerably across the distinct microbial habitats found in soil, including the rhizosphere, preferential water flow paths (including cracks in the soil), animal burrows, intra-aggregate and inter-aggregate environments, and surface versus deeper soil horizons (FIG. 1). For example, oxygen concentrations can vary from 20% to <1% from the outside to the inside of individual soil aggregates that are only a few millimetres in size[10], and the bacterial communities that are found in close proximity to a plant root or fungal hyphal network can differ substantially from those found in 'bulk' soil environments just a few centimetres away[11].

Rhizosphere
The soil located in close proximity to plant roots, which typically has more plant-derived carbon and a higher microbial biomass than the surrounding non-rhizosphere or 'bulk' soil.

Soil horizons
Distinct layers in a soil depth profile that are typically defined on the basis of physical or chemical characteristics.

Microbial survival and growth in the soil environment is often severely limited. There can be persistent abiotic stressors (for example, low water availability, limited availability of organic carbon substrates and acidic conditions), a high degree of competition with other soil microbial taxa (as exemplified by the widespread occurrence of antibiotic-producing and antibiotic-resistant soil bacteria[12]), frequent disturbances (for example, drying–rewetting events, predation by earthworms and other fauna, and freezing–thawing), and an uneven distribution of resources across space and time[13]. Numerous lines of evidence highlight just how difficult it is for microorganisms to survive and grow in the soil environment. First, even when soil is artificially inoculated with numerous bacteria, most of those bacteria are unlikely to persist in the soil for extended periods of time[14,15]. Second, despite the large amounts of microbial biomass found in soil (FIG. 2), far less than 1% of the available soil surface area is typically occupied by microorganisms[16], which suggests that there are biotic or abiotic constraints on the microbial colonization of soil surfaces. Third, many of the microorganisms found

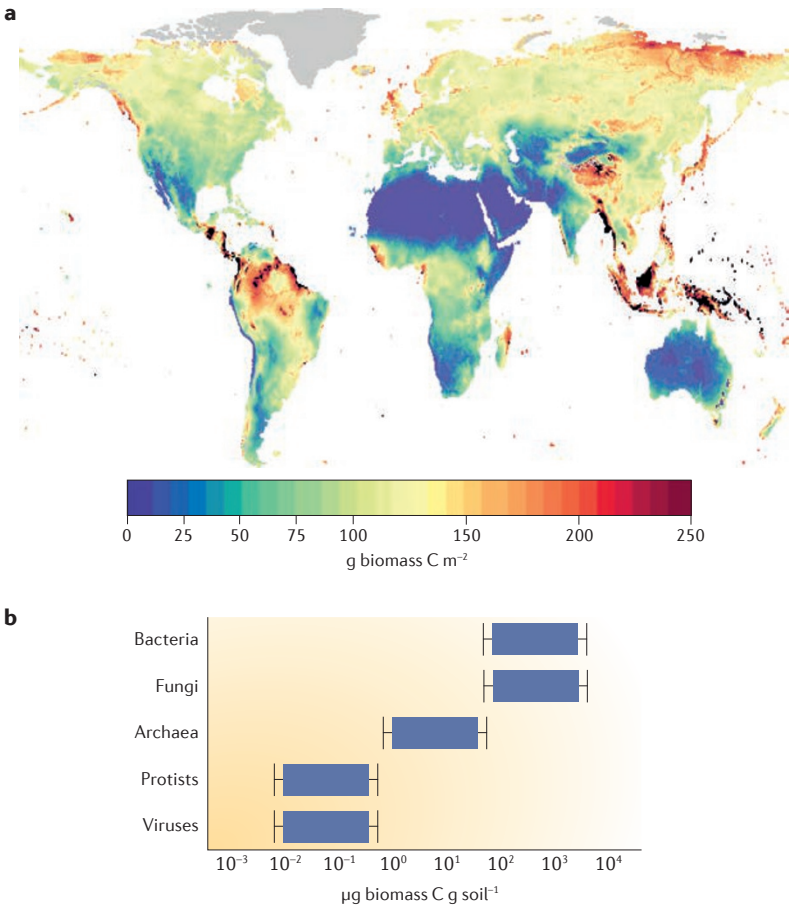


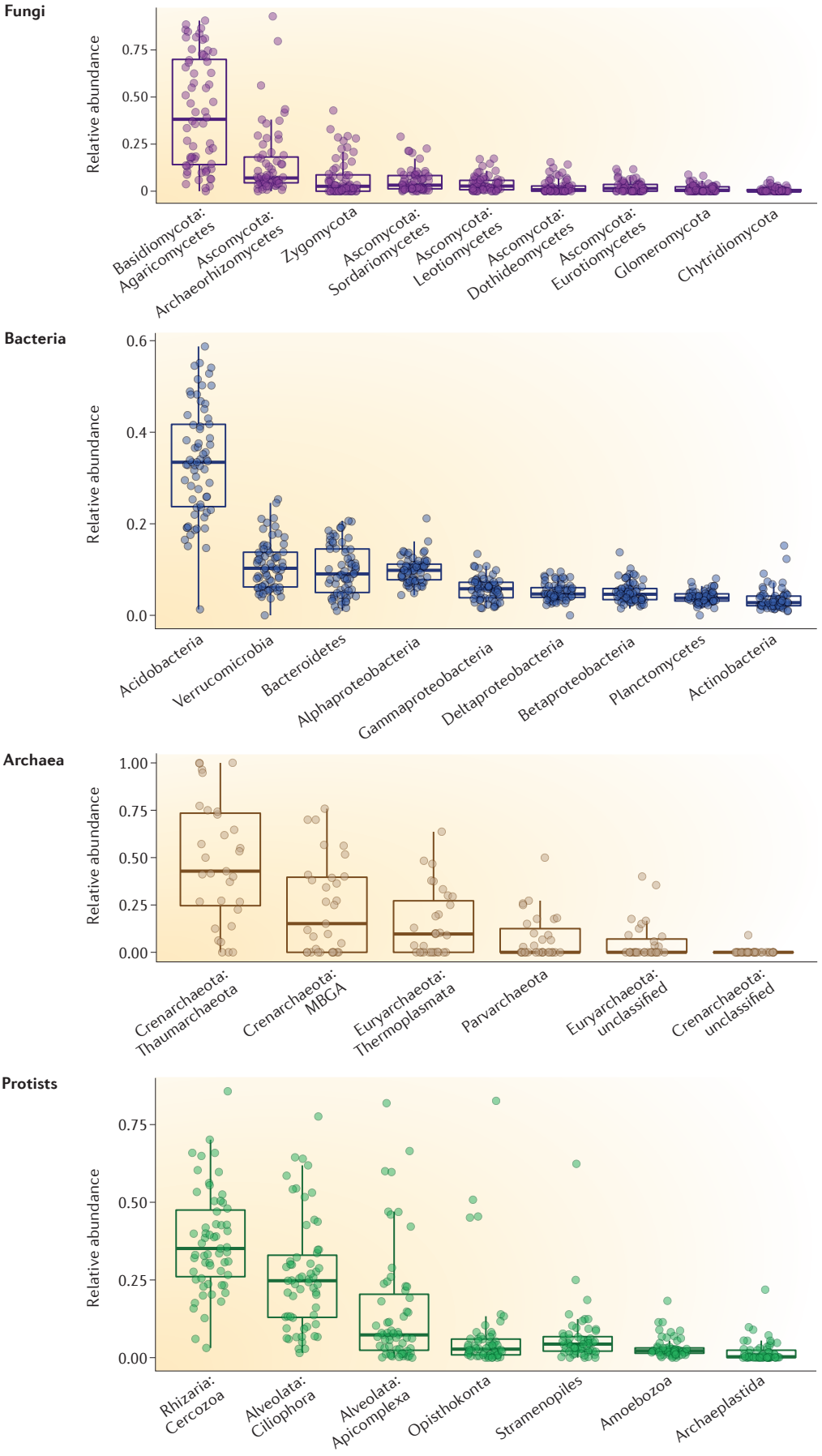
Figure 2 | **Global microbial biomass found in soil.** An estimation of how total soil microbial biomass (the sum of all microbial groups: bacteria, fungi, archaea, protists and viruses) varies across the globe (part **a**), and estimates of the contributions of the major microbial groups to this total soil microbial biomass pool (part **b**). The estimates in part **b** were compiled from information provided in REFS 104,124–130. These estimates are approximations; biomass can vary substantially across soils, and the biomass of protists and viruses is highly uncertain. The map in part **a** is adapted with permission from REF. 2, Wiley.

in soil are likely to be dormant, with >95% of the total microbial biomass pool represented by microorganisms that are inactive at a given point in time[17].

Characteristics of the soil microbiome. A range of biotic and abiotic factors, including the abundance of microbial predators (such as protists or nematodes) and the amount of available carbon, can influence the total amount of microbial biomass found in a soil at any given point in time. At the global scale, soil moisture availability is the best predictor of total soil microbial biomass; ecosystems that are wetter (for example, tropical rainforests) typically contain larger amounts of standing microbial biomass[2] (FIG. 2a). However, not all microbial taxa are equally abundant in soil. Bacteria and fungi are generally the dominant microorganisms found in soil; these groups usually have 10^2–10^4 times more biomass than the other major components of the soil microbiome (protists, archaea and viruses; FIG. 2b). A relatively small number of bacterial and archaeal phyla typically account for most of the 16S ribosomal RNA gene (16S rRNA gene) reads obtained from PCR-based surveys of prokaryotic diversity (FIG. 3). At finer levels of taxonomic resolution, most individual bacterial and archaeal species are rare, and relatively few are abundant in any given community[18]; this structure is similar to that observed in many plant and animal resident communities[19]. Although there are some notable exceptions (for example, some prairie soils, which are dominated by lineages in the Verrucomicrobia phylum[20]), the most abundant bacterial and archaeal species usually account for a relatively small percentage of the prokaryotic DNA recovered from individual samples. Most of these bacterial and archaeal taxa belong to lineages that remain undescribed. A good example of this is provided by an investigation of the diversity found in 596 soil samples that were collected from Central Park in New York City in the United States, which found that >80% of the individual bacterial and archaeal taxa found in soil have 16S rRNA gene sequences that do not match those found in reference databases[6]. The same patterns hold true for soil fungi and protists. Although a few major groups of fungi and protists typically dominate in soil (FIG. 3), many of the individual lineages remain undescribed. For example, Apicomplexa with poorly understood ecologies can dominate the protistan communities found in tropical soils[21]. Furthermore, even ubiquitous soil fungal groups can contain large amounts of novel diversity[22]. Although we are far from being able to describe the full extent of soil microbial diversity, and from understanding the metabolic capabilities and ecology of most soil microbial taxa, recent research efforts have greatly expanded our knowledge of the structure of soil microbial communities and their roles in terrestrial ecosystems. The soil microbiome is not necessarily a mysterious 'black box' that defies efforts to be opened.

There is no 'typical' soil microbiome. The relative abundances of major bacterial and archaeal taxa found in the soil microbiome can vary considerably depending on the soil in question (FIG. 3). This is true even if soil samples are collected from sampling sites that are just a few centimetres apart[23]. Part of this variation in the composition of the microbiome can be attributed to spatial variability in the soil environment and the specific characteristics of the sampling site. The importance of these factors is dependent on the taxa in question, the choice of soils analysed and the experimental methods used. For this reason, the literature on this topic can be confusing, as there is no single biotic or abiotic factor that is consistently the most important in determining the composition of the soil microbiome. Likewise, there is no single factor that consistently explains variability in plant and animal communities across global, regional and local scales. For example, when we analyse a collection of soils that represent a broad range of pH values (from pH 4 to pH >8), we often find that soil pH is the best predictor of bacterial and archaeal community composition[24,25]. However, these pH effects may not be apparent when soil samples span a narrower range of pH values, and not all taxa respond to changes in soil pH. There are clearly

16S ribosomal RNA gene (16S rRNA gene) A gene that encodes a subunit of bacterial and archaeal ribosomes, and that is commonly used for taxonomic and phylogenetic analyses of bacterial and archaeal communities.



Mycorrhizal fungi
Diverse groups of fungi that can live on or in plant roots. They extend the plant root system and often (but not always) confer benefits to the plant, including nutrient and water acquisition.

Nitrogen-fixing bacteria
Free-living or symbiotic bacteria (diazotrophs) that reduce atmospheric nitrogen gas to ammonia.

Relic DNA
DNA found in soil and other environments that is extracellular or found in cells with compromised cytoplasmic membranes, as opposed to DNA from living intact cells.

many factors that can directly or indirectly influence the spatial structure of soil microbial communities. However, there is an emerging consensus regarding which variables are most likely to have marked effects on the variety and abundance of soil microbial taxa (FIG. 4). In addition to soil pH, the most important factors that have notable influences on the structure of soil bacterial communities are most likely nitrogen availability[26], soil organic carbon content[27], temperature[28] and redox status[29].

One common assumption is that changes in the composition of the soil microbiome should be predictable from the composition of the aboveground plant community. Thus, we would expect that soils (either rhizosphere or bulk soils) with different overlying plant species should have distinct belowground microbial communities and that specific soil microbial taxa will preferentially associate with different plant species. Indeed, this is true for many mycorrhizal fungi, fungal plant pathogens and some nitrogen-fixing bacteria (for example, *Rhizobium* spp.), which typically only associate with a specific plant species[30,31]. In support of this, some observational studies have found that the overall structure of soil microbial communities can track changes in aboveground plant communities[32–34]. By contrast, other studies have found little to no effect of plant species on belowground communities[35–38]. The effect of specific plant species on the composition of the soil microbiome may often be context-dependent; a given plant species may associate with different microbial taxa depending on the soil type in question[39]. Furthermore, many soil bacterial taxa may be cosmopolitan and able to associate with a wide range of plant taxa, meaning that the effects of aboveground vegetation on the composition of microbial communities may take years to become evident[40]. Although plants can affect the structure of the soil microbiome, many other factors are involved. Thus, we cannot a priori predict soil microbial community composition by simply knowing what plant species are growing in each soil.

Sampling the soil microbiome. As the soil environment is so heterogeneous, when we analyse a soil core or a small amount of sieved soil we are effectively sampling a broad diversity of microhabitats. This is important for several reasons. First, it can make it difficult to link edaphic (soil) characteristics to microbial community composition. For example, high abundances of strict anaerobes, including methanogens, can be found even in well-aerated surface soils[41]. This is because there will be microhabitats (such as aggregates) in aerated soils that have localized low concentrations of oxygen. Second, by analysing individual soil samples that are typically several cubic centimetres in size, we may be obscuring potential interactions or co-occurrence patterns. Just as trees may exist in the same hectare plot without interacting directly, micrometre-sized bacteria that live in the same cubic centimetre of soil may be located too far apart to directly interact or exchange metabolites. Third, although soil microbial diversity may seem large by comparison to terrestrial plant and animal diversity, it is important to recognize that there is an enormous disparity between the size of the microorganisms and the comparatively large area sampled when a soil core is collected, which makes direct comparisons difficult[42]. Although soil microbial diversity can still be high, it is invariably lower when we examine the soil microbiome at spatial scales that are closer to the size of the organisms in question (for example, the diversity contained in individual soil aggregates[43]). The relevant spatial scale will depend on the questions being asked. For example, mapping the occurrence of soil ammonia oxidizers across an entire country[44] is useful for understanding landscape-level controls on their abundance, but this sampling scheme is not useful for quantifying the location of ammonia oxidizers within soil aggregates.

The full extent of the bacterial and archaeal diversity found in soil remains difficult to determine as there are methodological considerations that can lead to substantial mis-estimations of diversity. For example, the presence of relic DNA may increase diversity estimates by ≥40%[45]. Furthermore, PCR-based marker gene analyses are routinely used to characterize soil microbial diversity, even though we know that they can be biased against some bacterial and archaeal lineages[46,47]. Moreover, a combination of sequencing errors and constraints on the algorithms used to identify phylotypes may increase estimates of 'species'-level diversity[48]. In addition to the overestimation of diversity by PCR-based marker gene analyses, diversity may be underestimated when conducting shotgun metagenomic analyses if these analyses rely on incomplete reference genome databases[49]. More generally, diversity can vary depending on the sequencing depth, the sample size and the DNA extraction method[50]. In short, any attempts to estimate the richness or diversity of the soil microbiome are subject to a range of caveats, and therefore estimates of species-level or strain-level diversity should be interpreted with caution[51].

It is unclear what factors, or combinations of factors, are driving the apparent spatial variation in soil microbiome composition, as measured soil or site characteristics typically only explain a fraction of the variation[6,8,24,33]. This could be because we are not measuring the appropriate soil or site-level factors. For example, it is likely that organic carbon that enters soil from root exudates can be important in structuring rhizosphere soil microbial communities. However, accurate measurements of root exudate carbon inputs are difficult to conduct, even under controlled laboratory conditions[52]. In addition, measurements of bulk soil properties do not necessarily

◀ Figure 3 | **The general structure of the bacterial, archaeal, protistan and fungal communities found in soil.** The data show the range in proportional abundances for each major group across the 66 unique soil samples described by Crowther *et al.*[40]. Abundances were estimated by marker gene sequencing (16S ribosomal RNA (rRNA) for bacteria and archaea, internal transcribed spacer 1 (ITS1) for fungi and the 18S rRNA gene for protists). All soils were collected from relatively undisturbed sites (no cultivated soils) from across North America. The soils represent a wide range of ecosystem types (including both forests and grasslands), latitudes (from 18°N to 65°N), soil characteristics and climatic conditions. MBGA, Marine Benthic Group A.

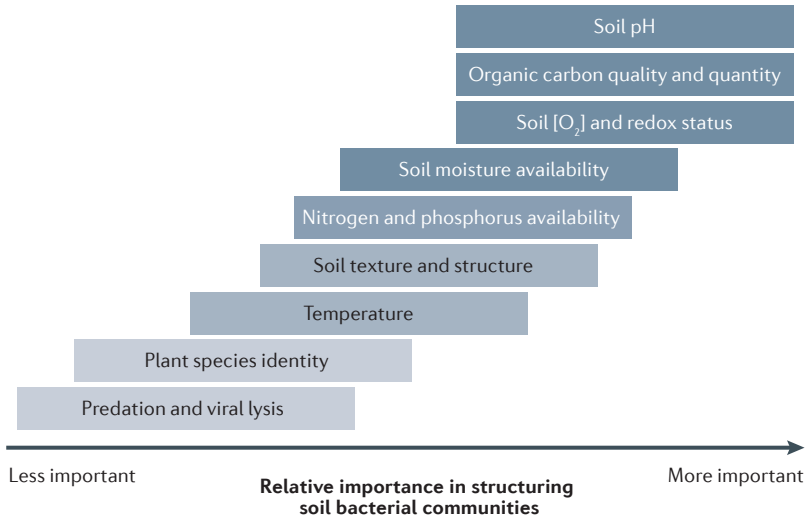


Figure 4 | **Biotic and abiotic factors that can influence the composition of soil bacterial communities.** A hierarchy of biotic and abiotic factors that can influence soil bacterial communities, and their relative importance in influencing the structure of soil bacterial communities across space or time. 'Importance' is defined here as the ease of detecting the effects of these factors on the overall composition of soil bacterial communities. These factors are not necessarily independent and can correlate with one another (for example, soil texture can influence soil moisture availability). Furthermore, the importance of these factors will depend on the soils under investigation and the bacterial lineage in question. The shading of each box qualitatively indicates how well we understand the specific effects of each factor on bacterial communities; darker shades highlight factors that have been reasonably well-studied. This hierarchy is based primarily on studies that have examined spatial patterns in soil microbial communities[24,25,33,58,131–133].

capture the micro-scale variation in soil properties that may drive spatial variation in soil microbial community composition (FIG. 1).

Even with a detailed characterization of the soil environment, it may still be difficult to explain all of the spatial variability in soil microbial communities, as some of that variation may be due to dispersal constraints. Although dispersal constraints are known to influence the structure of plant and animal communities[53], studies that investigate the soil microbiome across multiple sites and soil types often fail to detect an explicit effect of spatial distance (a proxy for dispersal constraints) on the composition of the soil microbiome. This means that soil or site conditions are often a better predictor of observed community patterns than geographical distance alone. However, our inability to detect the effects of dispersal constraints does not mean that dispersal is unimportant. The detection of dispersal constraints requires an appropriate sampling design that enables the effects of spatial distance to be disentangled from the degree of dissimilarity in soil environmental conditions. Notably, many of the commonly used methods for characterizing the soil microbiome, such as analyses of the 16S rRNA gene, often provide insufficient taxonomic resolution to detect the effects of dispersal constraints. When alternative approaches that provide more detailed taxonomic resolution are used (including whole-genome analyses), it becomes possible to identify dispersal constraints on the biogeographical patterns exhibited by specific strains of soil bacteria[54,55].

Much of the pre-existing literature has focused on spatial variability in the structure of the soil microbiome, and there are fewer studies that focus explicitly on temporal variability. One reason for this is that an investigation of true temporal variability would require the same location to be sampled repeatedly over time. As soil sampling is inherently destructive, the exact same location can never be sampled repeatedly, and sampling an immediately adjacent soil location may produce confounded results owing to the potentially large, fine-scale spatial heterogeneity that exists in soil microbial communities[23]. Accurately assessing the degree of temporal variability is important for answering both methodological questions (for example, is a sample collected at a single point in time sufficient to describe spatial patterns?) and for addressing more conceptual questions (for example, how quickly do soil microbiomes respond to environmental changes or changes in land-management practices?). In the few cases in which temporal variation has been explicitly quantified, it seems that temporal variation is typically lower than spatial variation[56–58]. However, the actual temporal variability in soil microbial communities may be underestimated owing to the presence of relic DNA[45]. In addition, temporal patterns may become more evident if analyses are focused only on living or actively metabolizing cells[59,60]. Moreover, not all taxa are likely to be equivalent in their temporal variability. Some taxa may be more sensitive than others to changing environmental conditions. For example, we would expect only a subset of soil bacterial taxa to be responsive to seasonal changes in soil temperature[28]. In addition, lineages of copiotrophic bacteria should be particularly sensitive to rapid fluctuations in soil carbon availability or soil moisture[13,61,62].

Functions of the soil microbiome

Although the diversity and spatiotemporal patterns in the soil microbiome are clearly of interest to microbiologists, such surveys of diversity are most useful when that information is directly relevant to other disciplines. It is important to understand how information about the soil microbiome can help us to predict the range of effects that soil microorganisms may have on both natural and managed ecosystems.

Effects of the microbiome on soil processes. Some of the soil processes that can be directly influenced by belowground microbial taxa are shown in FIG. 5. Microorganisms can produce and consume atmospheric trace gases (for example, hydrogen, carbon dioxide, nitric oxide, nitrous oxide, methane and other volatile organic compounds), influence soil acidity, regulate soil carbon dynamics and mediate the cycling of nutrients (for example, iron, sulfur, phosphorus and nitrogen) within the soil profile. FIGURE 5 does not include indirect mechanisms by which the microbiome can influence ecosystem and biogeochemical processes. For example, research suggests that soil-borne plant and animal pathogens can influence aboveground vegetation dynamics and animal populations, with far-reaching effects on the functioning of terrestrial ecosystems[30,63]. Furthermore, soil microorganisms may affect soil water

Dispersal constraints
The biotic or abiotic factors that restrict the movement of microorganisms across space.

Copiotrophic bacteria
Ruderal taxa that would be expected to preferentially consume increased quantities of labile carbon pools and have high maximum growth rates when resources are abundant.

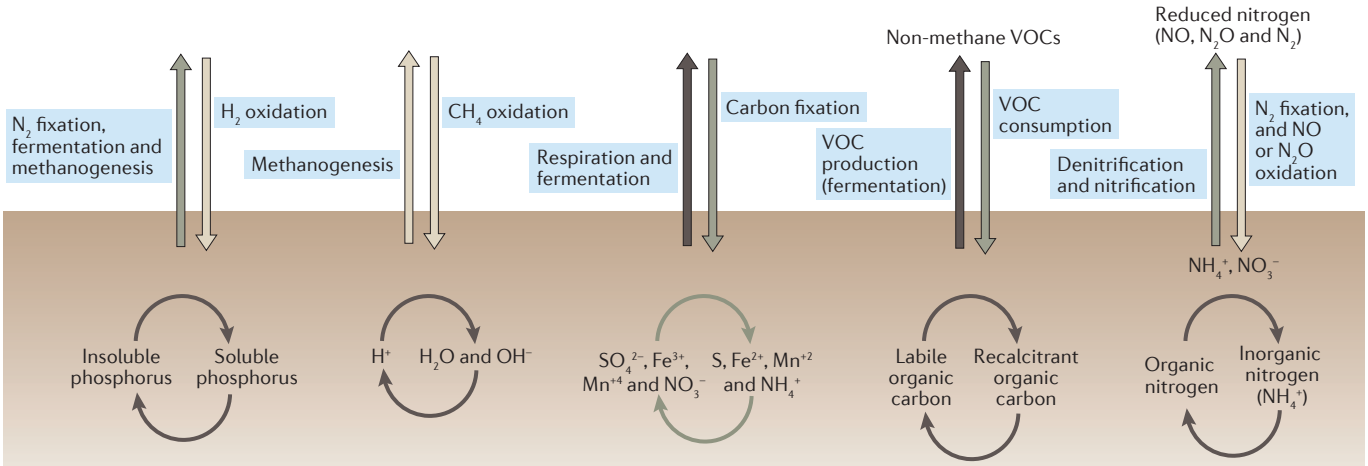


Figure 5 | **Soil biogeochemical processes that can be modulated by the soil microbiome.** A summary diagram that highlights a subset of the important soil biogeochemical processes that are directly modulated by soil microorganisms. The vertical arrows indicate microbial processes that are responsible for the production or consumption of trace gases at the soil–atmosphere interface. The curved arrows indicate some of the key microbial processes that can occur within soil, processes that can regulate soil acidity, the availability of nitrogen, phosphorus or other nutrients, and the lability (ease of consumption by microorganisms) of soil organic carbon pools. Non-methane volatile organic compounds (VOCs) include acetone, methanol, formaldehyde, isoprene and other organic compounds with low molecular weight. As indicated in the text, this Figure just highlights a subset of all possible soil microbial processes and does not highlight the inter-related nature of these processes, the specific metabolic pathways responsible, or the range of direct and indirect mechanisms by which soil microbial symbionts and pathogens can influence plants. The shading of the arrows indicates which processes are expected to be carried out by a relatively small subset of microbial taxa (light grey; 'narrow' processes), by an intermediate number of taxa (dark grey) and by a broad diversity of taxa (black; 'broad' processes).

availability by altering soil hydraulic conductivity and hydrophobicity[64,65]. In addition, there are many other processes that are mediated by microorganisms that are not included in FIG. 5 but can be important to soil functioning: for example, dissimilatory nitrate reduction to ammonium (DNRA[66]), the degradation of xenobiotic compounds, and metal chelation and detoxification[67].

As the soil microbiome can have direct or indirect effects on many ecosystem-level processes, it is important to identify taxa (to the highest resolution possible) that are responsible for specific processes. This could yield a better understanding of the biotic and abiotic factors that control these processes. For example, knowing which specific taxa are responsible for ammonia oxidation (nitrification) could improve our ability to predict rates of ammonia oxidation in the soil, as not all taxa share similar environmental constraints or enzyme kinetics[68]. In addition, efforts to predict how soil carbon dynamics may respond to ongoing climate change could benefit from a deeper understanding of the different growth physiologies and ecological strategies of soil microorganisms[69,70]. Soil microbial taxa are not functionally equivalent; they can clearly differ in their effects on soil processes and their responses to environmental conditions. The challenge remains how to use data on soil microbial community composition to improve our understanding of soil processes when these processes are difficult to predict a priori or measure directly.

Challenges in linking microbiome structure to function. Unfortunately, it is often difficult to identify specific links between the taxa found in soil and the functional capabilities of the soil microbiome. Thus, information about the microbial taxa found in soil is not often useful for predicting the rates of a given biogeochemical process or for identifying how key soil processes may shift in response to perturbation (for example, climate change or land-use change). This is even true if we ignore taxa, and instead just focus on the genes, transcripts or proteins found in soil[71–73]. There are many overlapping reasons why these difficulties persist. First, many of the microbial processes highlighted in FIG. 5 are not the product of a single metabolic pathway but the product of a myriad of integrated metabolic pathways that can be carried out by a broad range of taxa. For example, nearly all active taxa of heterotrophic bacteria in soil could potentially contribute to the rates of organic carbon catabolism or nitrogen mineralization. Even the catabolism of a single plant-derived compound, like cellulose, may require multiple metabolic processes to be carried out by a broad diversity of microbial taxa[74]. Second, the abundances of individual genes (both 16S rRNA genes and functional genes) from dormant or relatively inactive microorganisms[17] may confound efforts to link functional processes to specific microorganisms. This may even hold true for RNA-based (transcriptome) community analyses[75]. Third, there are methodological issues associated with linking specific taxa to specific metabolic processes; these include problems with accurate gene annotation[76], insufficient taxonomic resolution, and the rapid turnover of transcripts, proteins and metabolites[77]. Fourth, approaches that are based on DNA sequencing, which are widely used to quantify

Hydraulic conductivity
The ease with which pores of a saturated soil allow water movement.

DNRA
(Dissimilatory nitrate reduction to ammonium; also known as fermentative ammonification). A potentially important soil microbial process that, similar to denitrification, leads to nitrate reduction.

Nitrification
A process carried out by specific groups of bacteria and archaea (most of which are autotrophic) that can oxidize ammonia to nitrite or nitrate.

Heterotrophic bacteria
A general term for bacteria that cannot assimilate carbon from inorganic sources (such as carbon dioxide) and instead use organic carbon compounds for anabolism.

the abundances of taxa (for example, 16S rRNA gene sequencing) or functional genes in soil (for example, shotgun metagenomic sequencing) typically provide only information about the relative abundances of taxa or genes (that is, percentages) and not their absolute abundances. Soil processes are more likely to be associated with the absolute numbers of taxa, genes or gene products rather than with their relative percentages in a community. Inferring the absolute abundances of taxa, genes or gene products requires the use of alternative approaches (including quantitative PCR-based methods). Finally, even if we assume that there is a direct relationship between taxon, gene or gene product abundances and a process of interest, the prediction of process rates requires an understanding of specific enzyme kinetics and the environmental constraints on the taxa of interest. For example, distinct groups of bacterial methane oxidizers can have very different substrate affinities, even when grown under controlled laboratory conditions[78].

Given these constraints, it is tempting to assume that the 'omics' data being generated at an ever-increasing pace are useless for the purpose of predicting soil microbial processes. However, the situation is not so dire. As noted below, plant ecologists have faced similar problems for decades. Thus, the trait-based concepts developed by plant ecologists can be used to help us understand the relationship between microbial community composition and soil processes.

Strategies for elucidating the functions of the soil microbiome. How can we take advantage of the wealth of genomic, metagenomic and marker gene data to improve our understanding of the functions of the soil microbiome? Although there is no single solution, one fruitful direction uses the accumulation of molecular data to improve the delineation of functional groups of microorganisms or groups of microorganisms with shared lifestyles[79]. This approach has already proven valuable in plant ecology; in this field, plant species have been divided into groups that share similar traits, and this has proven effective for understanding and predicting many ecosystem-level processes[80]. If a similar strategy can be successfully applied to soil microbial communities, it would become easier to link the taxonomic or phylogenetic information about community composition to specific soil processes of interest.

For some soil processes, it is already known which taxa are the most probable drivers. For example, methanogenesis, nitrogen fixation and nitrification are processes that are carried out by reasonably well-characterized microbial taxa (as reviewed in REFS 41,68,81,82) (FIG. 5). Although it can still be difficult to link the abundances of specific taxa or genes to process rates[72], we can at least identify the specific taxa responsible for some 'narrow' processes (that is, processes that are carried out by a limited diversity of taxa[81]). However, it is important to acknowledge that there might be unexpected taxa involved in a given process, as exemplified by the discovery of nitrifying archaea[82]. Stable isotope probing-based methods are particularly useful for identifying which taxa are responsible for these relatively 'narrow' processes[83]. However, for many 'broad' processes — including the processes that drive soil carbon dynamics, or those that contribute to nitrogen mineralization and/or immobilization (FIG. 5) — it is far more difficult to link microbial community data to process rates[84]. This is because there are numerous individual processes and taxa associated with the metabolism of the thousands of organic compounds found in soil. This complexity makes it very difficult to predict soil function. If, for example, we want to know the fate of labile carbon compounds in soil (which is important in soil carbon models[85]), information about what taxa are present in a given soil sample is unlikely to be useful.

Instead of considering the soil microbiome as collections of genes or gene pathways, we can divide microbial taxa or lineages into broad categories on the basis of shared life-history strategies. This could be done by adopting a trait-based framework similar to that proposed in 1977 for plant taxa (the Grimes competitor–stress tolerator–ruderal (CSR) framework)[86] and modifying it to categorize the broad spectrum of soil bacterial lifestyles (FIG. 6). Briefly, such a framework would divide heterotrophic bacteria (which dominate most soil bacterial communities) into broadly defined groups that share similar functional capabilities and ecological strategies: namely, 'stress-tolerant' taxa that can persist under low-resource or suboptimal abiotic conditions, 'competitor' taxa that outcompete other soil microbial taxa for space or resources, and 'ruderal' taxa that can grow rapidly and exploit unoccupied niches generated as a result of biotic or abiotic disturbances. By adopting this framework, we can start predicting key traits for the majority of taxa that are difficult to study *in vitro*. For example, traits linked to growth rate[87], stress tolerance[88] and nutrient acquisition[89] can be inferred from genomic data. There are also other genes or gene categories that could be used as indicators of life-history strategies (FIG. 6). As demonstrated by recent studies that have used a similar approach to characterize soil methane oxidizer[90] and nitrifier[91] communities, microbial traits and the trade-offs between those traits are ultimately going to prove more useful for understanding the influence of microorganisms on soil processes than focusing on the numbers of different taxa, genes or gene products.

Managing the soil microbiome to improve soil quality. Just as human microbiome research is increasingly focused on manipulating our gut microbiomes to improve human health, soil microbial research is increasingly focused on leveraging our increasing understanding of the soil microbiome to improve the management of agricultural soils. This could be achieved by amending soils by adding specific microorganisms, the management of soils to promote the growth of beneficial microorganisms, or using microorganisms as 'bio-indicators' of soil conditions or processes that are difficult to measure directly. For example, there is a long history of research that highlights how

Methanogenesis
A metabolic process by which archaea reduce carbon dioxide or other single-carbon compounds to methane.

Stable isotope probing-based methods
Techniques that use isotopically labelled substrates (for example, ^{13}C or ^{15}N) to identify which microorganisms in an environmental sample are capable of taking up a given substrate and incorporating the isotopic label into their nucleic acids, proteins or membrane lipids.

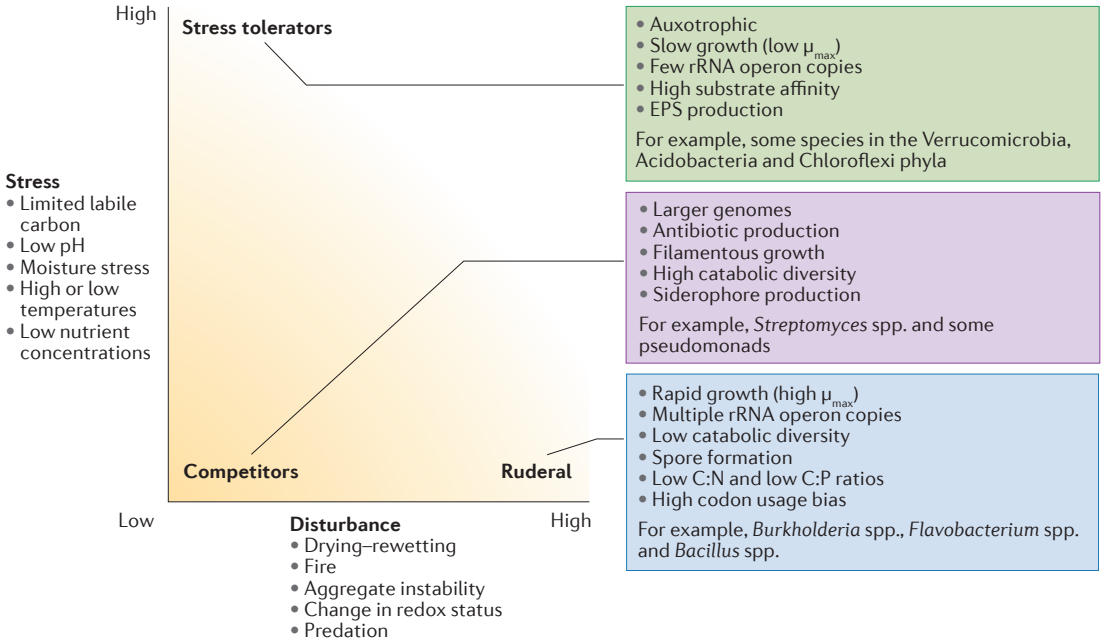


Figure 6 | **Grime's competitor–stress tolerator–ruderal framework applied to soil bacterial heterotrophs.** An outline of Grime's competitor–stress tolerator–ruderal (CSR) framework[86] and how it may apply to soil bacterial heterotrophs. Boxes include selected phenotypic or genomic attributes that may be associated with the three broad categories of life-history strategies along with examples of soil bacterial groups that are likely to fall into these categories. These categories are not meant to be discrete; rather, they represent a conceptual framework that helps to organize phenotypic or genomic information regarding the adaptive strategies that are used by soil bacterial taxa to survive in soil. μ_{max}, maximum potential growth rate; EPS, extracellular polymeric substances; rRNA, ribosomal RNA.

the addition of specific bacterial or mycorrhizal inocula to soil can promote plant growth[15,92]. Furthermore, there is evidence that we can actively manage the soil microbiome to promote the suppression of plant diseases[93], to reduce soil erosion[94] and to accelerate the remediation of heavy metal-contaminated soils[95]. In addition, we can use information about the abundances of ammonia oxidizers across a landscape to identify 'hotspots' where fertilizer applications should be avoided to decrease nitrate leaching[96].

Although there are clearly a range of ways in which agricultural productivity and sustainability could be improved by the management of the soil microbiome, it could be said that the 'devil is in the details', as this is an area of great complexity. There is no such thing as an 'ideal' soil community for crop production, just as a seemingly healthy human population can contain very distinct gut communities[97]. One reason for this is that a 'good' community is highly context-dependent. For example, it is unlikely that a single microbial community could universally promote crop growth, confer resistance to disease and mobilize limited nutrients. Moreover, microorganisms that might be beneficial under one set of conditions could prove to be pathogenic or detrimental under other conditions[98]. The definition of a beneficial community will depend on the following: how we define 'beneficial', the crop in question, the specific biotic or abiotic challenges facing those crops, and the specific soil conditions. An approach similar to that used in 'personalized medicine' (REF. 99) will be required, and simply knowing what microorganisms are found, or introduced into, a given soil will probably be of limited use if the context is ignored. These potential issues will clearly represent a challenge to the increasing number of companies that are trying to capitalize on the soil microbiome to improve agriculture.

The future of soil microbiome research

Any attempt to accurately predict the future of any scientific discipline is difficult. However, below is a selection of three key research directions that, in my opinion, will shape the future of both basic and applied studies of the soil microbiome.

Improved culturing strategies. We are rapidly compiling genomic data for the vast majority of undescribed soil microbial taxa, for which genomes from closely related strains are currently unavailable. These data are increasingly derived from cultivation-independent approaches, including single-cell genomics[100] and the assembly of individual genomes from metagenomes[20,101]. However, even if we had genomic information available for every soil microorganism, the gaps in our understanding of their functional attributes would persist, and assigning taxa to ecological categories on the basis of genomic data alone is risky. Although the cultivation of many soil microbial taxa — particularly slow-growing taxa — remains a difficult task[102], the utility of cultivating soil microorganisms is unquestionable if we want to assess environmental tolerances, improve gene annotations,

Horizontal gene transfer
The movement of genes from one independent, mature organism to another (either members of the same species or different species) through conjugation, transformation or transduction.

Xenobiotic degradation
The microbial breakdown and detoxification of compounds that are man-made and do not occur naturally in nature.

quantify enzyme kinetics, and identify novel antibiotics or probiotics. Strategies that use genomic data to identify conditions for the effective cultivation and isolation of recalcitrant microorganisms[103] will undoubtedly be useful as the field moves forwards.

Viruses and their role in the soil microbiome. Soils can contain 10^7–10^9 virus particles per gram[104,105], which is typically less than one phage per bacterial cell; this is a ratio far lower than what is typically observed in aquatic environments[106]. Unsurprisingly, soil viral communities are highly diverse, and most of the virus particles found in soil remain undescribed[104,107,108]. From work in marine systems, we know that phages are important drivers of carbon and nutrient dynamics, as they can kill a large percentage (probably 20–40%) of the microbial cells found in the water column[106]. In soil, there has been a lot of research into phages that target specific bacteria, including *Rhizobium* spp. and plant pathogens (for example, *Xanthomonas* spp.)[109,110], but the overall effects of viruses on the composition and activity of the soil microbiome remain poorly understood. Given that >90% of soil viruses seem to be strongly adsorbed to clays and other soil surfaces[109], it is unclear what percentage of the viruses that are found in soil are even capable of infecting their microbial prey. With recent advancements in viral metagenomics[111] and new approaches for the enumeration of viral populations[112], the stage is set to begin exploring viral communities and their effects on soil microbial populations and processes. More generally, it is clearly important to build a more holistic understanding of how all soil microbial taxa interact directly or indirectly, instead of just studying individual groups in isolation.

The importance of horizontal gene transfer. Soil microorganisms have the potential for high rates of horizontal gene transfer through transduction, transformation or conjugation[113–115]. Genes that encode traits — including antibiotic resistance[116], xenobiotic degradation[117], arsenic detoxification[118] and plant symbioses[119] — can move between soil microbial taxa (even distantly related taxa) via horizontal gene transfer. Mobile genetic elements can lead to the rapid evolution of novel phenotypic traits and can contribute to closely related strains having highly dissimilar genomes[120]. Notably, horizontal gene transfer can pose a problem when attempting to link specific genes (and the traits encoded by those genes) to specific phylogenetic lineages, as the genomes are not static across space or time. The specific controls on horizontal gene transfer, its prevalence in native soil microbial communities and the effects it may have on soil processes are topics that are ripe for exploration. This has been exemplified by recent work that explored the importance of mobile genes in the gut microbiome[121].

Conclusions and outlook

There is no shortage of knowledge gaps that limit our understanding of the soil microbiome. Even an answer to a question as simple as 'what is the average generation time of soil bacteria?' remains unknown. Furthermore, there is no shortage of new and emerging methodological approaches that can be used to further explore the phylogenetic and functional diversity of the microbiome. What is often missing is a conceptual framework that enables us to identify, and explain, patterns in the soil microbiome. Clearly it is not sufficient to characterize the soil microbiome using simple indices such as bacterial-to-fungal ratios[122] or phylum-level abundances, nor should we focus on basic diversity metrics that are often of limited use[51]. Instead, the field needs to move beyond simple descriptions of community diversity to identify patterns in this complexity and recognize when that complexity is important. This will enable us to make research on the soil microbiome of practical utility to human endeavours, from improving crop production to generating realistic predictions of how terrestrial ecosystems will respond to ongoing environmental changes.

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