

## Review article



# Soil structure and microbiome functions in agroecosystems

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## Abstract

Soil microbiomes drive key functions in agroecosystems, determining soil fertility, crop productivity and stress tolerance. The microbiome is intricately linked with soil structure, such as aggregation and pore connectivity, because this structure regulates the flow of water, oxygen and nutrients through the system. In this Review, we summarize the key functions of soil microbiomes in agroecosystems, highlight the dependence of these functions on the structural integrity of the soil, and discuss how agricultural practices influence the link between soil structure and microbiome functioning. System-level agricultural management practices can induce structural alterations to the soil, thereby changing the microbial processes occurring at the microscale. These changes have large-scale consequences, such as soil erosion, reduced soil fertility and increased greenhouse gas emissions. Sustainable approaches such as integrated soil fertility management and integrated pest management seek to improve soil structure and enhance microbial biodiversity, but we lack a mechanistic understanding of how multifaceted decisions at the farm level shape these context-dependent small-scale processes in the long term. Future research needs to bridge the microscale and field scale to inform agricultural management decisions for building climate-smart, resource-efficient and stress-resilient agroecosystems, and to harness the soil microbiome as a nature-based solution for sustainable agriculture.

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## Introduction

Agroecosystems are vital to global-scale ecological and economic networks, as they mediate the flow patterns of nutrients and energy, with direct feedback on human nutrition and the climate system<sup>1</sup>. However, balancing supply-side economics with ecosystem conservation and climate protection remains challenging<sup>2,3</sup>. Massive land conversion, mechanical soil perturbation, over-fertilization, intensive irrigation and incorrect pesticide application have led to soil degradation, environmental pollution, eutrophication and greenhouse gas emissions<sup>4</sup>. As a result, intensive agriculture is perceived as one of the greatest threats to global biodiversity and ecosystem services<sup>5,6</sup>. Therefore, sustainable agricultural management practices are required to meet global food demand, while preserving biodiversity and sustaining ecosystem functioning<sup>7,8</sup>.

A cornerstone of sustainable agriculture is proper stewardship of soil<sup>9</sup>. Soil provides fundamental ecosystem functions including decomposition and transformation of organic materials and toxic compounds, nutrient and water provisioning for plants, water purification, control of pests and diseases, and greenhouse gas mitigation<sup>10</sup>. Poor land use can rapidly degrade soil, impairing these functions; approximately 2 billion hectares (38% of the world's cropping area) has been degraded through soil sealing, erosion, compaction, salinization, acidification, waterlogging, pollution, organic matter loss and nutrient imbalance<sup>11</sup>. Degraded soils are particularly problematic because soil is a non-renewable resource characterized by extremely slow formation and regeneration rates<sup>12</sup>. Soil degradation can therefore have long-lasting impacts on agroecosystems.

Soil biodiversity in general, and microbial diversity in particular, is a driving force of the soil processes that are essential to sustaining agricultural production<sup>13</sup>. Biological data might even be better in predicting soil health metrics than physical or chemical soil properties<sup>14</sup>. However, these properties are interlinked, as microbiome structure and function are determined by and affect the physical and chemical properties of the soil<sup>15</sup>. Alterations in soil structure can have profound impact on the survival and metabolism of soil microorganisms, thereby influencing soil organic matter (SOM) and nutrient turnover, and the interaction of microorganisms with plants<sup>16</sup>. Soil disturbance associated with increasingly mechanized and intensified agriculture is a major risk to soil functioning<sup>17</sup> because it induces severe structural alterations, causing substantial and persistent damage that ultimately reduces land productivity and ecosystem health.

In this Review, we describe the link between soil microbiome, soil structure and management in agroecosystems and how this link must be protected, restored and promoted to improve the sustainability of future crop production systems. We examine the key functions of soil microorganisms in agroecosystems, highlight the role of soil structure as a basic driver of microbiome structure and function, and discuss how alterations in soil structure in response to different agricultural management practices are, in turn, influencing key microbial functions. We used an online network-based literature mapping tool, Research Rabbit (<https://www.researchrabbit.ai>), to identify and map the most relevant literature for the topics covered in this review.

## Key functions of the soil microbiome

The soil microbiome is essential to agroecosystem functioning (Fig. 1). Key roles played by the soil microbiome are described here.

### Climate regulation

Soil microbial processes play a central role in accelerating and mitigating climate change<sup>18</sup>, for example by regulating the terrestrial carbon flux<sup>19,20</sup>. Atmospheric carbon dioxide (CO<sub>2</sub>) fixation in soil is mainly

performed by higher plants. Some assimilation is mediated by auto-trophic soil microorganisms<sup>21,22</sup>, but the indirect effects of microbes on soil C cycling through processes influencing soil C turnover and retention are considered more important<sup>16</sup>. For example, most SOM is stored as microbial necromass (with less stored as plant-derived SOM or humic substances<sup>23</sup>) that cycles slowly because of sorption to soil minerals and formation of organo-mineral associations entrapped within soil aggregates<sup>24</sup>. Soil microbes contribute greatly to CO<sub>2</sub> emissions via litter decomposition and heterotrophic respiration; around half of the 120 Pg of carbon that is emitted per year on land is derived from heterotrophic microbial respiration<sup>18</sup>, causing up to nine times more emissions than the yearly global anthropogenic sources<sup>25</sup>.

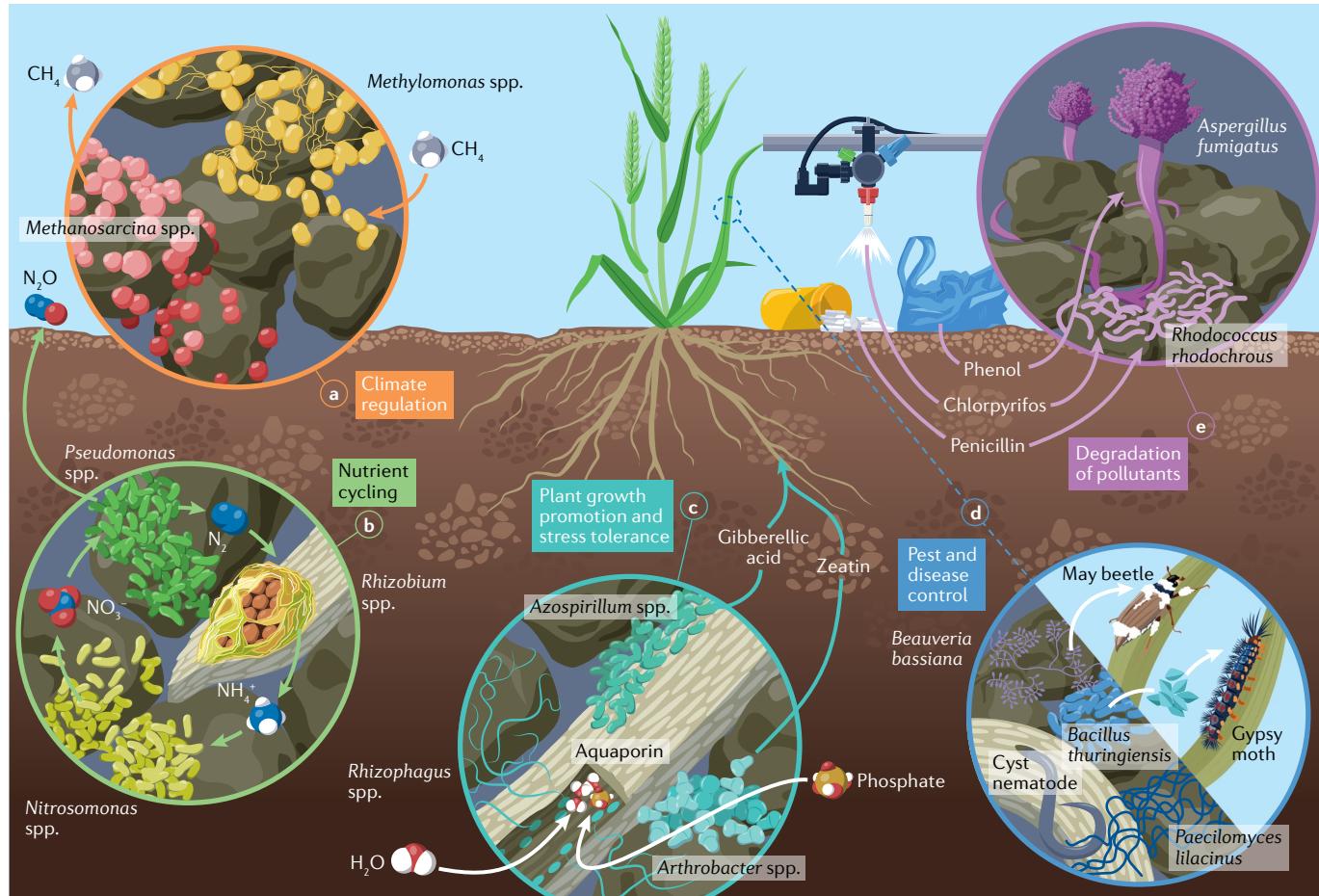
Microorganisms – specifically, facultative anaerobic methanogenic archaea such as *Methanosarcina*, *Methanosaeta* and *Methanocella*<sup>26</sup> – are responsible for around two-thirds of the 500–600 Tg of methane (CH<sub>4</sub>) emitted per year<sup>27</sup> (Fig. 1). A large part of this flux is natural (around 230 Tg of CH<sub>4</sub> per year) and primarily originates from wetlands, but biological methanogenesis is also responsible for a large fraction of the anthropogenic emissions through agriculture and landfills<sup>28</sup>. Aerobic methanotropic bacteria such as *Methylomonas*, *Methylococcus* or *Methylocystis* counteract this process by consuming large proportions of CH<sub>4</sub> before it escapes the source and oxidizing around 30 Tg of CH<sub>4</sub> directly from the atmosphere (by low-affinity and high-affinity methanotrophs, respectively)<sup>18</sup>. Altogether, methanotropic bacteria consume roughly 60% of the globally produced CH<sub>4</sub> (ref. <sup>29</sup>).

Emissions of nitrous oxide (N<sub>2</sub>O) are largely driven by microbial processes; nitrification and denitrification both contribute to N<sub>2</sub>O production and are mediated by ammonia-oxidizing bacteria and archaea, nitrite-oxidizing bacteria, heterotrophic denitrifiers, bacteria performing dissimilatory nitrate reduction to ammonia, and anaerobic ammonia-oxidizing bacteria<sup>30</sup>. Soils are the predominant natural and anthropogenic source of N<sub>2</sub>O emissions, contributing around 6.8 Tg N<sub>2</sub>O per year, or around 65% of the total global emissions<sup>30</sup>. The agricultural sector is responsible for around 52% of global anthropogenic emissions, owing to the application of mineral fertilizer and manure<sup>31</sup>.

### Nutrient cycling

Microorganisms decompose organic material to release plant-available inorganic nutrients, alter nutrient availability by oxidation, reduction, solubilization and chelation processes, and store and release nutrients in necromass<sup>32</sup>. These processes drive the global nutrient cycling<sup>33</sup> and regulate around 90% of the energy flux in soil<sup>34</sup>.

Nitrogen availability is one of the main limiting factors for plant growth in agroecosystems<sup>35</sup>. The availability of different nitrogen compounds is regulated by a complex network of metabolically versatile microorganisms that carry out transformations roughly grouped into six distinct processes: nitrogen fixation, nitrogen assimilation, ammonification, nitrification, denitrification and anaerobic ammonia oxidation<sup>36</sup> (Fig. 1). Biological nitrogen fixation is carried out by a wide range of diazotrophic bacteria and archaea<sup>37</sup>, such as plant-symbionts like *Rhizobium* and *Bradyrhizobium* or free-living diazotrophs like *Azospirillum* and *Azotobacter*. The ammonium resulting from nitrogen fixation and mineralization can then be taken up by plants, but in well-aerated neutral soils ammonium is quickly transformed into plant-available nitrate through ammonia- and nitrite-oxidizing bacteria and archaea such as *Nitrosospira*, *Nitrosomonas*, *Nitrososphaera*, *Nitrospira* and *Nitrobacter*<sup>38</sup>. Nitrate is finally converted back to dinitrogen under low partial oxygen pressures by bacterial, archaeal and fungal genera including *Pseudomonas*, *Rhizobium*, *Agrobacterium*,



**Fig. 1 | Microbial key functions in the plant–soil system.** Soil microorganisms play essential roles in ecosystem functioning. **a**, Climate regulation. **b**, Microbial nutrient cycling. **c**, Plant growth promotion and abiotic stress tolerance. **d**, Pest and disease control. **e**, Toxin and pollutant degradation.

*Burkholderia, Bacillus, Haloferax, Fusarium, Cylindrocarpon, Gibberella and Trichosporon*<sup>39,40</sup>.

Phosphorus is often a limiting nutrient in agroecosystems, especially tropical ones, so microbial phosphorus cycling affects plant growth and therefore the global carbon budget<sup>41</sup>. Soil microorganisms drive phosphorus availability through both mobilization (weathering, mineralization, solubilization) and immobilization (assimilation, precipitation) mechanisms<sup>41,42</sup>. Furthermore, microorganisms aid phosphorus foraging through enhanced root growth (phytostimulation) and root system extension (mycorrhizal network)<sup>43</sup>. Phosphate-solubilizing bacteria and fungi include *Bacillus*, *Pseudomonas*, *Enterobacter*, *Burkholderia*, *Penicillium* and *Aspergillus*, but many others are continually being identified<sup>44</sup>. Arbuscular mycorrhizal fungi, such as *Rhizophagus*, absorb and translocate solubilized phosphorus to the host plant<sup>45</sup> (Fig. 1). Soil microbial biomass itself is a substantial and dynamic phosphorus pool that responds rapidly to environmental changes and is therefore an important regulator of phosphorus availability<sup>46</sup>. Immobilization through assimilation into microbial biomass is relatively short-lived and is often called transitory immobilization, as phosphate stays in its original form and re-enters the reactive phosphate pool shortly after microbial cell death<sup>41</sup>.

## Plant growth and abiotic stress tolerance

Soil microorganisms interact with plants to stimulate plant growth and increase their tolerance towards abiotic stresses (Fig. 1). There is increasing evidence that plants recruit plant-growth-promoting microorganisms (PGPM) to the root system through the release of root exudates such as sugars, organic acids, amino acids and phenolics<sup>47</sup>. Once recruited, these PGPM can stimulate and regulate plant growth, not only by transforming and translocating nutrients, but also through a regulatory network of phytohormones including auxins, cytokinins, gibberellins, abscisic acid and ethylene that influence basic properties such as cell division and development in the host plant<sup>48</sup>. Bacteria such as *Pseudomonas*, *Bacillus*, *Arthrobacter* and *Azospirillum* and fungi such as *Trichoderma* and *Laccaria* can produce or alter the phytohormone levels in the rhizosphere and endosphere to modulate plant development and stress responses<sup>48</sup>. For example, the PGPM bacterial strain *Pantoea phytobeneficalis* MSR2 has genomic capabilities for nitrogen fixation, phosphorus solubilization, auxin and cytokinin biosynthesis, and jasmonic acid and ethylene regulation<sup>49</sup>.

Beneficial plant–microbe interactions become particularly important under abiotic stress conditions such as nutrient limitation, drought, flooding, extreme temperatures, high salinity and pollution<sup>50</sup>.

# Review article

## Glossary

### Agroecosystems

Sites or integrated regions that support food production while conserving biotic and abiotic resources and providing a balanced supply of ecosystem services.

### Antibiosis

A biological interaction between two or more organisms that is detrimental to at least one of them.

### Biochar

The product of thermal degradation of organic matter at high temperature in the absence of air (pyrolysis).

### Biologicals

Products consisting of either a living organism or a substance derived from it, providing protection against pests and diseases or promoting plant growth and stress tolerance.

### Coalescence

The mixing of different biological communities (for example through inoculation) that results in new metacommunities with potential novel functional attributes.

### Copiotrophs

Organisms that preferentially consume labile organic C pools, have high nutritional requirements and can exhibit high growth rates when resource conditions are abundant.

### Cover cropping

The planting of crops between periods of regular production of the main crop, with the goal of covering and protecting the soil rather than being harvested as cash crop.

### Endosphere

Internal environment of the host that can be colonized by microorganisms called endophytes.

### Host-mediated indirect selection

Using the host to select for beneficial microbes that improve host performance by selecting and propagating microbial communities associated with specific host-trait of interest (such as plant growth).

### Hyperparasitism

A relationship between two parasites in which one acts as parasite on the other.

### Integrated soil fertility management

A set of practices related to cropping, fertilizers, organic resources and other amendments on smallholder farms to increase crop production and input use efficiency.

### Integrated pest management

A set of best practices that focuses on long-term prevention or suppression of pests and diseases.

### Microbiome

A characteristic microbial community occupying a well-defined habitat with distinct physio-chemical properties, and its associated theatre of activity.

### Mycorrhizal fungi

Fungi that form mutualistic symbiotic relationships with roots of certain vascular plant species in which the fungus receives carbohydrates from the plant in exchange for mineral nutrients.

### Oligotrophs

Organisms that exhibit slow growth, low metabolic rates and generally low population density, but feature high substrate affinity under low nutrient availability.

### Phytohormones

Chemicals produced by plants that regulate their growth, development and reproduction, but are also used as signalling molecules to interact with microbes.

### Plant-growth-promoting microorganisms

Microorganisms that colonize various plant compartments such as the rhizosphere or endosphere and have positive effects on plant growth and stress tolerance.

### Rhizodeposition

Active and passive release of organic compounds from plant roots into the surrounding soil.

### Rhizosphere

Zone surrounding plant roots in which the chemistry and microbiology are influenced by root activity.

### Root exudates

A suite of substances that are secreted by the roots of living plants into the surrounding compartment.

### Secondary metabolites

Organic compounds produced by organisms that are not directly involved in normal growth, development or reproduction of the organism.

Plants experiencing stress can recruit PGPM from the bulk soil to the rhizosphere using various chemical stimuli to enhance their capacity to cope with stress conditions<sup>51,52</sup>. The recruited PGPM can increase abiotic stress tolerance of the host plant through the production of exopolysaccharides<sup>53</sup>, accumulation of osmolytes<sup>54</sup>, synthesis of antioxidants<sup>55</sup>, expression of aquaporins<sup>56</sup> and reduction of ethylene levels<sup>57</sup>. For example, arbuscular mycorrhizal fungi enhance plant growth and stress tolerance by improved access to nutrients and water through their hyphal network and stimulation of root hair growth, increased root hydraulic conductivity by modulating the expression

of root aquaporins, improved cell turgor through osmotic adjustments in plant cells, and enzymatic neutralization of reactive oxygen species in plant tissues<sup>58,59</sup>.

### Pest and disease control

Many soil microorganisms have beneficial regulatory effects on plant pathogens and pests. Root-associated microorganisms promote plant health through indirect mechanisms, such as competition with pathogens for physical space and resources or induction of systemic resistance and priming for enhanced defence, and through direct defence

responses such as antibiosis (production of antibiotics, lytic enzymes, volatiles) and hyperparasitism<sup>60</sup>. Many of the plant-mediated mechanisms are regulated by phytohormones. For example, jasmonates are often involved in the response to necrotrophic pathogens and herbivory whereas salicylates mediate defence responses to biotrophic pathogens<sup>48</sup>. The activation of the jasmonate and salicylate signalling pathways in response to herbivory can be triggered by the secretion of secondary metabolites such as benzoxazinoids through the plant root system and induction of microbially mediated soil feedback<sup>61</sup>.

Microorganisms have developed a large array of weapons to conduct warfare belowground through antibiosis and hyperparasitism<sup>62</sup>, thereby acting as biocontrol agents to regulate the occurrence and distribution of pests and pathogens (Fig. 1). For example, entomopathogenic fungi can infect and kill arthropod pests by penetrating the cuticle of the target arthropod and induce death by physiological starvation or mycotoxin production<sup>63</sup>. Common entomopathogenic fungi include *Beauveria*, *Metarrizium*, *Entomophthora* and *Verticillium*, which are increasingly used in commercial formulations against pests such as the gypsy moth, the European cockchafer, grasshoppers and aphids. Fungi can also infect and kill nematodes. These include nematode-trapping fungi that trap and kill nematodes by using modified hyphal elements to catch, penetrate and digest the nematodes; endoparasitic fungi that use their spores to invade and develop inside the nematodes; opportunistic fungi that invade nematode eggs and cysts with their hyphal tips; toxin-producing fungi that immobilize nematodes before invasion; and fungi that produce special sword-like structures to damage the cuticle and invade the nematode<sup>64</sup>. There are around 700 known nematopathogenic fungal species distributed across the major fungal phyla, including *Arthrobotrys*, *Dactyellina* and *Monacrosporium* (nematode-trapping), *Drechmeria*, *Hirsutella*, *Haploglossa* and *Catenaria* (endoparasitic), and *Pochonia*, *Paecilomyces* and *Verticillium* (egg-parasitic)<sup>65</sup>.

Bacteria also act as biocontrol agents to control pests<sup>66</sup>. The most important entomopathogenic bacteria belong to the genus *Bacillus*<sup>67</sup>, with *Bacillus thuringiensis* being the most widely used bacterial biocontrol agent against insects<sup>68</sup>. During sporulation, *Bacillus thuringiensis* forms crystal proteins that act as endotoxins against a wide range of insects such as Lepidoptera, Diptera, Coleoptera and Hymenoptera<sup>69</sup>, and even some nematodes<sup>70</sup>. Other well-known bacteria with biocontrol capabilities against insects and nematodes belong to the genera *Pseudomonas*<sup>71</sup> and *Streptomyces*<sup>72</sup>. Antagonistic effects are also frequently observed among soil bacteria and fungi, thereby ultimately controlling the abundance or distribution of microbial pathogens in agricultural soils<sup>73</sup>.

## Pollutant degradation

Contamination of agricultural soil and groundwater by pollutants (chemicals, pharmaceuticals, heavy metals and nanoparticles, for example) is introduced as pesticides, organic fertilizers (manure, compost, sewage sludge), irrigation water and plastic. This pollution is a global problem<sup>74–76</sup>. For example, around 64% (about 24.5 million square kilometres) of the global agricultural land is at risk of pesticide pollution<sup>77</sup>. Indirect inputs from industrial production and fossil fuel burning can further increase pollutant levels in agricultural soil.

Soil microorganisms that degrade organic pollutants such as synthetic polymers<sup>78</sup> or herbicides<sup>79</sup> are a promising tool for bioremediation of contaminated sites<sup>80</sup> (Fig. 1). Most naturally occurring organic pollutants are metabolized, and xenobiotics can often be transformed<sup>81</sup> as well, owing to the broad substrate specificity of many enzymes and

the rapid acquisition of new degradation pathways via genetic modification over short generation times. However, some pollutants are more hazardous after microbial transformation<sup>81</sup>, so it is important to understand complete degradation pathways. Soil microorganisms can also immobilize heavy metals and turnover mineral substances (for example leaching nitrogen fertilizers that contaminate groundwater), further affecting local pollution. The potential microbial degraders of environmental contaminants are as diverse as the contaminants themselves, and include bacterial genera such as *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Rhodococcus* and *Streptomyces*, and fungal genera such as *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma*<sup>82</sup>. Plants can recruit such microbes to the root system for degrading harmful pollutants<sup>83</sup>.

## Soil structure drives the microbiome

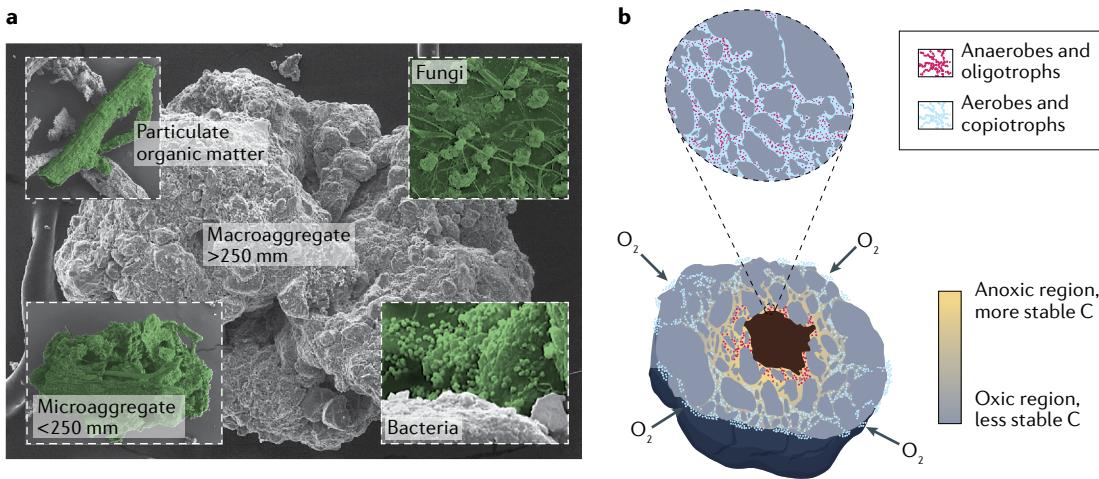
How microbial communities assemble in the soil matrix and the drivers of microbial processes at the relevant spatial scale (soil structural and textural units) are still poorly understood<sup>84</sup>. Soils consist of mineral particles and organic constituents packed into differently sized aggregates and organized as three-dimensional porous structures. The basic building blocks of soil are mineral soil particles that are classified based on their sizes into clay (<2 µm), silt (2–63 µm) and sand (63–2,000 µm)<sup>85</sup>. These soil particles bind with carbonates, metal oxides (Fe, Al), organic matter (particulate, humified, charred), biotic components and organic macromolecules (mucilage, polysaccharides, proteins, lipids, and agglomerations such as extracellular polymeric substances) into organo-mineral complexes to form silt-sized aggregates smaller than 50 µm or microaggregates of sizes 50–250 µm (ref. <sup>86</sup>); the forces holding the particles together in these aggregates are stronger than the forces between adjacent particles<sup>87</sup>.

Silt-sized aggregates and microaggregates are organized as larger and less stable macroaggregates (>250 µm) that are mainly held together by complexes of roots and fungal hyphae<sup>88</sup> (Fig. 2). Macroaggregates form first and function as a nucleus for microaggregate formation in the centre of the macroaggregate<sup>89,90</sup>. Decomposing plant and fungal debris or root-derived particulate organic matter become progressively encrusted with clay particles and microbial products to form the core of stable microaggregates and silt-sized aggregates<sup>91</sup>. Once the organic matter in the core is depleted, microbial activity and metabolite production is halted, which leads to a loss of aggregate stability and eventual breakdown of the aggregate. Therefore, primary particles organized as soil aggregates separated by voids are a relevant microhabitat for soil microorganisms.

Most soil microorganisms live as interconnected assemblages associated with these particles, so that the soil structure ultimately determines their resources through oxygen diffusion, water flow, organic matter accessibility and nutrient availability<sup>84,92</sup> (Fig. 2). Thus, understanding how agricultural management influences the link between soil structure and the soil microbiome is essential to anticipating changes in soil microbial communities and their ecosystem services under different agricultural management regimes. This section discusses the links between soil structure and the microbiome.

## Microorganisms and primary particles

Particle size fractions differ in their chemical composition, organic matter decomposability, cation exchange capacity, surface reactivity and sorption characteristics<sup>93</sup>; therefore, soil microbial communities are expected to differ across these fractions<sup>94–97</sup>. For example, independently of the agricultural background of the soil, a large proportion



**Fig. 2 | Abiotic and biotic interactions at the soil aggregate scale.** **a**, Scanning electron microscope image of a soil macroaggregate containing microaggregates, particulate organic matter and microorganisms such as fungi and bacteria. **b**, Cross-section through a soil aggregate visualizing the availability of water,

oxygen and nutrients, and the likely localization of different groups of microorganisms such as aerobes versus anaerobes and copiotrophs versus oligotrophs. Part **b** is adapted from ref.<sup>108</sup>, CC BY 4.0 (<https://creativecommons.org/licenses/by/4.0/>).

(47–69%) of the detected bacterial<sup>98</sup> and fungal<sup>99</sup> taxa has been shown to be associated with specific particle size fractions. In general, smaller size fractions carry larger (around 10-fold increase per size class) and more diverse (up to 48–52%) bacterial and archaeal<sup>98</sup> or fungal<sup>99</sup> loads. This difference is partially attributed to clay particles preferentially binding into soil aggregates, which in turn aids the association of microorganisms with clays. Ultimately, the microenvironment created by the different particle size fractions promotes variation in enzymatic activities<sup>95</sup> and the transformation of nitrogen<sup>100</sup>, carbon<sup>101</sup> and phosphorus<sup>102</sup> compounds, including pollutants<sup>96</sup>. Nevertheless, the relationships between primary particle characteristics (especially surface reactivity and sorption characteristics related to mineralogy) and microbial survival and functioning (especially climate regulation and pollutant degradation) are poorly constrained and thus remain an open field for further investigation.

### Soil aggregates as microbial habitats

The structure and stability of soil aggregates are important for the abundance, diversity and activity of soil organisms, and thus for plant growth, erosion susceptibility and organic matter turnover<sup>103</sup>. Indeed, the vast majority (~90%) of soil bacteria associate with microaggregates, with a dominant fraction (~70%) residing inside microaggregates<sup>104</sup> and surprisingly few organisms in direct contact with each other<sup>103,105</sup>. Only a minor fraction (<10%) of the microorganisms is located on the surfaces of macroaggregates directly exposed to large pores<sup>103</sup>. Communities in and on these aggregates are largely disconnected by distance and physical barriers, but become interconnected during re-wetting events in which soluble nutrients, metabolites and genetic information can be exchanged<sup>84</sup>. This spatial (and temporal) isolation by soil aggregates limits dispersal and promotes independent functional and evolutionary changes among the community residing inside an aggregate<sup>106</sup>. Aggregate formation, stability and breakdown are thereby important metrics determining the duration of these functional and evolutionary interactions among microbes in soils. However, the relationship between these aggregate dynamics and the microbial community is

rarely directly investigated<sup>105</sup> and, most importantly, not in the context of different agricultural interventions.

Microbial inhabitants are selected for by the prevailing physicochemical conditions in soil aggregates and their influence on SOM dynamics. Hence, the less stable macroaggregates characterized by more labile carbon compounds could select for fast-growing microbial copiotrophs that are adapted to easily available carbon sources<sup>107</sup>. In contrast, the more stable microaggregates characterized by recalcitrant carbon sources promote slow-growing and metabolically versatile oligotrophs that feature metabolic machineries to break down more complex compounds<sup>107</sup> (Fig. 2). It has therefore been proposed that external carbon inputs through cropping practices might predominantly shape carbon availability in the more labile pool of macroaggregates and thus the macroaggregate-associated microbial communities<sup>107</sup>. In summary, soil microbiome functions should largely be determined by the effects of environmental factors on soil aggregates and the voids in between them.

### Soil voids and the microbiome

Like the hierarchical concept of microaggregate formation within macroaggregates<sup>89</sup>, hierarchical categories of soil pores have been defined as: macropores (>75 um); pore space between macroaggregates; pores between microaggregates but within macroaggregates; and pores within microaggregates<sup>91</sup>. This hierarchical structure determines the organization of the food web at the micrometre scale, with microarthropods occupying the macropores; nematodes populating the pores between macroaggregates; protists, small nematodes and fungi inhabiting the pores between microaggregates; and bacteria and archaea being protected within microaggregate pores<sup>91</sup>. Smaller organisms can also occupy larger pores but not vice versa. Pore organization also leads to sharp gradients in oxygen and nutrient availability<sup>108</sup>, with larger pores between and within macroaggregates selecting for aerobic bacteria, and smaller pores within microaggregates providing both aerobic and anaerobic niches depending on dry–wet cycles<sup>94</sup> (Fig. 2). Oxygen limitation inside microaggregate pores provides a

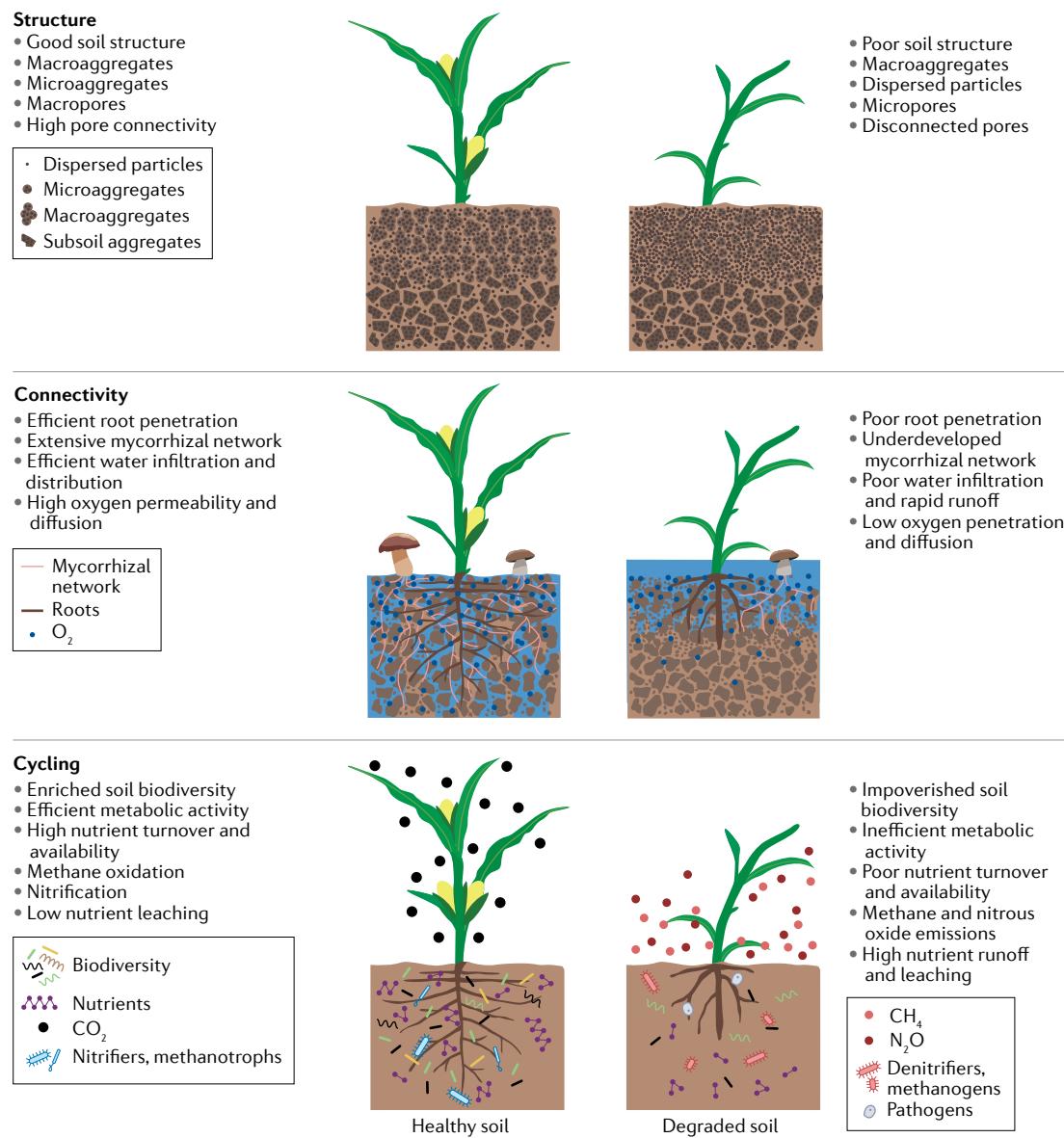
competitive advantage to anaerobically respiring microorganisms, such as denitrifiers<sup>109</sup>. Hence, the pores of microaggregates are likely to be simultaneously hotspots for soil carbon stabilization<sup>110</sup> and N<sub>2</sub>O emissions<sup>111</sup>.

Nevertheless, the trade-off between these two processes at the scale of pores and microaggregates in different soil types and across different degradation levels is not well understood, let alone quantified. It is clear that in structurally degraded soils, aggregates and their pore network collapse, leading to reduced infiltration and distribution of water and oxygen across the different soil structural forms. These changes ultimately limit soil biodiversity and activity relying on

these resources and negatively affect microbial functions including nutrient cycling, climate regulation, plant growth promotion, pest and disease control, and pollutant degradation. A degraded soil structure also limits the space for roots and soil fauna, reduces aerobic processes important for plant nutrition, and favours anaerobic microbial processes that lead to CH<sub>4</sub> and N<sub>2</sub>O production (Fig. 3).

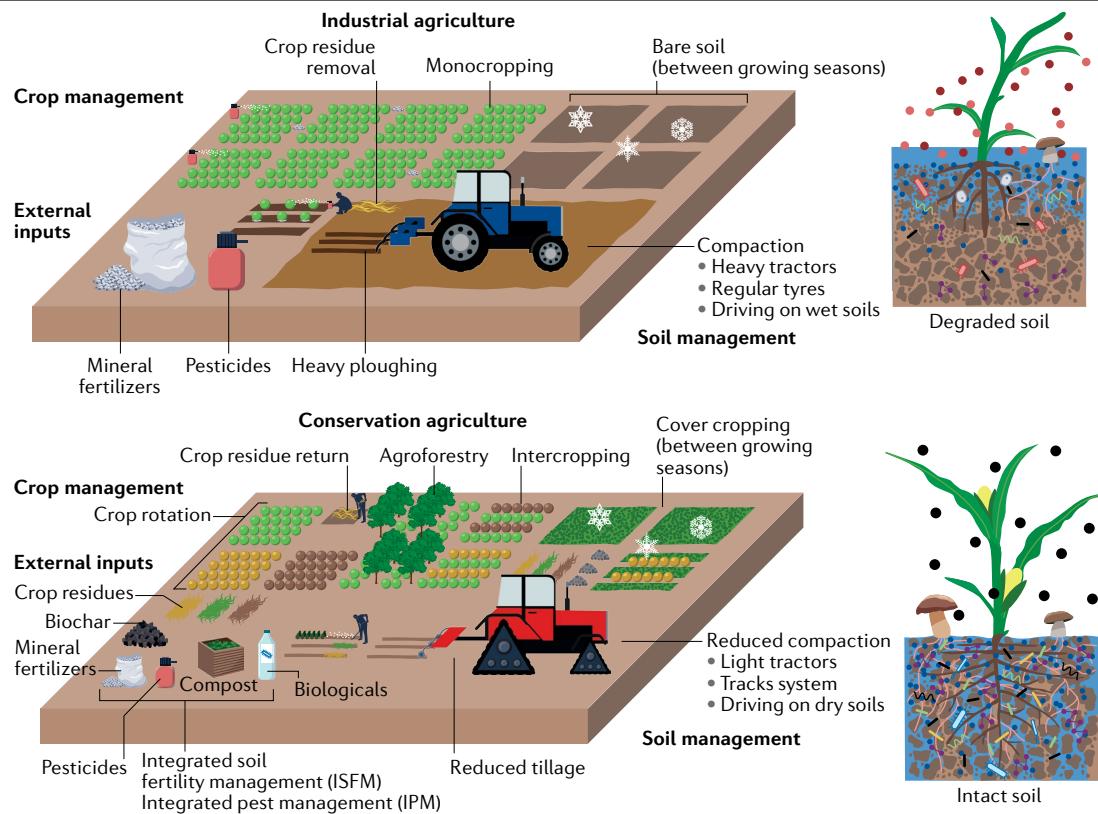
## Microbiome functions in agroecosystems

Industrial agriculture focuses on maximizing productivity by growing high-yielding crops in monocultures or simple rotations, under high inputs of agrochemicals and using intensive soil operations (Fig. 4).



**Fig. 3 | Differences in soil properties between structurally intact versus degraded soils.** Structurally intact soils are characterized by a well-developed soil structure (top left panel) that favours the development of a well-connected root and fungal network (middle left). This structure allows for efficient water and oxygen flow, thereby promoting soil biodiversity and activity, leading to

increased nutrient availability (bottom left). The disruption of aggregates and pores in structurally degraded soils (top right) limits the development of the root-fungal network and reduces the flows of water and oxygen (middle right), thereby negatively affecting soil biodiversity and activity, leading to reduced nutrient availability (bottom right).



**Fig. 4 | Differences in management practices between industrial and conservation agriculture.** Industrial agriculture (top left panel) focuses on maximizing yields and often relies on intensive soil management, chemical fertilizers and pesticides, and the use of highly productive plant material in simple cropping systems. Soil is degraded as a result (top right). Conservation agriculture (bottom left) features protective approaches in terms of soil management (reduced or no tillage), agricultural vehicles better protecting the soil), crop management (crop diversification, cover cropping) and external inputs (organic fertilizers and amendments, biologicals). These conservational

practices can have beneficial effects on soil structure, the flow of resources (oxygen, water, nutrients), and soil biodiversity and functioning (bottom right). Integrated soil fertility management and integrated pest management do not eliminate the beneficial use of targeted and microdosed application of agrochemicals. Instead, they combine these approaches with the application of organic fertilizers and other soil amendments, the use of biocontrol strategies, and the development of resource-efficient and disease-resistant plant germplasm. These strategies promote healthy, intact soil. See Fig. 3 for key to degraded and intact soil symbols.

This strategy is often associated with negative environmental impacts such as soil degradation, water pollution, greenhouse gas emissions and biodiversity loss<sup>112</sup>. Adapting agricultural management strategies to balance crop yield and resource protection is therefore key to sustaining food production for a growing and increasingly demanding global population<sup>113</sup>. Agricultural practices such as mechanical soil management (shallow and deep tillage practices), crop management (crop rotations, cover cropping, residue retention, intercropping, agroforestry) and external inputs (mineral and organic fertilizers, organic amendments, pesticides, biologicals) can exert a strong influence on the soil microbiome<sup>114–116</sup>, in part by altering soil structural properties (Fig. 4). Decisions at the agroecosystem level that are often focused on crop yield and health thereby link directly with processes at the microscale relevant for the functioning of the microbiome.

## Soil management

Agricultural management naturally includes mechanical operations on the field to prepare the land for the crops (tilling and seeding), support crop growth and health during the growing season (fertilization and

pest control), and harvest the final product (Fig. 4). These mechanical operations have direct influence on the soil structure with subsequent consequences for the soil microbiome.

Tillage operations incorporate crop residues, prepare the seedbed, alleviate compaction, improve nutrient mineralization, and reduce weeds, pests and pathogens<sup>117,118</sup>, resulting in a short-term increase in crop yield<sup>119</sup>. Conventional tillage (ploughing) continually exposes deeper soil to wet-dry and freeze-thaw cycles at the surface, thereby increasing macroaggregate turnover, disrupting the existing pore network and ultimately favouring soil erosion, but also accelerating SOM decomposition with a reduction in soil organic carbon stocks<sup>120</sup>. These alterations in soil structure have direct effects on the physical habitat of soil microbes, with fungi often being detrimentally affected through the destruction of their hyphal network. Bacteria living within microaggregates remain initially less affected<sup>121</sup>, but subsequently, the sudden release of nutrients aided by organic matter mineralization favours opportunistic commensal and fast-growing copiotrophs<sup>117,122</sup>.

Conservational management strategies such as reduced and no-tillage (direct seeding) operations aim to preserve the soil structure

(Fig. 4), thereby reducing soil erosion and soil organic carbon loss when compared with industrial agriculture. This strategy can be at the expense of reduced crop yield, but the reduction is often only moderate and specific to the type of crop, soil texture and climate<sup>119,123</sup>. Conservation tillage practices have been found to promote prokaryotic communities with oligotrophic lifestyles that are involved in the degradation of more complex organic compounds<sup>122,124,125</sup>. Furthermore, arbuscular mycorrhizae, mycoparasites and nematophagous fungi were promoted in soils without tillage, whereas fungal saprotrophs and plant pathogens dominated under conventional tillage<sup>125</sup>. Reduced tillage practices also seem to promote microbial taxa with the potential to produce exopolysaccharides and lipopolysaccharides<sup>126</sup>, which are biomolecules crucial for increasing aggregate stability and desiccation tolerance<sup>53</sup>.

These observations illustrate that tillage operations exert a strong influence on microbial nutrient cycling and the regulation of pest and diseases. However, the effect of soil tillage operations on greenhouse gas emissions is controversial<sup>127</sup>. CO<sub>2</sub> emissions generally increase under tillage owing to the breakdown of macroaggregates and the associated increase in decomposition and heterotrophic respiration<sup>128,129</sup>. Emissions of N<sub>2</sub>O generally increase upon cessation of tillage, at least in the initial years, but then decrease with long-term no-tillage<sup>130,131</sup>. This phenomenon could be related to an initial increase in bulk density, soil moisture and readily available C, which together favour denitrifiers and thus increased N<sub>2</sub>O emissions. With time under no-tillage, though, larger aggregates are formed, leading to more oxygen diffusion and better drainage, thereby reducing the anoxic conditions and N<sub>2</sub>O emissions in turn<sup>130</sup>. The effect on CH<sub>4</sub> emissions is less clear, but emissions generally decrease under conservation tillage operations owing to a more stable pore network that aids methanotrophic activity<sup>129</sup>. Therefore, reducing soil disturbance by conservation tillage practices has great potential to enhance microbial processes relevant for agroecosystems by improving soil aggregation and the soil pore network (Fig. 4).

Soil compaction is an undesirable but widespread side effect of agricultural intensification and has been recognized by the Food and Agriculture Organization of the United Nations (FAO) as one of the major threats to soil<sup>17</sup>. An estimated 68 million hectares of soil (about a quarter of the global agricultural land) is affected by soil compaction<sup>132</sup>. Compaction is caused by strong compressive forces on the soil owing to the increasing weight of highly mechanized agricultural equipment and frequent on-field operations during tilling, seeding, fertilizing, spraying and harvesting. As a result, there are severe alterations to the soil structure through increasing bulk density, destroying macroaggregates, and reducing macropores and pore connectivity with direct effects on water infiltration, nutrient flow, oxygen penetration and diffusion, rooting space and thus the physicochemical habitat of microbes<sup>133</sup> (Fig. 3). Soil compaction alters soil microbial communities by favouring anaerobically metabolizing prokaryotes and saprotrophic fungi at the expense of a reduction in microbial taxa with aerobic and plant-host-associated lifestyles in agroecosystems<sup>116</sup> and forests<sup>134</sup>. CH<sub>4</sub><sup>135</sup> and N<sub>2</sub>O<sup>136</sup> production increases as anaerobic microbial processes such as methanogenesis and denitrification are favoured while aerobic processes such as methanotrophy and nitrification are limited<sup>134</sup>.

The predominantly anaerobic conditions and physical space restrictions associated with severe compaction will also limit direct plant–microbe interactions. In turn, there could be impacts on crop nutrition and abiotic and biotic stress tolerance. However, more

moderate compaction might even have beneficial effects by aiding the contact between roots, associated microbes and soil nutrients<sup>137,138</sup>. Overall, soil compaction has the potential to impair microbiome functioning in the long term, and frequent passing with heavy vehicles especially under adverse conditions such as wet soils must be avoided, as natural recovery of compacted soil is slow<sup>139</sup> and active restoration measures can be costly<sup>132</sup>. Alongside SOM accumulation, reducing tractor weight, minimizing the number of passes, improving tyre pressure distribution, using controlled traffic systems to minimize the affected area, and avoiding unfavourable conditions during operation are conservation strategies to avoid rather than repair compacted soils<sup>140</sup>. In short, it is clear that conservation agriculture can reduce soil structural degradation – measured as an increase in soil aggregation and porosity – and thereby improve soil microbial functioning. Nevertheless, the trade-offs between plant growth and soil microbial functioning across different soil textures and mineralogies, climates and timescales are not fully understood from the soil microscale to the agroecosystem scale, limiting knowledge application.

## Crop management

Crop management practices such as crop diversification and cover cropping are another approach to positively influence soil structure and microbiome functions (Fig. 4). Crop diversification, either temporally staggered as crop rotations or simultaneously by intercropping, cultivar mixtures and agroforestry systems, can benefit biodiversity and ecosystem functioning, including positive effects on crop yield<sup>141,142</sup>. Complementarity effects such as facilitation and niche differentiation are generally assumed to explain positive plant diversity effects on soil biodiversity and ecosystem functions<sup>143</sup>. Crop diversification can directly alter the soil microbiome by supplying nutrients through root exudates and litter, and by providing physical space on root surfaces.

Effects can also be indirect, by altering physical and chemical soil properties. At the soil structural level, increased structural diversity of root systems and changes in SOM and nutrient inputs through plant litter and rhizodeposition can alter porosity, aeration and aggregate stability, providing more diverse niches for microbes<sup>144</sup>. Deep-rooting plants can change allocation of nutrients, water and oxygen along the soil depth profile. Increasing crop diversity generally increases microbial diversity, although not consistently<sup>145</sup>, which demonstrates the dependency of these effects on the plant species and the edaphic and climatic context<sup>146</sup>. A key benefit of crop diversification is the recruitment of beneficial microorganisms and the reduction in pathogen loads by interrupting their host-dependent lifecycles and recruiting natural antagonists<sup>147</sup>.

Cover cropping is increasingly applied to reduce soil erosion and nutrient leaching, improve soil structure, increase organic matter and nutrient availability, suppress weeds and balance moisture levels<sup>148</sup>. Root activity of the cover crop can increase or maintain porosity and pore connectivity, but these effects are crop-dependent, highlighting the need for careful selection of the cover crop<sup>149</sup>. For example, cereal rye is an excellent cover crop because it scavenges excess nitrogen in the soil (preventing nitrogen leaching), improves soil structure and prevents soil erosion through its deep and fibrous root system, builds up organic matter and suppresses weeds.

Cover crops will also steer abundance and compositions of microbial communities either directly through plant–microbe interactions or indirectly via soil structural alterations and modulation of physicochemical soil properties<sup>150,151</sup>. For example, they can have positive effects on microbial abundance, activity and diversity<sup>151</sup>, and increase

functional redundancy and complementarity<sup>152</sup>. Moreover, cover crops can promote specific microbial guilds such as arbuscular mycorrhizal fungi<sup>153,154</sup> and oligotrophic and/or metabolically versatile bacterial groups that are able to degrade complex carbon compounds incorporated into the soil during cover crop burial, such as Acidobacteria, Verucomicrobia, Actinobacteria and Firmicutes<sup>157</sup>. Soil-borne pathogens can be reduced and disease-suppressive microorganisms increased<sup>155</sup>.

Effects of cover cropping on greenhouse gas emissions are variable. Increased microbial activity and root respiration tend to increase CO<sub>2</sub> respiration, whereas alterations in CH<sub>4</sub> fluxes are usually low, and effects on N<sub>2</sub>O emissions are highly variable and depend on crop species (legume or not), fertilization regime and climatic conditions<sup>156,157</sup>. In fact, the climate mitigation potential of cover cropping might mainly arise from carbon sequestration rather than reduction in N<sub>2</sub>O emissions<sup>158</sup>.

Overall, increasing crop diversity in space and time and planting cover crops are valuable practices for improving soil structure and reducing soil erosion. In turn, there are beneficial impacts on microbial diversity and functions including nutrient cycling, disease control and climate regulation (Fig. 4). However, more research is needed to better understand how crop diversification alters the structural microscale environment in soil and the effects on beneficial microbes. In addition, a better understanding of how microbial interactions with various co-planted hosts drive cropping system performance is needed, to identify matching crop combinations with respect to microbial functions. The interplay between root traits and root microbiome remains particularly understudied and deserves more attention<sup>144</sup>.

## External inputs

The application of fertilizers, crop protection agents or soil conditioners influences the relationship between soil structure and microbiomes (Fig. 4). In contrast to industrial agriculture, integrated soil fertility management (ISFM)<sup>159</sup> and integrated pest management (IPM)<sup>160</sup> practices represent potentially more sustainable concepts. They both combine moderate and precise application of agrochemicals (mineral fertilizers and pesticides) during periods of high nutrient demand or pest and disease pressure with the use of more complex organic fertilizers, biological control agents, and resource-efficient and disease-resistant plant germplasm. Their goal is to sustain soil fertility, enhance soil biodiversity and improve soil structure over longer periods of time.

Mineral and organic fertilizers applied to agricultural fields are one of the main drivers of the soil microbiome<sup>114,161,162</sup>. Synthetic mineral fertilizers provide direct nutrition to the crops and thereby largely bypass the cycling through the soil microbiome. Unfertilized and mineral-fertilized soils are often characterized by slow-growing oligotrophic organisms commonly seen in resource-limited environments<sup>114,161</sup>. In contrast, organic fertilizers such as animal manure, slurry, sludge, compost and crop residues are complex substrates that require mineralization by soil microorganisms before nutrients become available to the plant. Organic fertilizers can enhance soil microbial abundance, activity and diversity<sup>163,164</sup> by increasing microbial taxa that respond rapidly to increased resource availability (copiotrophs) or are able to degrade a wide range of organic compounds<sup>114,161</sup>. Moreover, organic fertilizers can harbour a diverse microbial community that fuses with the local community (coalescence<sup>165</sup>), establishes in soil and alters soil functions<sup>161,162</sup>. However, the success of these inoculated communities in establishing long term and driving essential soil functions has not yet been thoroughly investigated<sup>124</sup>.

Mineral versus organic fertilization is a key determinant of soil structure formation<sup>16,166</sup>. Mineral fertilizer increases nutrient availability, leading to carbon limitation for soil microbial communities. In turn, microbial decomposition of organic binding agents that hold together soil aggregates is increased. In contrast, organic matter increases soil aggregate formation by acting as a binding agent, enhancing the development of fungal hyphal networks, and increasing the production of microbial by-products, such as polysaccharides, that also act as binding agents<sup>167</sup>. Furthermore, organic fertilizers promote biological activity of soil fauna (such as earthworms), which enhances soil aggregation and porosity through burrowing and production of aggregate binding agents<sup>168</sup>. Fertilization by incorporating crop residues after harvesting can also improve soil moisture levels, aggregate stability, porosity, cation exchange capacity, organic carbon content, nutrient availability and resistance to soil compaction<sup>169,170</sup>. These factors ultimately affect soil microbial diversity and activity, with subsequent impact on nutrient cycling and pathogen occurrence<sup>169,171</sup>.

The choice of fertilization regime influences microbiome functions. However, the effects are as diverse as the types of fertilizers that can be used. Organic fertilizers promote specific functional guilds involved in carbon, nitrogen and other cycles<sup>172</sup>, but the effects on nutrient cycling and greenhouse gas emissions vary strongly across studies. On the one hand, organic fertilizers generally increase heterotrophic respiration and nitrogen turnover in soil, leading to an increase in CO<sub>2</sub> and N<sub>2</sub>O emissions<sup>173,174</sup>. The effects are variable, though, and more stable organic fertilizers such as processed manure, compost and digestate can also reduce these emissions. On the other hand, long-term organic fertilization might strongly contribute to carbon sequestration and thus nitrogen stabilization, ultimately helping to mitigate climate change<sup>174</sup>. Organic fertilizers have also the potential to suppress soil-borne diseases<sup>175</sup>, but effects are again variable across fertilizers. Substrates that are suppressive to one pathogen are often ineffective or even conducive for other pathogens<sup>176</sup>. Finally, organic fertilizers such as compost and manure have the potential to boost microbial degradation of pollutants such as pesticides in agricultural soils<sup>177</sup>. Overall, versatile fertilization regimes are an important strategy for building alternative agroecosystems that preserve soil structure and promote microbial functions (Fig. 4).

Other abiotic, non-fertilizing amendments are used to improve soil health (Fig. 4). These amendments can include organic amendments such as wood chips, straw, sawdust and wood ash, and inorganic amendments such as vermiculite, perlite, gravel and sand. For example, biochar is applied to soil to increase carbon sequestration, retain nutrients through cation adsorption, elevate pH, regulate soil moisture and improve soil structure<sup>178,179</sup>. Biochar can also affect microbial biomass, composition and activity either directly by serving as a carbon source<sup>180</sup> or indirectly by altering the above-mentioned soil properties<sup>181</sup>. Hence, biochar can impact nutrient cycling by affecting specific functional guilds involved in nitrification and denitrification<sup>182,183</sup> or phosphorus solubilization<sup>184</sup>. However, biochar effects on soil microbiomes are variable and depend on the biochar and the edaphic properties, such as soil texture<sup>185</sup>.

Biochar tends to increase carbon dioxide (CO<sub>2</sub>) emissions, have negligible effects on CH<sub>4</sub> fluxes and show variable effects on N<sub>2</sub>O emissions<sup>186,187</sup>, although these effects largely depend on the fertilization regime<sup>188</sup> and cropping system<sup>187</sup>. Biochar has further the potential to regulate crop diseases by reducing plant pathogens and activate plant defence mechanisms<sup>181,189</sup>, reduce availability of antibiotics and presence of antibiotic resistance genes<sup>190</sup>, and stimulate microbial

degradation of pollutants such as pesticides<sup>191</sup>. However, the context dependency of these effects and the relative contributions of adsorption mechanisms and stimulation of microbial activity need further scrutiny<sup>182</sup>.

Soil amendments can also consist of biologicals (Fig. 4), which are products that consist of either the living organism itself or a product (biomolecule) derived from it. Biologicals can be broadly grouped into biocontrols (or biopesticides) that provide protection against pests and diseases, and biostimulants that promote plant growth and tolerance against abiotic environmental stresses<sup>192,193</sup>. Microbial inoculants are a promising tool to increase crop production while reducing the reliance on agrochemicals. For example, elite strains of *Bradyrhizobium japonicum* have been used in 80% of the soybean cultivation in Argentina to increase yields by around 190 kg ha<sup>-1</sup> (6%)<sup>194</sup>, and *Azospirillum brasilense* strains have been applied to around 5 million hectares in South America to improve yield of cereal crops and legumes by around 10%<sup>195</sup>. Many similar applications are emerging, but with mixed results and large financial investments from agro-industrial companies. As well as the many benefits of microbials for soil fertility improvement, plant growth promotion, pest and disease control, abiotic stress tolerance and bioremediation, such products might also be used for enhancing soil structure<sup>196</sup>. Microorganisms that contribute to soil aggregation, such as exopolysaccharide-producing bacteria<sup>53</sup> or mycorrhizal fungi<sup>197</sup>, have the potential to improve soil structure upon inoculation and indirectly steer ecological functions related to the availability of water, oxygen and nutrients, ultimately improving soil biodiversity and activity.

However, application of microbial inoculants also poses important challenges, as their establishment success, long-term survival in soil and ability to displace functionally relevant native species are important for soil functioning and for risk assessment<sup>198</sup>. Microbial inoculants can outcompete native species, change local species diversity and alter the functionality of resident soil communities, with unknown legacy effects in the long term<sup>199,200</sup>. Furthermore, the success of external inoculants in establishing in soil and inducing the desired function is very context-dependent<sup>201,202</sup>, making it difficult to predict the behaviour of the inoculant and develop universally applicable biofertilizers for improving agricultural performance. Therefore, a primary focus on engineering the soil microbiome to improve agroecosystem functioning should be on promoting beneficial native species – for example through mechanisms such as host-mediated indirect selection<sup>203</sup> – rather than introducing new species with unstable performance and poorly understood non-target effects. Overall, there is a need to better understand potential, challenges and risks associated with microbial inoculants, and contrast these findings with *in situ* engineering techniques of the native microbiome.

## Recommendations

Smart combinations of soil and crop management practices with system-tailored applications of external inputs will improve crop production systems sustainability by influencing the soil microbiome. In particular, the use of soil-protective agricultural vehicles, reduced tillage operations, crop diversification, cover cropping, and the application of high-quality organic fertilizers and soil amendments (Fig. 4) will sustain the physicochemical functioning of the soil matrix (Fig. 3) and harness the natural microbial functions (Fig. 1). However, current research often shows inconclusive results when comparing these different management factors, highlighting the context-dependent nature of many of these interventions.

An example of an integrated agricultural system that increased yield and decreased microbial N<sub>2</sub>O emissions has been developed in the Central Valley of California, USA<sup>204</sup>. To reduce operational costs, a tomato cropping system was developed that combined reduced tillage, drip irrigation with fertigation (injection of fertilizers in the irrigation system), and cover cropping with Trios triticale. In this system, the triticale cover crop with a large root system was specifically brought in to prevent compaction of the tomato bed in between tomato cropping seasons, owing to the reduced tillage, and allowed for the maintenance of the drip irrigation system to fertigate the tomato optimally. The combination of the optimum fertigation with an improved soil structure not only increased yield by around 53%, but also reduced N<sub>2</sub>O emissions from around 2.01 to 0.58 kg N<sub>2</sub>O-N ha<sup>-1</sup>. The system was operated on an area of 5 hectares, and there are no logistical barriers that would prevent operating it at larger scale.

Another example of an integrated agricultural system is the Quesungual slash-and-mulch agroforestry system (QSMAS) in the mountainous tropical dry forests of Central America<sup>205,206</sup>. The QSMAS was developed by farmers and FAO experts as an alternative to the traditional slash-and-burn systems (SB) that were leading to soil erosion and degradation. The main components of the QSMAS are: the ‘three sisters’ cropping system (maize, beans and squash); keeping native trees in the fields; pruning the trees and using the cuttings as a mulch instead of burning them; introducing livestock after several years of crop cultivation; and eventually returning the land to secondary forest before starting a new agricultural cycle. This system reduces soil erosion and improves yields in the longer term, and it increased earthworm populations (13.4 g m<sup>-2</sup> versus 0.8 g m<sup>-2</sup> in the QSMAS versus SB), tripled available phosphorus and increased soil carbon<sup>205</sup>. A more comprehensive macrofauna study also showed that QSMAS increased macrofauna (termites, ants, earthworms, beetles, centipedes and others) biomass compared with the secondary forest (from 4.3 g m<sup>-2</sup> to 24.8 g m<sup>-2</sup> in the dry season and from 13.1 g m<sup>-2</sup> to 41.9 g m<sup>-2</sup> in the wet season)<sup>206</sup>.

Collecting more data across soil types, climatic conditions and variations of different interventions will provide a better and more universal understanding of how these solutions need to be customized and combined for achieving optimal agronomic and ecological performance. Specifically, for many of these integrated agricultural systems, data on the dynamics and functioning of microbial communities and their link to the soil structural components are currently lacking, calling for further research activities. Ultimately, optimal combinations and trade-offs across different interventions need to be considered to fully harness the beneficial interplay between soil structure and microbial functions in agroecosystems.

## Summary and future perspectives

Soil microbiomes drive key functions in agroecosystems (Fig. 1). Soil particles, aggregates and pores represent the relevant scale for these functions, determining the physical habitat, the availability of essential resources, and the biotic interactions driving community assembly and dynamics (Fig. 2). Intensive agricultural management practices can induce structural alterations to the soil, including the breakdown of soil aggregates and the voids in between them. As a result, there are reduced water, oxygen and nutrient flows through the soil system, which promote undesirable microbial functions and limits crop production (Fig. 3). Alternative agricultural practices, such as conservation tillage, diversified cropping systems, and a partial replacement of agrochemicals by organic amendments and biologicals, need to be developed

and implemented on a large scale. These practices must promote good soil structure that supports a diverse set of beneficial microbial functions and ultimately contributes to more resource-efficient, climate-friendly and stress-resilient agroecosystems (Fig. 4).

The importance of the link between soil structure and microbiome functioning for agroecosystems is indisputable. However, most microbiome studies fail to interrogate the soil system at the relevant scales of soil particles, aggregates and pores, which are required to understand interactions of microbes with their abiotic and biotic environment. Top-down approaches that dissect the relevant fractions prior to analysis of microbial inhabitants, such as dry and wet sieving, flow cytometry or microdissection, have been successfully used to study microbial communities at the microscale<sup>84</sup>. Nevertheless, isolation of aggregates with intact microbial communities inside and on aggregates remains challenging. After aggregate dissection, many new nanoscale imaging techniques are available to study the localization and distribution of particles, cells and elements in soil aggregates<sup>84</sup>. For example, the combination of stable isotope tracing with nanoscale secondary ion mass spectrometry and Raman spectroscopy has the potential to study *in situ* incorporation and exchange of elements within aggregates and pores<sup>207,208</sup>. The combination of these imaging and tracing techniques with single-cell genomics and aggregate-scale gene expression studies will enable the exploration of microbial interactions at the relevant scales within, on and between particles and aggregates; however, the application of these emerging techniques is still in its infancy. Thus, the challenge will be to measure physicochemical gradients at the microscale inside and on soil aggregates, and link these changes with the presence, distribution and metabolic activity of individual microbial cells at the same scale.

Simultaneously, interrogations across scales – from agricultural interventions (reduced tillage, cover cropping, intercropping, agroforestry, ISFM and IPM) to the microscale – are needed. In particular, research must cover different soil types and climates to better understand the drivers of microbial functioning across scales. In fact, soil aggregate formation is controlled by different mechanisms in different soil types<sup>209</sup>. Clay type and content, soil organic carbon concentrations, the presence of carbonates and metal oxides, and cation exchange capacity and pH determine aggregate formation rates and stability. Furthermore, climate factors such as precipitation and temperature control soil aggregation through alterations in dry–wet and freeze–thaw cycles. All these properties determine the presence, abundance and activity of soil microorganisms, which, in turn, again influence soil aggregation. Therefore, agricultural interventions that aim at considering the interplay between soil structure and microbiome functioning need to consider the local or regional characteristics of the system. Future research should also focus more on developing countries, where such data are sparse but reliance on nature-based solutions is high. These efforts will be the foundation of building practical and feasible sustainable agroecosystems that allow farmers across the globe to optimally harness the natural functions provided by the soil microbiome.

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The authors declare no competing interests.

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