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Going back to the roots: the microbial ecology of the rhizosphere

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Abstract | The rhizosphere is the interface between plant roots and soil where interactions among a myriad of microorganisms and invertebrates affect biogeochemical cycling, plant growth and tolerance to biotic and abiotic stress. The rhizosphere is intriguingly complex and dynamic, and understanding its ecology and evolution is key to enhancing plant productivity and ecosystem functioning. Novel insights into key factors and evolutionary processes shaping the rhizosphere microbiome will greatly benefit from integrating reductionist and systems-based approaches in both agricultural and natural ecosystems. Here, we discuss recent developments in rhizosphere research in relation to assessing the contribution of the micro- and macroflora to sustainable agriculture, nature conservation, the development of bio-energy crops and the mitigation of climate change.

The rhizosphere, the narrow zone of soil that surrounds and is influenced by plant roots, is home to an overwhelming number of microorganisms and invertebrates and is considered to be one of the most dynamic interfaces on Earth. Organisms that are present in the rhizosphere microbiota can have profound effects on the growth, nutrition and health of plants in agro-ecosystems[1–3]. Rhizosphere microbiotas can also directly and/or indirectly affect the composition and biomass of plant communities in natural ecosystems[4,5]. Numerous organisms contribute to these processes, leading to countless interactions between plants, antagonists and mutualistic symbionts, both below ground and above ground[6–9].

Many of the current insights into interactions and processes in the rhizosphere have emerged from studies on agricultural or horticultural crop plants and model species such as *Arabidopsis thaliana* and *Medicago truncatula*. However, considerable progress is also being made in understanding the microbial ecology of the rhizosphere of non-cultivated plant species in natural ecosystems[10] and how microorganisms influence resource allocation, biodiversity and above-ground interactions with herbivores and their natural enemies[11]. To better understand the players and processes that operate in the rhizosphere, a variety of molecular techniques, such as metagenomics and stable-isotope probing, have been applied over the past decade[2,12–14]. At the plant community level, substantial progress has been made in studying intermingled root systems from different plant species[15,16]. Such studies on natural ecosystems are complementing and extending our current knowledge of the rhizosphere, as they resolve how multitrophic interactions may have co-evolved in the rhizospheres of plants grown in their native habitats as compared to in the rhizospheres of agricultural or exotic plant species introduced into new habitats[17].

Recent studies have further shown that, in non-cultivated ecosystems, plant community diversity and the genotypes of individual plants can influence the composition of their associated communities both above ground and below ground[18–21]. This might also explain why some plant species promote the decomposition of their own litter rather than that of other plant species or genotypes: it grants a 'home-field advantage' (REF. 22). Although the effects of decomposition and mineralization might seem to be less relevant in agricultural production systems, where mineral fertilizers can provide the majority of nutrient inputs, knowledge of the interlinkages between decomposers and soil-borne symbionts, and between antagonists and phytopathogens, in the rhizospheres of non-cultivated ecosystems might become more relevant when conventional agriculture becomes less dependent on external inputs of nutrients, biocides and fossil fuels. The rhizosphere microbiota can also be examined in relation to other ecological phenomena, such as natural succession. For example, in primary dune succession, one of the pioneer plant species, *Ammophila arenaria* (marram grass), is protected against plant parasitic nematodes by complex bottom-up and top-down interactions in the rhizosphere[23]. These

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natural controls might have got lost during breeding of crop species for high yield and because of management practices such as soil tillage and fertilization. Interestingly, the relationship between plant species diversity and productivity is also influenced by feedback interactions between individual plant species and microorganisms that reside in the rhizosphere, as evidenced by the decreased disease risk observed with increasing plant diversity[5,24]. Lessons from these studies might help to reduce the negative effects of soil-borne pathogens in rotational or mixed cropping systems in agriculture.

Here, we review recent progress in rhizosphere research and suggest that a conceptual framework is needed to stimulate a theory-based approach to studying the microbial ecology of the rhizosphere. For this purpose, we link knowledge from both agricultural and natural ecosystems, from single plants and multispecies plant communities, and from below-ground and above-ground multitrophic interactions. We consider this learning from nature as 'going back to the roots' of non-cultivated plant species, for which rhizosphere processes and microbial interactions might be more evolved than for most agricultural crops, which are under strong anthropogenic control.

The rhizosphere microbiota

Bacteria, fungi (including arbuscular mycorrhizal fungi (AMF)), oomycetes, viruses and archaea that live in the rhizosphere (FIG. 1) are attracted by and feed on rhizodeposits — that is, nutrients, exudates, border cells and mucilage released by the plant root (BOX 1; FIG. 2). Numerous studies have shown species-specific effects of plants on the composition and relative abundance of microbial populations in the rhizosphere of crops and of cultivated and native plant species[25–29]. For example, the relative abundances of 147 of the 1,917 bacterial taxa detected in the rhizosphere of the graminoid *Avena fatua* were found to be significantly different from the bacterial taxa in the bulk soil, with most of the rhizosphere species belonging to the phyla Firmicutes or Actinobacteria or to the class Alphaproteobacteria[30]. Many independent studies have depicted proteobacteria (for example, bacteria from the Pseudomonadaceae or Burkholderiaceae family) as dominant members of the rhizosphere microbiota[2,30–34] (FIG. 3). This is in line with proteobacteria being generally fast-growing r-strategists with the ability to utilize a broad range of root-derived carbon substrates. Indeed, providing $^{13}CO_2$ to plants revealed that most of the bacteria assimilating the labelled exudates were phylogenetically close to members of the order Burkholderiales[35]. However, bacteria do not monopolize the nutrient-rich rhizosphere niche, and fungi such as those in the phyla Ascomycota (for example, in the order Hypocreales) and Glomeromycota (for example, *Glomus* spp.) can also respond rapidly to rhizodeposits[35,36]. Next-generation sequencing is now frequently used to identify microbial taxa in the rhizosphere with considerable resolving power. Although a reduction of microbial diversity is often reported in the rhizosphere compared with in the bulk soil, providing a general description of the rhizosphere microbiome is difficult owing to large discrepancies between different studies, which might be due not only to biological variability, as detailed below, but also to practical issues related to the actual sampling of the rhizosphere[37]. Furthermore, in addition to descriptive analyses of the rhizosphere microbiome, there is a strong need to elucidate the mechanisms underlying the selection of specific populations of microorganisms among the soil-borne communities.

Mycorrhizal fungi
Fungi that form a mutualistic, symbiotic association with a plant.

r-strategists
Organisms that thrive in unstable or fluctuating environments where resources are abundant, unlike K-strategists, which are more competitive in stable environments with limited resources.

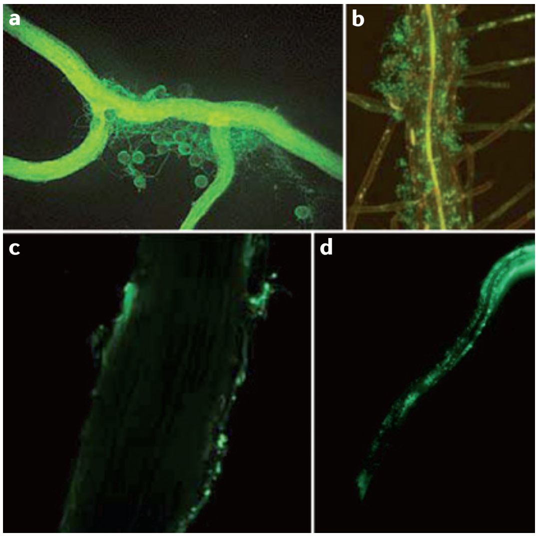


Figure 1 | **Microorganisms in the rhizosphere.**
a | Arbuscular mycorrhizal fungi (AMF) on a corn root.
b | Colonization of *Arabidopsis thaliana* by the plant growth-promoting rhizobacterium *Bacillus subtilis* FB17.
c | Bacteria on *A. thaliana* roots (imaged by catalysed reporter deposition fluorescence *in situ* hybridization of bacteria)[13]. **d** | GFP-tagged rhizobacteria on roots of *A. thaliana*. Image in part **a** is reproduced, with permission, from REF. 156 © (2003) Nature Education. All rights reserved. Image in part **b** is reproduced, with permission, from REF. 157 © (2012) American Society of Plant Biologists. Image in part **c** is reproduced, with permission, from REF. 13 © (2012) Macmillan Publishers Ltd. All rights reserved. Image in part **d** courtesy of J. van de Mortel, Wageningen University, The Netherlands.

Determinants of microbial community assembly in the rhizosphere. Microbial community assembly in the rhizosphere is governed by abiotic and biotic factors. Many studies have demonstrated that soil has a profound influence on the assembly of bacterial and mycorrhizal fungal communities in the rhizosphere[38–41]. The complex physico-chemical characteristics of soils affect plant physiology and root exudation patterns, which in turn influence the composition of the rhizosphere microbiota. Sequence-based analyses carried out on the bacterial microbiome of the rhizosphere of different *A. thaliana* ecotypes showed that soil type had a strong influence on microbiota composition[12,13]. By contrast, the actinobacterial communities in the rhizosphere of strawberry plants grown in different soils were more similar to each other than the bulk soil communities were to each other, indicating that in this case the plant

Box 1 | How do plants influence soil properties?

The properties of the soil in close vicinity to plant roots are modified by a range of processes occurring during plant growth, which in turn affect the rhizosphere microbiota. Roots release low-molecular-mass compounds (that is, sugars, amino acids and organic acids), polymerized sugar (that is, mucilage), root border cells and dead root cap cells. These rhizodeposits are used as carbon sources by soil microorganisms and represent approximately 25% of the carbon allocated to the roots in cereals and grasses[151]. Rhizodeposits also contain secondary metabolites, such as antimicrobial compounds, nematicides and flavonoids[78,152], which are involved in establishing symbiosis or in warding off pathogens and pests. Soil pH, another important driver of soil microbial communities[43,153], can increase or decrease by up to two units in the rhizosphere owing to the release and uptake of ions by roots[154]. Water uptake and root respiration affect soil oxygen pressure, thereby influencing microbial respiration. Finally, soil nutrient availability is modified in the rhizosphere by plant uptake and by the secretion of chelators, such as phytosiderophores, to sequester metallic micronutrients[155].

Disentangling the contribution of these different drivers is complex, as many of the processes are interwoven. In addition, the magnitude of the effects of roots on the properties of soil varies with the soil type, plant species and the feedback response of the rhizosphere microorganisms present. Characterization of the rhizosphere habitat is challenged by spatial and temporal variations of the soil properties along the root in relation to the age and physiological state of the plant. To overcome these hurdles, new analytical tools are being developed. For example, non-invasive two-dimensional imaging now offers exciting opportunities for quantitative visualization of the dynamics of specific soil properties or activities in the rhizosphere (FIG. 4).

is a stronger determinant of microbiota composition than the soil type[25].

The differences and similarities across studies can perhaps be best understood by considering the assembly of the rhizosphere microbiota as resulting from a hierarchy of events. First, the soil can be considered as a microbial seed bank[42], and the physico-chemical properties of the soil, together with biogeographical processes, structure this community[43]. Then, the location where plants are grown determines which indigenous biota the plant roots are exposed to. Finally, the plant species and genotype determine which members of this reservoir of microorganisms can grow and thrive in the rhizosphere.

Plant species can strongly influence the composition and activity of the rhizosphere microbiota, and differences in root morphology, as well as in the amount and type of rhizodeposits, between plants contribute greatly to this species-specific effect[44–49]. Specific metabolites released into the rhizosphere can trigger multiple responses in different soil microorganisms. For example, plant flavonoids can attract not only symbionts, such as *Bradyrhizobium japonicum,* but also pathogens, such as *Phytophthora sojae.* Flavonoids also stimulate mycorrhizal spore germination and hyphal branching, and influence quorum sensing, as has been shown for the flavonoids naringenin from legumes and catechin from the medicinal tree *Combretum albiflorum*[50–55]. Similarly, constitutive secondary defence metabolites, such as pyrrolizidine alkaloids, can affect the rhizosphere microbiota by favouring resistant or tolerant microorganisms or, in some cases, microorganisms that metabolize these compounds[56].

Not only the plant species but also the cultivar can affect the composition of the rhizosphere microbiota[41,57–60]. Characterization of the microbial community in the rhizosphere of three potato cultivars grown at two distant field sites revealed that, depending on the soil type, 4–9% of the operational taxonomic units detected by PhyloChip analysis were dependent on the cultivar[61]. Crop-breeding programmes are typically conducted in monocropping systems under fertile conditions and in the absence of soil-borne pathogens, thus minimizing the contribution of the rhizosphere microbiome to plant growth and health. In this context, it has

Microbial seed bank
A reservoir of dormant microorganisms.

Cultivar
A variety of plant that has been selected for specific traits.

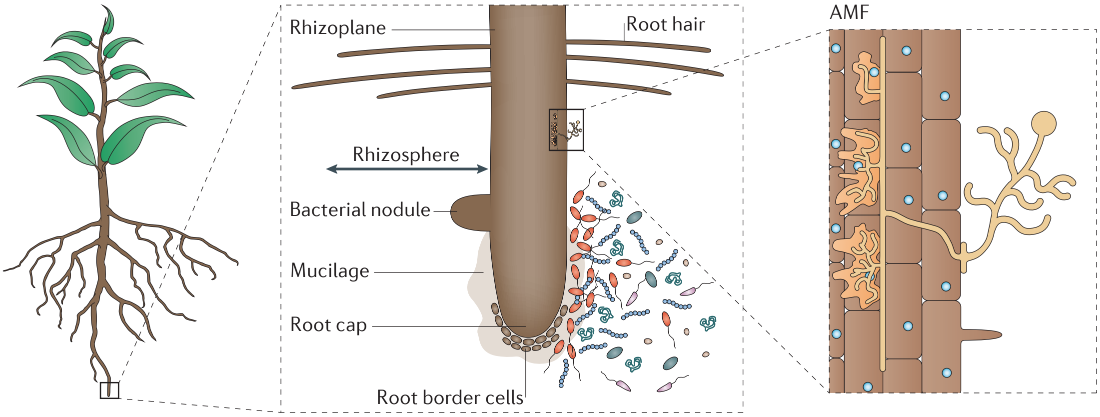


Figure 2 | **The rhizosphere.** The rhizosphere is a narrow zone of soil (a few millimetres wide) that surrounds and is influenced by plant roots. The schematic shows magnified pictures of the rhizosphere, containing saprophytic and symbiotic bacteria and fungi, including arbuscular mycorrhizal fungi (AMF). AMF inset modified, with permission, from REF. 158 © (2008) Macmillan Publishers Ltd. All rights reserved.

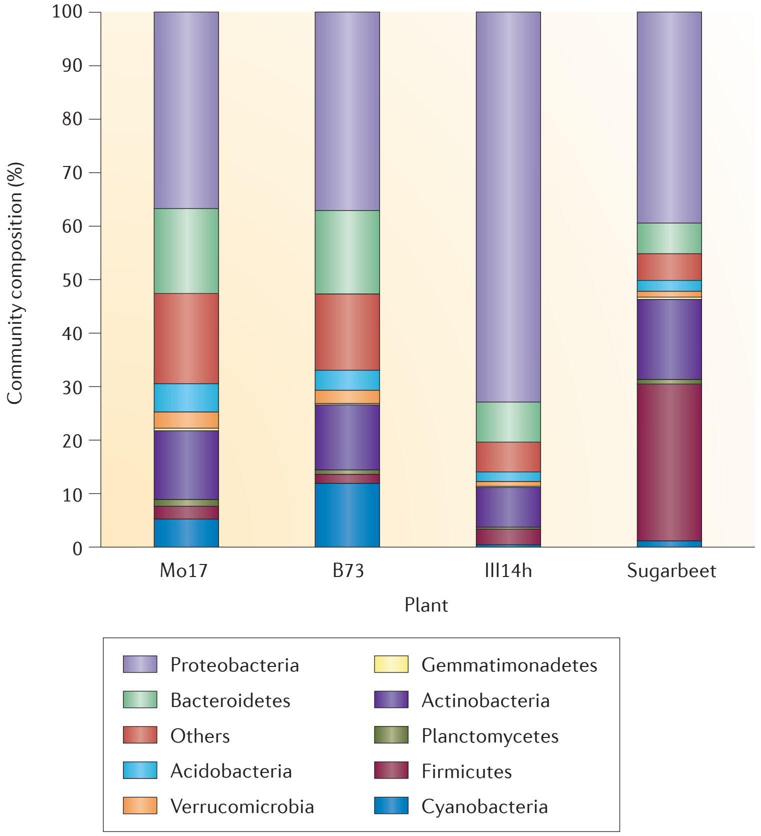


Figure 3 | **The composition of the bacterial community in the rhizosphere.** The figure shows examples of the composition of the bacterial community in the rhizosphere of three maize genotypes (Mo17, B73 and Ill14h) and of sugarbeet. The distribution of the different bacterial phyla is based on data obtained by 454 sequencing (maize)[2] and G3 PhyloChip analyses (sugarbeet)[33]. The bacterial community composition was characterized in the rhizosphere of 27 maize genotypes cultivated in five fields located in three states in the USA. Here, three genotypes displaying contrasted rhizosphere microbiota in a given field are depicted for illustration[33] and the sugar beet rhizosphere microbiota presented is from seedlings grown in a disease-conducive soil in The Netherlands[2].

Endophytic bacteria
Bacteria that live inside plant tissue without causing negative effects.

been hypothesized that modern plant breeding may have selected against plant traits that are essential for hosting and supporting beneficial microorganisms[62,63]. Investigation of the genetic basis in plants for interactions with beneficial rhizobacteria led to the identification of three quantitative trait loci in the tomato genome that were associated with the disease-suppressive capacity of a rhizobacterial strain of *Bacillus cereus*[63]. More recently, comparison of the bacterial diversity in the rhizosphere of inbred maize lines provided evidence of host genetic influences on the composition of the rhizosphere microbiota[33,60] (FIG. 3), but whether these variations differentially affect plant health and growth of the corresponding plant genotypes remain to be explored. Together, these findings illustrate that it might be possible to exploit genetic variation in host plant species in a manner that will allow the rhizosphere microbiota to be incorporated into plant breeding programmes to promote beneficial associations between plants and microorganisms.

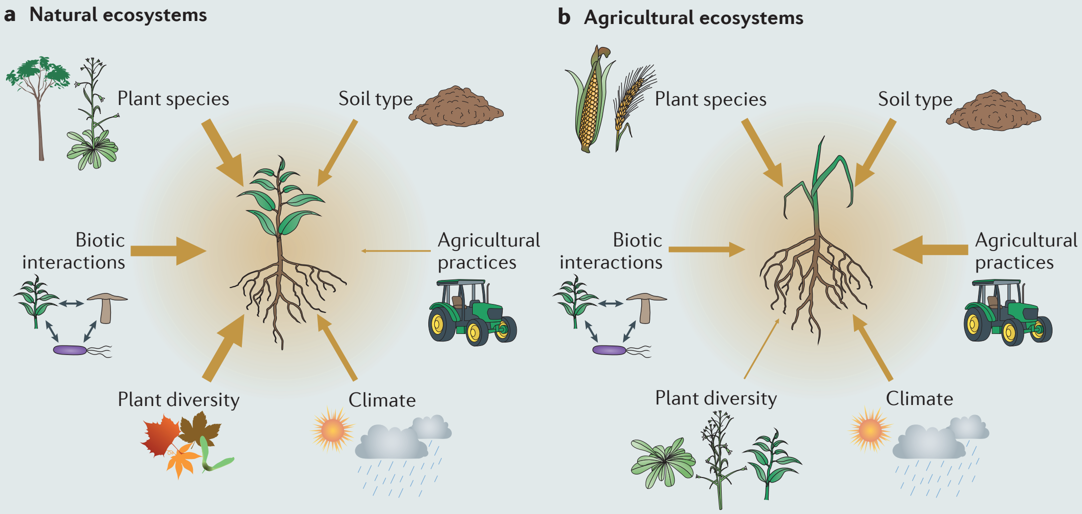
In addition to soil type and plant species and/or genotype, the microorganisms that are present on and in seeds[64] are also a potential source for the assembly of the rhizosphere microbiota. However, although numerous studies have been carried out to examine rhizosphere colonization by specific microorganisms introduced purposely onto seeds of crop plants[65], relatively little is known about the role of indigenous seed-borne microorganisms in the assembly of the rhizosphere microbiota, the so-called maternal effect[66]. Early studies showed that bacterial communities on the roots of cucumber seedlings resembled the soil microbial community more than the community from the seed coat[67], suggesting that bacterial communities associated with the seed surface have little or no effect on rhizosphere community assembly. However, this might be different for microorganisms living inside seeds, as an investigation of endophytic bacteria of wild and modern maize varieties revealed a conserved core microbiota of maize seed endophytes, with at least one member (*Enterobacter asburiae*) being able to exit the root interior to colonize the rhizosphere[68]. Recruitment of a core rhizosphere microbiota from seeds suggests that plants can transmit specific microorganisms from one generation to another. This carry-over effect on assemblage of the rhizosphere microbiota has important implications for the co-evolution of plant–microorganism interactions in natural ecosystems.

The relationship between plant community diversity and the composition of the rhizosphere microbiota is even more complex than for single plant species, and full coverage of this topic goes beyond the scope of this Review. Results from greenhouse studies and field experiments have shown that plant community composition influences the diversity of AMF[69] and nematodes[70], and the soil food web structure[71]. Recent work showed that the species richness of neighbouring plants altered the composition of the *Streptomyces* spp. community of several plant species, with a decline in *Streptomyces* spp. community similarities being observed as plant richness increased[72]. More generally, it is expected that the more diverse the plant community is, the more diverse the composition of plant residues and rhizodeposits, and consequently microbial diversity, will be. Intuitively, the influence of plant community diversity on the rhizosphere microbiota is probably more important in natural than in agricultural ecosystems, in which plant diversity and plant trait variation are limited. However, additional factors shaping the rhizosphere microbial community may also differ between these two ecosystems (BOX 2).

Multidimensionality of the rhizosphere microbiota. Both temporal and spatial scales are of great importance in the rhizosphere. Just as the amount and composition of rhizodeposits can vary between different plant species, they can also vary in time during growth and root development of a single plant species. It is well established that the structure of the rhizosphere microbial community can change during the plant life cycle[73–75]. For example, differences were observed in the bacteria and fungi present in rhizosphere soils sampled at several vegetative and reproductive stages of *M. truncatula*[76].

Box 2 | Drivers of the rhizosphere microbiota in natural and agricultural systems

Factors driving the assembly of the rhizosphere microbiota and its importance for plant communities in both natural and agricultural ecosystems (see the figure, parts **a** and **b**, respectively; the relative thicknesses of the arrows show the importance of each factor) can be analysed in a co-evolutionary framework. In natural ecosystems, plants are growing in their native soils with long-term co-evolution of plant–microorganism interactions, and therefore the plant species is likely to be a more important determinant of the rhizosphere microbial community than soil type. By contrast, in agricultural ecosystems, the same crops are cultivated in various soils, which has a strong impact on rhizosphere microbiota assembly. In natural ecosystems, plant diversity is generally higher than in agricultural systems, and therefore naturally co-evolved multitrophic interactions are likely to be more important for the rhizosphere microbiota. As an outcome of co-evolution, the fitness of wild plant species is expected to benefit more from the rhizosphere microbiota. This should lead to a stronger positive feedback on plant performance in natural than in agricultural ecosystems. In agricultural ecosystems, the importance of the rhizosphere microbiota for plant growth and health is lower compared with in natural ecosystems owing to the input of fertilizers and pesticides. However, in both natural and agricultural ecosystems, the rhizosphere microbiota has a strong cost for plant fitness related to the loss of photosynthates in the form of rhizodeposits.



Such microbial successions start in the early stages of plant development, with the release of carbon from seeds during germination[64]. Microorganisms are not homogenously distributed in the rhizosphere owing to differences in root types (primary and secondary) and zones (root cap, lateral meristem and border cells), as well as to movement through the soil as the roots grow. Comparison of rhizosphere samples from the bases and tips of the roots showed clear differences in the proportion of fast-growing bacteria, which could reflect the succession of r-selected and K-selected organisms[77]. Future studies of the rhizosphere should therefore integrate spatiotemporal variations of the microbiota according to plant development and to the different root zones expressing different functions and rhizodeposit patterns.

Multitrophic interactions in the rhizosphere. Direct and indirect interactions between hosts and their associated microbiota involve constitutive and inducible changes in secondary metabolism and morphological structures[78]. Communication through signalling molecules, such as flavonoids[52], strigolactones[79] and sesquiterpenes[80], is important for regulation of these interactions. For example, strigolactones released in low concentrations from plant roots induce the growth of both AMF and parasitic plants such as *Orobanche* spp.[81]. Originally, it was assumed by ecologists that below-ground multitrophic interactions were less specific than above-ground interactions. However, evidence is accumulating that below-ground multitrophic interactions are governed by similar mechanisms to those described for above-ground interactions[82].

Interactions in the rhizosphere with direct or indirect effects on plant health have been extensively documented. Pathogenic bacteria, fungi (including AMF), oomycetes, nematodes and microarthropods have adverse effects on plants. Viruses can also infect plants via the roots but require nematodes or fungi to penetrate the root tissue[83]. Rhizodeposits are important cues for germination, chemotaxis and directional growth of pathogens towards the plant roots. For example, the bacterial pathogen *Agrobacterium tumefaciens* is attracted by specific phenolic compounds (acetosyringones) released from wounded plant tissue[84]. Other phenolic compounds in root exudates, such as vanillic acids, can trigger spore germination of fungal pathogens[85]. For the oomycetes, it was demonstrated that electrotaxis is an important root-targeting mechanism for the motile zoospores[86]. Oomycetes and other pathogens can also hijack symbiotic signalling molecules, such as cutin monomers, to establish infections[87].

Rhizosphere microorganisms that are well known for their mutualistic interactions with plants include rhizobia and AMF, which facilitate plant nutrition through the acquisition of nitrogen and phosphorus, respectively, in exchange for photosynthetically fixed carbon[78,88,89]. AMF have also been suggested to affect the outcome of interactions between plants and herbivores in the rhizosphere[9,90]. In contrast to symbioses with the AMF, which occur in more than 80% of vascular plants, symbioses with nitrogen-fixing rhizobia are restricted to the legumes (members of the family Fabaceae) and to *Parasponia* spp., which belong to the family Cannabaceae. Both mycorrhizal and nitrogen-fixing symbioses have been extensively studied for decades and have been reviewed elsewhere[3,78,91–93]. Interestingly, recent studies have shown striking similarities between rhizobia and AMF in their crosstalk with the host plant. This is exemplified by the secretion of sulphated and non-sulphated lipochitooligosaccharides by *Glomus intraradices*; these diffusible symbiotic signals are structurally very similar to the nodulation factors released by rhizobia[94]. Non-symbiotic microorganisms can also facilitate the uptake of nutrients and trace elements. For example, bacteria and fungi can provide iron to plants via siderophores, such as the pyoverdines produced by fluorescent *Pseudomonas* species and rhizoferrin, produced by *Rhizopus arrhizus*[95,96]. It was also shown that the insect-pathogenic fungus *Metarhizium robertsii*, which is also a plant endophyte, can transfer nitrogen from the insect to the plant in a tripartite interaction[8].

Other multispecies interactions in the rhizosphere can be beneficial to plant growth. In this context, it was postulated that the rhizosphere microbiota acts as the first line of plant defence against soil-borne pathogens[97]. This protective effect of the rhizosphere microbiota is best exemplified in so-called disease-suppressive soils; that is, “soils in which plants do not suffer from certain diseases or where disease severity is substantially reduced even though a virulent pathogen is present and the host plant is susceptible to the disease” (REF. 98). In most of these soils, disease suppression is microbial in origin and ascribed to consortia of soil and rhizosphere microorganisms that inhibit the growth or activity of the pathogen at some stage in the life cycle. The mechanisms underlying pathogen suppression in disease-suppressive soils include competition for nutrients and microsites, along with parasitism, mycoparasitism and amensalism[2]; amensalism (or antibiosis) in the rhizosphere refers to the production, by microorganisms, of secondary metabolites with specific or broad-spectrum antimicrobial activities[99]. However, the significance of amensalism in microbial interactions in the rhizosphere is still subject to debate. Interestingly, the finding that antimicrobial compounds can have other effects on microorganisms at subinhibitory concentrations, including a role in intercellular signalling, motility and biofilm formation[99–101], led to a renewed interest in the phenomenon of hormesis, the concentration-dependent, differential effects of antimicrobial compounds[102]. The interactions between bacteria and AMF can also be beneficial such that the bacteria help establish the mycorrhizal symbiosis, although the mechanisms involved are still unclear[103,104]. Reciprocally, AMF can have an impact on bacterial colonization[105] and diversity, with bacteria from the family Oxalobacteraceae being preferentially promoted[106,107].

Amensalism
An association between two different species that is detrimental to individuals of one species but not to those of the other. The secretion of chemical compounds by one species, thus damaging or killing the other species, is the most common mechanism of amensalism.

Phyllosphere
The above-ground parts of plants, mostly the leaves.

Below-ground–above-ground interactions. Rhizosphere microbiotas can reduce the competitiveness of the dominant plant species or enhance the competitiveness of rare and subordinate plant species, and can thus influence plant community diversity[108]. There is an increasing number of studies, often referred to as plant–soil feedback experiments, showing such effects of rhizosphere microbiotas on plant community composition[93]. These interactions could involve symbionts, pathogens, herbivores and decomposers. A few studies have related plant community diversity to the rhizosphere community[109], but most other studies have focused on the effects of soil diversity on plant productivity[110,111]. These diversity effects can be either positive or negative, depending on the plant–symbiont combination[21]. Therefore, the effects of rhizosphere microbiota diversity on plant community diversity could be strongly context dependent.

An increasing number of studies are showing how below-ground interactions can influence above-ground communities of herbivores, carnivores, mutualists and symbionts. These below-ground–above-ground interactions can be due to altered nutrient or water uptake by the plant, or altered plant defences[112,113]. Both fungal inhabitants of the rhizosphere, such as mycorrhizal fungi, and rhizobacteria, including *Bacillus* and *Pseudomonas* spp., can induce a systemic resistance response in the plant that is typically effective against multiple pathogens and insect pests[114]. For example, the plant response to above-ground herbivory might depend on the association of the plant with a mycorrhizal fungus[6]. Reciprocally, defence responses that are induced in the phyllosphere can spread systemically to the roots and affect the rhizosphere microbiota[115], with soil legacy effects that can affect above-ground multitrophic interactions on succeeding plants[116]. Above-ground and below-ground plant–herbivore interactions are also influenced by diluting soil microbial communities, which suggests that rare soil microorganisms can play a substantial part in above-ground and below-ground plant defence[7]. The systemic resistance triggered by several non-pathogenic rhizobacteria primes the plant for activation of various cellular defence responses that are induced on pathogen attack[117]. The systemic resistance responses are, depending on the inducing microorganism, regulated by the plant hormones jasmonic acid, salicylic acid and ethylene, leading to an oxidative burst, the production of secondary metabolites and cell wall reinforcement. For example, the level and composition of secondary metabolites that are toxic to, and/or deterrents for, herbivores were influenced in plant roots and shoots by the soil microbial community[118]. Rhizobacteria not only enhance the production of known defence metabolites (glucosinolates) but can also trigger the production of plant metabolites for which the structures and functions are as yet unknown[119]. Collectively, these studies demonstrate that members of the rhizosphere microbiota have profound effects on plant metabolism and resistance to pathogens and insect pests.

The effect of the rhizosphere on biogeochemical cycling. Plant roots modulate biogeochemical cycles both indirectly and directly. Analysis of the stability of organic carbon revealed that mineralization of ancient soil organic matter is stimulated by root-derived carbon[120]. Conversely, it was recently demonstrated that 50–70% of the humus build-up in boreal forest ecosystems is derived from roots and root-associated microorganisms, highlighting the role of mycorrhizal fungi in sequestering carbon in the soil[121]. Nitrogen cycling in the rhizosphere has also received considerable attention, as nitrogen is the nutrient most often limiting to plant growth. It is argued that roots affect several abiotic and biotic factors, such as oxygen pressure and carbon and nitrogen availability (BOX 1; FIG. 4), which in turn influence nitrogen transformations by soil microorganisms. For example, plant roots were reported to increase denitrification rates in the rhizosphere up to 22-fold[122]. It is generally accepted that roots promote denitrification because they consume oxygen, thereby increasing the anaerobic volume of the soil. The higher carbon availability in the rhizosphere is another important factor stimulating denitrification and emissions of the greenhouse gas N_2O (REF. 123). Unravelling the plant traits that affect microbial guilds involved in nitrogen-cycling emissions holds great potential for breeding new cultivars that could decrease nitrogen losses through leaching and greenhouse gas emissions[124]

Nitrification can be either inhibited or stimulated in the rhizosphere[125,126], which can lead to shifts in the relative amount of ammonium and nitrate available for nitrogen nutrition in the plant. As plant species-specific nitrogen preferences might influence species coexistence and dominance[127], the ability to control nitrification can be an important trait for invasive plants. This was shown by the increased nitrification rates in soil invaded by exotic grasses[128], with doubled rates in Californian soil in part owing to increased abundance and changes in the composition of ammonia-oxidizing bacteria[125]. By contrast, the Australian savannah-invasive species *Andropogon gayanus,* which prefers ammonium over nitrate as a nitrogen source, inhibits nitrification but stimulates ammonification[129]. Modelling the preferences of plants for ammonium versus nitrate indicated that the ability to control nitrification modifies the patterns of coexistence between plants[130]. Some studies reported that invasive plants could also modify denitrification and nitrogen fixation[131,132]. Together, these studies show that the plants can both cause and benefit from altered microbial nitrogen transformation, which is of importance for ecosystem functioning and plant community structure.

Future directions

Ecological engineering and nature restoration. Recent studies on the rhizosphere ecology of non-cultivated plant species has increased awareness that the building of soil structure by soil biota (ecological engineering) and the restoration of former natural ecosystems to conserve biodiversity depend on interactions between plants and soil biota. Traditionally, plant–soil relationships were considered mainly from the perspective of resource utilization and competition. However, it is becoming more evident that pathogenic and mutualistic–symbiotic organisms, and decomposers in the soil biota, influence plant community diversity and succession[133]. Further progress is to be expected if we can understand how rhizosphere interactions influence the legacy effects of soil biotic conditions and how these legacy effects influence priority effects in plant community composition[134]. Thus, current drivers of plant community composition often seem to be reflections of rhizosphere interactions that took place in the previous plant generation or in even earlier generations. A key problem is how these historical events or legacy effects can be managed during nature restoration or ecological engineering. For example, the colonization and proliferation of soil-borne fungi might be a factor in the transition of bacterium-dominated to more fungus-dominated soil food webs, which influences ecosystem responses to drought events[135]. Also, the removal of introduced exotic plant species might not be sufficient to restore the original plant communities of invaded sites, as the exotics might have changed the community composition of soil biota[136] and other soil characteristics[137]. Therefore, enhancing our knowledge of the rhizosphere interactions of successive plant species and of the ecological

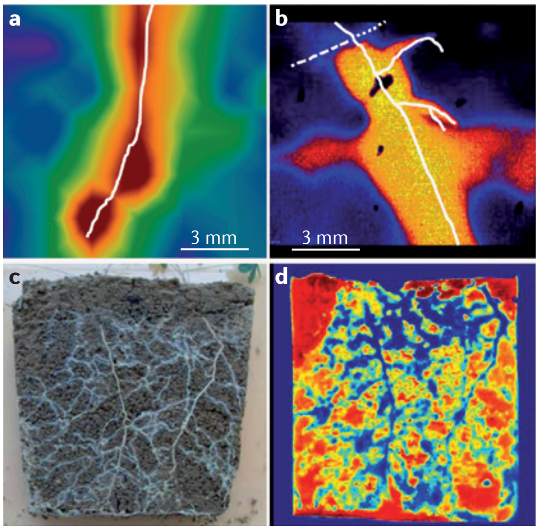


Figure 4 | **Root-induced changes in the rhizosphere.** **a** | Oxygen profiles across a growing root of *Juncus effusus* (in white). **b** | pH profiles across growing roots of intercropped durum wheat (dashed white) and chickpea (solid white). **c**,**d** | Soil rooted by *Lupinus polyphyllus* (part **c**), and the distribution of protease activity in this soil (part **d**). Rhizoboxes were trapezoid in shape, with a height of 13 cm, an upper base of 14 cm and a lower base of 10.5 cm. Images in parta **a**,**b** are reproduced, with permission, from REF. 159 © (2011) Elsevier and REF. 160 © (2013) Annals of Botany Company, respectively. Images in parts **c**,**d** are reproduced, with permission, from REF. 161 © (2013) Elsevier.

Denitrification
A microbial anaerobic respiratory pathway that consists of the sequential reduction of soluble nitrate and nitrite to the nitrogen gases NO, N_2O and N_2.

Nitrification
A two-step aerobic process consisting of the oxidation of ammonia to nitrite, which is carried out by the ammonia-oxidizing betaproteobacteria and thaumarchaeotes, and the subsequent conversion of nitrite to nitrate, which is carried out by nitrite-oxidizing bacteria.

Ecological engineering
The application of ecological principles for sustainable management of ecosystems, including preservation, restoration and creation, to integrate human society with its natural environment for the benefit of both.

consequences during vegetation development is pivotal to making ecological engineering and nature restoration more effective.

Climate change. Climate change can have various consequences, ranging from global warming to local cooling, increased extreme weather events and shifting vegetation zones. All these changes will indirectly affect the microbial ecology of the rhizosphere. The rise of CO_2 levels, a factor that is considered to be a main driver of climate change, can also have direct effects on rhizosphere interactions by altering exudation patterns and by changing soil food web composition and functioning[138–140]. The composition of the soil food web itself can have an important role in mediating the effects of extreme weather events[135]. An unresolved problem here is how rhizosphere communities will respond to the various aspects of climate change. Soil microorganisms might have a greater capacity to evolve than their host plants. Moreover, owing to their enormous biodiversity, soil microorganisms might contain taxa that are adapted to warmer conditions. Furthermore, the dispersal and spread of soil microorganisms might enable immigration of species from warmer climates into locally warming soils. Whether the composition of the rhizosphere microbiota under ongoing climate change depends on dispersal of microorganisms or on their rapid genetic adaptation is still a key question that needs to be addressed to predict whether the rhizosphere communities can keep up with changes induced by global climate change.

Sustainable agriculture. The sustainability of agriculture depends, in part, on reduced inputs of mineral nutrients and pesticides. One major line of research relies on the selection and cultivation of plant genotypes that take advantage of biotic and abiotic soil resources. This corresponds to a major paradigm shift proposed in agroecology, involving a move towards adapting plant genotypes to the environment rather than the environment to plant genotypes, for which high genetic performance (for example, increased yield) relies on high chemical input. This selection can be achieved by engineering plants with a higher level of resistance to pathogens via diverse mechanisms. For example, plants expressing bacterial acyl-homoserine lactonase, an enzyme that degrades quorum sensing molecules[141], and plants with increased production of 5-*O*-glucosyltransferase[142] showed significantly enhanced resistance to *Pectobacterium carotovorum* infection. A possible limitation of this strategy might be a high fitness cost[143]. Another research strategy for enhancing agricultural sustainability is the identification of plant traits that influence either nitrogen losses through leaching or greenhouse gas emissions by soil microorganisms. This knowledge can be used to breed or select new generations of plant cultivars that have the potential to mitigate these emissions[124]. However, our fundamental knowledge of root exudation is still limited, and a better understanding of the chemistry and spatiotemporal exudation patterns involved is needed to identify novel targets for plant breeding and rhizosphere engineering. One major challenge is to take advantage of the substantial fraction of photosynthates released by the plant as rhizodeposits by promoting microbial populations and activities, which could support specific ecosystem services and allow the use of chemical inputs to be decreased.

Bioenergy crops. Recently, various programmes have been launched worldwide to promote a bio-based economy to reduce societal dependence on fossil fuels. There is increasing concern that bioenergy crops might affect several crucial ecosystem services of soil, including greenhouse gas emission, nutrient leaching, and outbreaks of pests and emerging pathogens[144,145]. One of the key questions is whether bioenergy crops indeed have a negative effect on these ecosystem services. Although they are increasing in number, relatively few studies have focused on the rhizosphere ecology of (second generation) bioenergy crops. Metagenomic approaches are being adopted to provide new insights into the various functions that rhizosphere microorganisms carry out in association with a wider range of potential bioenergy crop species[146]. The ultimate challenge of these studies will be to find potential bioenergy crops for specific soils and for other environmental conditions that will enhance soil ecosystem services and functions, instead of deteriorating them. When possible, it might be revealed that, besides trade-offs for biomass production, there are also multiple other benefits that optimize the provisioning of a number of ecosystem services, rather than maximizing only a few.

Concluding remarks

Although considerable progress has been made in our understanding of the microbial ecology of the rhizosphere, rhizosphere ecologists face several major challenges. These include the development of new crops and cropping systems to produce sufficient biomass for food, feed, fibre and bioenergy at low environmental costs. Production methods need to focus on efficient recycling of nutrients and effective control of pests and pathogens. The acknowledgement that above-ground pest control is influenced by below-ground interactions in the rhizosphere has opened up new avenues for integrated crop management. The restoration and conservation of above-ground biodiversity, as well as the prevention of exotic plant invasions, require insight into the microorganisms and fundamental processes in the rhizosphere. Studies using crop-based systems are now increasingly being complemented by studies on non-cultivated plant species, which will provide insight into the rhizosphere ecology of the co-evolved communities in natural ecosystems. In these systems, associated microbial communities play a major part in plant adaptation to biotic and abiotic stresses. This might account for the conservation of rhizodeposit release in all plant species on all continents, when these photosynthates could have been used instead for plant flowering and seed dissemination[147]. Thus, the promotion of plant growth and health by the rhizosphere microbiota balances the cost represented by rhizodeposits. Better knowledge of how plants differ in their patterns of rhizodeposition, both in quality and quantity, according to genotypes, traits, environmental parameters and cropping systems is a challenging field of research to promote beneficial interactions in the rhizosphere. In this context, progress in understanding signalling not only between plants and microorganisms but also among the plants themselves, through microorganisms transporting signalling compounds to neighbouring plants, is another important challenge[148]. Indeed, it was recently demonstrated that the mycorrhizal mycelial network can function as a messaging system, allowing uninfested plants to invoke herbivore defences before attack by aphids[149].

There is a need for integrative studies in soil microbiology, and a holistic consideration of the various mechanisms at play in the rhizosphere would undoubtedly improve prediction and management of the rhizosphere microbiota[150]. Despite these challenges, it is clear that 'going back to the roots' of natural plant communities holds great promise to further improve the sustainability of crop production for food, feed, fibre and fuel.

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Competing interests statement

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