COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Who's who in the plant root microbiome?

Penny R Hirsch & Tim H Mauchline

Metagenomics reveals the identities of the microorganisms that make up the endophytic and rhizosphere microbiomes of the model plant *Arabidopsis thaliana*.

The term 'rhizosphere' was coined by Lorenz Hiltner in 1904 to describe the influence of root exudates on the proliferation of soil microorganisms around and inside roots[1]. Since then, much has been learned about the interactions between soil microorganisms, rhizosphere colonists and plant hosts[2–4]. But to understand more fully how rhizosphere microorganisms are recruited from soil and either benefit or harm plant growth, nutrition and health, we need to compare complete inventories of the microorganisms that are present, which can be problematic as many bacteria, archaea and fungi cannot be cultured. In recent articles in *Nature*, two groups have used metagenomics to describe in the most detail reported to date the core rhizosphere and endophytic microbiomes of the model plant *Arabidopsis thaliana*[5,6].

The 19th century pioneers of microbiology identified the importance of soil bacteria in the major nutrient cycles and were aware of special relationships between plant roots and microorganisms. They studied mycorrhizal fungi and the nitrogen-fixing symbioses of root nodule–inducing rhizobia with legumes and of actinobacteria with various shrubs. Subsequently, the importance of the whole plant microbiome (below and above ground, including seeds) was demonstrated[2–4]. Soil structure constrains bacterial movement, and rhizosphere bacteria are recruited from the immediate vicinity of the root[2], although some are bequeathed from previous generations via the seed. Spatial aspects of the plant microbiome are difficult to resolve, but *in situ* hybridization of roots with fluorescently labeled DNA probes has localized some bacterial groups[5,6]. However, whether microorganisms present in the root microbiome are recruited stochastically from the local soil community or actively through selection by plant exudates remains unclear. Similarly, although some endophytes are beneficial[3,4], the identities and functions of the majority of microbial species that inhabit root intercellular spaces are unknown.

The discrepancy between the numbers of bacterial cells observed by direct microscopy of stained soil samples and of those growing on agar plates has long been recognized. The much less abundant, slow-growing soil archaea have not been detected using agar plate culture, and there are obvious inaccuracies using culture-based methods to assess the abundance of fungal mycelium and spores[7]. It was unclear to soil microbiologists whether the excess bacterial cells are moribund relatives of the community that can be cultured or of different phyla that cannot be cultured. The use of DNA-based and RNA-based metagenomic sequencing for analyzing soil microbial communities *en masse* has revealed that the excess cells most likely represent uncultured bacteria, with the 10^9 cells per gram in typical soil comprising up to 10^6 taxa[7]. Notably, archaeal species and recently described bacterial phyla, including Acidobacteria, Gemmatimonadetes and Verrucomicrobia, are ubiquitous in soil[8].

Lundberg *et al.*[5] and Bulgarelli *et al.*[6] used 454 sequencing (Roche) of 16S rRNA gene amplicons to compare soil, rhizosphere and endophytic bacterial communities (**Fig. 1**) from different *Arabidopsis* genotypes grown in pots under controlled conditions in various soils. Lundberg *et al.*[5] sampled eight *Arabidopsis* lines grown in two soils of pH 5.9–6.4 differing in sand and loam content at two growth stages (one plant per pot), whereas Bulgarelli *et al.*[6] compared two ecotypes in sandy or loamy soils of pH 6.9–7.3 collected in both spring and autumn (nine plants per pot, pooled at sampling). Both groups used at least ten replicate pots for each treatment and used similar methods to extract bacterial DNA and separate the different plant compartments. They collected rhizosphere bacteria (defined as those adhering to the root surface and inhabiting soil up to 1 millimeter from it; **Fig. 1**) before removing the outer root cortex by sonication, which enabled extraction of both endophyte and host-plant DNA. In the two studies the researchers amplified different variable regions of the bacterial 16S rRNA gene, but, as reported previously[8], soil community structure at the phylum level was remarkably similar for all the soil samples from plant-free pots: dominated by Proteobacteria, with Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes and Gemmatimonadetes forming substantial proportions.

In both studies, comparison of species richness in the different compartments required normalization of the number of sequences, which entailed pooling replicates of the endophytic compartments (where bacteria are much less abundant) and using rarefaction to generate 1,000 reads per sample. In both studies, soil type defined the composition of the rhizosphere microbiome, and the endophytes were a smaller, distinct group (50% fewer species identified than in the rhizosphere) dominated by Actinobacteria followed by Proteobacteria; Firmicutes, Bacteroidetes and Cyanobacteria were also present. The families and genera detected have been described previously as effective root colonizers; some, including *Streptomyces*, have been detected in seeds[9]. The relative abundance of sequences identified as *Streptomyces* sp. was surprising but possibly biased by the extraction method as these actinobacteria have robust spores. Bulgarelli *et al.*[6] also investigated the colonization of non-live, exudate-free plant material by inserting wooden sticks into soil. They found that some members of the Proteobacteria and Bacteroidetes colonized both *Arabidopsis* roots and wooden sticks, indicating that the lignocellulose nature of the surface may be more important than root exudates in selecting these particular groups.

Both Lundberg *et al.*[5] and Bulgarelli *et al.*[6] conclude that there is a typical 'core' microbiome for *Arabidopsis* that is recruited from common soil bacteria and selected by the ability of community members to grow in root exudates; a distinct subset of the bacteria that colonize the

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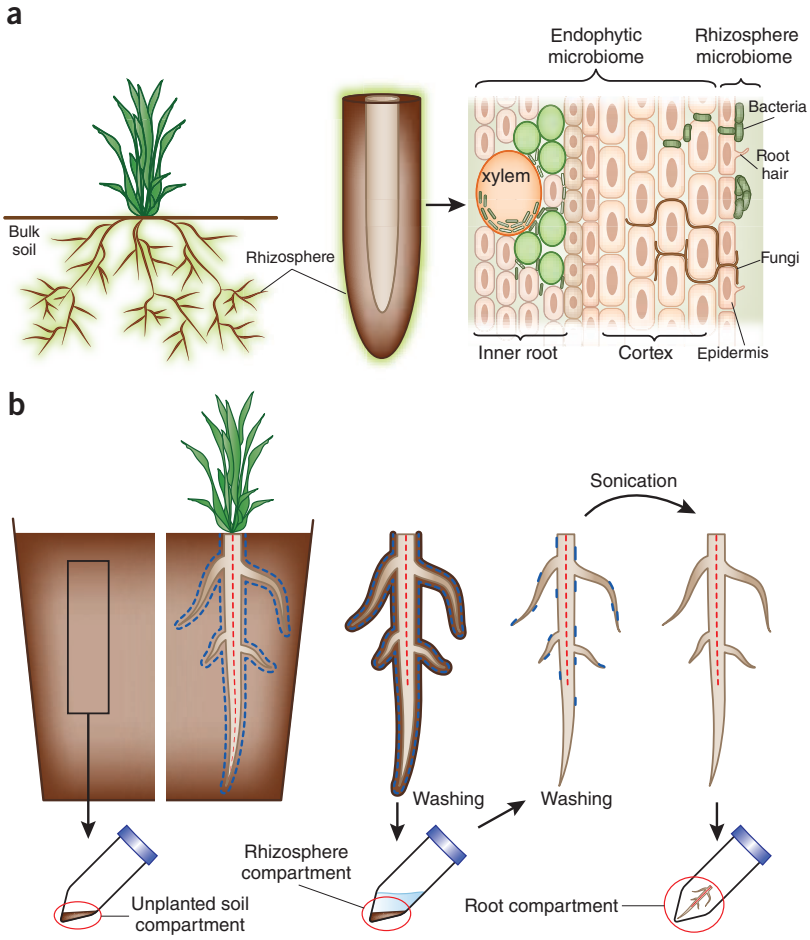

Figure 1 Separating out root-associated microbiomes for metagenomic analyses. (**a**) The rhizosphere microbiome includes bacteria and fungi that are recruited from bulk soil and colonize the root surface. The endophytic microbiome includes species that infiltrate the root cortex and live as endophytes until their release back into the soil upon root senescence. Some microorganisms can colonize the seed. (**b**) The fractionation protocols used in both studies (adapted from ref. 6). Briefly, roots are shaken to remove adherent soil, and rhizosphere microorganisms are collected by washing. Sonication is used to remove epidermal cells, leaving the root compartment, which contains the endophytic microbiome. DNA is extracted from soil, rhizosphere and endophytic compartments for metagenomic sequencing.

root surface can enter the interior of the root and survive as endophytes. As both studies compared only 16S rRNA genes, the relative importance of the ability to grow rapidly in common components of root exudates, catabolize unusual substrates or respond to specific signal molecules can only be partially discerned. However, from a principal coordinate analysis of their sequence data, Lundberg *et al.*[5] concluded that plant genotype and age were less important than soil type in defining the assemblage of species present. Bulgarelli *et al.*[6] estimated that only 60% of root colonists were stimulated by root exudates, with the remainder responding to the lignocellulose surface. To date, no other plant species microbiome has been subjected to such intensive sequencing, so it is difficult to compare the influence of different genotypes in one species with the selective ability of different plant species overall, but many studies attest to gross differences in the composition of exudates and also of rhizosphere communities[2–4].

A recent study of sugar beet grown in soil suppressive or conducive for the fungal pathogen Rhizoctonia[10] revealed similar assemblages of rhizosphere bacteria to those reported in *Arabidopsis*, using a different method (phlyochip microarrays rather than 16S rRNA amplicon sequencing). Although some of the proteobacteria present in disease-suppressive soils produced an antifungal compound, this was only one factor in controlling disease, and the authors concluded that the presence of a complex community was also important in protecting roots. This illustrates the advantage to the plant in attracting and cultivating a diverse rhizosphere community and explains the observation that it is difficult to introduce root-colonizing bacteria and fungi with beneficial properties[2–4]. Unless they are especially fast-growing and aggressive or occupy a specific niche (as do the root-nodule bacteria), they cannot compete with the existing community adapted to the receiving soil, influenced by factors including climate, local mineralogy and soil pH (the major influence on the composition of soil communities)[11].

The work of Lundberg *et al.*[5] and Bulgarelli *et al.*[6] provides the most comprehensive picture to date of the *Arabidopsis* root microbiome and its relationship with the soil microbial community. It also provides a set of protocols for examining the rhizosphere in *Arabidopsis* and other small-rooted plants, which will facilitate future comparisons with other genotypes—for example, to study the interaction of endophytes and plant gene expression. However, full metagenomic sequencing will be required to pinpoint which bacterial genes are selected in surface and internal root colonization of *Arabidopsis*, as well as transcriptomics and proteomics to indicate when and where these genes are expressed. Such studies may reveal the contribution of rhizosphere bacteria to both disease resistance and nutrient cycling in this model plant system. Ultimately, we look forward to the application of these approaches to crop plants, leading to a full understanding of the plant-microorganism-soil system that will enable us to optimize plant health, nutrition and yields in sustainable agriculture.

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