

## REVIEW

# The plant microbiome: The dark and dirty secrets of plant growth

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## Social Impact Statement

Terrestrial life depends on a thin layer of top-soil measuring no more than a few cm to around a meter. This nutrient rich zone supports the growth of plants by providing minerals and water they need for growth and photosynthesis. It is in the dark underworld of soil that plant roots interact with perhaps the most complex microcosm of organisms, from bacteria, archaea and fungi to single-celled animals as well as multicellular animals such as nematodes and earth worms. This soil microbiome profoundly alters plant growth and in turn depends on root secretion and is shaped by plant immunity.

## Summary

This article is about life underground. More specifically it is about the rhizosphere, the zone where plant roots meet the soil. The whole of terrestrial life depends on a few inches of soil. Soil is complicated. The nature of the soil is shaped by the presence of roots. In turn, the microbes in the soil immediately surrounding roots have a profound influence on plant growth and development. The soil microbiome is the community of soil microorganisms. Sampling and analyzing the soil microbiome and the way in which microbes and roots recognize and associate with each other are technically challenging. Here, we discuss new ways to reveal what is happening hidden beneath the soil surface.

## KEYWORDS

biofilms, microbiome, nitrogen fixation, plant growth, rhizosphere, root endosphere

## 1 | RHIZOSPHERE AND ENDOSPHERE

The rhizosphere is the 1 or 2 mm of soils adhering to roots. The endosphere is the name given to the zone that is inside the root itself but not inside the cells of the root. Rhizosphere and endosphere are the two important regions for plant-microbe interaction underground. A large proportion of photosynthate (estimated to be from 2%–4% (Jones et al., 2004) to even 50% (Kuzakov & Domanski, 2000) ends up in the rhizosphere. Roots utilize the sucrose transported from the green above-ground parts to make exudates which act as sources of energy and carbon, allowing the plant to farm the rhizosphere

by engineering the microbiome. The presence of roots enhances the microbial population of the rhizosphere: it is estimated that soil in general contains up to  $10^9$  bacteria per gram and that there is a remarkable ten-fold enrichment of bacterial numbers in the rhizosphere zone (Tkacz et al., 2020; Wang et al., 2020). Interestingly, there appears to be a link between the soil and shoot microbiomes. The microbiome turns over quickly, in about a week or two. It is not clear how the microbiome might get from the soil to the shoot. Perhaps by splashes, or insects, or even by specialized microbes travelling up through the xylem. But at present we do not have an answer.

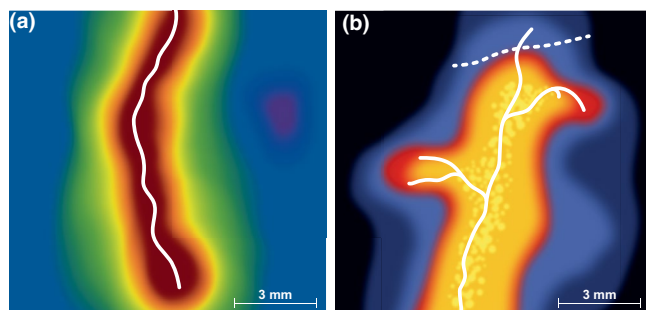
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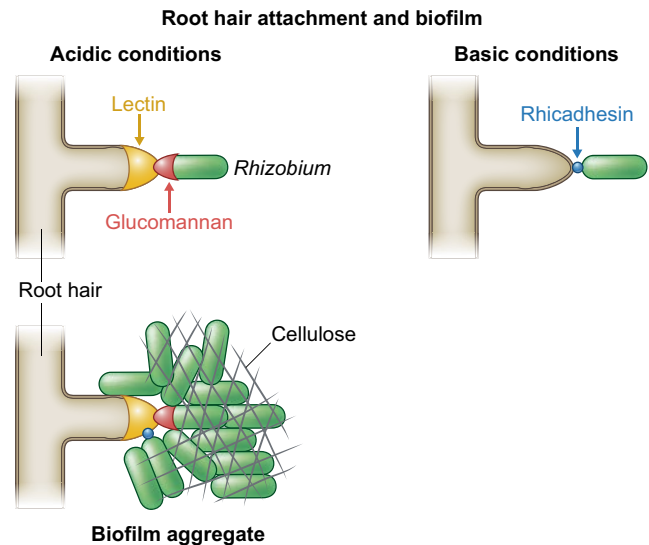
## 1.1 | Cross-talk in the rhizosphere

The complexity of the soil-root association is reflected in the compositional heterogeneity of the rhizosphere in space and time. This is illustrated in Figure 1a, which shows the profile of  $O_2$  across the root and rhizosphere of the common rush, *Juncus effusus*. Roots influence soil pH, as depicted in the rhizosphere of durum wheat (*Triticum durum*) and chickpea (*Cicer arietinum*) in Figure 1b. Plant roots are constantly secreting small molecules, including sugars and vitamins, and complex proteins. The root-soil interactions illustrated in Figure 1 are among the major factors determining the nature of the rhizosphere microbiome.

Bacteria associate with roots as biofilms, aggregations of cells that stick to each other and to surfaces. The capacity of pathogenic bacteria to form biofilms that attach to the cells of food plants can have serious health consequences, exemplified by the Shiga toxin-producing *E. coli* infections linked to alfalfa sprouts occurring in the USA during 2016 (FDA, 2016). As a nitrogen-fixation focused lab we are especially interested in rhizobacterial attachment to plant roots and we will describe it as an example for general bacterial attachment to the surface of other organisms. Figure 2 illustrates the nitrogen-fixing bacterial species *Rhizobium leguminosarum* forming biofilms in vitro and in vivo. Bacteria make proteins and polysaccharides that enable them to stick on roots. Mutants defective in exopolysaccharide production, and wild-type cells in which export of proteins via the *prsDE*-encoded Type I secretion system has been blocked, fail to form biofilms (Russo et al., 2006). Figure 2 presents a model for *R. leguminosarum* attachment and subsequent biofilm formation on legume root hairs. Under acidic conditions, a plant lectin is localized on the root hair tips and binds to the glucomannan polysaccharide expressed at the pole of *R. leguminosarum*. Under basic conditions, the root lectin is solubilized from the root hair tip and an alternative mechanism of attachment occurs. It has been claimed this is due to an extracellular rhizobial protein rhicadhesin, which attaches to the rhizobial cell surface and the root hair in a calcium-dependent manner (Laus et al., 2006). However, this has



**FIGURE 1** Plant interaction with the soil environment. (a) Oxygen profiles across a growing root of *Juncus effusus* (in white); and (b) pH profiles across growing roots of intercropped durum wheat (dashed white) and chickpea (solid white). Warm colors indicate high oxygen and lower pH, respectively. Figure redrawn from Blossfeld et al., 2011 (panel a) and Blossfeld et al., 2013 (panel b)



**FIGURE 2** Root attachment and biofilm formation of *Rhizobium leguminosarum* is pH dependent. Figure redrawn from Downie, 2010

never been proven and the proteins or proteins remain elusive. The bacteria then aggregate on the root hair, forming a biofilm or a cap, a structure that requires cellulose (which is not necessary for the in vitro biofilm).

## 1.2 | Model plants

Plant-microorganism interactions in the rhizosphere are complex. To study them, we need simplified systems. We can use model plants and apply statistical methods similar to those employed by population biologists studying fish and other populations to determine the microbial community structure associated with each individual model plant species.

*Arabidopsis thaliana* is not only a model in plant genetics for its ease in genetic alternation but also in plant-microbe interactions thanks to the availability of natural accessions and engineered mutants. A Genome Wide Association Study (GWAS) based on 196 wild accessions showed that genes involved in plant immunity as well as root and root hair development are mainly responsible for shaping the plant bacterial and fungal microbiome (Bergelson et al., 2019). The role of the plant immune response to soil-borne pathogens is difficult to study due to the fact that rhizosphere bacteria interact both with the plant as well as with the pathogens. Berendsen et al. developed an experimental system of applying a foliar pathogen to study the immune response of *Arabidopsis* in shaping the rhizosphere microbiota. Three strains of *Xanthomonas*, *Stenotrophomonas*, and *Microbacterium* were shown to interact and form a dense biofilm on plant root protecting their host from other soil borne attacks. Crucially, these strains needed their partners, as individually they were unable to establish on roots indicating the presence of a potential quadruple symbiotic interaction

between plant and these strains (Berendsen et al., 2018). One of the possible mechanisms of attracting beneficial microbes is secreting various metabolites as a part of root exudates. For example, the benzoxazinoid breakdown product called MBOA, which can accumulate in cereal rhizospheres can act both on the microbial community and as a signal to trigger plant immunity (Hu et al., 2018). Some microbes have evolved the ability to use plant immunity to their benefit. Some rhizobacteria are able to induce transcription of MYB72, a plant transcription factor regulating secretion of iron-mobilizing coumarins. While the microbial inducers are tolerant to these compounds, some fungal pathogens are highly susceptible. Hence, this interaction is both beneficial for the plant, reducing the pathogen load and for the selected rhizobacteria allowing them to establish on the host root (Stringlis et al., 2018). We are just starting to understand the biosynthesis and the influence on the microbiota of other root secreted compounds. Biosynthetic pathways for three triterpene compounds have been solved and shown that plants lacking the necessary biosynthetic genes select for Bacteroidetes while suppressing Delta-proteobacteria (Huang et al., 2019).

### 1.3 | Soil responses to plant breeding

To place this in a practical context, a project at the National Institute of Agricultural Botany (NIAB) to recapitulate the evolution of modern wheat from its undomesticated progenitors has shown how wheat influences soil microbiome. Modern wheat varieties are highly biased against associating with certain kinds of microbes and nematodes. Plant breeding clearly does not only select the plant, it selects the microbes too, with profound consequences for plant growth (Tkacz et al., 2020). High-throughput DNA sequencing technology has revealed that suppression of certain kinds of soil microbes by modern bread wheat is related to particular parts of the wheat genome. Different plant species have a different proportion of prokaryotic to eukaryotic microorganisms in their rhizospheres. Wild-type oat is associated with a much higher proportion of eukaryotic microorganisms (mostly fungi) than wheat, and the eukaryote soil microbial population accompanying the growth of mutant oat and peas is even greater (Turner et al., 2013).

## 2 | FUNCTIONS OF SOIL MICROORGANISMS

The microbial communities in soils are facilitators of plant processes. For example, they secrete hormones, such as auxins that change root growth. Some microbes take up iron or solubilize phosphorus and make these nutrients available to the plant. Particular non-pathogenic microorganisms may alter plant immune responses, thereby giving protection against pathogens. And, of course, nitrogen fixers provide N for plant growth by converting atmospheric  $N_2$  to  $NH_3$ .

### 2.1 | Antibiotics

Microbes also interact with each other by secreting antibiotics. Antibiotics are usually thought of in a medical context, but soil microorganisms deploy them in order to survive in a highly competitive environment (Cornforth & Foster., 2015). Actinomycetes, ubiquitous bacteria that play important roles in soil ecology, are sources of 70% of clinical antibiotics—a widely used example is streptomycin. A pressing current problem is the issue of antibiotic resistance, a consequence of the scale of use in medicine and animal husbandry. Genes encoding pathways of antibiotic resistance are frequently organized in clusters. In the case of Actinomycetes, the genome very often contains 16 or 17 resistance clusters but maybe only one is observed to be expressed at any given time. Antibiotic resistance is probably active at very particular points during colonization of roots and soil and only when certain other microorganisms are present. For this reason, resistance to antibiotic molecules has not built up in natural ecosystems, even over timescales of hundreds of millions of years. The organisms that make antibiotics must always have a resistance mechanism, otherwise they will kill themselves. Resistance develops when antibiotics are over-used, stimulating resistance gene clusters to get moved around between organisms by horizontal DNA transfer.

### 2.2 | Suppressive soils

Some soils are suppressive; that is, they antagonize (i.e. suppress) the development of plant diseases. In sugar beet rhizosphere suppressiveness is accompanied by an increased abundance of Burkholderia (Mendes et al., 2011). Moreover root colonizing Bacteroidetes strains were shown to actively fight the pathogenic fungus by already being inside the root, which suggests a new frontier for plant immunity (Carrión et al., 2019).

When wheat has been planted in soil where it has not been grown for some time, farmers often observe the first harvest to be good, the second to be poor and the third to be worse still; but if sowing in the same soil persists, by the 9th or 10th harvest the wheat can be yielding well again. Soil microbiome-mediated suppressiveness is known to reduce take-all disease caused by the wheat root pathogen *Gaeumannomyces graminis* var. *tritici*, even in the presence of the pathogen, a susceptible host and a favorable environment (Schlatter et al., 2017). “Good”, antibiotic-producing, microbes such as Pseudomonads and Actinobacteria progressively build up and the soil becomes suppressive. The microbiome is changing over time because plants are somehow able to select the rhizosphere microorganisms that antagonize pathogen growth.

## 3 | THE RHIZOBIUM-LEGUME SYMBIOSIS

Nitrogen, together with phosphorus, is the main limiter of plant growth. Global agricultural production is absolutely dependent on

fertilizer N. An estimated 50% of N in food comes from the Haber-Bosch process, which uses fossil fuel energy to convert atmospheric  $N_2$  into  $NH_3$ . So great is the requirement of N for crop growth that there are pipelines in the mid-west of the United States delivering  $NH_3$  direct from sites of Haber-Bosch manufacture to farmland every day. A serious environmental health concern arising from agriculture's demand for fertilizer is the excessive N that ends up in drinking water in crop-growing regions and leaches into waterways and coastal zones, causing excessive algal growth and depletion of  $O_2$ . There is, however, an alternative and possibly more sustainable biological source of N: 50%–60% of nitrogen in the biosphere comes from N fixation by rhizobium bacteria in legume root nodules. The one unusual example is the non-legume tropical tree *Parasponia* that forms nodules with rhizobia (Santi et al., 2013). However, a number of non-legume species also fix nitrogen through symbiosis with Actinobacteria (Gtari et al., 2012). There is an opportunity here to use comparative genomics to look at the evolution of nodulation (Griesmann et al., 2018; van Velzen et al., 2018; Werner et al., 2014). It will enable us to identify the plant genes needed specifically for nodulation, which is important if the long-standing dream of engineering nitrogen-fixing wheat is ever to be realized (Charpentier & Oldroyd, 2010).

### 3.1 | Nitrogen fixation and plant productivity

The nodule is a specialized root structure occupied by N-fixing bacteroids. It consists of a gradient of cells at different stages of development within which four zones may be recognized: a meristematic region of active cell division; a zone where cells become infected with rhizobia and differentiate; the main nitrogen-fixing tissue, where  $N_2$  is converted to  $NH_3$  by the enzyme nitrogenase; and finally a zone of senescence. The red color of the nodule is due to leghemoglobin, which is structurally similar to the hemoglobin of blood but which has arisen independently by convergent evolution. The function of leghemoglobin is to maintain oxygen tension in the nodule at a level that sustains the rhizobia, which are obligate aerobes, but which prevents  $O_2$  from inhibiting the oxygen-sensitive nitrogenase reaction.

### 3.2 | Signaling between roots and *Rhizobia*

Bacteria move towards roots by chemotaxis. Roots are producing chemicals all the time and bacteria will swim up gradients of compounds they find attractive. Flavonoids are among the most important of these signaling chemicals. *Rhizobia* respond by producing their own signaling molecules (i.e. lipochitinosaccharides or LCOs) which in turn induce the plant to make nodules.

Early responses to the exchange of signals include transfer of genetic elements around the bacterial population. Such a process of trading plasmids is another factor in antibiotic resistance. Subsequently bacteria stick to roots, by specific molecular mechanisms and form a biofilm. Questions arise from this picture of

plant-microbe interaction. Where do bacteria appear from to stick to root hairs; is it the soil or the root surface? How are biofilms and nodules formed? To address these matters requires a range of experimental approaches. For example, the same bacteria could be presented to different plants and gene expression observed (Ramachandran et al., 2011). Which genes are expressed? What genes are specific for the host, which are specific for legumes and which are non-specific?

### 3.3 | Lighting up the legume root system

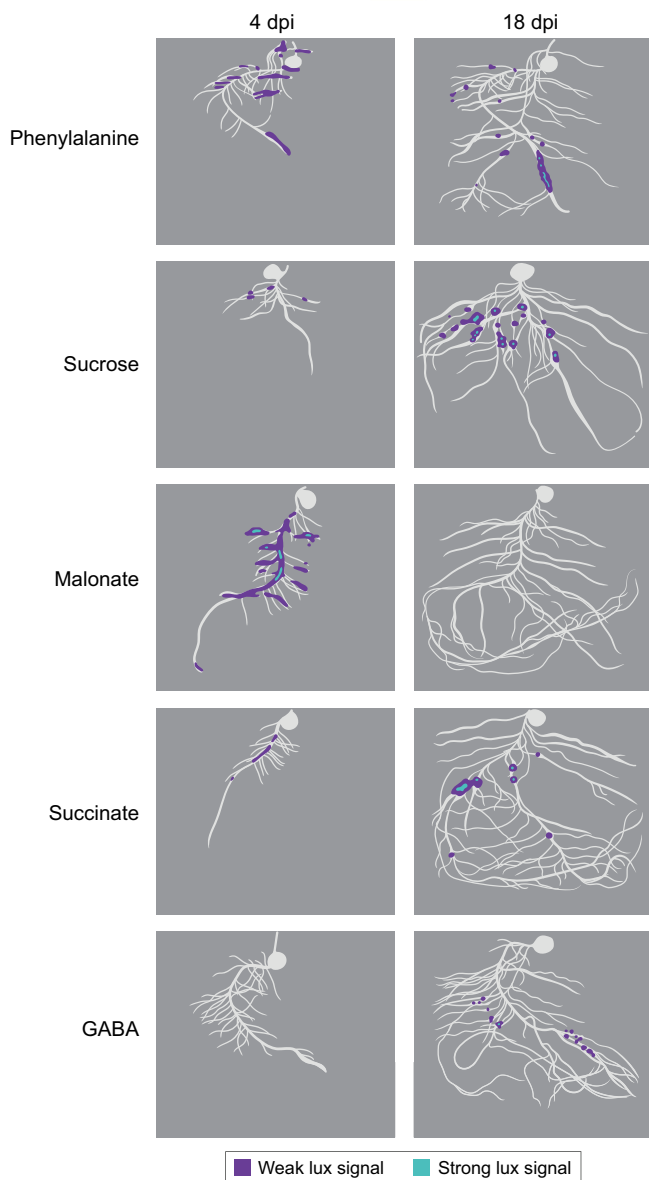
The production and uptake of chemical signals requires high-specificity transport systems. There is a great variety and species distribution of rhizosphere transporters (Mauchline et al., 2006; Ramachandran et al., 2011). These studies have revealed that tartrate utilization may be particularly important in legume rhizospheres. They also suggest that transporters can be markers for what bacteria are seeing in their environment and may be exploited for lighting up the root system.

Rhizobia have been engineered to glow in response to the compounds made by pea roots. Roots inoculated with such rhizobia produce light and allow secretion of different root compounds to be mapped in space and time (Pini et al., 2017; Figure 3). Peas begin to form nodules at about 12 days post-inoculation (dpi). Malonate is released by roots into the rhizosphere early on, giving a strong signal 4 dpi. Sucrose, delivered through the phloem to the root from the shoot, is metabolized via the TCA cycle and is a major energy source for N fixation. It, like succinate and GABA, gives a strong signal at 18 dpi inside  $N_2$ -fixing nodules (but not in the rhizosphere), later than phenylalanine. We would also like to observe how this secretion pattern changes in presence of other bacteria and especially a native microbial community. In contrast to other systems, where plants need to be uprooted, soil medium collected and analyzed chemically to gain an insight into the root secretion accumulation, Lux-based analysis allows for non-destructive semi-quantitative measurement of root secretion one compound at a time. Moreover in the typical chemical systems, microbes present in the soil quickly utilize the majority of the secreted compounds and hence their soil accumulation is probably skewed towards less metabolically active compounds.

The light production system in *Rhizobia* can be linked to nitrogenase so that when bacteria make the enzyme they light up (Mendoza-Suárez et al., 2020). In this way effective strains that are good at fixing N can be selected, for use with crops. There are programs underway in the UK, USA and Africa to exploit this technology, and some improved bacterial strains are currently undergoing field trials.

## 4 | IMPLICATIONS FOR AGRICULTURE

Studying microbiomes holistically rather than one microbe at a time (using tracking systems, for example) has implications for agriculture. Most crop plants have been selected under high nutrient conditions.



**FIGURE 3** Using light for mapping root secretion. Illustrations of pea plants at 4- and 18-days post inoculation with bacterial symbiont (*Rhizobium leguminosarum* bv. *viciae* 3,841) carrying Lux cassette driven by a corresponding promotor induced by the tested compounds. Figure redrawn from Pini et al. 2017

In terms of environmental sustainability, it makes sense to select for plants that use nutrients efficiently and associate well with their microbiomes. Consider the case of wheat. The wheat D genome confers strong interaction with mycorrhizal fungi. Backcrossing to modern wheat varieties is associated with a fall in mycorrhizal interaction. Thus, breeding modern varieties has inadvertently selected against mycorrhizal fungi (and against nematodes, which can be pathogenic) (Tkacz et al., 2020). The new methods for studying the soil microbiome can reveal these changes where before they would have been invisible and incidental. Biotechnology companies are investing greatly in N-fixing bacteria and other beneficial microbiome organisms, to make inocula that will improve yield.

## AUTHOR CONTRIBUTIONS

PP and AT substantially contributed to the discussion of content, PP wrote the initial version of the article while AT reviewed and edited the manuscript before and after the submission.

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