

# Plant microbiomes and sustainable agriculture

*Deciphering the plant microbiome and its role in nutrient supply and plant immunity has great potential to reduce the use of fertilizers and biocides in agriculture*

Philip Hunter

Bacteria had been around long before multi-cellular eukaryotes evolved and quickly began to colonize these new life forms as another suitable habitat and potential source of nutrients. Nearly all higher organisms across taxa now have bacterial communities living on and within them—from mutually beneficial symbioses to antagonistic parasitic conflicts. They have co-evolved with their microbiota and come to depend on it for a range of functions in immunity, metabolism and nutrient absorption. In humans, the microbiota, especially bacteria in the gut, play important roles in all areas, by breaking down indigestible polysaccharides, providing vitamins, mediating nutrient absorption in the gut and helping train the immune system. While research on the human microbiome has stolen the limelight, plant scientists have also been making progress towards elucidating the composition and function of plant microbiomes. Their work has great potential for the future of humanity, as it could help to establish more sustainable agriculture with less dependence on fertilizers, pesticides and herbicides, while increasing yield and nutrient content.

Research on the plant microbiome actually predates the animal and human counterparts because microorganisms play an obvious and crucial role in fixing nutrients for the plant, especially nitrogen and phosphorus. Given their vital importance for protein and DNA synthesis and energy production, many plants have evolved close symbiotic relationships with soil bacteria and fungi that supply them with both elements in convenient forms with

minimum energy expenditure. Indeed, the close relationships with two specific families of microorganisms set apart the plant microbiome from its animal counterpart: rhizobia nitrogen-fixing bacteria and mycorrhizal fungi, which greatly enhance the ability of roots to extract various nutrients from the soil. Neither group can survive without the host plant that in return supplies oxygen and products of photosynthesis, mainly proteins and carbohydrates.

## A three-way relationship

The relationship with mycorrhizal fungi seems to be the closest and oldest, and involves more than 80% of terrestrial plants, according to Euan James from the Ecological Sciences Group at The James Hutton Institute of Ecological Sciences in Aberdeen, UK. Evidence from molecular clock sequence analysis shows that the relationship first arose about 1 billion years ago with green algae and then facilitated plants' colonization of the land around 300 million years later by providing a ready source of soil nutrients [1]. The fungi increase the effective surface area of plant roots 100–1,000 times by building out extended filament networks which release enzymes that dissolve tightly bound nutrients including organic nitrogen, iron and especially phosphorus.

Subsequently, rhizobia evolved in tandem with plants, especially legumes, to fix nitrogen directly from the air and so help the plants spread to soils with low levels of organic forms of the element. In return, these bacteria depend on the host not just

for carbon-based compounds but actually some of the genes required for the nitrogen fixation itself; their relationship with the host is now virtually as inter-dependent as that of mycorrhizal fungi.

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In fact, legumes evolved a three-way symbiosis with rhizobia and mycorrhiza as the most efficient way of obtaining nutrients. “The ‘tripartite’ symbioses between legumes, rhizobia and mycos are very common in natural ecosystems”, James said. “The nitrogen fixing symbiosis is highly energy demanding, and so requires a lot of phosphorus for ATP. This phosphorus is often supplied by mycos”. James noted that although mycorrhiza enhance the ability of plant roots to uptake or scavenge phosphorus from soils, they also help them take up many other minerals, including nitrogen. The key point though is that only the bacteria can fix nitrogen from the air, which gives the plants scope to colonize a wider range of soils.

James also noted that while these relationships are usually symbiotic, they can also turn parasitic. “This can occur if the

two partners are ‘incompatible’ and/or if, in the case of rhizobia, they are ‘cheaters’, such that they gain access to the legume nodule, but do not fix any nitrogen”, he explained. So far this has mostly been demonstrated in the laboratory only, without clear evidence whether this occurs extensively under cultivation in the field or is a significant cause of disease in plants.

### Plant immunity

Science is also making progress in understanding the role of the plant microbiome in immunity even if it is not as clear-cut as the role of mycorrhiza and rhizobia in nutrient fixation in plants, or that of enteric microbiota in animals. “Relatively speaking, very little is known about how the endogenous microbiome collectively interacts with plant immunity in a community context”, commented Sheng Yang He, head of a group studying disease susceptibility in plants at Michigan State University in the USA. “In mammals, the use of germ-free mice and other gnotobiotic systems reveals that endogenous microbiota play important roles in many host processes, including the maturation of host immunity. It is not well understood whether a microbiome-mediated immune maturation process also occurs in plants”.

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He’s team has devised a system called FlowPot (<http://www.phytobiomes.org/activities/Documents/2015PhytobiomesAbstractBooklet.pdf>), a sterile soil for subsequent microbial colonization under controlled conditions. “Using FlowPot, we initially grew microbe-free *Arabidopsis* in parallel with *Arabidopsis* colonized by several microbial communities from distinct soils across North America”, said James Kremer, a researcher in He’s team and inventor of the FlowPot system. “When we performed RNA sequencing to examine global host gene expression, it was clear that microbe-free plants have an abnormally low level of many defense-associated transcripts, compared to

microbiome-colonized plants, irrespective of microbiome community composition. Furthermore, microbe-free plants are compromised in many important immune responses, such as defense marker gene expression, cell wall reinforcement, oxidative burst and MAPK phosphorylation cascades. Essentially, we can conclude that the presence of a normal endogenous microbiota is necessary for the development of immunocompetence, better equipping the host plant to recognize and respond to pathogens”. The results suggests that the plant microbiome may play a similar role to its mammalian counterpart in helping the immune system to adapt, although more research is needed on both fronts to elucidate the underlying mechanisms and pathways. At the moment, understanding is more at the level of correlation than causation.

The functional overlaps between plant and animal microbiomes has prompted research to compare the two, and one of the most comprehensive studies concluded that the two have relatively little in common in terms of composition, except between fish and plants [2]. It found that there was no overlap of abundant bacterial taxa between the microbiota of the mammalian gut and plant roots, whereas taxa do overlap between fish gut and plant root communities. While the reasons for the striking differences in microbiota’s composition in independently evolved, yet functionally related, organs in plants and animals, are unclear, the study suggested that this may reflect the very different start inoculum to which plants and animals are exposed, along with niche-specific factors such as oxygen levels, temperature, pH and organic carbon availability. Indeed, it ultimately reflects the fundamental differences between plants’ and animals’ experience. Plants are rooted to the spot and so utterly depend on their immediate environment, while animals move around which exposes them to a larger variety of microbes in a wider ecosystem.

### Environmental factors

For animals, the principle source of microbes is the foot they eat, while for plants it is the soil in which they are rooted. Most microorganisms thus enter via the rhizosphere, the area of the roots, and migrate to the other regions of the plant: the

phyllosphere, comprising the plant’s aerial surfaces, and the endosphere, the internal tissues. The roots, comprising root exudes and the thick grey polysaccharide mucilage produced by all plants, is a region of rich microbial diversity and slowly changes over time, whereas the phyllosphere is relatively nutrient poor and subject to extremes of temperature, light and moisture. The endosphere is somewhere in between and the region where the plant has the most direct control over its microbiome. Even so, given that the rhizosphere predominates, only about 5% of the microbiome’s composition can be accounted for by the plant itself, commented Klaus Schlaeppli, Project Leader at the Institute for Sustainability Sciences at the Federal Research Institute Agroscope in Zurich, Switzerland. “The effect of the plant genotype is an absolute maximum of 10%”, he said. “It’s even the environment which defines the compartments most of the microbes inhabit, whether in the soil, or close to the roots, or on the root surface, or up in the plant. It’s predominantly the physical and chemical properties of the micro-habitat which are the primary determinants for the microbial surface. The habitat determines the stresses and conditions for the microbes”.

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This is not the end of the story though, because the exchange of microbes between soil and plant is not a one-way street. The plant can control the composition of its microbiome through emissions into the soil, including anti-microbial compounds and nutrients. This is less effective in sparsely populated areas, such as desert scrubland or arctic tundra, although even there the immediate microhabitat is determined by the decaying organic material from plants. But in more densely populated areas, such as forests or grasslands, soil composition will be mediated by multiple plant varieties and so would not tend to correlate with any specific plant genome. But it leads to the

idea that not just the plant's microbiome but also the immediate soil environment can be regarded as an extended phenotype that is subject to natural selection and that plays an intimate part in key plant phenotypes.

Some evidence for this view has come from several studies associating the microbiome with various aspects of flowering, especially timing and biomass. One study developed a system for comparing multiple generations of *Arabidopsis thaliana* Col, observing flowering times and the associated microbiome composition [3]. After the 10th generation, the microbiomes that were now clearly associated with early and late flowering were used to inoculate soils in which three additional *A. thaliana* genotypes had been planted, along with a related crucifer, *Brassica rapa*. With the exception of one variety, all plants shifted their time of flowering, which was earlier when inoculated with the early-flowering microbiome and vice versa.

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Furthermore, plants grown with late-flowering-associated microbiomes produced flowers with greater biomass and, in one case, had greater total biomass. This increase was correlated with a two- to five-fold enhancement of microbial extracellular enzyme activities associated with nitrogen mineralization in soils. The authors concluded that this reproducibility of flowering phenotype across plant hosts suggested that plant microbiomes can be subject to selection by the plant host and even to mediate the resources available in the soil. The implication is that the wider extended genotype—comprising the plant and surrounding microbial genomes—is subject to mutual selection, which, to a large extent, determines the soil's composition.

#### The crucial role of soil

This is highly relevant for the phenomenon known as disease-suppressive soils, where plants suffer less from specific soilborne

pathogens than elsewhere owing to the activity of certain microorganisms in the vicinity of their roots. This has not yet been widely studied despite the obvious potential for conferring disease resistance to a field through inoculation. As a result, the microbes and mechanisms involved in such pathogen suppression are largely unknown, but one study identified some of the bacterial taxa and genes involved in suppressing a particular fungal root pathogen [4]. The study analysed more than 33,000 bacterial and archaeal species in total and found that *Proteobacteria*, *Firmicutes* and *Actinobacteria* were consistently associated with disease suppression. Members of the  $\gamma$ -*Proteobacteria* family were shown to have disease-suppressive activity governed by non-ribosomal peptide synthetases. These are produced by bacteria and fungi and known as secondary metabolites that are not directly involved in the organism's growth, development or reproduction.

The authors argue that their data indicate that plants can almost deliberately exploit microbial consortia in the soil for protection against fungal root infections. Schlaeppi suggested that such disease suppression can build up in soils planted with monocultures after the ecosystem was repeatedly exposed to the relevant pathogen—almost like adaptive immunity in vertebrates. “But that does not occur in all soils, so there must be some cue that makes this occur in some but not others”, he said. The mechanisms that enable such resistance to develop are yet unknown, but it seems as if the microbes conferring the suppression have themselves adapted to the presence of the pathogen that proliferated as a result of the crop being planted. The secondary metabolites produced by those microbes are then available to, or recruited by, the plant for defence against that particular pathogen.

Naturally, such research into the microbiome generally should not just help to combat disease but also enhance growth and potentially nutrient content. But, as Schlaeppi noted, there is a problem in that benefits occurring under laboratory conditions have to date not been replicated in the field where the environment is more complex. “We know from study of individual microbes that there is enormous potential to support plant growth or health”, Schlaeppi said. “But most of that work is from binary or mono associations, where we have one strain in a sterilised soil that gives

fantastic effects. But when we do it in a native soil or field, many of those benefits disappear”.

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One reason might be that inoculating a native soil with a supposedly beneficial microbe disturbs the equilibrium between the existing microbe population. The focus now is therefore on manipulating the whole microbiome of a field rather than targeting individual pathogens. “Conceptually I liken that to personalized medicine in humans”, Schlaeppi commented. “You would pre-diagnose the soil microbiome and then target the inoculation of the organism to that particular field”.

#### Applications for sustainable agriculture

Such work has yet to deliver in the field so to speak, but there may be more immediate applications at a different level to exploit the well-understood three-way symbiosis between plants, rhizobia bacteria and mycorrhizal fungi. These symbioses are widespread in legumes but do not occur in most cereal crops although the underlying signalling pathways still exist. If cereals could therefore be engineered to produce the relevant components that enable these symbioses to develop—particularly the root nodules for mycorrhizal fungi and rhizobia bacteria—they should be able to obtain nitrogen and phosphorus without fertilizer. Research to understand these signalling mechanisms with beneficial microorganisms and apply this information to transfer the nitrogen-fixing capability from legumes to cereal crops is being conducted under Giles Oldroyd at the John Innes Centre at Norwich in the UK. According to Oldroyd, the main focus at present is helping small-holder farmers in sub-Saharan Africa who cannot afford fertilizer and would benefit greatly from automatic nitrogen fixing, but the work has clear potential for application globally. It could also help win over a public who

remain opposed to genetic modification, given that in this case, the objective would be merely to restore lost capabilities rather than add novel ones.

Indeed, one important focus of applied work on plant microbiomes is in helping cereal crops and other commercial plants to exploit beneficial microbes in the soil, as many commercial varieties have lost this capability as a result of extensive use of pesticides and fertilizers. “One route is to identify and breed in the old traits where plant lines had the capacity to interact with

such beneficials”, Schlaeppli explained. “That would be one avenue towards a more sustainable agriculture”.

### References

1. Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB (2001) Molecular evidence for the early colonization of land by fungi and plants. *Science* 293: 1129–1133
2. Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R *et al* (2015) Microbiota and host nutrition across plant and animal kingdoms. *Cell Host Microbe* 17: 603–616
3. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J* 9: 980–998
4. Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PA *et al* (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332: 1097–1100