

# Role of the root microbiota in plant productivity

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## Statement of novelty

Soil microorganisms are essential for maintaining and increasing plant productivity. Future prospects are bright for the use of bespoke rhizosphere and root endophytic microbiota to control pathogens and plant health.

## Keywords

rhizosphere, microbiota, plant productivity, metagenomics, plant-microbe interactions, endophytes

## Abstract

The growing human population requires increasing amounts of food but modern agriculture has limited possibilities for increasing yields. New crop varieties may be bred to have increase yields and be more resistant to environmental stress and pests. However, they still require fertilization to supplement essential nutrients that are normally limited in the soil. Soil microorganisms present an opportunity to reduce the requirement for inorganic fertilization in agriculture.

Microorganisms due to their enormous genetic pool are also a potential source of biochemical reactions that recycle essential nutrients for plant growth. Microbes that associate with plants can be considered to part of the plants pan-genome. Therefore it is essential for us to understand microbial community structure and their “metagenome” and how different soil types and crop varieties influence it. In the future we may be able to modify and better utilize the soil microbiota potential for promoting plant growth.

## Introduction

The human population has grown 7-fold since the beginning of the XIX century (Speidel et al., 2009). This has lead to the planets natural resources being over-exploited, with a massive biodiversity loss, climate change and disturbance of the nitrogen cycle (Rockstrom et al., 2009). Biodiversity reduction and climate change have become major issues for social and political consideration (Lenton, 2011). However, the disturbance to the nitrogen cycle is a global problem that requires closer attention. While the food demand of a growing human population has so far been met by increased crop yields (Godfray et al., 2010), this agricultural revolution has had a massive impact on the global biogeochemical cycle of nitrogen. Addition of nitrogen fertilizers, is now estimated at  $\sim 10^{11}$  kg annum<sup>-1</sup> (Glass, 2003; Schmer et al., 2014). However, as around 60% of the synthesised nitrogen fertilizer is not absorbed by plants, most of it leaches into groundwater. Nitrogen is normally one of the limiting nutrients for

34 cyanobacterial and algal blooms and once released into groundwater it migrates  
35 into seas causing dramatic changes in marine microbial populations, effecting the  
36 whole marine food chain (Conley, 2012). Fertilizers are normally over-used in  
37 developed countries and plants are able to reach their current yield potential.  
38 However, developing countries have to substantially improve their yield per  
39 hectare (Mueller et al., 2012). This is why it is crucial to understand how can we  
40 improve plant growth with reduced dependency on expensive and  
41 environmentally harmful synthetic fertilizers.

42 In the optimistic scenario that crop yield per hectare will double by 2050 (Ray et  
43 al., 2013), it will still not be enough to feed a growing population demanding  
44 more animal based food in their diets (Robinson et al., 2014). Even assuming this  
45 optimistic scenario some sacrifices in the land coverage of natural habitats will  
46 have to be made. The best known example of on-going deforestation is the  
47 Amazon basin, where there are infrastructure (Fraser, 2014), urbanization and  
48 agricultural stresses on the forest (Ellis et al., 2013). This region is critical to  
49 climate change and recently a lot has been proposed and introduced to stop  
50 these negative processes (Galford et al., 2013). However, deforestation is a  
51 temporary solution for increasing crop production in countries like Indonesia,  
52 Malaysia, Paraguay, Bolivia, Zambia and Angola with the overall loss of the forest  
53 estimated at 1.5 million km<sup>2</sup> since the beginning of the XXI century (Hansen et al.,  
54 2013).

55 It is important that we are able to increase yields from land that has already been  
56 converted into fields. One of the most sustainable ways to achieve this is to focus  
57 research on the natural abilities of plants to increase yields. More than 100 years  
58 ago it was noticed that the soil around the plant roots is extremely rich with  
59 microbes and the term rhizosphere was coined (Hartmann, 2008). These  
60 microbes were extensively studied for their role in the plant health and with  
61 increasing understanding of the processes that take place in the rhizosphere we  
62 may start to utilize these relations to increase plant growth in an  
63 environmentally sustainable way. Harnessing the ability of microbes to provide  
64 plants with essential micro and macro-nutrients is an important goal of  
65 rhizosphere plant-microbe studies. In this review we consider selected features  
66 of these interactions with a focus on nitrogen-fixation as well as phosphorous

and iron sequestration by soil microbes. We present the current understanding of microbial community structure and how environmental factors and plant hosts shape this. Finally, we consider future directions in the field and the possibilities for better understanding and use of the large soil microbiota.

## Plant – mycorrhizal fungi interactions

The limiting factors for plant growth are often phosphorus and nitrogen, and to a lesser extent iron. These are nutrients that plants are able to obtain from soil either directly or by “using” microorganisms as fixers or “soil scavengers”. Perhaps the most ubiquitous and important examples of this in the mutualistic interaction between mycorrhizal fungi and plant roots, which is particularly important in providing water and phosphorous for the plant host in exchange for carbon for the fungus (Augé, 2001). Phosphorous often limits plant growth even though abundant in soil because it is normally bound with aluminum and iron (forming strengite and varescite) or with calcium (forming apatite) in acidic or alkaline conditions, respectively. Plants require phosphate in a soluble form, either as  $\text{H}_2\text{PO}_4^-$  or  $\text{HPO}_4^-$  (Schachtman et al., 1998). Some bacteria release organic acids able to chelate the cations bound to phosphate, thus releasing it into soil (Vassilev et al., 2006). However, this is insufficient for plants to obtain all the necessary phosphate (especially in acidic soils) making its acquisition via mycorrhizal fungi particularly important (Smith et al., 2003).

Mycorrhiza evolved during the Early Devonian period (Pirozynski and Malloch, 1975). The early occurrence of this relationship is well documented in the fossil record, such as in the sedimentary rocks of the Rhynie Chert in Scotland (Krings et al., 2007), in paleobotanical data (Berbee and Taylor, 2007) and by phylogenetic analysis based on DNA sequencing (James et al., 2006). It is estimated that ~300,000 plant species have been found to interact with arbuscular mycorrhizal (AM) fungi (Bouwmeester et al., 2007). AM fungi thrive in soil as spores until they detect a plant. They germinate and release hyphae through the soil in search of a host plant root with hyphal branching stimulated

in response to plant strigolactones (Akiyama et al., 2005). After contact with the plant, fungi form appressoria, through which they gain an access to the intracellular space of the root using LCO signals (sulphated and non-sulphated lipochitooligosaccharides) (Akiyama et al., 2005; Maillet et al., 2011). Ultimately the fungus form branched hyphae (arbuscules) inside cortical cells (Harrison, 2005), where they are surrounded by the plant plasma membrane. Plants supply the hyphae with a carbon source and in turn receive phosphate (Harrison et al., 2002).

The AM fungi – plant host cross-talk is similar to nodulation and many steps are conserved in what has been termed the symbiotic common pathway (SYM pathway) (Capoen and Oldroyd, 2008; Gutjahr and Parniske, 2013; Oldroyd, 2013). There must be an initial specific recognition of mycorrhiza or rhizobia but both pathways then generate calcium ion oscillations in and around the plant cell nucleus that are decoded by a calcium- and calmodulin-dependent kinase CCaMK, with subsequent steps specific for nodulation or mycorrhization (Oldroyd et al., 2005).

The *ram1* (Required for Arbuscular Mycorrhization) gene encodes a mycorrhizal specific GRAS-domain transcription factor (GRAS stands for GIBBERELLIC-ACID INSENSITIVE (GAI), REPRESSOR of GAI (RGA) and SCARECROW (SCR)). RAM1 regulates the expression of the mycorrhization specific *ram2* (Gobbato et al., 2012), which encodes a GPAT protein (glycerol-3-phosphate acyl transferase), involved in cutin and suberin biosynthesis. Cutin was suggested to be involved in signalling role in enhancing fungal appressoria formation. Moreover, it was found that addition of C16:0 monomer (one of the building blocks of cutin) to the *ram2* mutant rescued mycorrhization (Wang et al., 2012). Mutation in either of these genes causes the plant to be impaired in mycorrhization but does not interfere with nodulation. Oomycetes also use part of the SYM pathway in order to infect plants, suggesting this pathway is widely used to “communicate” with the soil microbiota.

Nodulation as a plant solution for nitrogen deficiency

Some bacteria belonging to the order Rhizobiales of the Alphaproteobacteria as well as some members of Betaproteobacteria subphylum (predominantly Burkholderiales) form nodules on leguminous plant roots, inside which they convert atmospheric N<sub>2</sub> into plant-available NH<sub>3</sub> and in return for carbon compounds released by the plant (Gyaneshwar et al., 2011; Oldroyd et al., 2011). There is also a distinct group of actinorrhizal plants, such as Alder and Casuarina that form nodules in association with N-fixing actinobacteria of the genus *Frankia*. However, many bacteria also exist as free-living bacteria in the soil or endophytes in roots (e.g. Azotobacteraceae, Cyanobacteria) and some of these may fix significant amounts of N<sub>2</sub> (reviewed in (Turner et al., 2013a). Legume nodulation appeared for the first time approximately 100 million years ago (Doyle, 2011), which is more than 300 million years later than mycorrhizal infection suggesting nodulation is a modification of the mycorrhizal pathway.

The ubiquity of the common SYM pathway in plant – microbe interactions begs the question of whether all soil microorganisms (both symbionts and pathogens) use it to gain entrance into inter- and intracellular root compartments? Oomycetes use the SYM pathway to gain entry into the plant and cause diseases (Wang et al., 2012). However, rice mutants defective in the SYM pathway show that at least some endophytic bacteria such as *R. leguminosarum* *bv. trifolii* can still colonize plant roots (Chen and Zhu, 2013), suggesting that the SYM pathway is not the only pathway for microorganisms to colonize plants. Plants may also be able to detect specific pathogen using the SYM pathway. The Nod Factor Receptor (NFR) in *Medicago truncatula* is important in the plant immune response against fungal and oomycetes infection (Rey et al., 2013).

Growing interest in plant endophytes ability to fix nitrogen

Nodulation is a highly effective method of nitrogen assimilation and has been reviewed extensively (Oldroyd et al., 2011; Udvardi and Poole, 2013). However, (see chapter 13), it is restricted to a subset of legumes and actinorrhizal plants present in the eurosid clade. Unfortunately, the most important crop plants,

cereals, cannot acquire nitrogen through nodulation. However, some bacteria enter root tissues through cracks caused by lateral root emergence and wounds acquired by the movement through the soil (Gaiero et al., 2013). There are also other ways of bacterial entry into the plant and for an extensive review on the topic of rhizobia entry as an example of this process please refer to (Masson-Boivin et al., 2009). Some of these bacteria promote plant growth and a subset of them may fix N<sub>2</sub> (Santi et al., 2013). Even though direct proof that endophytic N-fixers provide their plant hosts with nitrogen compounds is often lacking it is widely accepted that such a process is likely. For example a *nif* mutant of *Gluconacetobacter diazotrophicus* unable to fix nitrogen has reduced ability to promote growth of its plant host sugarcane compared to the wild type (Sevilla et al., 2001).

One of the best-studied nitrogen fixing endophytes is *Pseudomonas stutzeri* A1501. It was isolated from rice roots in China, where it is used as a field inoculant (Vermeiren et al., 1999). It probably acquired genes encoding nitrogenase and later gained genes required to adapt the enzyme activity to appropriate environment conditions (aerobic, microaerobic or anaerobic). *P. stutzeri* has a single 49 kb nitrogen fixation cluster containing 59 genes. This region has a distinct G + C ratio and has probably been horizontally transmitted into this strain (Yan et al., 2008). *P. stutzeri* has also been studied in order to understand the control of nitrogenase expression and activity. After addition of ammonia to the growth media N-fixing bacteria switch off nitrogen-fixation. There are many genes that become strongly down regulated between these two conditions. *nif* genes are required for nitrogen-fixation and their transcription is repressed by addition of ammonia. Interestingly, *P. stutzeri* can switch between denitrification, nitrification and nitrogen-fixation under anaerobic, aerobic and microaerobic conditions, respectively. A global transcriptome study revealed a new gene involved in nitrogen fixation called *pnfA*. *pnfA* is chromosomally linked to and regulated by the same sigma factor as *nifHDK* (encoding nitrogenase). Even though mutation in *pnfA* did not directly altered expression of these genes, the mutant strain has reduced nitrogenase activity under microaerobic conditions (Yan et al., 2010).

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199 *Azoarcus* sp. BH72 colonizes the root of Kallar grass (Hurek et al., 2002).  
200 Furthermore, wildtype BH72 increased the dry weight of Kallar grass grown  
201 under nitrogen starvation by 60% relative to a *nifK* mutant strain of BH72.  
202 Interestingly, the bacteria may undergo irreversible changes between the free-  
203 living and endophytic states so that endophytic colonies of *Azoarcus* sp. BH72  
204 could not be re-isolated from roots. Indeed, further studies revealed substantial  
205 gene expression changes under nitrogen-fixing conditions (Sarkar and Reinhold-  
206 Hurek, 2014). *Azoarcus* is a plant growth promoting bacteria as it fixes nitrogen  
207 that its host appears to be able to access but lacks the usual genetic components  
208 involved in plant pathogenicity (for example type III and IV secretion) (Krause et  
209 al., 2006). *Azoarcus* along with the nitrogen-fixing *Azospirillum* has been found to  
210 be a common root colonizer of rice. Plants clearly exert some control of the  
211 endophytic N-fixing community as wild rice species were preferably colonized by  
212 *Azoarcus* while modern cultivars selected *Azospirillum* (Engelhard et al., 2000).

213

214 Legume nodulation involves a sophisticated plant-microbe communication,  
215 explaining why only a very limited number of bacterial species nodulate a given  
216 plant (Mutch and Young, 2004). It seems plausible that endophytic interactions  
217 are less stringent, with nitrogen-fixing endophytes able to colonize a broader  
218 array of plant hosts. This characteristic makes it especially valuable as an  
219 endophyte studied with a model plant but may be applied to crop plants. For  
220 example, *Rhizobium* sp. IRBG74 and *Azorhizobium caulinodans* were isolated  
221 from the wetland plants *Sesbania aculeata* and *Sesbania rostrata*, respectively  
222 but are also able to infect rice roots (Christiansen-Weniger, 1996; Tan et al.,  
223 2001). Strain IRBG74 has also been isolated from nodules of *Sesbania cannabina*,  
224 but it is noteworthy that it is unable to fix atmospheric nitrogen as an endophyte  
225 as it lacks some key *nif* genes such as *nifV* (Crook et al., 2013). Based on  
226 *Rhizobium* sp. IRBG74 16S rRNA, *fusA* and *rpoB* gene sequences and the absence  
227 of Ti plasmid this strain has been reclassified from the *Agrobacterium* to  
228 *Rhizobium* genus (Cummings et al., 2009). The strain carries a sym plasmid with  
229 *nifH* and *nodA* genes (later confirmed by genome sequencing (Crook et al., 2013))  
230 and is able to colonise a wide variety of *Sesbania* species.



231 *A. caulinodans* ORS571, a *Sesbania rostrata* nodule symbiont is able to infect rice  
232 and fix nitrogen as an endophyte (Christiansen-Weniger, 1996). *A. caulinodans*  
233 can enter roots via cracks and particularly in regions treated with 2,4-  
234 dichlorophenoxyacetic acid, which induces “nodule-like” tumors. *A. caulinodans*  
235 differs from *Rhizobium* sp. IRBG74 as it is able to fix atmospheric nitrogen in a  
236 free-living state and presumably in soil as well (Gebhardt et al., 1984). This  
237 ability may explain why *A. caulinodans* is able to fix nitrogen as a rice root  
238 endophyte, making it one of the most ubiquitous N-fixers discovered so far.

239 More plant species would have to be tested for *A. caulinodans* endophytic  
240 colonization and N-fixing properties in order to determine if this ability is  
241 reserved for this plant species or is it a common feature. In order to determine  
242 whether it is the plant that initiates the nitrogen-fixation in its bacterial  
243 symbiont (as in case of nodulation) a common SYM pathway rice mutant should  
244 be tested for its ability to form endophytic symbiosis with ORS571 (Chen and  
245 Zhu, 2013; Venkateshwaran et al., 2013). Based on the genome sequence, *A.*  
246 *caulinodans* ORS571 acquired nodulation genes through horizontal gene transfer  
247 (Lee et al., 2008). Due to its plant colonization ubiquity (root and stem nodules,  
248 grass root endophyte) the genes involved in colonization and nodulation have  
249 been extensively studied. A large-scale *Azorhizobium* mutant screen identified  
250 many genes involved in plant colonization, stress tolerance and nodulation  
251 ability (Suzuki et al., 2007). It is important to distinguish which of these genes  
252 are uniquely involved in *Azorhizobium* – *Sesbania* symbiotic system and which  
253 genes are universally required for rhizobia – legume interactions.

254  
255 Another well-studied root endophyte is *Herbaspirillum seropedicae*. It colonizes  
256 roots of wheat, rice, sugarcane, corn and sorghum. Under limiting soil-free  
257 nitrogen and oxygen level it fixes atmospheric nitrogen, thus supporting the  
258 growth of its plant host. All the genes required for the plant colonization and  
259 nitrogen fixation have been identified (Pedrosa et al., 2011). Similarly to other  
260 non-rhizobia species *H. seropedicae* probably acquired its nitrogen fixation  
261 ability through horizontal gene transfer. What is striking about this particular  
262 species is that although it possesses all the genetic machinery for the type I, II, III, V,  
263 VI and IV pili secretion (as some other pathogenic species of this genus), it does

not cause plant diseases, but rather uses these systems in order to better “communicate” with its plant host (Schmidt et al., 2012). The Type III secretion system has been identified to play a vital role in the initial signal communication between *Rhizobium* sp. strain NGR234 and *Bradyrhizobium elkanii* and their plant hosts (Marie et al., 2001; Okazaki et al., 2013), in contrast to its pathogenic role as a virulent factor transporter in other bacterial species.

Sugarcane is a nutrient demanding, fast growing, C4 photosynthetic plant. Because of its high biomass increase and sugar content it has become an important biofuel crop. *Gluconacetobacter diazotrophicus* Pal5 is a model endophyte in sugarcane roots, stem and leaves. It belongs to the same bacterial subphylum as rhizobia - Alphaproteobacteria, but to a different order (Rhodospirillales). This strain not only fixes atmospheric nitrogen, but also has antifungal and antibacterial properties against plant pathogens like *Fusarium sp.* and *Xanthomonas albilineans* (Blanco et al., 2005; Mehnaz and Lazarovits, 2006). There are also studies focusing on the ability of *Gluconacetobacter* to produce plant hormones (Cavalcante et al., 2007) and solubilise phosphate (Crespo, 2011), making this species a truly plant growth promoting rhizobacteria (PGPR) for the growth of non-legume plants (Lugtenberg and Kamilova, 2009). Genome sequencing confirmed that *Gluconacetobacter diazotrophicus* Pal5 is able to promote plant growth and probably due to its relatively small genome size is not a common soil bacterium but instead closely relies on its plant host (Bertalan et al., 2009)

Our interest in endophytes and their role in plant health is not purely academic. These organisms may be used to increase plant biomass and nutrient uptake. However, we still have to understand a lot about them and choose the most promising microbial strains, which are likely to be plant and soil specific. In order to commercially use endophytes it is also essential to determine their impact on the environment. Using them to enhance growth of crops would also require a detailed knowledge about their potential influence on human health (Berg et al., 2005). Many of the PGPR species have close relatives that are human opportunistic pathogens. The easy assay to test for their potential pathogenicity

is their ability to grow at 37°C (Alavi et al., 2014). Comparative genomics can unravel the differences between pathogenic and PGPR strains. Plant associated *Stenotrophomonas maltophilia* R551-3 and *S. rhizophila* DSM14405T, even though they exhibit a high level of genomic similarity with the human pathogenic *S. maltophilia* K279a, have genes responsible for spermidine synthase, biodegradation of bacterial and plant cell wall, iron uptake and salinity stress (Alavi et al., 2014).

Several other nitrogen-fixing endophytes also have close relatives among human pathogenic species. *Klebsiella pneumonia* Kp342, a nitrogen-fixing endophyte of rice, maize, sugarcane and banana has a human pathogenic relative – strain MGH78578. The main difference between these two strains is the ability of Kp342 to fix atmospheric nitrogen. The other important difference is the lack of genes coding for the global secondary messenger c-di-GMP in the endophytic strain, involved in the regulation of biofilm formation and virulence factors. In total 4205 proteins (putative orthologs with the average identity of 96%, based on CDS prediction) were shared between these two strains, and 1107 proteins were unique to the plant associated Kp342. Interestingly, none of the predicted CDS were uniquely shared between the Kp342 and already described and sequenced *Azoarcus* sp. BH72 (Fouts et al., 2008).

#### Iron sequestration with the help from soil bacteria

Apart from nitrogen and phosphorous, iron is another element, which plants can acquire via soil microorganisms. A group of PGPR sequester the insoluble form of  $\text{Fe}^{3+}$ , from the rhizosphere environment using siderophores (Jin et al., 2014). Plants take up iron bound by bacterial siderophores even though they secrete their own siderophores these have a lower affinity for binding iron. This acquisition of iron via microbial siderophores reduces iron availability in the rhizosphere, leading to slower growth of other microorganisms (especially fungi) that may be parasitic toward the plant (Bal et al., 2013; Finlay, 2007; Shippers et al., 1987; Traxler et al., 2012). In iron poor soil plants grow better in

non-sterile rather than sterile soil, supporting the idea that microbes help the plant in obtaining this scarce macronutrient (Masalha et al., 2000).

Plant secretion as a form of communication with the soil microbiota

Up to 21% of the carbon fixed by plants is secreted by roots (Lugtenberg and Kamilova, 2009). This suggests that plants may “fuel” plant – microbe interactions. Thus plants can actively secrete compounds and modify the rhizosphere microbiota. When *Arabidopsis thaliana* ABC transporters are mutated, the bacterial and fungal microbiota structure in the rhizosphere changes (Badri et al., 2009). In this study the elevated phenolic and decreased sugar content in plant exudates was responsible for the observed microbial changes. When different groups of compounds were added directly into the soil it was observed that organic acids rather than sugars are responsible for the major shifts in microbial richness and structure (Shi et al., 2011). A more comprehensive study showed, that among *Arabidopsis thaliana* exudates, it was phenolic compounds followed by amino acids, sugar alcohols and sugars that alter the soil microbiota (Badri et al., 2013). It may be that plants are using metabolite secretion to recruit beneficial microbes and suppress pathogens. Tomato is able to change its secretion profile depending on whether the pathogen *Fusarium oxysporum* f.sp. *radices-lycopersici* or *Pseudomonas fluorescens* WCS365 (a natural biocontrol agent against the fungus) is present (Kamilova et al., 2006).

Much research has focused on comparative studies of the structure of the rhizosphere microbiota of different plant species. A detailed rhizosphere microbiota structure has been obtained for potato, rice, maize, wheat, oat and pea and an array of less economically significant plants. Betaproteobacteria and *Pseudomonas* are selected in the potato rhizosphere (Inceoglu et al., 2011), while rice selects for Actinobacteria (Aslam et al., 2013). Maize selects for Burkholderiales, Oceanospirillales and Shingobacteriales (Peiffer et al., 2013), wheat has affinity towards *Dyadobacter*, Fibrobacteriaceae *Verrucomicrobium*

and Firmicutes, oat for Actinobacteridae and pea selects for *Masillia*, *Dyadobacter*, *Flavobacterium* and *Streptomyces* (Turner et al., 2013b).

Once the rhizosphere microbiota started to be elucidated, research was focused on the plant root endosphere as microorganisms in this environment may have an even stronger impact on plant health. In general the endosphere is enriched with Proteobacteria; at the order level: Burkholderiales, Oceanospirillales and Sphingobacteriales (Peiffer et al., 2013) and at the genus level: *Sphingomonas*, *Rhizobium*, *Pseudomonas* and *Variovorax* (Schreiter et al., 2014) and also Actinobacteria and Bacteroidetes (Schlaeppli et al., 2014).

With the new high-throughput sequencing methods a wave of studying the rhizosphere microbiota is emerging. When only a few years ago DNA fingerprinting was a common practise (Fisher and Triplett, 1999; Jones and Thies, 2007) it is now possible to sequence multiple samples with a great depth at a fraction of the price (Fadrosh et al., 2014).

#### Selected problems with DNA based soil metagenomics

Phylogenetic studies prior to sequencing require DNA to be amplified using specially designed primers, for example; V4 region of prokaryotic 16S rRNA subunit (Caporaso et al., 2012) or ITS region of rRNA operon in fungi (Buee et al., 2009). The use of two or more different sets of PCR primers produces independent data sets that cannot be correlated. However, recent research focusing on the influence of wheat, oat and pea used RNA rather than DNA to study the rhizosphere microbiota. Plants not only shift the microbial population within each domain of life, but also there are significant changes at this level, i.e. pea supports more of the eukaryotic population than oat, wheat and bulk soil (Turner et al., 2013b). Future research into soil microbiota should also show the ratio of prokaryotes to eukaryotes as this may be a key element in plant selection. There are two methods to do this. The first is based on amplification of DNA using domain/kingdom specific primers, sequencing and estimating the relative abundance of these groups against each other. In order to do that a series of qPCR reactions would have to be performed on the environmental DNA. The other method based on metatranscriptomics is described in (Turner et al.,

2013b). Briefly, environmental RNA, which is >95% rRNA, was reverse transcribed into cDNA and sequenced using Illumina HiSeq (normally cDNA reads would be relatively short). In this method a total PCR-unbiased microbiota structure was obtained. It is worth remembering that RNA based research focuses on metabolically active microorganisms rather than the total population.

#### Factors controlling soil microbiota

It was shown that pH (Lauber et al., 2009), land use and land history (Osborne et al., 2011), vegetation cover (Buee et al., 2009) and soil type (Berg and Smalla, 2009) all influence the rhizosphere community. Given the strength of these environmental interactions the question arises of whether plants establish a core microbiome. It was thus essential to identify the core microbial community of the plant rhizosphere and endosphere.

*A. thaliana* due to its ubiquitous use in plant genetics was chosen as a model plant in studying plant-microbe interactions in the soil environment. The rhizosphere community, is recruited from the bulk soil and at least in the case of *Arabidopsis* it closely resembles the bulk soil community (Bulgarelli et al., 2012; Lundberg et al., 2012). Interestingly, Proteobacteria seem to be attracted to the endosphere simply by the presence of cellulose, while Actinobacteria are clearly selected for by the endosphere habitat. It was found that among Actinobacteria, the Streptomycetaceae were especially abundant in the root endosphere (Bulgarelli et al., 2012). One possible reason for this enrichment is that Streptomycetaceae lack flagella. Plants can recognize flagella using their MAMP recognition system (Roux et al., 2011) and subsequently trigger an immune response. Streptomycetaceae, which lack flagella, would have a clear advantage over flagellated, motile bacteria.

#### Bespoke field soil microbiota

For new plant breeding programs we need to understand their responses to the soil microbiota (Donn et al., 2014). The efficiency of plants in selecting for

beneficial microorganisms in the rhizosphere and/or endosphere may be an important trait in plant nutrient assimilation and pathogen resistance. In order to understand the plant genetic influence on the soil microbiota it is necessary to study the impact of closely related plant lines/accessions on soil communities.

Even though research conducted on *Arabidopsis* accessions cultured in growth rooms clearly showed that plant genotype controls the soil microbiota (Micallef et al., 2009), this is less clear when applied to field grown maize. Here 27 inbred lines of maize were grown under 5 different field conditions (Peiffer et al., 2013). In the natural environment it is the soil, or factors that influence soil conditions like climate, that has the major influence on the maize rhizosphere microbiota. Even though there were significant differences between the plant lines no relationship between host genetic diversity and its rhizosphere microbial structure was found. An extra dimension to complexity of the root – soil microbiota interactions is the fact that different parts of root of oat exert a subtle but statistically significant effect on the microbial community (DeAngelis et al., 2009).

Microbiota structure is a heritable trait as demonstrated for the wheat rhizosphere (Donn et al., 2014), so in theory it is possible to microbiologically prepare the field for the optimum crop. Heritability of the microbiome was shown indirectly in a much earlier study, where *Arabidopsis* was grown for multiple generations (Swenson et al., 2000). After the initial generation, soil that supported plants with the highest and lowest biomass was re-used as separate microbial inocula for the next generation of plants. This was repeated for 16 generations and after 8 generations onward the changes in the plant biomass between the high and low biomass lines became statistically significant (Swenson et al., 2000). This indicates that separate populations of microbes are being selected that either enhance or retard plant growth.

Soil microorganisms play a vital role in plant health and this has been extensively explored in the phenomenon of soil suppression (Mendes et al., 2011). It was noticed that in some soils plants that are initially susceptible to fungal attack and suffer reduced yields, could become resistant to attack in subsequent years. It was also noticed that inoculation of this “suppressive” soil into a different plot

was successful in promoting plant health. Fungal plant pathogens appear to be the cause of the reduced plant yield and this pathogen abundance is reduced in the suppressive soil. A comprehensive study was performed to establish whether the soil microbiota is responsible for pathogen suppression. It was found that suppressive soil has elevated abundance of Gamma and Beta- Proteobacteria and Firmicutes. The study also found that a nine-amino acid chlorinated lipopeptide produced by *Pseudomonas* sp. in the suppressive soil might be responsible for *R. solani* inhibition (Mendes et al., 2011). Likewise, it has been shown that the plant microbiota changes during plant monoculture. Oilseed rape yield declined over 4 years of monoculture and the possible reason for that was the build up of the specific plant host pathogens *Olpidium brassicae* and *Pyrenochaeta lycopersici*. Interestingly bacteria from the order of Burkholderiales (Betaproteobacteria) and species of *Pseudomonas fluorescens* also become more abundant possibly initiating the soil suppression effect (Hilton et al., 2013).

Taking these findings together it becomes theoretically possible to investigate soil communities in the field and based on that choose the crop that would grow best. Of course it is not only the soil microbiota that defines crop yields, but focusing on the spatial and temporal changes in its structure and activity would give an advantage in controlling soil pathogens and possibly reduce the need for fungicide and fertilizers. Such an approach would require far better understanding of plant-microbe interactions and requires rapid and cheap screening of the soil microbiota.

#### Future of agriculture science

Plant roots are clearly crucial to nutrient acquisition and productivity but in the future we need to pay much closer attention to their interaction with the soil microbiota. While a lot is already known about soil microorganisms, most of this research comes from studying bacteria, fungi and oomycetes in laboratory conditions. More focus on field conditions is needed in order to decipher plant-microbe interactions. However, with advances in sequencing technology (metagenomics/metatranscriptomics) it becomes possible to follow changes in



the soil microbiota and their impact on plants with great temporal and spatial resolution. This suggests we should be able to incorporate plant responses to the soil microbiota into future breeding programs to select for genotypes that favour beneficial interactions.

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