Journal of Experimental Botany, Vol. 66, No. 8 pp. 2167–2175, 2015
doi:10.1093/jxb/erv157

REVIEW PAPER

Role of root microbiota in plant productivity

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Received 14 January 2015; Revised 9 March 2015; Accepted 12 March 2015

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 increased 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 be part of the plant's pan-genome. Therefore, it is essential for us to understand microbial community structure and their 'metagenome' and how it is influenced by different soil types and crop varieties. In the future we may be able to modify and better utilize the soil microbiota potential for promoting plant growth.

Key words: Endophytes, metagenomics, microbiota, plant–microbe interactions, plant productivity, rhizosphere.

Introduction

The human population has grown 7-fold since the beginning of the 19th century (Speidel *et al.*, 2009). This has led to the planet's natural resources being overexploited, 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 nitrogen cycle. Addition of nitrogen fertilizers is now estimated at ~10^{11} kg $year^{-1}$ (Glass, 2003; Schmer *et al.*, 2014). However, as ~60% of the synthesized nitrogen fertilizer is not absorbed by plants, most of it leaches into groundwater. Nitrogen is normally one of the limiting nutrients for cyanobacterial and algal blooms and, once released into the groundwater, it migrates into the sea, causing dramatic changes in marine microbial populations, affecting the whole marine food chain (Conley, 2012). Fertilizers are normally overused in developed countries and plants are able to reach their current yield potential. However, developing countries have to improve their yield per hectare substantially (Mueller *et al.*, 2012). This is why it is crucial to understand how can we improve plant growth with reduced dependency on expensive and environmentally harmful synthetic fertilizers.

In the optimistic scenario that crop yield per hectare will double by 2050 (Ray *et al.*, 2013), it will still not be enough to feed a growing population demanding more animal-based food in their diets (Robinson *et al.*, 2014). Even assuming this optimistic scenario, some sacrifices in the land coverage of natural habitats will have to be made. The best known example of ongoing deforestation is the Amazon basin, where there are infrastructure (Fraser, 2014), urbanization, and agricultural stresses on the forest (Ellis *et al.*, 2013). This region is critical to climate change and recently many options have been proposed and introduced to stop these negative processes (Galford *et al.*, 2013). However, deforestation is a temporary solution for increasing crop production in countries such as Indonesia, Malaysia, Paraguay, Bolivia, Zambia, and Angola, with the overall loss of the forest estimated at

1.5 million km^2 since the beginning of the 21st century (Hansen *et al.*, 2013).

It is important that we are able to increase yields from land that has already been converted into fields. One of the most sustainable ways to achieve this is to focus research on the natural abilities of plants to increase yields. More than 100 years ago it was noticed that the soil around the plant roots is extremely rich with microbes, and the term rhizosphere was coined (Hartmann, 2008). These microbes have been extensively studied for their role in plant health and, with increasing understanding of the processes that take place in the rhizosphere, we may start to utilize these relationships to increase plant growth in an environmentally sustainable way. Harnessing the ability of microbes to provide plants with essential micro- and macronutrients is an important goal of rhizosphere plant–microbe studies. In this review, we consider selected features of these interactions with a focus on nitrogen fixation as well as phosphorus and iron sequestration by soil microbes. We present the current understanding of microbial community structure and how this is shaped by environmental factors and plant hosts. Finally, we consider future directions in the field and the possibilities for better understanding and use of the large soil microbiota.

Interactions between plants and mycorrhizal fungi

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 example of this is the mutualistic interaction between mycorrhizal fungi and plant roots, which is particularly important in providing water and phosphorus for the plant host in exchange for carbon for the fungus (Augé, 2001). Phosphorus often limits plant growth even though it is abundant in soil because it is normally bound to aluminium and iron (forming strengite and varescite) or to calcium (forming apatite) in acidic or alkaline conditions, respectively. Plants require phosphate in a soluble form, as either $H_2PO_4^-$ or HPO_4^- (Schachtman *et al.*, 1998). Some bacteria release organic acids able to chelate the cations bound to phosphate, thus releasing it into the 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 access to the intracellular space of the root using LCO signals (sulphated and non-sulphated lipochitooligosaccharides) (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 a 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 that 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 the Betaproteobacteria subphylum (predominantly Burkholderiales) form nodules on leguminous plant roots, inside which they convert atmospheric N_2 into plant-available NH_3 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 as endophytes in roots (e.g. Azotobacteraceae, Cyanobacteria), and some of these may fix significant amounts of N_2 (reviewed in Turner *et al.*, 2013*a*).

Legume nodulation appeared for the first time ~100 million years ago (Doyle, 2011), which is > 300 million years later than mycorrhizal infection, suggesting that 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 *Rhizobium 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 (NFP) in *Medicago truncatula* is important in the plant immune response against fungal and oomycete infection (Rey *et al.*, 2013).

Growing interest in the ability of plant endophytes 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, it is restricted to a subset of legumes and actinorhizal 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 routes 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_2 (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 with 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 has probably acquired genes encoding nitrogenase and later it gained genes required to adapt the enzyme activity to appropriate environment conditions (aerobic, microaerobic, or anaerobic). There is clear spatial gene expression under low levels of fixed nitrogen and under ammonium shock (Hartmann *et al.*, 1986). *Pseudomonas 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). *Pseudomonas 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 free living, 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 alter expression of these genes, the mutant strain has reduced nitrogenase activity under microaerobic conditions (Yan *et al.*, 2010).

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

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

Azorhizobium caulinodans ORS571, an *S. rostrata* nodule symbiont, is able to infect rice and fix nitrogen as an endophyte (Christiansen-Weniger, 1996). *Azorhizobium caulinodans* can

enter roots via cracks and particularly in regions treated with 2,4-dichlorophenoxyacetic acid, which induces 'nodule-like' tumours. *Azorhizobium. caulinodans* differs from *Rhizobium* sp. IRBG74 as it is able to fix atmospheric nitrogen in a free-living state and presumably in soil as well (Gebhardt *et al.*, 1984). This ability may explain why *A. caulinodans* is able to fix nitrogen as a rice root endophyte, making it one of the most ubiquitous N fixers discovered so far.

More plant species would have to be tested for *A. caulinodans* endophytic colonization and N-fixing properties in order to determine if this ability is reserved for this plant species or if it a common feature. In order to determine whether it is the plant that initiates the N_2 fixation in its bacterial symbiont (as in case of nodulation), a common SYM pathway rice mutant should be tested for its ability to form endophytic symbiosis with ORS571 (Chen and Zhu, 2013; Venkateshwaran *et al.*, 2013). Based on the genome sequence, *A. caulinodans* ORS571 acquired nodulation genes through horizontal gene transfer (Lee *et al.*, 2008). Due to its plant colonization ubiquity (root and stem nodules, grass root endophyte), the genes involved in colonization and nodulation have been extensively studied. A large-scale *Azorhizobium* mutant screen identified many genes involved in plant colonization, stress tolerance, and nodulation ability (Suzuki *et al.*, 2007). It is important to distinguish which of these genes are uniquely involved in the *Azorhizobium–Sesbania* symbiotic system and which genes are universally required for rhizobia–legume interactions.

Another well-studied root endophyte is *Herbaspirillum seropedicae*. It colonizes roots of wheat, rice, sugarcane, corn, and sorghum. Under limiting soil-free nitrogen and oxygen level, it fixes atmospheric nitrogen, thus supporting the growth of its plant host. Many genes required for plant colonization and nitrogen fixation have been identified (Pedrosa *et al.*, 2011). Similarly to other non-rhizobial species, *H. seropedicae* probably acquired its nitrogen fixation ability through horizontal gene transfer. What is striking about this particular species is that although it possesses all the genetic machinery for type I, II, III, V, VI, and IV pili secretion (as do 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 of *Rhizobium* sp. strain NGR234 and *Bradyrhizobium elkani* with 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, C_4 photosynthetic plant. Because of its high rate of 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 such as *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 rhizobacterium (PGPR) for the growth of non-legume plants (Lugtenberg and Kamilova, 2009). Genome sequencing confirmed that *G. 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 use endophytes commercially, 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. An 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 walls, iron uptake, and salinity stress (Alavi *et al.*, 2014).

Several other N_2-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 orthologues with the average identity of 96%, based on coding sequence (CDS) prediction] were shared between these two strains, and 1107 proteins were unique to the plant-associated Kp342. Interestingly, none of the predicted CDS was uniquely shared between the Kp342 and the already described and sequenced *Azoarcus* sp. BH72 (Fouts *et al.*, 2008).

Iron sequestration with the help of soil bacteria

Apart from nitrogen and phosphorus, iron is another element which plants can acquire via soil microorganisms. A group of PGPR sequestrate the insoluble form of 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 (Shippers *et al.*, 1987; Finlay, 2007; Traxler *et al.*, 2012; Bal *et al.*, 2013). 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 the study of Badri *et al.*, 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 *A. 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 has affinity towards Actinobacteridae, and pea selects for *Masillia*, *Dyadobacter*, *Flavobacterium*, and *Streptomyces* (Turner *et al.*, 2013*b*).

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 (Schlaeppi *et al.*, 2014).

With the new high-throughput sequencing methods, a wave of studies on the rhizosphere microbiota are emerging. Whereas only a few years ago DNA fingerprinting was a common practice (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 required DNA to be amplified using specially designed primers, for example the V4 region of the prokaryotic 16S rRNA subunit (Caporaso *et al.*, 2012) or the ITS (internal transcribed spacer) region of the 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 there are also significant changes at this level; that is, pea supports more of the eukaryotic population than wheat, and bulk soil (Turner *et al.*, 2013*b*). 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 by which this can be done. 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 this, a series of quantitative PCRs would have to be performed on the environmental DNA. The other method based on metatranscriptomics is described in Turner *et al.* (2013*b*). In brief, 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.

Arabidopsis 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, it was the Streptomycetaceae that 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 the flagella using their MAMP (microorganism-associated molecular pattern) recognition system (Roux *et al.*, 2011) and subsequently trigger an immune response. Streptomycetaceae, which lack flagella, would have a clear advantage over any flagellated, motile bacterial species.

Bespoke field soil microbiota

For new plant breeding programmes, 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 the first research conducted on *Arabidopsis* accessions grown in growth rooms clearly showed that plant genotype controls the soil microbiota (Micallef *et al.*, 2009), this is less clear when the research was applied to field-grown maize. Here 27 inbred lines of maize were grown under five different field conditions (Peiffer *et al.*, 2013). In the natural environment, it is the soil, or factors that influence soil conditions such as 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 of the complexity of the root–soil microbiota interactions is the fact that different parts of the 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 prepare the field microbiologically 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 eight generations, 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 suppressiveness (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 the pathogen suppression. It was found that the 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 a soil suppresiveness effect (Hilton *et al.*, 2013).

Taking these findings together, it becomes theoretically possible to investigate the soil community in the field and, based on that, to 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.

The 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 is becoming possible to follow changes in the soil microbiota and their impact on plants with great temporal and spatial resolution. This suggests that we should be able to incorporate plant responses to the soil microbiota into future breeding programmes to select for genotypes that favour beneficial interactions.

Acknowledgement

We are grateful to Barney Geddes, Francesco Pini and Alison East for help with reviewing this manuscript.

References

Akiyama, K, Matsuzaki, K, Hayashi, H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435,** 824–827.

Alavi P, Starcher MR, Thallinger GG, Zachow C, Muller H, Berg G. 2014. Stenotrophomonas comparative genomics reveals genes and

functions that differentiate beneficial and pathogenic bacteria. *BMC Genomics* **15,** 482.

Aslam Z, Yasir M, Yoon HS, Jeon CO, Chung YR. 2013. Diversity of the bacterial community in the rice rhizosphere managed under conventional and no-tillage practices. *Journal of Microbiology* **51,** 747–756.

Augé RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11,** 3–42.

Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM. 2013. Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *Journal of Biological Chemistry* **288,** 4502–4512.

Badri DV, Quintana N, El Kassis EG, Kim HK, Choi YH, Sugiyama A, Verpoorte R, Martinoia E, Manter DK, Vivanco JM. 2009. An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. *Plant Physiology* **151,** 2006–2017.

Bal HB, Das S, Dangar TK, Adhya TK. 2013. ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. *Journal of Basic Microbiology* **53,** 972–984.

Berbee ML, Taylor JW. 2007. Rhynie chert: a window into a lost world of complex plant–fungus interactions. *New Phytologist* **174,** 475–479.

Berg G, Eberl L, Hartmann A. 2005. The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environmental Microbiology* **7,** 1673–1685.

Berg G, Smalla K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* **68,** 1–13.

Bertalan M, Albano R, de Padua V, *et al*. 2009. Complete genome sequence of the sugarcane nitrogen-fixing endophyte Gluconacetobacter diazotrophicus Pal5. *BMC Genomics* **10,** 450.

Blanco Y, Blanch M, Pinon D, Legaz ME, Vicente C. 2005. Antagonism of Gluconacetobacter diazotrophicus (a sugarcane endosymbiont) against Xanthomonas albilineans (pathogen) studied in alginate-immobilized sugarcane stalk tissues. *Journal of Bioscience and Bioengineering* **99,** 366–371.

Bouwmeester HJ, Roux C, Lopez-Raez JA, Becard G. 2007. Rhizosphere communication of plants, parasitic plants and AM fungi. *Trends in Plant Science* **12,** 224–230.

Buee M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F. 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist* **184,** 449–456.

Bulgarelli D, Rott M, Schlaeppi K, *et al*. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* **488,** 91–95.

Capoen W, Oldroyd G. 2008. How CYCLOPS keeps an eye on plant symbiosis. *Proceedings of the National Academy of Sciences, USA* **105,** 20053–20054.

Caporaso JG, Lauber CL, Walters WA, *et al*. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal* **6,** 1621–1624.

Cavalcante JJ, Vargas C, Nogueira EM, Vinagre F, Schwarcz K, Baldani JI, Ferreira PC, Hemerly AS. 2007. Members of the ethylene signalling pathway are regulated in sugarcane during the association with nitrogen-fixing endophytic bacteria. *Journal of Experimental Botany* **58,** 673–686.

Chen C, Zhu H. 2013. Are common symbiosis genes required for endophytic rice–rhizobial interactions? *Plant Signaling and Behavior* **8,** e25453.

Christiansen-Weniger C. 1996. Endophytic establishment of Azorhizobium caulinodans through auxin-induced root tumors of rice (Oryza sativa L.). *Biology and Fertility of Soils* **21,** 293–302.

Conley DJ. 2012. Ecology: save the Baltic Sea. *Nature* **486,** 463–464.

Crespo J, Boiardi J, Luna M. 2011. Mineral phosphate solubilization activity of Gluconacetobacter diazotrophicus under P-limitation and plant root environment. *Agricultural Sciences* **2,** 16–22.

Crook MB, Mitra S, Ane JM, Sadowsky MJ, Gyaneshwar P. 2013. Complete genome sequence of the Sesbania symbiont and rice growth-promoting endophyte Rhizobium sp. strain IRBG74. *Genome Announcements* **1,** e00934–13.

Cummings SP, Gyaneshwar P, Vinuesa P, *et al*. 2009. Nodulation of Sesbania species by Rhizobium (Agrobacterium) strain IRBG74 and other rhizobia. *Environmental Microbiology* **11,** 2510–2525.

DeAngelis KM, Brodie EL, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK. 2009. Selective progressive response of soil microbial community to wild oat roots. *ISME Journal* **3,** 168–178.

Donn S, Kirkegaard JA, Perera G, Richardson AE, Watt, M. 2014. Evolution of bacterial communities in the wheat crop rhizosphere. *Environmental Microbiology* (in press).

Doyle JJ. 2011. Phylogenetic perspectives on the origins of nodulation. *Molecular Plant-Microbe Interactions* **24,** 1289–1295.

Ellis EC, Kaplan JO, Fuller DQ, Vavrus S, Klein Goldewijk K, Verburg PH. 2013. Used planet: a global history. *Proceedings of the National Academy of Sciences, USA* **110,** 7978–7985.

Engelhard M, Hurek T, Reinhold-Hurek B. 2000. Preferential occurrence of diazotrophic endophytes, Azoarcus spp., in wild rice species and land races of Oryza sativa in comparison with modern races. *Environmental Microbiology* **2,** 131–141.

Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J. 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* **2,** 6.

Finlay RD. 2007. *Modern soil microbiology* . CRC Press, Taylor&Francis Group.

Fisher MM, Triplett EW. 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Applied Environmental Microbiology* **65,** 4630–4636.

Fouts DE, Tyler HL, DeBoy RT, *et al*. 2008. Complete genome sequence of the N2-fixing broad host range endophyte Klebsiella pneumoniae 342 and virulence predictions verified in mice. *PLoS Genetics* **4,** e1000141.

Fraser B. 2014. Deforestation: carving up the Amazon. *Nature* **509,** 418–419.

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE. 2013. Inside the root microbiome: bacterial root endophytes and plant growth promotion. *American Journal of Botany* **100,** 1738–1750.

Galford GL, Soares-Filho B, Cerri CE. 2013. Prospects for land-use sustainability on the agricultural frontier of the Brazilian Amazon. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368,** 20120171.

Gao X, Lu X, Wu M, Zhang H, Pan R, Tian J, Li S, Liao H. 2012. Co-inoculation with rhizobia and AMF inhibited soybean red crown rot: from field study to plant defense-related gene expression analysis. *PLoS One* **7,** e33977.

Gebhardt C, Turner GL, Gibson AH, Dreyfus BL, Bergersen FJ. 1984. Nitrogen-fixing growth in continuous culture of a strain of Rhizobium sp. isolated from stem nodules on Sesbania rostrata. *Journal of General Microbiology* **130,** 843–848.

Glass ADM. 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. *Critical Reviews in Plant Sciences* **22,** 453–470.

Gobbato E, Marsh JF, Vernie T, *et al*. 2012. A GRAS-type transcription factor with a specific function in mycorrhizal signaling. *Current Biology* **22,** 2236–2241.

Godfray HC, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C. 2010. Food security: the challenge of feeding 9 billion people. *Science* **327,** 812–818.

Gutjahr C, Parniske M. 2013. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cell and Developmental Biology* **29,** 593–617.

Gyaneshwar P, Hirsch AM, Moulin L, *et al*. 2011. Legume-nodulating betaproteobacteria: diversity, host range, and future prospects. *Molecular Plant-Microbe Interactions* **24,** 1276–1288.

Hansen MC, Potapov PV, Moore R, *et al*. 2013. High-resolution global maps of 21st-century forest cover change. *Science* **342,** 850–853.

Harrison MJ. 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review of Microbiology* **59,** 19–42.

Harrison MJ, Dewbre GR, Liu J. 2002. A phosphate transporter from Medicago truncatula involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *The Plant Cell* **14,** 2413–2429.

Hartmann A, Fu H, Burris RH. 1986. Regulation of nitrogenase activity by ammonium chloride in Azospirillum spp. *Journal of Bacteriology* **165,** 864–870.

Hartmann A, Rothballer M, Schmid M. 2008. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil* **312,** 7–14.

Hilton S, Bennett AJ, Keane G, Bending GD, Chandler D, Stobart R, Mills P. 2013. Impact of shortened crop rotation of oilseed rape on soil and rhizosphere microbial diversity in relation to yield decline. *PLoS One* **8,** e59859.

Hurek T, Handley LL, Reinhold-Hurek B, Piche Y. 2002. Azoarcus grass endophytes contribute fixed nitrogen to the plant in an unculturable state. *Molecular Plant-Microbe Interactions* **15,** 233–242.

Inceoglu O, Al-Soud WA, Salles JF, Semenov AV, van Elsas JD. 2011. Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. *PLoS One* **6,** e23321.

James TY, Kauff F, Schoch CL, *et al*. 2006. Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature* **443,** 818–822.

Jin CW, Ye YQ, Zheng SJ. 2014. An underground tale: contribution of microbial activity to plant iron acquisition via ecological processes. *Annals of Botany* **113,** 7–18.

Jones CM, Thies JE. 2007. Soil microbial community analysis using two-dimensional polyacrylamide gel electrophoresis of the bacterial ribosomal internal transcribed spacer regions. *Journal of Microbiological Methods* **69,** 256–267.

Kamilova F, Kravchenko LV, Shaposhnikov AI, Makarova N, Lugtenberg B. 2006. Effects of the tomato pathogen Fusarium oxysporum f. sp. radicis-lycopersici and of the biocontrol bacterium Pseudomonas fluorescens WCS365 on the composition of organic acids and sugars in tomato root exudate. *Molecular Plant-Microbe Interactions* **19,** 1121–1126.

Krause A, Ramakumar A, Bartels D, *et al*. 2006. Complete genome of the mutualistic, N2-fixing grass endophyte Azoarcus sp. strain BH72. *Nature Biotechnology* **24,** 1385–1391.

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ. 2007. Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *New Phytologist* **174,** 648–657.

Lauber CL, Hamady M, Knight R, Fierer N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied Environmental Microbiology* **75,** 5111–5120.

Lee KB, De Backer P, Aono T, *et al*. 2008. The genome of the versatile nitrogen fixer Azorhizobium caulinodans ORS571. *BMC Genomics* **9,** 271.

Lenton T. 2011. 2 degrees C or not 2 degrees C? That is the climate question. *Nature* **473,** 7.

Lugtenberg B, Kamilova F. 2009. Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* **63,** 541–556.

Lundberg DS, Lebeis SL, Paredes SH, *et al*. 2012. Defining the core Arabidopsis thaliana root microbiome. *Nature* **488,** 86–90.

Maillet F, Poinsot V, Andre O, *et al*. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* **469,** 58–63.

Marie C, Broughton WJ, Deakin WJ. 2001. Rhizobium type III secretion systems: legume charmers or alarmers? *Current Opinion in Plant Biology* **4,** 336–342.

Masalha J, Kosegarten H, Elmaci Ö, Mengel K. 2000. The central role of microbial activity for iron acquisition in maize and sunflower. *Biology and Fertility of Soils* **30,** 433–439.

Masson-Boivin C, Giraud E, Perret X, Batut J. 2009. Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? *Trends in Microbiology* **17,** 458–466.

Mehnaz S, Lazarovits G. 2006. Inoculation effects of Pseudomonas putida, Gluconacetobacter azotocaptans, and Azospirillum lipoferum on corn plant growth under greenhouse conditions. *Microbial Ecology* **51,** 326–335.

Mendes R, Kruijt M, de Bruijn I, *et al*. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* **332,** 1097–1100.

Micallef SA, Shiaris MP, Colon-Carmona A. 2009. Influence of Arabidopsis thaliana accessions on rhizobacterial communities and natural variation in root exudates. *Journal of Experimental Botany* **60,** 1729–1742.

Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N, Foley JA. 2012. Closing yield gaps through nutrient and water management. *Nature* **490,** 254–257.

Mutch LA, Young JP. 2004. Diversity and specificity of Rhizobium leguminosarum biovar viciae on wild and cultivated legumes. *Molecular Ecology* **13,** 2435–2444.

Okazaki S, Kaneko T, Sato S, Saeki K. 2013. Hijacking of leguminous nodulation signaling by the rhizobial type III secretion system. *Proceedings of the National Academy of Sciences, USA* **110,** 17131–17136.

Oldroyd GE. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* **11,** 252–263.

Oldroyd GE, Harrison MJ, Udvardi M. 2005. Peace talks and trade deals. Keys to long-term harmony in legume–microbe symbioses. *Plant Physiology* **137,** 1205–1210.

Oldroyd GE, Murray JD, Poole PS, Downie JA. 2011. The rules of engagement in the legume–rhizobial symbiosis. *Annual Review of Genetics* **45,** 119–144.

Osborne CA, Zwart AB, Broadhurst LM, Young AG, Richardson AE. 2011. The influence of sampling strategies and spatial variation on the detected soil bacterial communities under three different land-use types. *FEMS Microbiology Ecology* **78,** 70–79.

Pedrosa FO, Monteiro RA, Wassem R, *et al*. 2011. Genome of Herbaspirillum seropedicae strain SmR1, a specialized diazotrophic endophyte of tropical grasses. *PLoS Genetics* **7,** e1002064.

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences, USA* **110,** 6548–6553.

Pirozynski KA, Malloch DW. 1975. The origin of land plants: a matter of mycotrophism. *Biosystems* **6,** 153–164.

Ray DK, Mueller ND, West PC, Foley JA. 2013. Yield trends are insufficient to double global crop production by 2050. *PLoS One* **8,** e66428.

Rey T, Nars A, Bonhomme M, *et al*. 2013. NFP, a LysM protein controlling Nod factor perception, also intervenes in Medicago truncatula resistance to pathogens. *New Phytologist* **198,** 875–886.

Robinson TP, Wint GR, Conchedda G, Van Boeckel TP, Ercoli V, Palamara E, Cinardi G, D'Aietti, L, Hay SI, Gilbert M. 2014. Mapping the global distribution of livestock. *PLoS One* **9,** e96084.

Rockstrom J, Steffen W, Noone K, *et al*. 2009. A safe operating space for humanity. *Nature* **461,** 472–475.

Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones, A, Holton N, Malinovsky FG, Tor M, de Vries S, Zipfel C. 2011. The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *The Plant Cell* **23,** 2440–2455.

Santi C, Bogusz D, Franche C. 2013. Biological nitrogen fixation in non-legume plants. *Annals of Botany* **111,** 743–767.

Sarkar A, Reinhold-Hurek B. 2014. Transcriptional profiling of nitrogen fixation and the role of NifA in the diazotrophic endophyte Azoarcus sp. strain BH72. *PLoS One* **9,** e86527.

Schachtman DP, Reid RJ, Ayling SM. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiology* **116,** 447–453.

Schlaeppi K, Dombrowski N, Oter RG, Ver Loren van Themaat E, Schulze-Lefert P. 2014. Quantitative divergence of the bacterial root microbiota in Arabidopsis thaliana relatives. *Proceedings of the National Academy of Sciences, USA* **111,** 585–592.

Schmer MR, Vogel KP, Varvel GE, Follett RF, Mitchell RB, Jin VL. 2014. Energy potential and greenhouse gas emissions from bioenergy cropping systems on marginally productive cropland. *PLoS One* **9,** e89501.

Schmidt MA, Balsanelli E, Faoro H, *et al*. 2012. The type III secretion system is necessary for the development of a pathogenic and endophytic interaction between Herbaspirillum rubrisubalbicans and Poaceae. *BMC Microbiology* **12,** 98.

Schreiter S, Ding GC, Heuer H, Neumann G, Sandmann M, Grosch R, Kropf S, Smalla K. 2014. Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. *Frontiers in Microbiology* **5,** 144.

Sevilla M, Burris RH, Gunapala N, Kennedy C. 2001. Comparison of benefit to sugarcane plant growth and 15N2 incorporation following

inoculation of sterile plants with Acetobacter diazotrophicus wild-type and Nif– mutants strains. *Molecular Plant-Microbe Interactions* **14,** 358–366.

Shi S, Richardson AE, O'Callaghan M, DeAngelis KM, Jones EE, Stewart A, Firestone MK, Condron LM. 2011. Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiology Ecology* **77,** 600–610.

Shippers B, Bakker A, Baker P. 1987. Interactions of deletorious and beneficial microorganisms and the effect on cropicing practices. *Annual Review of Phytopathology* **25,** 339–358.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133,** 16–20.

Speidel JJ, Weiss DC, Ethelston SA, Gilbert SM. 2009. Population policies, programmes and the environment. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364,** 3049–3065.

Suzuki S, Aono T, Lee KB, Suzuki T, Liu CT, Miwa H, Wakao S, Iki T, Oyaizu H. 2007. Rhizobial factors required for stem nodule maturation and maintenance in Sesbania rostrata–Azorhizobium caulinodans ORS571 symbiosis. *Applied Environmental Microbiology* **73,** 6650–6659.

Swenson W, Wilson DS, Elias R. 2000. Artificial ecosystem selection. *Proceedings of the National Academy of Sciences, USA* **97,** 9110–9114.

Tan Z, Hurek T, Vinuesa P, Muller P, Ladha JK, Reinhold-Hurek B. 2001. Specific detection of Bradyrhizobium and Rhizobium strains colonizing rice (Oryza sativa) roots by 16S–23S ribosomal DNA intergenic spacer-targeted PCR. *Applied Environmental Microbiology* **67,** 3655–3664.

Traxler MF, Seyedsayamdost MR, Clardy J, Kolter R. 2012. Interspecies modulation of bacterial development through iron competition and siderophore piracy. *Molecular Microbiology* **86,** 628–644.

Turner TR, James EK, Poole PS. 2013*a*. The plant microbiome. *Genome Biology* **14,** 209.

Turner TR, Ramakrishnan K, Walshaw J, Heavens D, Alston M, Swarbreck D, Osbourn A, Grant A, Poole PS. 2013*b*. Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *ISME Journal* **7,** 2248–2258.

Udvardi M, Poole PS. 2013. Transport and metabolism in legume–rhizobia symbioses. *Annual Review of Plant Biology* **64,** 781–805.

Vassilev N, Vassileva M, Nikolaeva I. 2006. Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. *Applied Microbiology and Biotechnology* **71,** 137–144.

Venkateshwaran M, Volkening JD, Sussman MR, Ané J-M. 2013. Symbiosis and the social network of higher plants. *Current Opinion in Plant Biology* **16,** 118–127.

Vermeiren H, Willems A, Schoofs G, de Mot R, Keijers V, Hai W, Vanderleyden J. 1999. The rice inoculant strain Alcaligenes faecalis A15 is a nitrogen-fixing Pseudomonas stutzeri. *Systematic and Applied Microbiology* **22,** 215–224.

Wang E, Schornack S, Marsh JF, Gobbato E, Schwessinger B, Eastmond P, Schultze M, Kamoun S, Oldroyd GE. 2012. A common signaling process that promotes mycorrhizal and oomycete colonization of plants. *Current Biology* **22,** 2242–2246.

Yan Y, Ping S, Peng J, *et al*. 2010. Global transcriptional analysis of nitrogen fixation and ammonium repression in root-associated Pseudomonas stutzeri A1501. *BMC Genomics* **11,** 11.

Yan Y, Yang J, Dou Y, *et al*. 2008. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated Pseudomonas stutzeri A1501. *Proceedings of the National Academy of Sciences, USA* **105,** 7564–7569.