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Understanding and exploiting plant beneficial microbes

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After a century of incremental research, technological advances, coupled with a need for sustainable crop yield increases, have reinvigorated the study of beneficial plant–microbe interactions with attention focused on how microbiomes alter plant phenotypes. We review recent advances in plant microbiome research, and describe potential applications for increasing crop productivity. The phylogenetic diversity of plant microbiomes is increasingly well characterized, and their functional diversity is becoming more accessible. Large culture collections are available for controlled experimentation, with more to come. Genetic resources are being brought to bear on questions of microbiome function. We expect that microbial amendments of varying complexities will expose rules governing beneficial plant–microbe interactions contributing to plant growth promotion and disease resistance, enabling more sustainable agriculture.

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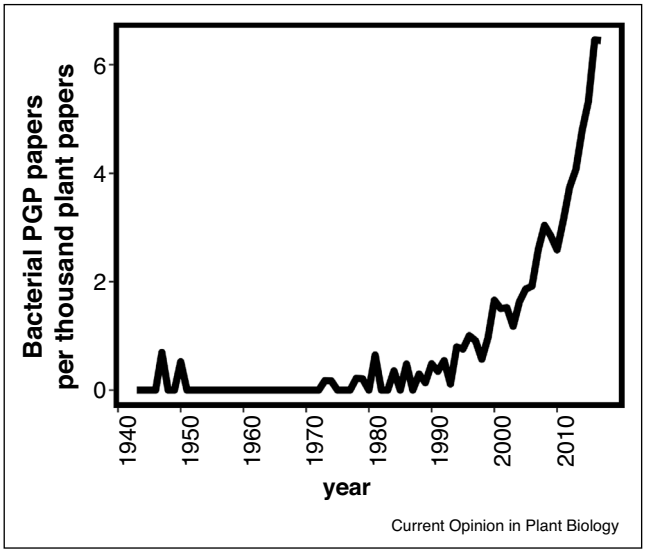
Introduction

The manipulation of soil microbiomes to optimize crop productivity is an ancient practice; records can be traced to ~300 BC [1]. It is interesting to note that although soil microbiomes are now touted as a cornerstone of the next green revolution [2•], the first commercial bioinoculant, 'nitrogin', was patented in 1896 [3], during the golden age of microbiology and preceding the Haber–Bosch process by 15 years. Currently, the Organic Materials Review Institute (OMRI) lists 174 products under the category of 'microbial inoculants' and 274 products under the category 'microbial products', either as crop fertilizers or as crop management tools. The number of publications associated with plant growth promoting (PGP) microbes, has been growing exponentially since the 1990s (Figure 1). Few, if any, of these are associated with mechanistic studies or modes of action; exceptions being biological nitrogen fixation by rhizobia on legumes [4], and auxin [5] or ACC-deaminase [6] -mediated phytostimulation. However, the lack of broad host ranges and variable field efficacy have sharply limited their widespread deployment. We therefore need to forge a deeper understanding of (a) the mechanisms governing microbial invasion and persistence into standing heterogeneous communities in diverse locations, soils and hosts; and (b) the genetics, in both partners, that drives colonization and delivery of plant phenotypes by microbes. The advent of culture-independent microbial ecology, powered by development of high-throughput analytic technologies, has enabled increasingly systematic study of the plant-associated ecological context in which microbial inoculants could be applied; and of mechanisms of plant control over colonization by beneficial microbes. However, novel approaches are needed in order to bridge current gaps between plant-productivity phenotypes and understanding of the underlying mechanisms [7–9].

Screening of large isolate collections

The limited taxonomy of plant-associated microbes, compared with the vast diversity of soil microorganisms [9–11], suggests that plants are a highly selective microbial niche and thus that general rules may be inferred for plant colonization by microbes. Shotgun metagenomics to compare plant-associated microbiome functions can be used to search for plant colonization markers [12,13]; this can be complemented by read-binning and assembly of bacterial genomes from plant-associated environments [14]. However, metagenomic datasets from different rhizospheres exhibit little overlap in plant-enriched functions [13,15]. On the other hand, plant-associated microbiomes contain a relatively high cultivable fraction of microbes, particularly bacteria [16•,17–19]. It is therefore feasible in plant microbiome research to mitigate the limitations of culture independent methods by generating and studying taxonomically and functionally representative culture collections from plant-associated

Figure 1



The number of articles about bacterial plant growth promotion per year per thousand plant-related papers, found in the PubMed database, using the search term ((“plant development”[MeSH Terms] OR (“plant”[All Fields] AND “development”[All Fields]) OR “plant development”[All Fields] OR (“plant”[All Fields] AND “growth”[All Fields]) OR “plant growth”[All Fields]) AND promoting[All Fields] AND (“microbiology”[Subheading] OR “microbiology”[All Fields] OR “bacteria”[All Fields] OR “bacteria”[MeSH Terms])) OR ((“plant development”[MeSH Terms] OR (“plant”[All Fields] AND “development”[All Fields]) OR “plant development”[All Fields] OR (“plant”[All Fields] AND “growth”[All Fields]) OR “plant growth”[All Fields]) AND promoting[All Fields] AND rhizobacteria[All Fields]).

habitats. Returning to culture-dependent microbial surveys allows the construction of increasingly complex experimental ecology systems for understanding plant–microbe interactions, while providing material for the discovery of potential PGP inoculants. Large-scale isolation, genome sequencing and functional screening efforts are underway in both academic and industrial settings (http://news.monsanto.com/press-release/corporate/novozymes-and-monsanto-complete-closing-bioag-alliance). The definition of large scale, in fact, has changed rapidly from hundreds [16•] to tens of thousands [20] of strains. Recent plant-associated bacterial and fungal isolate collections (summarized in Table 1) are derived from sugarcane [20]; grapevine [21–23]; potato [24]; tomato [25]; eucalyptus [25]; rice [26,27]; ancient wheat ancestors [19]; lettuce [28]; Arabidopsis [16•,29]; poplar [29]; and from plants growing in an arsenic-contaminated soils [30•]. The increasing volume of isolate collections will tax existing repositories; yet the genomic diversity contained in the bacterial isolates that are being obtained is not nearing saturation [16•]. Mechanisms to curate, share and standardize metadata for strains from these collections are needed.

While ongoing attempts exist to screen isolates in the field (see Supplementary Table 1 summarizes of recent PGP experiments in laboratory and field settings), the most common approach is to utilize a pre-screening strategy to select candidate strains for further analysis. Pre-screening strategies include *in vitro* screening for known PGP-related activities such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase [31], phosphate solubilization [32], nitrogen fixation [33], or enhancement of plant immune system function [34,35•,36]. Of the 1151 bacterial strains screened in [21,22,27,29], 332 strains solubilized phosphate, 229 strains produced auxin; ACC deaminase activity was found in 85 of 729 strains and bacterial nitrogen fixation was measured in 54 of 229 strains. These screening methods, however, are more likely to confirm the known, rather than to discover novel mechanism of PGP. Furthermore, none of these traits are actually correlated with the magnitude of PGP. Thus the suite of PGP traits that are commonly tested does not predict plant-associated phenotypes, and suggests that untapped mechanisms await discovery.

Genome sequencing of strain collections (Figure 2) [16•,37•] might provide a richer screening tool for sets of PGP traits that could be readily detected in genomes [38]. For example, the presence of minimal *Nif* and full *Phn* gene cassettes and genes required for indole acetic acid (IAA) production corresponded to the respective phenotypes in *Paenibacillus polymixa* genomes, albeit at variable levels [39]. This indicates that identification of appropriate genomic markers and screening of genome collections might provide a faster and less labor-intensive alternative to physiological screening, while also providing the opportunity for the discovery of correlated and novel PGP-associated genes.

Ecological considerations for plant beneficial function of microbes in the field

Ultimately, beneficial phenotypes will need to be operative in the field. A successful microbial inoculant has to invade and persist in the context of indigenous microbes and local abiotic conditions in variable settings, and to establish a compatible interaction with the host that includes molecular détente with the plant immune system. Studies of successional dynamics of plant microbiota suggest that upon emergence, initial seed microbiomes rapidly give way to different, soil-derived communities that are still changing days following emergence [40]. Throughout the growing season, this soil-derived community undergoes continuous succession in both above-ground [41,42] and below-ground [43•] fractions of the plant. Thus, even if PGP inoculants colonize the plant initially, their persistence over time is not guaranteed. Measuring persistence of bacterial inoculants in soil poses technical difficulties, as the inoculant needs to be identified from within a complex community. Heterologous bacterial inoculants can persist in soil for

Table 1

Summary of recent microbial culture collections from plant-associated environments

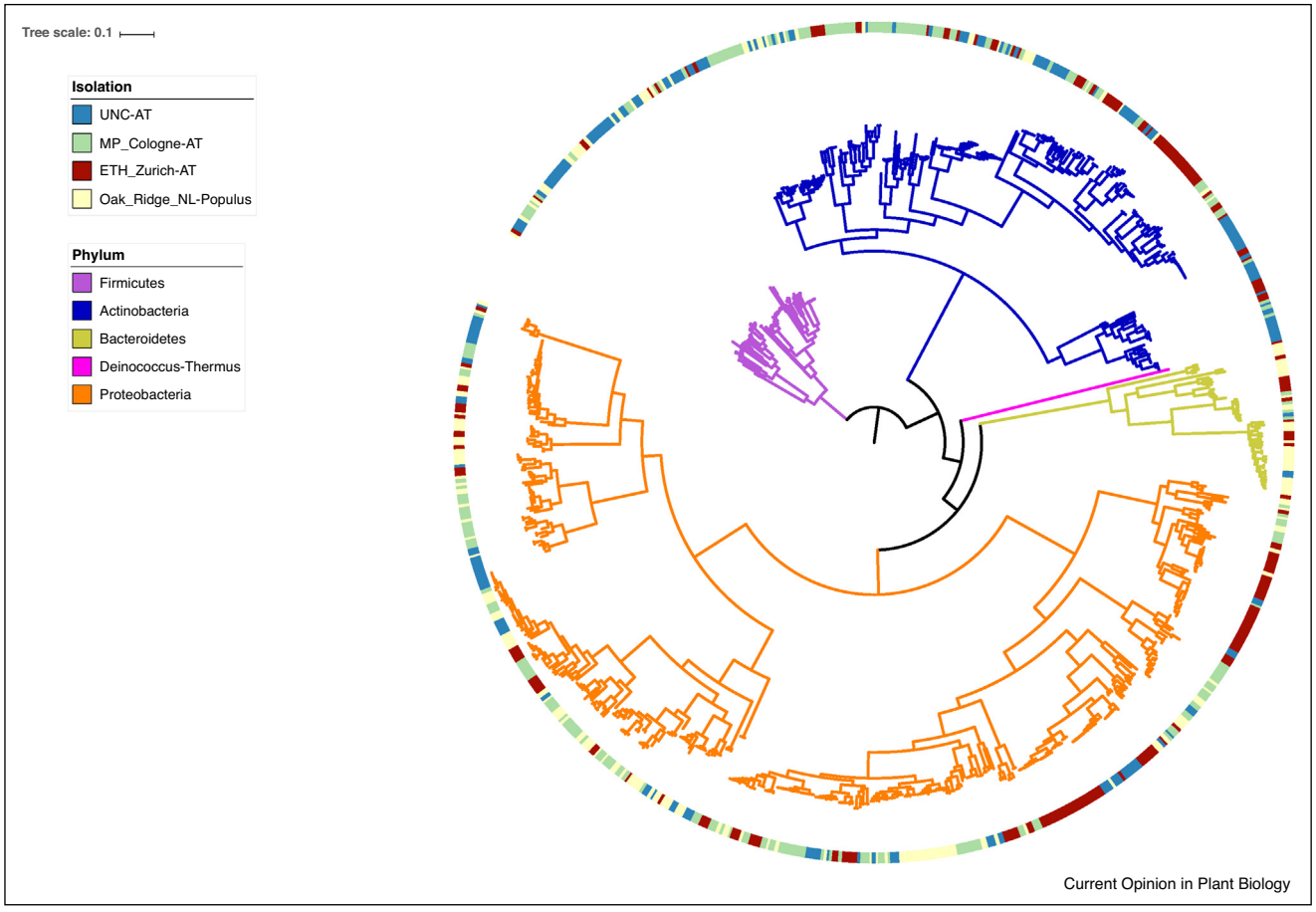
Specimen	Plant compartment	No. of isolates	Domain	Analyses performed	Reference
Arabidopsis thaliana	Shoot, root and soil	7976	Bacteria	16S rRNA sequenced 432 genomes sequenced	[16•]
Arabidopsis thaliana	Root	196	Bacteria	Genomes sequenced	[29]
Populus deltoides	Root	203	Bacteria	Genomes sequenced	[29]
Eucalyptus	Rhizosphere and rhizoplane	298	Bacteria	Control of bacterial wilt in *Eucalyptus* and *Solanum lycocersicum* caused by *Ralstonia solanacearum*	[25]
Oryza sativa	Phyllosphere	86	Bacteria	Indole acetic acid (IAA) production, nitrogen fixation	[26]
Oryza sativa	Root, stem and leaves	1318	Bacteria	Automated Ribosomal Intergenic Spacer Analysis 762 members 16S rRNA sequenced 689 members identified at the genus level The 228 members of the working collection were analyzed for *in vitro* PGP and plant immunity traits	[27]
Saccharum officinarum	Rhizosphere, endophytic root and endophytic stalk compartments	5137 colony communities	Bacteria	16S rRNA sequenced	[20]
Solanum tuberosum	Phylloplane and interiors, soil and tubers surface	243	–	Interaction assays with plant pathogens	[24]
Soil	Soil within the rhizosphere of different autochthonous plants	80	Bacteria	Arsenic resistance Arsenate reductase activity	[30•]
Triticum dicoccoides *Aegilops sharonensis* *Triticum aeastivum*	Stems and seeds	686	Fungi	514 members ITS sequenced Categorization into cOTUs at 97% sequence similarity	[19]
Vitis vinifera *Lepidium draba* L.	Rhizosphere and root	500	Bacteria	A range of bacterial features known to contribute to PGP, stress tolerance or bio-control	[22]
Vitis vinifera	Root, shoot and leaves	381	Bacteria	377 members evaluated for features known to contribute to PGP. Evaluation of their effects on root development in *Arabidopsis thaliana*	[21,98]
Vitis vinifera	Rhizosphere and endosphere	510	Bacteria	8 strains examined for an array of PGP abilities *in vitro*, focusing both on conventional and drought-related PGP traits	[23]

up to seven weeks [23,27,44,45••,46], but whether they are at levels necessary to continuously provide PGP activity is not clear. Methods to detect persistence include culture-based enumeration using re-isolation of antibiotic resistant inoculants [27,44], or culture-independent measurement of relative abundance of the inoculant's 16S rRNA gene in the soil, via DGGE [23,44]; amplicon sequencing [45••] or by metagenomic sequencing [46].

Diversity of the inoculum

The diversity of a microbial inoculum can widen available plant-associated niches and enhance productivity [47–50]. However, interpreting the effect of microbial (and plant) diversity on microbiome function must be done in a nuanced and context-dependent manner [51]. Complex inocula can provide plants with stronger disease resistance [52,53•,54–57] and growth promotion [58,59] than single strains. Use of a complex inoculum, rather than single strains, improved arsenic sequestration efficiency of the hyper-accumulator fern *Pteris vittata* [30•], tripling phytoremediation efficiency. In other cases, however, consortia were equal to, or worse than, some individual strains tested, as demonstrated by the growth of grapevines under drought stress [23]. Consortia can consist of either closely related strains used to expand the niche breadth of a certain trait [54,55], or of distantly related strains providing PGP via different mechanisms, thus contributing to an overall additive effect [59]. However, increasing strain richness, for example, within the biocontrol species *Pseudomonas fluorescens*, can cause community collapse and subsequent loss of plant protection [60]. Multispecies inocula can be used to exploit positive microbe–microbe interactions. For instance, bacteria can enhance germ tube elongation and hyphal branching in arbuscular mycorrhizal fungi (AMF), promoting the symbiotic development of the AMF on potato plants [61].

Figure 2



Diversity of genome-sequenced plant associated bacterial isolates from Arabidopsis and Populus currently available on IMG/JGI.
An approximately-maximum-likelihood phylogenetic tree of 831 plant-associated bacteria isolated from *Arabidopsis thaliana* roots (blue and green bars) and shoots (red bars); and *Populus deltoides* roots (yellow bars). Tree branches are colored by Phylum. Purple: Firmicutes, Blue: Actinobacteria, Yellow: Bacteroidetes, Pink: Deinococcus-thermus and Orange: Proteobacteria. The tree was constructed using a concatenated filtered alignment of 31 single copy genes [99,100]. The genome assemblies of each of the three different sequencing projects can be accessed via the IMG/JGI portal by using the following project IDs:
"Genome sequencing of Arabidopsis leaf and root microbiota representing the majority of bacterial species in their natural communities." *A. thaliana.* Max Planck Cologne and ETH Zurich.
"Plant associated metagenomes-Microbial community diversity and host control of community assembly across model and emerging plant ecological genomics systems." *A. thaliana*. University of North Carolina.
"Populus root and rhizosphere microbial communities from Tennessee, USA"—*P. deltoides.*
The tree can be visualized and downloaded on iTOL [101] using the following link: http://itol.embl.de/tree/1522317731415721485965060 or via the user Understanding_the_plant_microbiome_COPB.

The prospect of inoculating crops with consortia rather than single strains exponentially increases the complexity of experimental screening systems. This demands solid design and an analytic framework that is experimentally tractable. Synthetic bacterial communities consisting of up to hundreds of strains have been shown to colonize plants in a reproducible pattern under gnotobiotic conditions [16•,37•,57], providing a powerful research tool to study microbial PGP and microbe–microbe interactions.

Plant–microbe interactions in soil

Any plant microbiome is a direct function of the microbial meta-community found in the soil around it [10,11,43•], a community that in turn can be deeply impacted by agricultural practices [62,63]. Continuously growing crops in agricultural soils can result in pathogen buildup [56] but also in the emergence of disease-suppressive soils: soils that convey resistance to plant pathogens and can contain biocontrol agents within their resident bacterial community [36]. The observation of disease suppressive soils has been linked to shifts in microbial community composition and activity [56,64–66]. Microbial communities in the soil can induce other phenotypes in plants. Artificial selection experiments [67] have shown that iteratively selecting soil slurries can alter plant biomass [68]. Biotic plant–soil feedback was

recently shown to be dependent on the type of mycorrhizal fungi or biologically nitrogen fixing (BNF) bacteria that associate with plants [69••,70••]. Plants associating with AMF or with BNF exhibited conspecific growth inhibition, a long standing observation that can be linked to density-dependent predation or disease [71]. In contrast, plants associating with ectomycorrhizal fungi (EF) exhibited the opposite trend: local conspecifics facilitate growth linked to stronger protection from pathogens provided by EF. EF fungi preferentially link to, and transfer carbon between, kindred trees in ectomycorrhizal networks [72], potentially explaining conspecific facilitation. As the spatial density of monoculture, agricultural crops resembles that of EF-associated plants, the mechanisms that evolved to protect from pathogens in EF-associated species may be relevant to how biological disease suppression is applied to crops. Ultimately, both sides of this ancient interaction need to be understood if we are to harness them for agricultural productivity.

Genetic control of beneficial plant–microbe interactions

Plants assemble distinct root [10,11,73] and shoot [18,74,75] microbiomes from the surrounding soil and air. Significant differences in microbe community composition were detected between plant species [13,73,76–79], and between natural accessions of the same species, though the intraspecific genetic contribution to microbiome assembly is quite low [10,11,15,18,74,80,81••]. Nevertheless, these findings demonstrate that host genetics contribute to plant microbiome assembly; whether the observed heritability will be actionable with respect to plant breeding using genetic approaches based on natural variation to identify causal host genes remains to be demonstrated.

Plants detect microbes via pattern-recognition receptors that bind microbe-associated molecular patterns (MAMPs), triggering a basal defense sufficient to halt the growth of most pathogenic microbes [82,83]. Most non-pathogenic bacteria and fungi associated with plants are sure to produce their own MAMPs, which prompts the question of how beneficial microbes and plants manage to avoid elimination of the microbes via an immune response. Plants can presumably discriminate pathogens from non-pathogens and respond by either resisting microbial growth, ignoring it, or actively supporting it on or within plant tissues. The transcriptional response of Arabidopsis leaves differs when inoculated with different non-pathogenic members of its natural microbiota [35•]. While *Methylobacterium extorquens* induces almost no transcriptional response, *Sphingomonas melonis* activates the expression of defense related genes that partly overlap with those triggered by the pathogen *Pseudomonas syringae* DC3000 [35•]. This may represent a mechanism of plant defense priming [84] driven by the plant microbiome. The response pattern to non-pathogenic bacteria can differ both across plant species [13] and across accessions within a single species [45••]. While some Arabidopsis accessions are colonized by, and establish a beneficial relationship with *Pseudomonas fluorescens*, other accessions actively inhibit growth of the same strains in their roots.

Given the critical function of defense phytohormones in the plant immune system, it is not surprising that plant microbiome composition is influenced by defense phytohormone signaling. Experiments using a set of mutants with altered defense phytohormone synthesis and/or perception demonstrated that salicylic acid and/or salicylic acid-mediated events influence the root microbiome composition at multiple taxonomic levels [37•]. These data suggest that microbial inoculants will not act as 'one size fits all', and may need to be specifically tested down to the host genotype level [74].

The plant microbiome structure changes upon infection [12,53•]. This could represent a general response to the plant defense mechanisms, but may also reflect changes made to the habitat by the pathogen [85•,86••]. Antifungal traits are enriched in barley following infection with *Fusarium graminearum*, potentially via changes in exudate composition [87]. A study of tomato plants challenged with the pathogen *Ralstonia solanacearum* revealed that the root exudation profile changed upon pathogen infection, increasing the secretion of phenolic compounds. Exudates of infected plants were correlated with changes in soil microbial abundances, and this response could be emulated *in vitro* using caffeic acid, one of the phenolic compounds secreted [88]. Similar patterns of pest-inhibition were shown in response to insect herbivores. Inoculation of Arabidopsis with *Pseudomonas fluorescens* WCS417r altered the plant volatile emission profile in response to an herbivorous caterpillar; this correlated with recruitment of an insecticidal parasitoid wasp [89].

Plant defense mechanisms also impact other drivers of plant–microbe interactions, like plant nutrition [90]. Mutant analysis in *Lotus japonicus* demonstrated that the root nodulation pathway plays a role not only in nitrogen-fixing symbiosis, but also in the establishment of taxonomically diverse root microbiome [79]. Modern molecular approaches are also being applied to understanding nitrogen-fixing symbioses in non-nodulating plants. Use of dual host-microbe transcriptomics demonstrated that the capacity of a nitrogen-fixing *Burkholderia* strain to form microaerobic biofilms on sugarcane roots is preceded by reduced motility and immunogenicity, followed by metabolic adaptation to the sugar-rich plant environment. The plant does not activate an immune response, but does change its root morphology, and supplies the bacterium with photosynthates [91], a

response pattern that is analogous to the process of infection by BNF in legumes [92].

Regulatory overlap exists between the plant immune system and nutritional stress, as recently demonstrated in the context of the plant phosphate starvation response (PSR) [93]. Signaling dependent on the phytohormone jasmonic acid (JA) and JA accumulation is induced during PSR in Arabidopsis. The Arabidopsis *pho1* mutant, deficient in shoot phosphate accumulation, exhibited an activation of JA signaling leading to increased resistance to insect herbivory [94]. PSR signaling and concomitant dampening of the immune system can also regulate beneficial fungal colonization [95••,96]. Fungus-mediated PGP also requires PEN2-dependent indole glucosinolate metabolism, a component of plant defense [95••]. Experiments using both wild soil and a synthetic bacterial community demonstrated that Arabidopsis PSR mutants assemble a different root microbiome than wild type plants, even when grown in phosphate-sufficient soil. Further, the master transcriptional regulator of PSR directly integrates this nutritional stress response and immune system outputs [97••]. These early examples suggest that the coordination of defense and nutrition is critical to driving microbiome function.

Conclusions

The study of plant microbiomes has benefited from holistic ecological studies on one-hand and reductionist mechanistic discoveries on the other. Both schools of thought are yielding increasingly profound insight into the ecological processes that govern plant–microbe interactions as well as the molecular mechanisms that facilitate them. The generation of large isolate collections and the study of synthetic microbial communities in combination with plant genetic resources, will allow us to bridge this gap and to conduct reductionist, hypothesis driven studies in increasingly complex ecological contexts up to field tests. These advances have the potential to transform our understanding of plant–microbe interactions in nature and in agriculture, and will contribute significantly to the next green revolution.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at **http://dx.doi.org/10.1016/j.pbi.2017.04.018**.

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