MOLECULAR ECOLOGY

Molecular Ecology (2014) **23**, 1571–1583 doi: 10.1111/mec.12571

SPECIAL ISSUE: NATURE'S MICROBIOME

Diffuse symbioses: roles of plant–plant, plant–microbe and microbe–microbe interactions in structuring the soil microbiome

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Abstract

A conceptual model emphasizing direct host–microbe interactions has dominated work on host-associated microbiomes. To understand plant–microbiome associations, however, broader influences on microbiome composition and functioning must be incorporated, such as those arising from plant–plant and microbe–microbe interactions. We sampled soil microbiomes associated with target plant species (*Andropogon gerardii, Schizachyrium scoparium, Lespedeza capitata, Lupinus perennis*) grown in communities varying in plant richness (1-, 4-, 8- or 16-species). We assessed *Streptomyces* antagonistic activity and analysed bacterial and *Streptomyces* populations via 454 pyrosequencing. Host plant species and plant richness treatments altered networks of coassociation among bacterial taxa, suggesting the potential for host plant effects on the soil microbiome to include changes in microbial interaction dynamics and, consequently, co-evolution. Taxa that were coassociated in the rhizosphere of a given host plant species often showed consistent correlations between operational taxonomic unit (OTU) relative abundance and *Streptomyces* antagonistic activity, in the rhizosphere of that host. However, in the rhizosphere of a different host plant species, the same OTUs showed no consistency, or a different pattern of responsiveness to such biotic habitat characteristics. The diversity and richness of bacterial and *Streptomyces* communities exhibited distinct relationships with biotic and abiotic soil characteristics. The rhizosphere soil microbiome is influenced by a complex and nested array of factors at varying spatial scales, including plant community, plant host, soil edaphics and microbial taxon and community characteristics.

Keywords: coassociation networks, soil microbiome, species interactions, *Streptomyces*

Received 16 April 2013; revision received 20 September 2013; accepted 20 September 2013

Introduction

A frontier for new biological discovery, soil harbours vast biodiversity (Torsvik & Øvreås 2002), much of which has only recently been made available to study by advancing technological capacity (Carvalhais *et al.* 2012). The diversity of life in soil is predominantly microbial, and there is a great deal that we do not yet understand about the structure and functioning of the soil microbiome (Little *et al.* 2008), the full suite of microorganisms present in soil and their genetic capacity. However, plants have been a central focus among possible determinants of the composition and structure of the rhizosphere microbiome as they are the source of the majority of carbon that supports heterotrophic life in soil.

Plants interact extensively with soil micro-organisms, with reciprocal impacts on fitness. Impacts of the soil microbiome on plants span a continuum from beneficial to detrimental and are relevant in both managed and natural habitats. For instance, productivity of

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agricultural crops can be impacted positively through the activities of soil organisms that suppress disease (Wiggins & Kinkel 2005b) or enhance access to nutrients (Richardson *et al.* 2011). Conversely, plant productivity can be reduced by microbial pathogens (Strange & Scott 2005) or through microbial activities that reduce the availability of nutrients (Cameron *et al.* 2013). Plants also exert reciprocal effects on the soil microbiome (Hartmann *et al.* 2009), influencing soil microbial community structure. For instance, even small changes in plant genotype sometimes lead to distinct differences among soil microbial communities (Castaldini *et al.* 2005; Lundberg *et al.* 2012).

It has been commonly assumed that host plant selective effects result from direct plant–microbe interactions. For instance, a great deal of attention has been given to the potential for characteristics of plant root exudates to preferentially enrich distinct soil populations (Walker *et al.* 2003; Bais *et al.* 2006). Despite the intensive focus on the significance of pairwise plant–microbe interactions to the soil microbiome, this view of tightly coupled plant–microbe interactions is artificially simple and perhaps irrelevant to the majority of microbial taxa in soil. This focus probably arose because the microbial functional groups that have received the most study are those that participate in relatively tightly coupled symbioses. Yet the vast majority of members of the soil microbiome do not engage in such close interactions. Depending upon the microbial taxon or functionality of interest, it may be necessary to change the conceptual lens through which we study and understand plant–microbiome linkages. Specifically, the roles of both larger-scale (landscape, plant community) and smaller-scale (within-community microbial interactions) factors in modifying soil microbiome structure and function require further study.

Plants serve as the primary carbon source for the soil microbiome, but plant-driven effects on microbial composition or relative abundance may be indirect for most microbial taxa, including taxa that have significant effects on plant fitness. In particular, localized interactions among soil micro-organisms are likely to be critical to microbial community dynamics (Czaran *et al.* 2002; Hibbing *et al.* 2010; Kinkel *et al.* 2011), perhaps as much or more than plant–microbe interactions. Crucially, the outcomes of interactions between species are influenced by characteristics of the environment (Drakare 2002; Tétard-Jones *et al.* 2007). For the soil microbiome, plants play a keystone role in shaping the soil environment via their impacts on soil chemistry and the quantity and characteristics of soil organic matter. In this way, plants set the stage upon which the drama of microbial interactions plays out. For instance, soil microbes engage in intense competition for nutrients (Little *et al.* 2008). Plants provide the resources, but microbe–microbe interactions are critical to community dynamics, structure and function (Kinkel *et al.* 2011).

In a similar fashion, the assumption of tightly coupled plant–microbe interactions underestimates the complexity of plant effects on the soil microbiome. Plants are embedded in communities that can range from simple (e.g. agricultural monocultures) to highly complex (e.g. temperate grasslands and tropical forests). Plant productivity, nutrient allocation and tissue chemistry can vary significantly depending on the identity of neighbouring individuals (Gersani *et al.* 2001; Murphy & Dudley 2009; Broz *et al.* 2010). This suggests that the effects of a given plant 'host' on the soil microbiome may be substantially mediated by the community context of that host, as previous work has shown (Bakker *et al.* 2013a,b; Schlatter *et al.* submitted).

Furthermore, studies of the soil microbiome need to move beyond assessments of composition or structure to incorporate functional aspects of soil microbiomes (Torsvik & Øvreås 2002). Indeed, connecting microbial community structure and functioning is a primary goal of microbial ecology. We need to understand not only what forces shape microbial community composition, but also how those forces influence the functions or services provided by those communities. However, it can be difficult to assign functions of interest to particular members of the microbiome. Many microbiome studies consider only the totality or aggregate microbial community, either because of limits to taxonomic resolution or in an attempt to be comprehensive. This approach may make it challenging to draw linkages between microbiome characteristics and functional attributes, which are unlikely to be evenly distributed among taxa. To draw clear linkages between specific community functions and microbial composition or structure, it will be important to consider both broad-scale microbiome characterization as well as more targeted assessments of particular taxa hypothesized to be significant to key microbiome functions.

One important function of soil microbial communities that has significant feedbacks to plant fitness is the suppression of plant pathogens. The *Streptomyces* (phylum Actinobacteria, order Actinomycetales, family Streptomycetaceae) have been studied extensively in regard to plant disease (Kinkel *et al.* 2012). *Streptomyces* have been used in inoculative biocontrol of plant pathogens (Yuan & Crawford 1995; Jones & Samac 1996; Liu *et al.* 1996; Xiao *et al.* 2002) and have also been a focus of efforts to manage indigenous microbial communities for pathogen suppression (Wiggins & Kinkel 2005a,b; Perez *et al.* 2008). *Streptomyces* are particularly notable for their prodigious production of antibiotic compounds (Challis & Hopwood 2003), and these are believed to be vital to

suppression of plant pathogens in soil (Rothrock & Gottlieb 1981). Antibiotics may also mediate a wide array of other organismal interactions, including microbe–microbe and plant–microbe interactions in soil (Linares *et al.* 2006; Seipke *et al.* 2012).

The maintenance of antibiotic-producing phenotypes among *Streptomyces* is hypothesized to be a result of selection via competitive interactions with other microbes (Kinkel *et al.* 2011, 2012), including interactions both among *Streptomyces* and with other microbial taxa. Like plant–microbe interactions, microbe–microbe interactions have reciprocal effects, with the potential for both partners to influence one another's fitness. The abundance and ubiquity of *Streptomyces* in soil, their broad nutrient utilization capacities (Schlatter *et al.* 2013) and their ability to inhibit a diverse array of soil fungi and bacteria (Bakker *et al.* 2010) suggest that *Streptomyces* may interact in significant ways with the broader soil microbiome. Indeed, there is a growing recognition that microbial communities are highly interactive entities and that positive and negative interactions among distantly related microbes are common (Linares *et al.* 2006; Schrey *et al.* 2007; Wu *et al.* 2012). Thus, understanding the composition, diversity and functioning of *Streptomyces* communities in soil requires explicit consideration of the networks of interactions occurring within the microbiome (Barberan *et al.* 2012).

In this work, we integrate *Streptomyces* community structure (assessed via targeted sequencing; Bakker *et al.* 2013a) with the broader bacterial community (assessed via nontargeted sequencing; Schlatter *et al.* submitted) and with measures of *Streptomyces* functioning (in vitro antagonistic activity; Bakker *et al.* 2013b). Furthermore, our experimental design gives explicit consideration to the broader plant and microbial context within which plant–microbiome interactions occur (Fig. 1). We investigate patterns of composition, structure, coassociation and function among soil microbiomes, and how these change with target host plant species and across plant richness treatments.

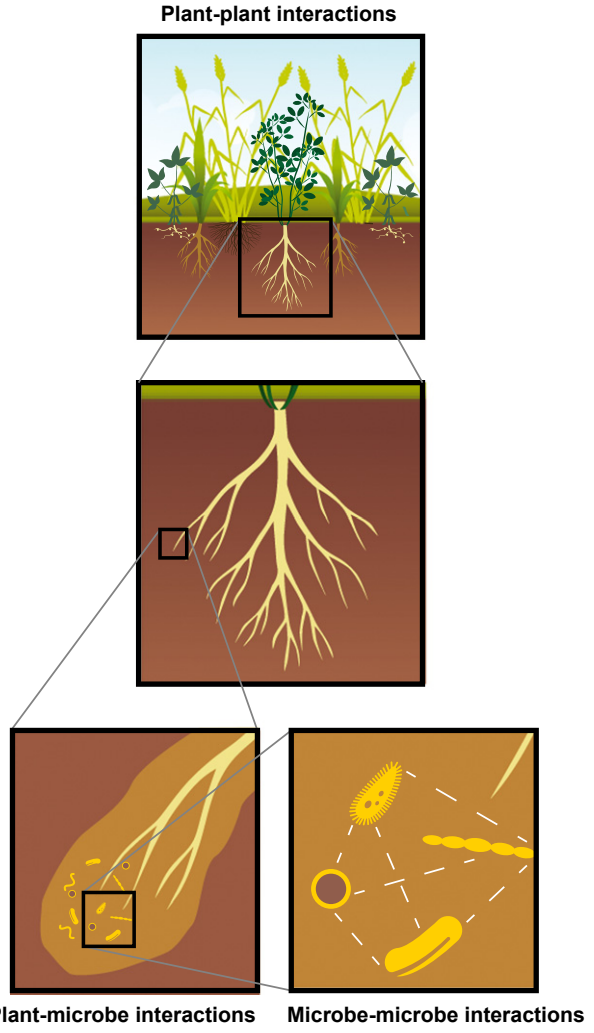

Fig. 1 Species interactions at diverse scales can impact soil microbiome composition, structure and functioning. Most attention to date has been given to direct plant–microbe interactions. However, microbe–microbe interactions, taking place in an environment under plant influence, are vital in shaping microbiome structure and functioning. Furthermore, plant–plant interactions can alter host plant impacts on associated soil microbiomes.

Materials and methods

Sampling design

We sampled soil microbiomes under the dominant influence of four perennial prairie plant species (two C4 grasses: *Andropogon gerardii* and *Schizachyrium scoparium*; two legumes: *Lespedeza capitata* and *Lupinus perennis*), with each species grown in communities planted to 1-, 4-, 8- or 16-species mixtures (Tilman *et al.* 2001). There were 48 soil samples collected in total (4 plant species × 4 plant richness treatments × 3 replicates). Detailed methods on sample collection and processing have been reported elsewhere (Bakker *et al.* 2013a).

Streptomyces antagonistic activity

Streptomyces antagonistic activity was assessed in vitro as an estimate of the potential for suppression of plant pathogens. Briefly, antagonistic activity was measured as the density [log colony-forming units (CFU)/g] and proportion of colonies on a *Streptomyces*-selective medium that created visible zones of inhibition when overlaid with an indicator organism. We used three

different *Streptomyces* isolates (Davelos *et al.* 2004) as indicators and analysed mean values across indicator strains. The intensity of inhibition was assessed as the mean radius of inhibition zone sizes across inhibitory colonies for a given soil sample. Detailed methods, reporting and interpretation of these data have been presented elsewhere (Bakker *et al.* 2013b). Here, we link these microbial community functional data to detailed characterization of community composition and structure.

Sequencing and sequence processing

Our microbiome sequence data gave particular attention to the *Streptomyces*, but included taxa across the domain Bacteria. We took this approach because we expect that interactions with phylogenetically distinct taxa may be important to the generation and maintenance of the inhibitory phenotypes that contribute to pathogen suppression by *Streptomyces*. Our data set included amplicon sequencing from PCR using sets of 16S rRNA gene primers that were either universal and nonselective ('bacteria' data set) or selective for *Streptomyces* ('*Streptomyces*' data set). Detailed descriptions of the generation and processing of these sequence data have been reported elsewhere (Bakker *et al.* 2013a; Schlatter *et al.* submitted). Both sequence data sets were processed similarly, and operational taxonomic units (OTUs) were defined using a 97% sequence similarity criterion. The sample of bacterial communities consisted of 476 573 high-quality sequence reads, which were distributed across 26 153 OTUs belonging to 16 different phyla. The *Streptomyces*-targeted data set consisted of 59 184 high-quality sequence reads, which were distributed across 409 OTUs. The vast majority of these sequence reads (>90%) belonged to the *Streptomyces*, although other taxa within the phylum Actinobacteria were also represented at low relative abundances (Bakker *et al.* 2013a).

Coassociation networks

To investigate coassociation among bacterial taxa, we combined the total bacterial and the *Streptomyces*-specific data sets by concatenating OTU occurrence tables. Because different primer sets were used for sequencing targeted to *Streptomyces* vs. for universal coverage across bacterial taxa, OTUs could not be matched between data sets. Co-occurrence among common OTUs (those found in at least 50% of samples; $n = 626$ OTUs; see Table S1, Supporting information) was tested using the SparCC method (Sparse Correlations for Compositional data; Friedman & Alm 2012). The significance of SparCC correlations was assessed with a permutation test, using 99 permutations of the data table. OTUs having observed SparCC correlations that were positive and of greater magnitude than in any random permutation ($P < 0.01$) were input into network analyses. Networks of co-occurring OTUs were visualized using the software Gephi ver. 0.8.2 (Bastian *et al.* 2009). Network modules, or clusters of coassociated taxa, were defined with the default parameters in Gephi (with randomization, uniform edge weighting, resolution = 1.0). Other network characteristics (average degree, graph density, average clustering coefficient, network diameter, average path length) were also calculated in Gephi.

Statistical analyses

Statistical analyses were performed in R (R Development Core Team 2011) and PRISM ver.6.0c for Mac (GraphPad Software, Inc.). OTU abundance tables were rarefied to the depth of the shallowest sample in each data set. There was no filtering performed based on taxon rarity prior to calculating diversity statistics or pairwise distances. The diversity (Shannon index) and richness (Chao1 estimate) of bacterial and of *Streptomyces* communities were calculated using MOTHUR (Schloss *et al.* 2009). Patterns of similarity among bacterial and *Streptomyces* communities were evaluated with pairwise Bray–Curtis distances, using the vegdist function of the vegan package for R (Oksanen *et al.* 2011). Changes in the frequencies of phyla within network modules, relative to the input data set as a whole, were assessed with chi-square tests. Correlations between OTU relative abundances and *Streptomyces* inhibitory activities were explored using Pearson's correlation coefficient. Inhibitor densities were log-transformed prior to analysis, to meet assumptions of normality. Measured soil edaphic properties have been reported elsewhere (Bakker *et al.* 2013a). Spatial distances among plots were estimated as the Euclidean distances among all plot pairs using a gridded map of field plots (http://www.cedarcreek.umn.edu/research/data/). Samples from the same plot were considered to have a distance of zero. Spatial distances among all plot pairs were correlated with the absolute value of differences in antagonistic activity, using Mantel tests with 999 permutations to assess significance.

Results

Bacterial taxa coassociate within complex networks and have consistent relationships with microbial community interaction phenotypes

We generated a coassociation network comprised of common OTUs whose relative abundances were positively correlated across samples (Fig. 2). There were a total of 4057 such correlations, among 618 of the 626

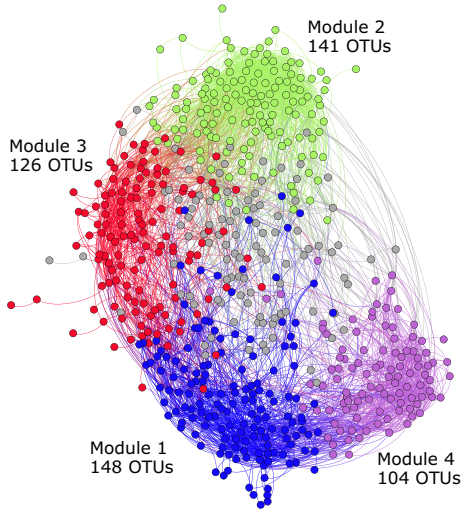

Fig. 2 Coassociation network of common bacterial and *Streptomyces* operational taxonomic units (OTUs; those present in at least 50% of samples), across host plant species and plant richness treatments (n = 48 samples). Nodes correspond to OTUs, and connecting edges indicate correlations in abundance across samples. Modules of coassociated OTUs containing at least 50 OTUs are coloured and labelled (Module 1–Module 4). For OTU identifiers associated with each module, see Table S1 (Supporting information).

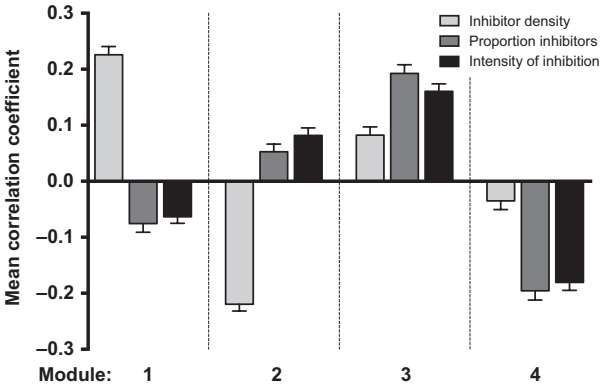

Fig. 3 Mean correlations (±SE) between operational taxonomic unit relative abundance and measures of *Streptomyces* inhibitory activity, for each network module shown in Figure 2.

common OTUs in the data set. Across host plant species and plant richness treatments, bacterial and *Streptomyces* OTUs formed modules of coassociated taxa, four of which were populated >50 OTUs each (Fig. 2). Although the distribution of sequences among phyla differed for modules compared with the data set as a whole (chi-square test, $P < 0.001$; Table S2, Supporting information), no modules were dominated by a single phylum. Rather, modules were consistently comprised of diverse taxa belonging to a range of phyla including Actinobacteria, Proteobacteria, Acidobacteria and unclassified phylum (Table S2, Supporting information). Thus, co-occurrence of OTUs within network modules was not primarily driven by the presence of closely related taxa that might be expected to share similar environmental preferences.

We assessed the importance of microbial species interactions in structuring soil microbial communities by relating OTU abundance across samples to *Streptomyces* inhibitory activities (Fig. 3). There was substantial variation among modules in mean correlation between OTU abundance and *Streptomyces* inhibitory activity (Fig. 3), with all four of the largest modules showing unique relationships to *Streptomyces* inhibitory activity. For instance, network Modules 1 and 2 exhibited opposite patterns of relationship (sign of mean correlation coefficient) with *Streptomyces* inhibitory activity; the abundance of OTUs in Module 1 tended to be strongly positively correlated with inhibitor density and weakly negatively correlated with the proportion of inhibitors and intensity of inhibition. In contrast, the abundance of OTUs in Module 2 tended to be strongly negatively correlated with inhibitor density and weakly positively correlated with the proportion of inhibitors and intensity of inhibition (Fig. 3). Modules 3 and 4 similarly showed opposite patterns of correlation with *Streptomyces* inhibitory activity (Fig. 3). These differences among modules suggest variation in sensitivity or responsiveness of OTUs in different modules to competitive inhibitory interactions.

Microbial coassociation networks vary among plant hosts and plant richness treatments

We divided the data set by host plant species or by plant richness treatment and re-assessed patterns of coassociation among OTUs to test whether the same microbial OTUs associated with one another in the microbiomes of different plant hosts or in plant communities of differing richness. To assess the consistency of microbial coassociation, we first defined network modules (groups of coassociated taxa) for samples from *A. gerardii*. We tracked the identity of each OTU across the coassociation networks for the other plant hosts (Fig. 4). Bacterial OTUs that were coassociated in a given plant treatment rarely shared patterns of coassociation under different plant treatments. For instance, among the OTUs that clustered into modules in the *A. gerardii* network, on average, only 25% remained clustered together in the network of another host plant species (Table 1). The largest observed overlap in module composition between host plant species was for *A. gerardii* network Module 4, for which 44% of

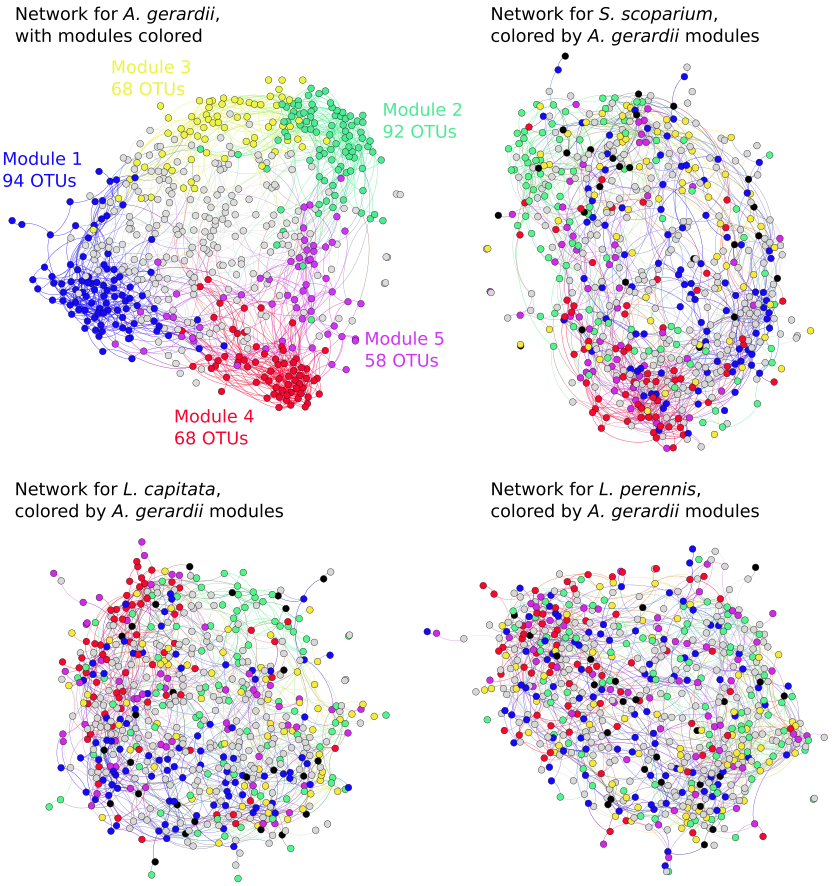

Fig. 4 Coassociation networks of common bacterial and *Streptomyces* operational taxonomic units (OTUs), for each host plant species (n = 12 samples per host plant species). Nodes correspond to OTUs, and connecting edges indicate correlations in abundance across samples. Nodes are coloured in each panel according to their membership in network modules defined for the *A. gerardii* samples. For OTU identifiers associated with each module, see Table S1 (Supporting information).

Table 1 Overlap in operational taxonomic unit (OTU) membership for modules present in networks formed by host plant species or plant richness treatment

Andropogon gerardii network module		1	2	3	4	5
Largest proportion of OTUs remaining grouped together, for samples collected from:	OTU count	94	92	68	68	58
	Schizachyrium scoparium	0.26	0.38	0.31	0.44	0.24
	Lespedeza capitata	0.16	0.21	0.19	0.26	0.16
	Lupinus perennis	0.20	0.23	0.21	0.28	0.24

Monoculture network module		1	2	3	4	5	6
Largest proportion of OTUs remaining grouped together, for samples collected from:	OTU count	105	95	88	80	69	54
	4 species plots	0.21	0.31	0.23	0.20	0.30	0.28
	8 species plots	0.22	0.32	0.26	0.18	0.17	0.30
	16 species plots	0.34	0.21	0.49	0.26	0.19	0.28

component OTUs remained clustered together in the network of *S. scoparium* (Table 1).

Similarly, we defined network modules for the monoculture samples and tracked individual OTUs across the microbial coassociation networks for the other plant richness treatments (Fig. S1, Supporting information). Again, most modules of coassociated taxa from the monoculture samples were broadly distributed among modules from other plant richness treatments; on average, only 26% of OTUs from monoculture modules remained clustered together in the network of another plant richness treatment (Table 1). The largest observed overlap in module composition between plant richness treatments was for monoculture network Module 3, for which 49% of component OTUs remained clustered together in the network from the 16 spp plots (Table 1).

Together, these results indicate that associations among the majority of OTUs differ fundamentally within the rhizosphere of different plant hosts, or within the rhizosphere of plant hosts in communities of different plant richness. This finding illustrates that explicit consideration of how the plant context alters patterns of microbe–microbe associations, and consequently potential for interactions, will be required to understand the factors structuring the composition, structure and functions of plant–microbiome associations.

To the extent that patterns of coassociation reflect environmental filtering due to abiotic environment or biotic interactions, altered patterns of coassociation among OTUs may suggest that the relative influence of distinct environmental drivers varies under the influence of different plant hosts or under communities of different plant richness. Among samples from a given plant species or plant richness treatment, the OTUs comprising individual modules commonly showed consistent relationships between relative abundance and *Streptomyces* inhibitory activity (Fig. 5, *A. gerardii* modules; Fig. S2, Supporting information, monoculture modules). However, in other host plant or plant richness treatments, the same OTUs showed no consistent correlation, or a different pattern of correlation, between relative abundance and *Streptomyces* inhibitory activity (Fig. 5, Fig. S2, Supporting information). For instance, the abundances of OTUs belonging to *A. gerardii* Module 1 were, on average, positively correlated with the proportion of inhibitory *Streptomyces*. Under the influence of other host plants, however, the correlation of these OTUs with the frequency of inhibitory *Streptomyces* was approximately zero (Fig. 5b). This result suggests that the environmental factors (whether biotic or abiotic) that structure the relative abundance of specific OTUs in the microbiome vary among plant hosts.

In aggregate, these results suggest that both plant host and plant community context alter population-limiting factors and patterns of coassociation among microbial taxa. Thus, microbe–microbe interactions may be fundamentally different and mediated by distinct environmental factors in soils associated with different plant hosts or in plant communities of varying species richness. Furthermore, the relative influence of environmental drivers on the abundance of individual OTUs varies with host plant species and plant richness across the landscape. Interestingly, plant richness treatments displayed more OTU coassociations than did plant hosts (mean of 6.09 correlations per OTU across plant richness treatment networks vs. mean of 5.34 correlations per OTU across host plant species networks; *t*-test, $P = 0.016$; Table S3, Supporting information). This may suggest that plant species richness has a larger impact on soil microbial species interactions than does host plant identity.

Responses to potential drivers of composition and structure differ between aggregate bacterial vs. Streptomyces communities

This work focuses on antibiotic-mediated inhibition of plant pathogens and specifically on antagonistic activities associated with soilborne *Streptomyces*. Thus, in attempting to identify factors that structure the composition and function of the soil microbiome, we were especially interested in determining whether broad microbiome characterization across all bacterial taxa, and more focused characterization of *Streptomyces* communities, yield comparable insights. As a first step, we considered patterns of diversity and richness between bacterial and *Streptomyces* communities among samples. Aggregate bacterial vs. *Streptomyces* richness and diversity were not correlated among samples (Chao richness estimate: $R = 0.022$, $P = 0.88$; Shannon diversity index: $R = 0.26$, $P = 0.07$). Neither were aggregate bacterial vs. *Streptomyces* richness and diversity significantly correlated among samples for individual plant hosts or within individual plant species richness treatments ($P > 0.05$; data not shown). Such a lack of consistency in richness and diversity suggests that the factors generating or maintaining diversity differ between the *Streptomyces* and the aggregate bacterial communities. This finding is reinforced by the distinct relationships between diversity and soil edaphic characteristics for bacterial vs. *Streptomyces* communities. Bacterial diversity (Shannon index) was negatively correlated with soil organic matter, nitrogen and carbon ($P < 0.05$; Table S4, Supporting information); more nutrient-rich soils supported less diverse bacterial communities. In contrast, soil nutrients were not significant correlates of *Streptomyces* diversity ($P > 0.05$; Table S4, Supporting information). Bacterial richness was significantly negatively correlated with soil organic matter, nitrogen and carbon, while *Streptomyces* richness was significantly positively correlated with organic matter, nitrogen, carbon, potassium and pH ($P < 0.05$; Table S4, Supporting information). Thus, soil nutrients were broadly positively correlated with *Streptomyces* richness and negatively correlated with bacterial richness. This suggests that environmental drivers of taxon richness differ for overall bacterial communities vs. for our target lineage, the *Streptomyces*. More broadly, there are unlikely to be simple predictors of phylogenetic richness or diversity among diverse microbial taxa in soil.

Despite the traditional emphasis on diversity as a community metric, the importance of microbial diversity per se for the functioning of soil microbial communities is not clear. As an alternative, patterns of similarity in community structure may better reflect consistency in community responses to environmental

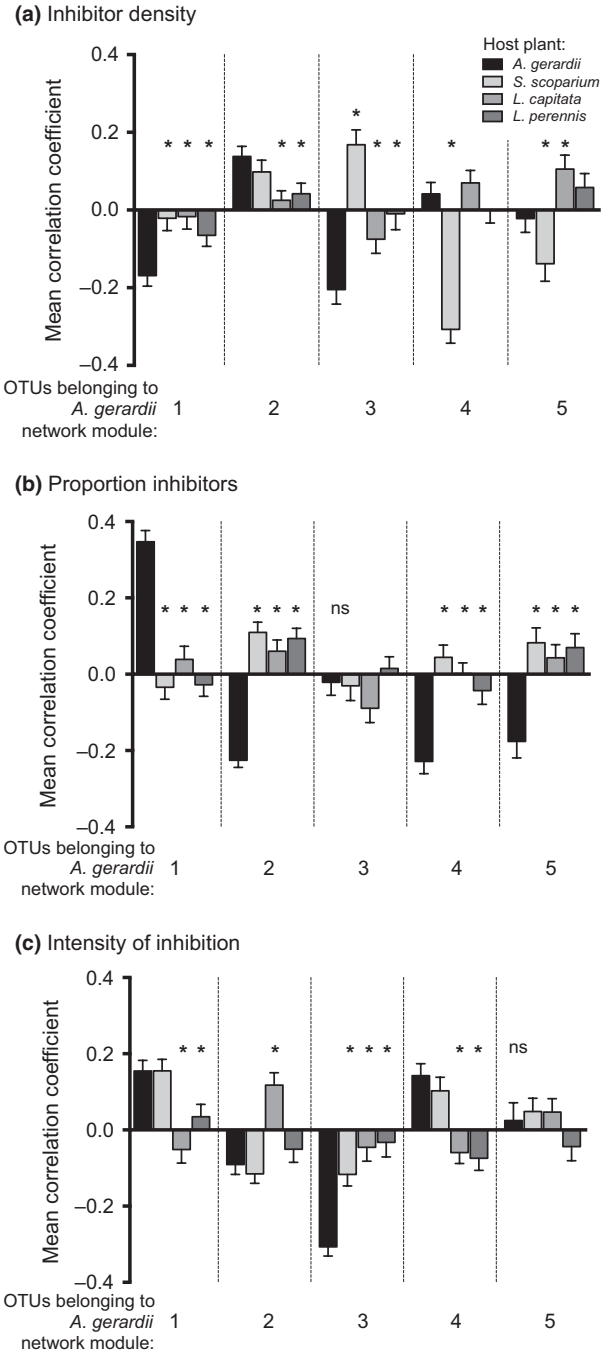

Fig. 5 Patterns of correlation between operational taxonomic unit (OTU) relative abundance and *Streptomyces* inhibitory activity, between network modules and between plant hosts for given sets of OTUs. Inhibitory activity is divided into (a) Inhibitor density; (b) Proportion inhibitors; (c) Intensity of inhibition. Shown are the mean (±SE) Pearson correlation coefficients for OTUs within each *A. gerardii* network module, and for the same OTUs across samples from the other host plant species. *Significant difference relative to the corresponding sample from *A. gerardii* (ANOVA with Dunnett's test, $P < 0.05$).

drivers, or to community processes such as competitive interactions, dispersal and local extinction. Contrasting community composition and structure may also more accurately predict differences in microbiome functional potential. Among these microbiomes, patterns of similarity among bacterial communities were significantly positively correlated with patterns of similarity among *Streptomyces* communities ($R = 0.55$, $P = 0.001$; Mantel test on Bray–Curtis distance matrices). This suggests some consistency in the factors that structure overall bacterial and *Streptomyces* community composition, although 70% of the variation in community structure among samples in one data set remained unexplained by variation in community structure in the other data set (bacteria vs. *Streptomyces*).

Community similarity varies with spatial distance

Spatial distance between plots was significantly correlated with pairwise community dissimilarity, for both total bacterial and *Streptomyces* data sets (Mantel test: $R = 0.37$ and $R = 0.20$, respectively; $P < 0.001$). Thus, independent of plant host or plant community richness, plots that were farther away from each other tended to have more dissimilar microbial community structure than plots located more closely together. Spatial distance between samples also explained significant variation in the density of inhibitory *Streptomyces* (Mantel test: $R = 0.29$, $P \leq 0.001$), but did not explain significant variation in the proportion of inhibitory *Streptomyces* or the mean intensity of inhibition ($P > 0.4$, data not shown).

Bacterial and *Streptomyces* communities were also significantly more similar when originating from the same vs. from different plots (Fig. 6), even though different samples drawn from the same plot were associated with different host plant species. This suggests that landscape-scale factors are also important in determining microbiome dynamics in soil, although our ability to infer the relative importance of spatial separation is hampered by a confounding with plant richness in the present case. Increased within-plot similarity was observed for 4-, 8- and 16-species plant richness treatments (Fig. 6), but could not be tested for monocultures due to a lack of replicate within-plot sampling. *Streptomyces* communities were markedly more similar to one another than were bacterial communities both among samples from the same and from different plots (Fig. 6). Thus, bacterial communities exhibited greater spatial heterogeneity at both plot- and field scales. However, the change in dissimilarity between communities from the same vs. different plots was dramatically greater for *Streptomyces* than for bacterial communities (Fig. 6).

This may suggest that *Streptomyces* are much more plant richness responsive than most other bacterial taxa, or that *Streptomyces* dispersal is relatively more effective within plots vs. between plots, compared with the effectiveness of dispersal for bacterial communities as a whole. More broadly, these data illustrate that the relative significance of landscape-scale, plant community, plant host, soil edaphic and microbial factors in structuring the rhizosphere microbiome differs among microbial taxa.

Discussion

There has been increasing interest in the structure and functioning of host-associated microbiomes as their significance to host physiology (Badri *et al.* 2013), phenotype (Friesen *et al.* 2011) and to the provisioning of ecosystem services (Turnbaugh *et al.* 2006; Marasco *et al.* 2012) has become evident. Relationships between host plants and rhizosphere or soil microbiomes have received much attention (Berendsen *et al.* 2012; Lundberg *et al.* 2012). However, plant–microbiome associations have typically been conceptualized as the simple additive outcome of direct and tightly coupled plant–microbe interactions (Walker *et al.* 2003; Bais *et al.* 2006). In contrast, we have outlined an approach that considers the influence of soil properties, plant–plant, plant–microbe and microbe–microbe interactions on plant-associated soil microbiomes. Our data illustrate that the structure and functioning of soil microbiomes arise from complex and interactive effects between the host plant, the surrounding plant community, the soil environment and the network of microbial coassociations within the microbiome.

Many studies have documented the effects of host plant species or genotype on the soil microbiome (Innes *et al.* 2004; Marschner *et al.* 2004; Garbeva *et al.* 2006). Research has also characterized the effects of plant community richness or diversity on soil microbial communities (Carney & Matson 2006; Lamb *et al.* 2010), although the majority of such studies confound effects of plant diversity with sampling effects. In this work, we sampled the same plant hosts across a plant richness gradient, which permits us to distinguish the effects of plant species richness from the effects of host plant species identity on the soil microbiome. Previous work has demonstrated a significant effect of plant richness on bacterial and *Streptomyces* community structures (Bakker *et al.* 2013a; Schlatter *et al.* submitted).

There are a variety of mechanisms through which plant community richness, and more specifically plant–plant interactions within communities, may lead to shifts in host-specific microbiomes. Competition for resources among plants can lead to significant shifts in

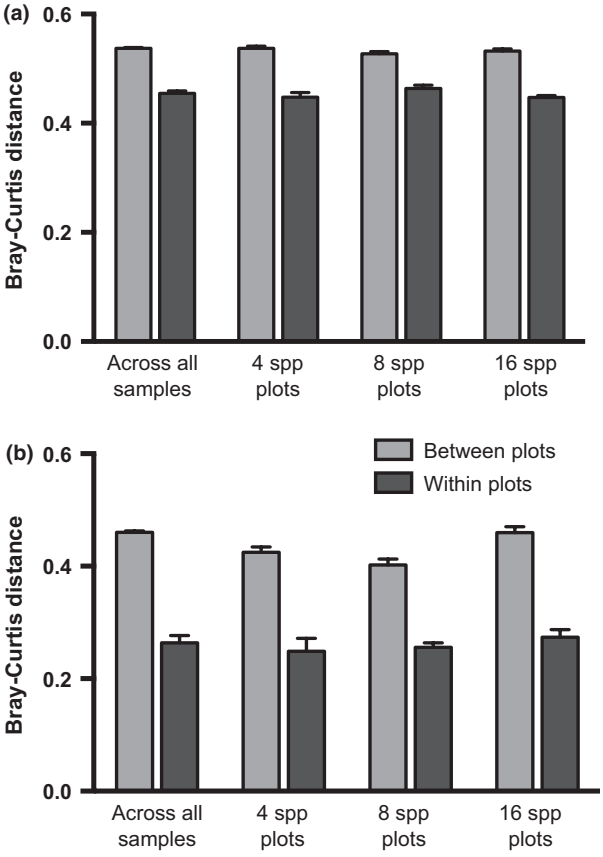

Fig. 6 Both (a) bacterial and (b) *Streptomyces* communities were more similar to each other if they originated from the same plot vs. from different plots. Shown are the mean (+SE) pairwise distances between samples. All pairwise contrasts (between vs. within plots) were statistically significant (*T*-test, $P < 0.001$).

both belowground and aboveground plant phenotypes (Gersani *et al.* 2001; Murphy & Dudley 2009; Broz *et al.* 2010). Shifts in plant structure and responses to competitors are likely to lead to alterations in the quantity, chemical nature or temporal availability of resources supplied by a host plant to its associated microbiome. Moreover, species-rich plant communities accumulate greater soil carbon over time (Tilman *et al.* 2001), suggesting that the total availability of carbon to soil microbes is different within soil microbiomes in species-rich vs. species-poor plant communities. Such variation in resource availability is likely to contribute to differences among microbiomes associated with a given host plant species when that host is embedded in plant communities that differ in plant species richness. Alternatively, in species-rich plant communities, spatial proximity of plants that support disparate microbiomes could lead to substantial shifts in the microbial immigrant pools to which a target plant is exposed. Such shifts may alter the composition and dynamics of the

microbiome over time. Further work is needed to clarify the mechanisms most important for generating plant richness-associated variation in the plant microbiome.

While scaling 'up' from plant–microbe interactions highlights the effects of interactions within the plant community on the soil microbiome, scaling 'down' suggests a focus on the role of microbial interactions in structuring the soil microbiome. Our data reveal complex patterns of coassociation among microbial taxa within the soil microbiome. Intriguingly, we found a significant effect of host plant species and host plant richness on the patterns of coassociation among microbial taxa, not merely on OTU identity and relative abundances. Under different host plant species, bacterial OTUs differed in strength of coassociation and the identity of OTUs with which they were coassociated. This suggests that different host plant species set different environmental contexts, which generate microbiome structures and patterns of microbial coassociation (and presumably networks of microbial interactions) that are fundamentally different. There are several mechanisms that could give rise to these effects. For example, microbe–microbe interactions may have different outcomes under different abiotic environmental conditions (Drakare 2002; Tétard-Jones *et al.* 2007). Through influences on the soil chemical and physical environment, plants may change competitive dynamics among microbes, leading to shifts in microbiome organization. Alternatively, plant-driven impacts on particular populations of keystone bacterial taxa may have cascading effects that result in reorganized networks of interaction among taxa. Regardless of the mechanism, the observation that plant-driven impacts on soil microbiomes manifest themselves in a restructuring of microbial coassociation or interaction networks deserves greater attention. This is a conceptual departure from traditional understandings of host plant effects on soil microbial communities and suggests the need for novel approaches to studying plant-associated microbiomes. For instance, rather than merely tracking the relative abundance of particular microbial taxa among plant hosts, research should attempt to uncover the implications of different interaction partners and co-evolutionary dynamics within microbiomes associated with different plant hosts.

Changes in microbial community association networks as a function of host plant species or plant richness treatment will have profound implications for microbiome dynamics and functioning. Species interactions, including competition, gene exchange, signalling, parasitism and predation, occur only among species that coexist in soil. If plant hosts fundamentally alter the network of microbial taxa within which a target taxon coexists, quite different competitive and co-evolutionary dynamics may be generated within soil microbiomes. For instance, antagonistic activity, the functional trait assessed here, is promoted and maintained by selection for phenotypes that are successful under strong resource competition. If networks of association among microbes shift in ways that alter the intensity of resource competition, this will influence selection for phenotypes such as antibiotic production, which are critical to suppressing pathogens. Determining the specific factors that contribute to variation in microbial association networks among plant species has important implications for understanding the competitive and co-evolutionary dynamics (and consequent functional attributes) of plant-associated microbiomes.

A common hypothesis suggests that plants may enrich for particular microbial taxa in soil, because of resulting fitness benefits (Hartmann *et al.* 2009; Bakker *et al.* 2012; Berendsen *et al.* 2012). It is possible that some microbial functions of importance to plant performance may be responsive to selection through direct plant–microbe interactions. However, for many functions performed by the soil microbiome, there is no clear mechanism by which plants might enrich the responsible organisms. The plant-driven restructuring of microbial coassociation networks observed here suggests an alternative: plants may 'manage' microbial co-evolutionary dynamics within the soil microbiome to foster advantageous microbial phenotypes. By homogenizing variation in abiotic environmental factors or biotic associations, plants may force a sustained and consistent engagement between particular microbial taxa, driving co-evolution. For instance, resource competition among microbes could drive selection for antagonistic phenotypes (production of inhibitory chemicals). Such microbial phenotypes could improve plant fitness through control of pathogens, but it is not clear how plants could select directly for inhibitory microbial phenotypes. Through influences on soil chemical variation, or through chemical characteristics of resource inputs, plants may create conditions under which microbe–microbe interactions promote inhibitory phenotypes that are advantageous to the plant.

Considering the vast diversity that exists within and between locations in the soil environment, managing co-evolutionary dynamics may be more effective for plants than developing strategies to preferentially enrich particular taxa. For instance, dispersing plants initially associate with unknown soil communities, and there is no guarantee that any particular microbial taxon will be present (Aleklett & Hart 2013). At the same time, high functional redundancy among microbial taxa, even as community composition and diversity vary widely, may allow plants to support microbial interaction networks whose competitive and co-evolutionary dynamics will

generate functional outcomes favourable to the plant, unconstrained by the specific taxa available for colonization in a particular soil location.

We argue that biotic interactions are important in explaining spatial patterns of composition, function or diversity in soil microbiomes. For instance, patterns of co-occurrence among bacteria may reflect shared responsiveness to interactions among species, such as antibiotic-mediated inhibition. This may be especially true for those modules of coassociated OTUs for which OTU relative abundances were strongly correlated with *Streptomyces* community antagonistic activity (density and proportion of inhibitors, or intensity of inhibition; Fig. 3). Additional categories of microbial species interactions may also be relevant to explaining patterns of coassociation. For instance, chemical signalling (Davis *et al*. 2008), syntrophy (Orphan 2009) and synergistic antagonism or competitive strategies (de Boer *et al*. 2007) are all likely to influence patterns of microbial coassociation. It is also important to note that historical and stochastic effects, or larger-scale processes, may have a significant influence on host-associated microbiomes. Among the microbiomes considered here, spatial distance between samples had a significant influence on the similarity of communities.

Our results indicate that the drivers of diversity or abundance for a given microbial population may vary across the landscape, with host plant species identity and plant community richness. At the same time, the significance of distinct drivers also varies among microbial taxa. For instance, *Streptomyces* communities exhibited different patterns of richness and diversity, and different relationships with environmental correlates, than bacterial communities as a whole. This finding underscores the importance of considering microbiome functioning in relation to relevant taxa or subsets of the community; tracking changes across the aggregate bacterial microbiome may not provide reliable indicators of changes to taxa that are vital to functions of interest.

In sum, this work argues for expanding the boundaries of consideration beyond pairwise plant–microbe interactions when studying plant–microbiome associations. Our data show that the structure and function of the rhizosphere soil microbiome are influenced by a complex array of factors at varying spatial scales, including plant species richness, host plant identity and microbial taxon and community characteristics. We also highlight a novel role for host plant species and plant community richness in fundamentally shifting the patterns of coassociation among microbial taxa in the soil microbiome, with significant implications for understanding plant effects on rhizosphere microbiome dynamics and functioning. Simultaneous consideration of diverse factors occurring at multiple scales of space and time is needed to achieve comprehensive understanding of the ecology and evolutionary biology of host-associated microbiomes.

Acknowledgements

AJ Lange contributed technical support and Amy Sheflin provided assistance creating Fig. 1. Soil samples were collected from long-term plots in an experiment designed and maintained by D. Tilman. M. Bakker is supported by a USDA NIFA AFRI Postdoctoral Fellowship Grant (2011-67012-30938). Research funding was provided by grants from the USDA (2011-67019-30200 and 2006-04464) and the NSF Long-Term Ecological Research Network (0620652).

References

Aleklett K, Hart M (2013) The root microbiota—a fingerprint in the soil? *Plant and Soil*, **370**, 671–686.

Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM (2013) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytologist*, **198**, 264–273.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, **57**, 233–266.

Bakker MG, Glover JD, Mai JG, Kinkel LL (2010) Plant community effects on the diversity and pathogen suppressive activity of soil streptomycetes. *Applied Soil Ecology*, **46**, 35–42.

Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil*, **360**, 1–13.

Bakker MG, Bradeen JM, Kinkel LL (2013a) Effects of plant host species and plant community richness on streptomycete community structure. *FEMS Microbiology Ecology*, **83**, 596–606.

Bakker MG, Otto-Hanson L, Lange AJ, Bradeen JM, Kinkel LL (2013b) Plant monocultures produce more antagonistic soil *Streptomyces* communities than high-diversity plant communities. *Soil Biology and Biochemistry*, **65**, 304–312.

Barberan A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal*, **6**, 343–351.

Bastian M, Heymann S, Jacomy M (2009) Gephi: An open source software for exploring and manipulating networks. *International AAAI Conference on Weblogs and Social Media*.

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. *Trends in Plant Science*, **17**, 478–486.

de Boer W, Wagenaar AM, Gunnewiek PJAK, van Veen JA (2007) In vitro suppression of fungi caused by combinations of apparently non-antagonistic soil bacteria. *FEMS Microbiology Ecology*, **59**, 177–185.

Broz A, Broeckling CD, De-la-Peña C *et al.* (2010) Plant neighbor identity influences plant biochemistry and physiology related to defense. *BMC Plant Biology*, **10**, 115.

Cameron KC, Di HJ, Moir JL (2013) Nitrogen losses from the soil/plant system: a review. *The Annals of Applied Biology*, **162**, 145–173.

Carney KM, Matson PA (2006) The influence of tropical plant diversity and composition on soil microbial communities. *Microbial Ecology*, **52**, 226–238.

Carvalhais LC, Dennis PG, Tyson GW, Schenk PM (2012) Application of metatranscriptomics to soil environments. *Journal of Microbiological Methods*, **91**, 246–251.

Castaldini M, Turrini A, Sbrana C *et al.* (2005) Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. *Applied and Environmental Microbiology*, **71**, 6719–6729.

Challis GL, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proceedings of the National Academy of Sciences*, **100**, 14555–14561.

Czaran TL, Hoekstra RF, Pagie L (2002) Chemical warfare between microbes promotes biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 786–790.

Davelos AL, Xiao K, Flor JM, Kinkel LL (2004) Genetic and phenotypic traits of streptomycetes used to characterize antibiotic activities of field-collected microbes. *Canadian Journal of Microbiology*, **50**, 79–89.

Davis MJ, Mondal SN, Chen H, Rogers ME, Br Iansky RH (2008) Co-cultivation of *Candidatus Liberibacter asiaticus* with Actinobacteria from citrus with Huanglongbing. *Plant Disease*, **92**, 1547–1550.

Drakare S (2002) Competition between picoplanktonic Cyanobacteria and heterotrophic bacteria along crossed gradients of glucose and phosphate. *Microbial Ecology*, **44**, 327–335.

Friedman J, Alm EJ (2012) Inferring correlation networks from genomic survey data. *PLoS Computational Biology*, **8**, e1002687.

Friesen ML, Porter SS, Stark SC *et al.* (2011) Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 23–46.

Garbeva P, Postma J, van Veen JA, van Elsas JD (2006) Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. *Environmental Microbiology*, **8**, 233–246.

Gersani M, Brown JS, O'Brien EE, Maina GM, Abramsky Z (2001) Tragedy of the commons as a result of root competition. *Journal of Ecology*, **89**, 660–669.

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. *Plant and Soil*, **321**, 235–257.

Hibbing ME, Fuqua C, Parsek MR, Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. *Nature Reviews Microbiology*, **8**, 15–25.

Innes L, Hobbs PJ, Bardgett RD (2004) The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. *Biology and Fertility of Soils*, **40**, 7–13.

Jones CR, Samac DA (1996) Biological control of fungi causing alfalfa seedling damping-off with a disease-suppressive strain of *Streptomyces*. *Biological Control*, **7**, 196–204.

Kinkel LL, Bakker MG, Schlatter DC (2011) A coevolutionary framework for managing disease-suppressive soils. *Annual Review of Phytopathology*, **49**, 47–67.

Kinkel LL, Schlatter DC, Bakker MG, Arenz BE (2012) Streptomyces competition and co-evolution in relation to plant disease suppression. *Research in Microbiology*, **163**, 490–499.

Lamb EG, Kennedy N, Siciliano SD (2010) Effects of plant species richness and evenness on soil microbial community diversity and function. *Plant and Soil*, **338**, 483–495.

Linares JF, Gustafsson I, Baquero F, Martinez JL (2006) Antibiotics as intermicrobial signaling agents instead of weapons. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 19484–19489.

Little AEF, Robinson CJ, Peterson SB, Raffa KF, Handelsman J (2008) Rules of engagement: interspecies interactions that regulate microbial communities. *Annual Review of Microbiology*, **62**, 375–401.

Liu D, Anderson NA, Kinkel LL (1996) Selection and characterization of strains of *Streptomyces* suppressive to the potato scab pathogen. *Canadian Journal of Microbiology*, **42**, 487–502.

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

Marasco R, Rolli E, Ettoumi B *et al.* (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. *PLoS ONE*, **7**, e48479.

Marschner P, Crowley D, Yang CH (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant and Soil*, **261**, 199–208.

Murphy GP, Dudley SA (2009) Kin recognition: competition and cooperation in *Impatiens* (Balsaminaceae). *American Journal of Botany*, **96**, 1990–1996.

Oksanen J, Blanchet FG, Kindt R *et al.* (2011) *Vegan: Community Ecology Package. R Package Version 2.0*. http://cran.r-project.org/web/packages/vegan/

Orphan VJ (2009) Methods for unveiling cryptic microbial partnerships in nature. *Current Opinion in Microbiology*, **12**, 231–237.

Perez C, Dill-Macky R, Kinkel LL (2008) Management of soil microbial communities to enhance populations of *Fusarium graminearum* antagonists in soil. *Plant and Soil*, **302**, 53–69.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Richardson AE, Lynch JP, Ryan PR *et al.* (2011) Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*, **349**, 121–156.

Rothrock CS, Gottlieb D (1981) Importance of antibiotic production in antagonism of selected *Streptomyces* species to two soil-borne plant pathogens. *Journal of Antibiotics*, **34**, 830–835.

Schlatter DC, Davelos-Baines AD, Xiao K, Kinkel LL (2013) Resource use of soilborne *Streptomyces* varies with location, phylogeny and nitrogen amendment. *Microbial Ecology*, **66**, 961–971.

Schlatter DC, Bakker MG, Bradeen JM, Kinkel LL (submitted) Plant species, plant community richness, and microbial interactions structure bacterial communities in soil.

Schloss PD, Westcott SL, Ryabin T *et al.* (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, **75**, 7537–7541.

Schrey SD, Salo V, Raudaskoski M *et al.* (2007) Interaction with mycorrhiza helper bacterium *Streptomyces* sp AcH 505 modifies organisation of actin cytoskeleton in the ectomycorrhizal fungus *Amanita muscaria* (Fly Agaric). *Current Genetics*, **52**, 77–85.

Seipke RF, Kaltenpoth M, Hutchings MI (2012) *Streptomyces* as symbionts: an emerging and widespread theme? *FEMS Microbiology Reviews*, **36**, 862–876.

Strange RN, Scott PR (2005) Plant disease: a threat to global food security. *Annual Review of Phytopathology*, **43**, 83–116.

Tétard-Jones C, Kertesz MA, Gallois P, Preziosi RF (2007) Genotype-by-genotype interactions modified by a third species in a plant-insect system. *The American Naturalist*, **170**, 492–499.

Tilman D, Reich PB, Knops J *et al.* (2001) Diversity and productivity in a long-term grassland experiment. *Science*, **294**, 843–845.

Torsvik V, Øvreås L (2002) Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology*, **5**, 240–245.

Turnbaugh PJ, Ley RE, Mahowald MA *et al.* (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, **444**, 1027–1131.

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. *Plant Physiology*, **132**, 44–51.

Wiggins BE, Kinkel LL (2005a) Green manures and crop sequences influence alfalfa root rot and pathogen inhibitory activity among soil-borne streptomycetes. *Plant and Soil*, **268**, 271–283.

Wiggins BE, Kinkel LL (2005b) Green manures and crop sequences influence potato diseases and pathogen inhibitory activity of indigenous streptomycetes. *Phytopathology*, **95**, 178–185.

Wu J-H, Wu F-Y, Chuang H-P *et al.* (2012) Community and proteomic analysis of methanogenic consortia degrading terephthalate. *Applied and Environmental Microbiology*, **79**, 105–112.

Xiao K, Kinkel LL, Samac DA (2002) Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. *Biological Control*, **23**, 285–295.

Yuan WM, Crawford DL (1995) Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Applied and Environmental Microbiology*, **61**, 3119–3128.

M.G.B. and L.L.K. designed the experiments, M.G.B., D.C.S., L.O.H generated the data, M.G.B., D.C.S., L.O.H., L.L.K analysed the data and wrote the study.

Data accessibility

Raw sequence data are available in the NCBI Sequence Read Archive as accessions SRA019985 and SRR786944. Aligned sequence files, OTU abundance tables and records of sequence data processing are available through Dryad (http://datadryad.org/) as doi: 10.5061/dryad.2fc8m/3. *Streptomyces* antagonistic activity data are available through the data portal of the Cedar Creek Ecosystem Science Reserve (http://www.cbs.umn.edu/cedarcreek/research/data), under Experiment #e284.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Coassociation networks of common bacterial and *Streptomyces* OTUs, for each plant richness treatment (n = 12 samples per plant richness treatment). Nodes correspond to OTUs, and connecting edges indicate correlations in abundance across samples. Nodes are coloured in each panel according to their membership in network modules defined for the monoculture samples. For OTU identifiers associated with each module, see Table S1.

Fig. S2 Patterns of correlation between OTU relative abundance and *Streptomyces* inhibitory activity, between network modules and between plant richness treatments for given sets of OTUs. Shown are the mean (±SE) Pearson correlation coefficients for OTUs within each monoculture network module, and for the same OTUs across samples from the other plant richness treatments. * indicates a significant difference compared with the corresponding sample from monoculture (ANOVA with Dunnett's test, $P < 0.05$).

Table S1 List of OTUs included in network analyses, their consensus taxonomic identification and placement in network modules for the whole data set, for the *A. gerardii*-specific network and for the monoculture-specific network.

Table S2 Chi-square test for the distribution of sequences among phyla, comparing module composition to the composition of the input data set as a whole.

Table S3 Characteristics of coassociation networks for bacterial OTUs, across all samples, or by plant host or plant richness treatment.

Table S4 Bacterial and *Streptomyces* diversity and richness were sometimes significantly correlated with soil edaphic factors, but patterns of relationship differed between data sets (bacterial vs. *Streptomyces*).