RESEARCH ARTICLE

Effects of plant host species and plant community richness on streptomycete community structure

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Abstract

We investigated soil streptomycete communities associated with four host plant species (two warm season C4 grasses: *Andropogon gerardii*, *Schizachyrium scoparium* and two legumes: *Lespedeza capitata*, *Lupinus perennis*), grown in plant communities varying in species richness. We used actinobacteria-selective PCR coupled with pyrosequencing to characterize streptomycete community composition and structure. The greatest pairwise distances between communities were observed in contrasts between monocultures of different plant species, indicating that plant species exert distinct selective effects on soil streptomycete populations. Increasing plant richness altered the composition and structure of streptomycete communities associated with each host plant species. Significant relationships between plant community characteristics, soil edaphic characteristics, and streptomycete community structure suggest that host plant effects on soil microbial communities may be mediated through changes to the soil environment. Co-occurring streptomycete taxa also shared consistent relationships with soil edaphic properties, providing further indication of the importance of habitat preference for taxon occurrence. Physical distance between sampling points had a significant influence on streptomycete community similarity. This work provides a detailed characterization of soil streptomycete populations across a field scale and in relation to plant host identity and plant community richness.

Introduction

Elucidating the forces that structure and maintain microbial diversity is one of the central tasks of the discipline of microbial ecology. While the importance of abiotic environmental characteristics, biotic interactions, and stochastic events (e.g. immigration, colonization sequence) have been highlighted (Palacios *et al.*, 2008; Chase, 2010; Caruso *et al.*, 2011), our understanding of the determinants of microbial community structure and composition remains limited. For soil microbial communities, pH (Fierer & Jackson, 2006), parent material (Ulrich & Becker, 2006), and plant community or host plant genotype (Innes *et al.*, 2004; Marschner *et al.*, 2004; Garbeva *et al.*, 2008) have all been shown to be important correlates of community structure. In this work, we extend the focus on host plant effects to test the hypothesis that plant community characteristics modulate the impacts of a given host plant species on associated soil microbial communities.

Impacts of changing plant diversity on microbial community composition have been documented previously (Carney & Matson, 2006; Lamb *et al.*, 2010). However, it has been difficult to distinguish between effects due to diversity *per se* and effects due to the increasing likelihood of the presence of particular plant species having a strong effect on soil microbes [the so-called 'sampling effect' (Wardle, 1999)]. One way around this dilemma is to investigate the impacts of particular plant species across a gradient of plant richness; if host species have consistent impacts on soil microbial communities regardless of the richness or diversity of the surrounding plant community, then the impacts of plant diversity may be limited to the additive effects of individual host species across the landscape. On the other hand, if the impact of individual plant hosts on soil microbes can be altered by the richness or identity of neighboring plant species, then plant diversity may be an important variable in its own right for understanding soil microbial community structure and dynamics.

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Plants exert selection on associated soil microbes through both direct and indirect mechanisms. For example, the provision of specific chemical compounds may offer a selective advantage to organisms with the optimal enzymatic capabilities for accessing those substrates, while bioactive molecules in root exudates may directly inhibit particular microbial taxa (Broeckling *et al.*, 2008; Badri *et al.*, 2009; De-la-Pena *et al.*, 2010). Plant compounds may also act as signals that trigger changes in microbial gene expression (Mark *et al.*, 2005) and impact fitness or alter outcomes of competitive interactions among microbes.

Importantly, the mechanisms by which plants impact associated soil microbes may themselves be sensitive to neighboring plants. For instance, the production of defense-related phenolic compounds varies according to the identity of neighboring individuals (Broz *et al.*, 2010), as do plant morphology, flowering, and biomass production (Schmidtke *et al.*, 2010). Root traits relevant to interactions with rhizosphere microbes may also vary with neighboring plant diversity. Indeed, surrounding plant community characteristics have been shown to influence host plant effects on soil microfauna (Bezemer *et al.*, 2010). Theoretically, incentives may also exist for adaptive plant responses that alter interactions with soil microbial partners depending on the identity of neighboring plants. For example, if the development of a beneficial microflora requires costly inputs, there may be selection against such investment where neighboring plants could share the benefits without incurring costs, as has been observed in other systems involving common goods (Strassmann *et al.*, 2000; West *et al.*, 2002).

To address the possibility of neighboring plants impacting interactions between a host plant and its associated soil microbes, we characterized streptomycete community composition and structure in soils associated with four host plant species grown in plant communities varying in species richness. The actinobacteria, among which streptomycetes are the most abundant, are among the dominant members of soil bacterial communities (Lauber *et al.*, 2009) and are well known for their role in organic matter (OM) decomposition and for the production of diverse antibiotics and other secondary metabolites (Genilloud *et al.*, 2011). Streptomycetes can also have significant influences on plant health and productivity via both pathogen suppression and through the production of plant growth-promoting hormones (Tuomi *et al.*, 1994; Doumbou *et al.*, 2001). However, little is known about the specificity of plant host effects on streptomycete community composition or structure.

We sampled streptomycete communities associated with four prairie plant species belonging to two functional groups: the warm season C4 grasses (*Andropogon gerardii*, *Schizachyrium scoparium*) and the legumes (*Lespedeza capitata*, *Lupinus perennis*). Understanding the interactions of these dominant, long-lived perennial plants with soil microbial communities may provide insight into both plant and microbial community dynamics in natural settings, and may have implications for agricultural plant species and management practices.

Materials and methods

Soil sampling

Sampling was performed in July of 2009, from a long-term plant richness manipulation (Tilman *et al.*, 2001) at the Cedar Creek Ecosystem Science Reserve, which is part of the National Science Foundation Long-Term Ecological Research network. Soil at this site belongs to the Zimmerman series and is a fine sand resulting from glacial outwash. Fertility is low, while permeability is high (Grigal *et al.*, 1974). We targeted soil under the dominant influence of each of four different plant species (two C4 grasses: *A. gerardii*, *S. scoparium*; two legumes: *L. capitata*, *L. perennis*) by collecting soil cores from the base of individual plants. Each sample consisted of four bulked soil cores (5 cm diameter, 30-cm depth) collected from different individuals of the same host species within the same plot and homogenized by hand. Each plant species was sampled in five different plant richness treatments (monoculture and assemblages of 4, 8, 16, or 32 species). There were three plot-level replicates per host plant richness combination, except for monocultures of *A. gerardii* and *L. perennis*, for which only two plot-level replicates were available. Two independent soil samples were processed from one of the plots in these cases. Thus, we had a total of 60 soil samples (4 plant hosts × 5 plant community diversity levels × 3 replicates). Soil samples were sieved through a 2-mm mesh and stored at 4 °C until processing.

Soil pH, potassium (K+), phosphorus (P), nitrogen (N), carbon (C), and OM were measured at the University of Minnesota soil-testing lab using standard procedures (http://soiltest.cfans.umn.edu/). Realized plant diversity, percent cover, and above- and below-ground plant biomass data for the long-term experimental plots sampled for this work were accessed through the CCESR Long-Term Ecological Research network database (http://www.cedarcreek.umn.edu/research/data/).

Determination of culturable streptomycete densities

A portion of each soil sample was used to determine culturable streptomycete density. For this purpose, soil was dried at room temperature overnight under sterile cheesecloth in a fume hood. Five grams of dry soil were dispersed in

50-mL water by shaking (175 r.p.m., 1 h, 4 °C) and then serially diluted. Soil dilutions were spread onto water agar plates and then covered with 5 mL of cooled, molten starch-casein agar. This method allows filamentous streptomycetes to grow through the overlay medium, while suppressing the growth of many other bacteria (Wiggins & Kinkel, 2005). Colonies were counted from three replicate plates, after 5 days of growth at 28 °C.

DNA extraction and sequencing

A subsample of each soil sample was used for DNA extraction, using the PowerSoil DNA kit (MO BIO, Carlsbad, CA). The manufacturer's protocol was modified with extended bead beating and sonication to enhance recovery of DNA from streptomycete spores (Schlatter *et al.*, 2010). Selective primers were used to amplify a portion of the 16S rRNA gene. We used StrepB (Rintala *et al.*, 2001) as our forward primer, and the reverse complement of Act283 (McVeigh *et al.*, 1996) as our reverse primer, each at a final concentration of 200 nM. Both primers are selective and together amplify a fragment of approximately 165 nucleotides, encompassing the V2 variable region of the 16S rRNA gene, which is referred to as the 'gamma variable region' in older studies of streptomycetes (Stackebrandt *et al.*, 1991). Primers were modified to contain one of 30 different 10mer identifying barcodes (Parameswaran *et al.*, 2007). PCRs consisted of 10 ng of template DNA in a 50-μL reaction volume using PCR Supermix High Fidelity (Invitrogen, Carlsbad, CA). PCR conditions consisted of an initial denaturation step of 30 s at 94 °C, followed by 30 cycles of 30 s 94 °C, 30 s 57 °C, 60 s 70 °C. Products of PCRs were passed through the QIAquick PCR Purification Kit (Qiagen, Valencia, CA), quantified by spectrophotometry, diluted with elution buffer to approximately 15 ng μL^{-1}, and quantified by fluorometry (Quant-iT dsDNA HS assay kit; Invitrogen). Thirty samples, each with a unique primer barcode, were combined in equimolar amounts to form each of two pooled amplicon samples. Emulsion PCR and sequencing were performed using a GS FLX emPCR amplicon kit according to the manufacturer's protocols (454 Life Sciences, Branford, CT). Each pooled sample was run on one region of a picotitre plate on the GS FLX sequencing system (Droege & Hill, 2008) at the University of Minnesota BioMedical Genomics Center. Resulting sequence data have been submitted to the NCBI Sequence Read Archive as accession SRA019985.

Sequence processing

Sequence data were processed through the program AmpliconNoise, version 1.24 (Quince *et al.*, 2011) for the detection and correction of probable errors. The data set was processed on a per-sample basis, with the raw flowgram signals as the input to AmpliconNoise. Initial processing tested for a perfect match to the forward primer, truncated flowgrams at 225 flows and discarded any reads that did not reach this length threshold. The PyroNoise algorithm was run with parameters set at $s = 1/60$, $c = 0.01$. The SeqNoise algorithm was run with parameters set at $s = 1/30.3$, $c = 0.08$. The AmpliconNoise output provides a mapping of input sequences to de-noised output sequences. We generated a single output file per sample by duplicating each output sequence according to the number of input sequences mapping to that output sequence in order to retain information on the relative abundance of sequence variants.

Subsequent processing was performed with the program MOTHUR, version 1.25.1 (Schloss *et al.*, 2009). Quality screening removed sequences containing ambiguous bases or homopolymeric stretches of greater than six nucleotides. Sequences were aligned to the Silva reference database (Pruesse *et al.*, 2007) using kmer searching with a ksize of 8 to find the best template sequence and the gotoh alignment method with a reward of +1 for a match and penalties of −1 for a mismatch, −2 for opening a gap, and −1 for extending a gap. Aligned sequences were screened for chimeric sequences using the UChime method (Edgar *et al.*, 2011), and sequences belonging to phyla other than *Actinobacteria* were removed. Sampling effort was equalized to the size of the smallest sample (1233 reads) by random subsampling. Sequences passing these quality criteria were clustered into operational taxonomic units (OTUs) using a 3% sequence dissimilarity criterion and the average neighbor clustering method.

Statistical analyses

Statistical analyses were performed in R (R Development Core Team, 2011). Richness estimates, diversity indices, and pairwise community similarities were calculated in MOTHUR and with the VEGAN package in R (Oksanen *et al.*, 2011). Differences in soil properties and in streptomycete density, richness, and diversity were tested with analysis of variance (ANOVA) using the Tukey method for *post hoc* multiple contrasts. Pearson correlations with false discovery rate (FDR) multiple test correction were used to test for significant relationships of streptomycete community characteristics with plant and soil characteristics. For comparisons of community composition and structure, species abundance tables were relativized, and pairwise similarities were calculated with the Bray–Curtis index (or the inverse for distance). Nonmetric

multidimensional scaling (NMDS) was used to produce graphical summaries of distance matrices, using the metaMDS function in the VEGAN package. Enrichment or preferential association of particular OTUs with plant host or plant richness treatment was tested with indicator species analysis (Dufrene & Legendre, 1997), using the LabDSV package in R (Roberts, 2010). Significance of indicator values was assessed with 9999 iterations and a threshold probability of 0.05. Patterns of co-occurrence among common OTUs (those consisting of at least 10 sequence reads; $n = 183$) were observed by testing for correlations in relative abundance across samples (Ravel *et al.*, 2010). Modules of co-occurring OTUs were defined by network analysis using the software Gephi (Bastian *et al.*, 2009), with connections among nodes defined by significant ($P < 0.05$, with FDR multiple test correction) Pearson correlation coefficients > 0.5. Correlations between OTU abundance (as proportion of sequence reads) and soil edaphic characteristics were used to draw inferences about habitat preference among OTUs.

Results

Approximately 230 000 partial 16S rRNA gene fragment sequence reads were collected from 60 soil samples. After equalizing sampling effort, 73 980 sequence reads were retained for analysis. Twenty-five different genera were detected within the order *Actinomycetales*, but 90% of all sequence reads belonged to a single genus, the *Streptomyces* (data not shown). Because of the difficulty of accurately assigning short sequence reads from 16S rRNA gene fragments to finer taxonomic categories than the genus, we based our analysis on OTUs defined on the basis of sequence similarity (3% dissimilarity cutoff, with average neighbor clustering).

Across all samples, sequence reads clustered into 445 OTUs. Rarefaction analysis suggested that further sampling and deeper sequencing would have continued to reveal additional diversity (Supporting Information, Fig. S1). Indeed, over 300 additional OTUs were detected in the full data set (i.e. including reads that were omitted to equalize sampling effort; data not shown). On average, there were 110 OTUs per sample (Chao estimate; range 62–193). The Shannon diversity index ranged from 2.04 to 3.80 among samples, with a mean value of 3.35. Culturable streptomycete density averaged 1.6×10^6 colony forming units/g soil (range $0.49–2.9 \times 10^6$) and was significantly positively correlated with observed OTU richness ($r^2 = 0.26$, $P < 0.001$) and diversity ($r^2 = 0.19$, $P = 0.002$). Thus, larger communities (greater streptomycetes densities per g of soil) had greater OTU richness and diversity.

Variation in streptomycete communities associated with plant species richness and identity

In comparisons of streptomycete community structure, samples from monocultures of different plant species showed the greatest pairwise distance (two-way ANOVA, $P < 0.01$; Fig. 1). This indicates that host plant genotype impacts soil streptomycete populations. However, such effects were not visible in simple metrics of density, richness or diversity (two-way ANOVA with host species and plant richness as main effects; $P > 0.05$).

We performed an indicator species analysis to test for significant enrichment or preferential association of streptomycete OTUs with each of the host species and plant richness treatments. Indicator OTUs were found for each plant host and plant richness treatment (Table S1). Among plant hosts, one OTU was a significant indicator for each of *A. gerardii* and *S. scoparium*, while three OTUs were significant indicators of samples associated with *L. capitata*. The greatest number and diversity of indicator OTUs was found for *L. perennis*, with ten indicator OTUs having consensus classifications in *Streptomyces*, *Amycolatopsis*, and *Saccharopolyspora* (Table S1). Seven OTUs were significant indicators of monoculture plant treatments, while 1, 4, 14, and 3 OTUs were significant indicators of treatments planted with 4, 8, 16 or 32 species, respectively. In addition to *Streptomyces*,

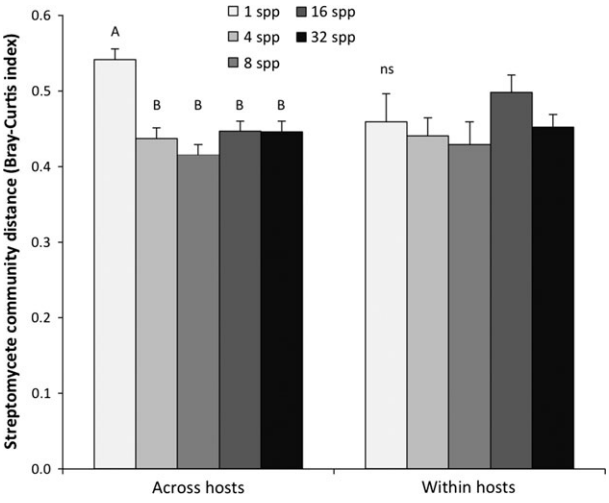

Fig. 1. Mean (+SE) pairwise Bray–Curtis distance (a measure of variation among samples) was greatest for contrasts across monocultures of different plant species (two-way ANOVA, $P < 0.01$ with Tukey contrasts), suggesting host-specific impacts on soil streptomycete populations. Contrasts between plant species within a given plant richness treatment are represented as 'Across hosts.' Contrasts between samples from the same plant species within a given plant richness treatment are represented as 'Within hosts.' Different letters indicate that means differ significantly among treatments. ns, No significant differences.

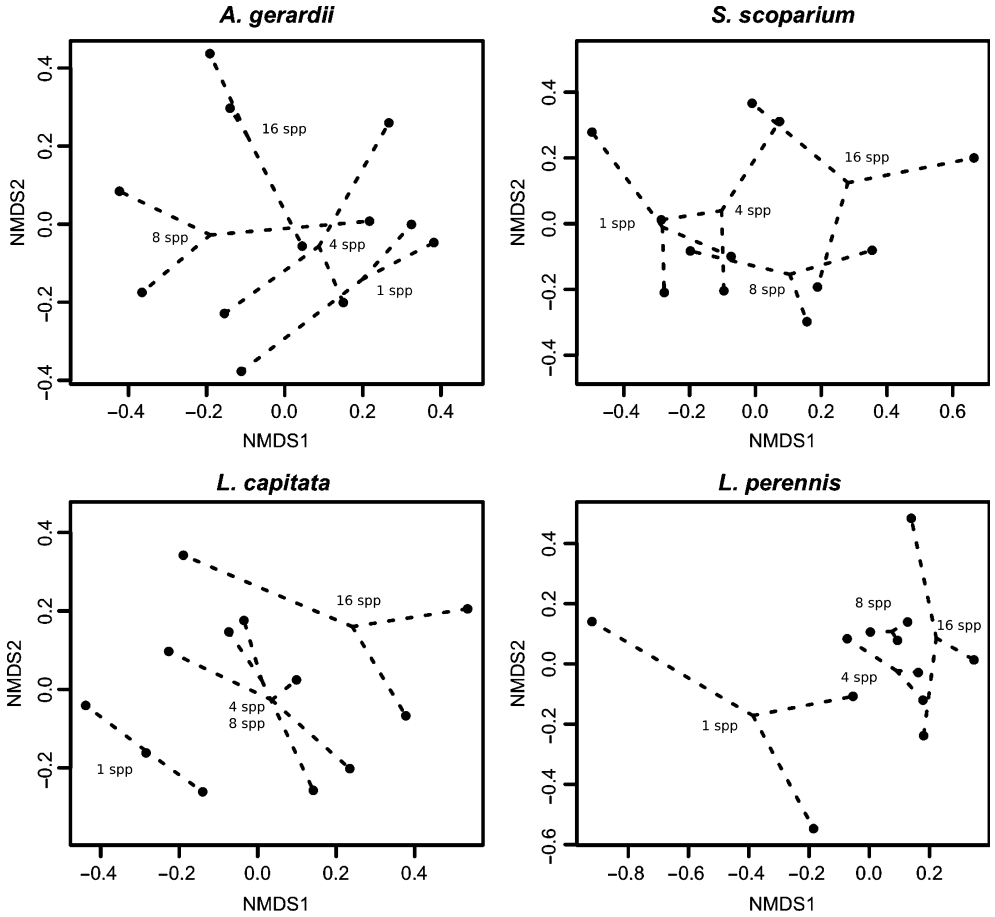

Fig. 2. The structure of streptomycete communities associated with a given host plant species shifted with surrounding plant richness. Each panel shows a NMDS of a Bray–Curtis distance matrix summarizing pairwise distances in streptomycete community structure. Host plant species are shown in separate panels, while connecting lines and labels distinguish the plant richness treatments.

these indicator OTUs included members of *Kitasatospora*, *Kribbella*, *Streptacidiphilus*, and *Saccharopolyspora*. Identifiers and consensus classification for these indicator OTUs are provided in Table S1. Consensus classification for all OTUs (to the genus level) is provided in Table S2.

Importantly, host plant identity and plant species richness interactively impacted streptomycete community structure. For each host species, streptomycete communities became increasingly dissimilar to the monoculture community as surrounding plant richness increased (Fig. 2). This suggests that host plant effects may be variable according to the identity or diversity of neighboring plants.

Variation in soil edaphic characteristics associated with plant treatment and related to streptomycete community characteristics

Soil edaphic characteristics sometimes varied significantly among plant species or plant richness treatments. Samples from *L. perennis* had higher pH than *A. gerardii* samples, and higher K+ than *S. scoparium* samples (one-way ANOVA, $P < 0.05$; Table 1). Samples from plant communities with a richness of 16 species had higher soil C, N, OM, and K+ than samples from monocultures. Samples from plant communities with a richness of 32 species had higher K+ than samples from plant communities consisting of 1, 4, or 8 species (one-way ANOVA, $P < 0.05$; Table 1). Thus, both plant host and plant community richness treatment significantly altered the soil chemical environment.

These changes may be a primary driver of plant species- or plant richness-specific effects on associated streptomycete communities. Culturable streptomycete density was significantly positively correlated with plant diversity and with soil pH, K+, N, and OM (Table 2). Streptomycete OTU richness was significantly positively correlated with soil pH and with aboveground productivity, while streptomycete OTU diversity was significantly positively

Table 1. Variation in soil edaphic characteristics across plant host species and plant richness treatments

	pH	Potassium (p.p.m.)	Phosphorus (p.p.m.)	Nitrogen (%)	Carbon (%)	OM (%)
A. gerardii	5.95 a	42.1 ab	42.1 ns	0.037 ns	0.56 ns	1.11 ns
S. scoparium	6.10 ab	38.1 a	35.1	0.034	0.49	1.03
L. capitata	6.03 ab	44.9 ab	34.9	0.035	0.51	1.05
L. perennis	6.17 b	52.4 b	34.4	0.042	0.54	1.09
Monocultures	6.01 ns	34.1 a	32.7 ns	0.028 a	0.42 a	0.88 a
4 plant spp	6.03	42.0 ac	34.4	0.034 ab	0.51 ab	1.06 ab
8 plant spp	6.16	41.6 ac	40.5	0.035 ab	0.51 ab	1.06 ab
16 plant spp	6.05	57.4 b	43.2	0.052 b	0.68 b	1.31 b
32 plant spp	6.08	46.8 bc	32.2	0.035 ab	0.51 ab	1.05 ab

Different letters indicate significant differences between mean values within a given comparison (one-way ANOVA with Tukey contrasts, $P < 0.05$). OM, soil organic matter.
ns, No significant differences.

Table 2. Pearson correlation coefficients (*P*-values) for relationships between streptomycete community characteristics and various plant community and soil edaphic characteristics ($n = 60$)

	Culturable density	OTU richness (observed)	OTU diversity (Shannon index)
Belowground plant biomass	0.22 (0.20)	0.23 (0.17)	0.12 (0.50)
Total plant cover	0.29 (0.06)	0.23 (0.14)	0.13 (0.40)
Realized plant diversity	0.44 (0.007)*	0.23 (0.17)	0.08 (0.64)
Aboveground plant biomass	0.32 (0.06)	0.34 (0.04)*	0.09 (0.58)
Soil pH	0.33 (0.03)*	0.39 (0.006)*	0.35 (0.02)*
Soil K+	0.33 (0.03)*	0.23 (0.14)	0.09 (0.58)
Soil P	0.26 (0.09)	0.16 (0.28)	0.12 (0.41)
Total soil N	0.30 (0.05)*	0.23 (0.14)	−0.04 (0.80)
Total soil C	0.25 (0.11)	0.18 (0.23)	−0.05 (0.73)
Soil OM	0.33 (0.03)*	0.19 (0.23)	−0.02 (0.87)

*Correlations significant at $P < 0.05$.

correlated only with soil pH (Table 2). However, the possibility also exists that shifts in soil edaphic characteristics may be an indirect effect of plant host species or plant richness, perhaps mediated through the activity of soil microbes.

Co-occurrence of streptomycete OTUs among samples

Streptomycete OTUs co-occurred across samples in nonrandom patterns, with both significant positive and negative associations among different OTUs. Among the collection of 60 samples, six distinct modules of co-occurring OTUs were defined by network analysis of positive correlations in relative abundance (Fig. 3). Relationships with soil edaphic characteristics varied among OTUs associated with different network modules (Fig. 4); for example, OTUs in both network modules 1 and 6 showed similar positive correlations of abundance with soil pH and K, and lack of correlation with soil N and C. However, these two network modules could be distinguished by different relationships with soil P (Fig. 4). Differences in relationship with soil edaphic characteristics among modules of co-occurring OTUs suggest that patterns of co-occurrence may be driven at least in part by habitat preferences.

Streptomycete communities across space

Because multiple host species were sometimes sampled within the same experimental plot, we were able to compare the variation in streptomycete composition among plant hosts from the same vs. different plots. Samples collected from different plant species within the same plot showed greater similarity relative to the corresponding comparison for samples drawn from different plots of the same plant richness treatment (*t*-test, $P < 0.01$; Fig. 5). Higher community similarity within than among plots suggests a local spatial structure to streptomycete communities, reflecting possible roles of historical factors, landscape-scale variation in soil edaphic characteristics, initial colonization events, or dispersal limitation in structuring streptomycete communities.

Discussion

We investigated the structure of streptomycete communities in soils associated with different plant host species and across a gradient of plant community richness. Previous work has indicated that soil microbial communities are differentially impacted by host plant species (Mazzola *et al.*, 2004; Funnell-Harris *et al.*, 2010) and by communities of varying diversity (Chung *et al.*, 2007; Lamb *et al.*, 2010). However, our data provide evidence for interaction between these two factors, with plant community characteristics altering the influence of individual host species on soil microbes. This concept deserves

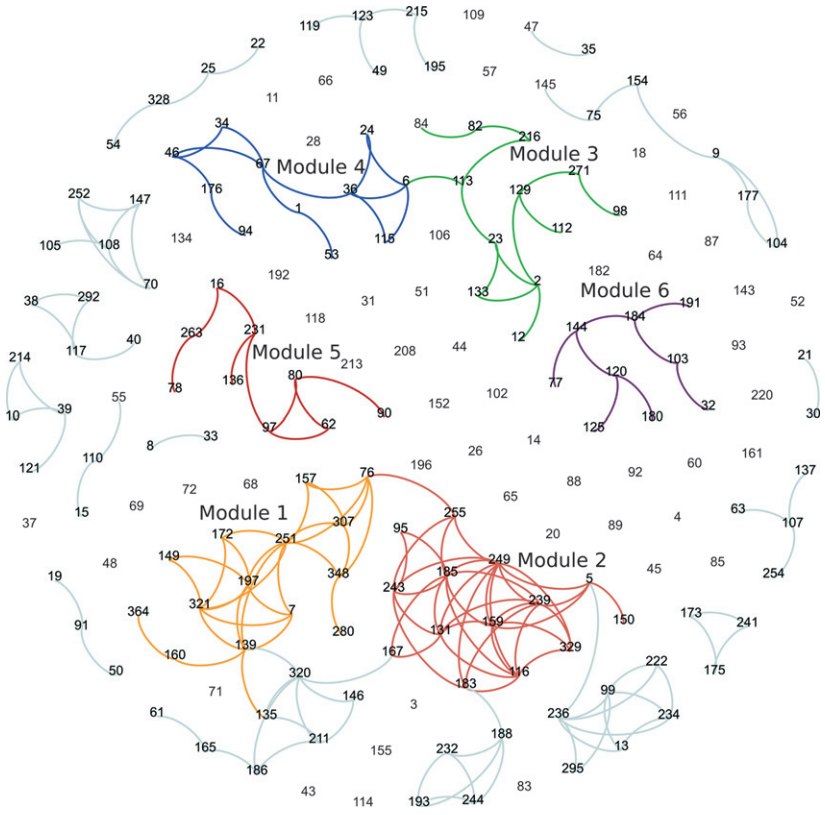

Fig. 3. Streptomycete OTUs co-occurred in nonrandom patterns. Network diagram shows significant positive correlations (Pearson; $r > 0.5$, $P < 0.05$ with FDR multiple test correction) in relative abundance of OTUs (consisting of at least 10 sequence reads; $n = 183$) across samples. Node labels show OTU numbers. Edges connect OTUs whose relative abundance was significantly positively correlated. Modules containing at least nine OTUs are colored and labeled (Module 1–Module 6).

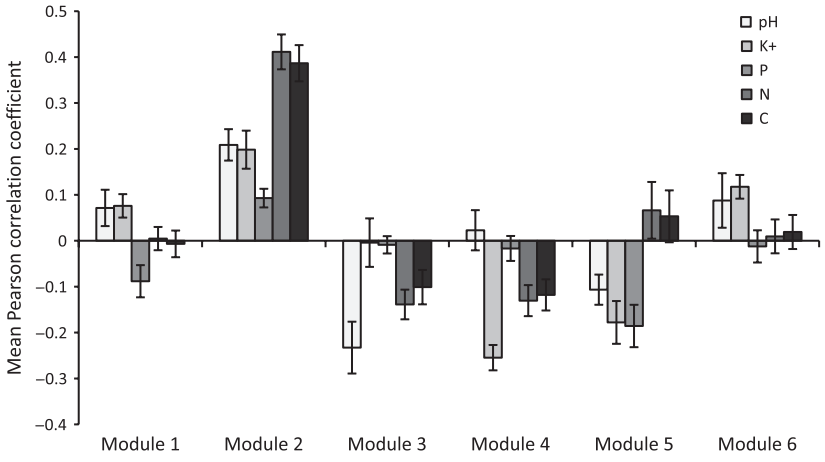

Fig. 4. Patterns of correlation between OTU relative abundance and soil pH, potassium (K+), phosphorus (P), nitrogen (N), and carbon (C) differed between network modules (Fig. 3). Shown are the mean (±SE) Pearson correlation coefficients for OTUs within each network module.

further attention, as many previous studies of plant diversity effects on soil microbial communities confound the effects of diversity with the effects of sampling multiple distinct plant host species. This work documents and distinguishes the effects of diversity from those of individual plant host species on soil microbial communities.

As surrounding plant richness increased, streptomycete communities became increasingly dissimilar to the monoculture community for each host plant tested. This suggests a dose response in plant-mediated impacts on soil streptomycete communities, as a function of surrounding plant richness. However, further work will be needed to clarify the mechanisms that underlie these interactions. For example, plant diversity may directly alter host species effects on soil microbial communities if root exudation or litter chemistry shifts in response to the presence of heterospecific neighbors. Alternatively, mixing of individual plant host effects may result in a composite plant effect on soil microbes. These mechanisms carry distinct implications for understanding the role of feedbacks in influencing plant and

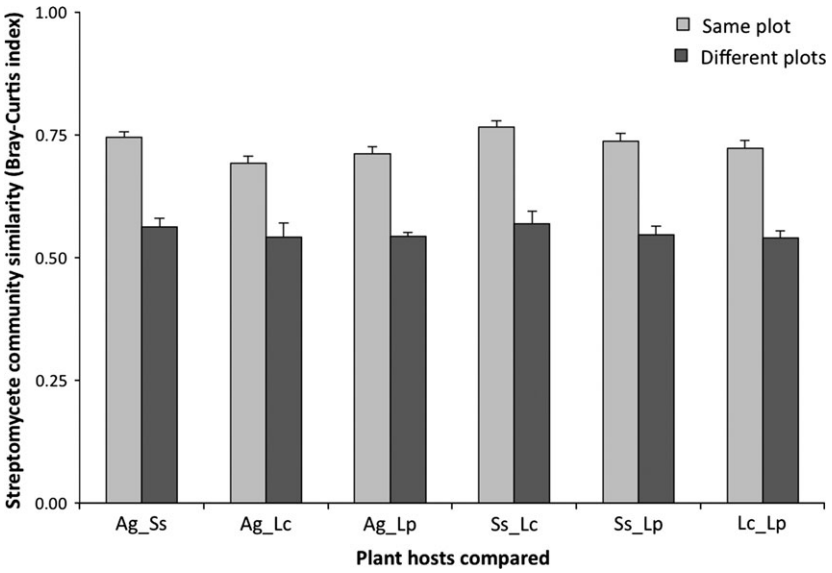

Fig. 5. Streptomycete communities across plant hosts were more similar when hosts were sampled in the same experimental plot compared with sampling across plots. Only pairwise comparisons within the same plant richness treatment are included. Ag, *Andropogon gerardii*; Ss, *Schizachyrium scoparium*; Lc, *Lespedeza capitata*; Lp, *Lupinus perennis*. All comparisons showed significant differences (*t*-test, $P < 0.01$).

microbial fitness. In particular, future work should explore whether plants deliberately alter investment in their rhizosphere microflora in response to competitors vs. whether altered host plant effects in diverse plant stands result mainly from dilution of host plant impacts.

The *Actinobacteria* and the streptomycetes have received attention in other studies of plant genotype-driven impacts on soil microbial populations. For instance, Actinobacterial communities from the rhizosphere of strawberry and of oilseed rape could be distinguished by denaturing-gradient gel electrophoresis (DGGE) of selectively amplified genes (Costa *et al.*, 2006). It is notable, however, that rhizosphere- and host genotype-specific effects may not be as strong for streptomycetes as for other bacterial groups. For instance, rhizosphere and bulk soil Actinobacterial DGGE fingerprints could not be distinguished in several cases (Costa *et al.*, 2006), and fewer host plant genotype effects were seen in Streptomycetaceae compared with other bacterial groups (Weinert *et al.*, 2009). Thus, our observation of relatively modest host plant effects on soil streptomycete community structure is consistent with previous studies. Although we sampled from a long-term experiment, allowing adequate time for host-driven impacts to become evident, it should be noted that our sampling occurred at a single point in time. Patterns of similarity among treatments may shift over time, and it has been observed previously that the strength of host genotype effects on soil microbial communities can vary over time (Weinert *et al.*, 2009). Although limited in temporal scope, this work provides what may be the most in-depth characterization of streptomycete community structure at the field scale.

Our work also sheds light on possible mechanisms underlying host plant effects on soil microbes. One mechanism by which plants and plant communities may exert selection on soil microbial populations is by modifying resource availability. In this work, streptomycete density and richness increased with measures of plant productivity, including percent cover and above ground biomass. This is consistent with a mechanism of plants impacting soil microbial communities through the quantity of resource inputs provided (De Deyn *et al.*, 2010). At the same time, more diverse chemical inputs accompanying a rise in plant species richness may provide a greater array of nutrients, enhancing the potential for niche differentiation among soil microbes. Unfortunately, plant richness and productivity are confounded in the plots from which we sampled: more species-rich communities have consistently greater above- and below-ground productivities (Zak *et al.*, 2003). Thus, it is not possible in this study to distinguish microbial community responses to resource quantity vs. resource composition or diversity, although the two aspects of resource provision may carry distinct implications for ecological interactions among microbes (Kinkel *et al.*, 2011).

Provision of carbon substrates via rhizodeposition is commonly assumed to be a primary mechanism for plant-driven impacts on soil microbial communities. However, indirect and nonnutritive impacts, such as physiological stresses, are also likely. The interactive impacts of plant and soil factors on soil microbial communities have been highlighted previously (Berg & Smalla, 2009; Nunes da Rocha *et al.*, 2009). Several distinct analyses of our data provide consistent support for plant-mediated changes to the soil

chemical environment as an important driver of soil streptomycete community structure. First, we found that soil chemical properties differed among plant species and plant richness treatments. Secondly, modules of co-occurring streptomycete OTUs showed consistent patterns of correlation with these chemical properties. Finally, streptomycete indicator OTUs often belonged to modules that were characterized by consistent positive correlation between OTU abundance and the soil properties that were enhanced by those treatments. For example, the 16 plant species treatment enriched soil K+, N, and C (Table 1). The abundance of OTUs belonging to network module 2 was consistently positively correlated with these soil properties (Fig. 4), and four of the indicator OTUs for the 16 plant species treatment belonged to this network module (Table S1). Similarly, samples from *L. perennis* had higher soil pH and K+ (Table 1). The abundance of OTUs belonging to network modules 1 and 6 was consistently positively correlated with these soil properties (Fig. 4), and six of the indicator OTUs for *L. perennis* samples belonged to these network modules (Table S1).

Consistent relationships with soil edaphic characteristics among co-occurring taxa observed here suggest that shared habitat preferences may be responsible for similarities in patterns of occurrence. If soil nutrient concentrations correspond to resource availability for microbes, OTUs showing strong positive correlations with soil K+, P, N, and C may consist of copiotrophic organisms. In contrast, OTUs showing strong negative correlations with soil K, P, N, and C may represent oligotrophic organisms. Of course, we did not measure the full suite of soil properties that could influence relative fitness among soil streptomycetes. Other factors related to soil, or to plant or microbial species interactions, may play a role in determining streptomycete community structure or composition. For example, growth of some organisms has been shown to be dependent upon exogenous signals produced by other community members (Nichols *et al.*, 2008).

Our results revealed extensive diversity among the streptomycetes, suggesting that 100 or more taxa may be present in a given gram of soil. Streptomycete community structure and composition were more similar among samples from different hosts growing in the same experimental plot (located within a few meters of each other) than samples from different plots (tens to hundreds of meters apart). Previous work (Davelos *et al.*, 2004) also showed substantial spatial structure in streptomycete community composition, but at much smaller spatial scales (10–50 cm). In aggregate, these data confirm potentially significant roles for immigration, dispersal, local selection and landscape-scale processes in structuring streptomycete communities.

Plant–microbe interactions and feedbacks do not take place in isolated pair-wise systems, but in complex communities that can modulate those interactions. We document that surrounding plant community richness shifts the impacts of particular host plant species on soil streptomycete communities. This work adds nuance to our understanding of plant-driven impacts on soil microbial communities and has implications for our understanding of plant-soil feedbacks, plant invasions, and the importance of plant diversity in mediating ecosystem functions. Studies of plant–microbe feedbacks must be designed in ways that capture the influences of surrounding community characteristics. What is more, conceptual models of plant–microbe interactions must be able to incorporate effects operating at vastly different spatial scales. For instance, this work suggests that landscape-scale organization of plant community richness and diversity can influence very small-scale microbial community organization.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Rarefaction curves for streptomycete OTUs detected by pyrosequencing.
Table S1. Indicator OTUs (consisting of at least 10 sequence reads) that were enriched or preferentially associated with particular plant hosts or with plant community richness treatments.
Table S2. Consensus classification for each OTU, to the genus level.

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