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Plant community richness and microbial interactions structure bacterial communities in soil

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Abstract. Plant species, plant community diversity and microbial interactions can significantly impact soil microbial communities, yet there are few data on the interactive effects of plant species and plant community diversity on soil bacterial communities. We hypothesized that plant species and plant community diversity affect soil bacterial communities by setting the context in which bacterial interactions occur. Specifically, we examined soil bacterial community composition and diversity in relation to plant "host" species, plant community richness, bacterial antagonists, and soil edaphic characteristics. Soil bacterial communities associated with four different prairie plant species (*Andropogon gerardii*, *Schizachyrium scoparium*, *Lespedeza capitata*, and *Lupinus perennis*) grown in plant communities of increasing species richness (1, 4, 8, and 16 species) were sequenced. Additionally, soils were evaluated for populations of antagonistic bacteria and edaphic characteristics. Plant species effects on soil bacterial community composition were small and depended on plant community richness. In contrast, increasing plant community richness significantly altered soil bacterial community composition and was negatively correlated with bacterial diversity. Concentrations of soil carbon, organic matter, nitrogen, phosphorus, and potassium were similarly negatively correlated with bacterial diversity, whereas the proportion of antagonistic bacteria was positively correlated with soil bacterial diversity. Results suggest that plant species influences on soil bacterial communities depend on plant community diversity and are mediated through the effects of plant-derived resources on antagonistic soil microbes.

Key words: bacterial diversity; microbial antagonism; plant diversity; plant richness; plant–soil interactions; soil bacteria.

Introduction

Plant–soil feedbacks have been widely studied and are hypothesized to play important roles in the ecological and evolutionary dynamics of both plants and microbes (van der Putten et al. 2013, Bakker et al. 2014, Schweitzer et al. 2014). Soil microbial communities can influence plant fitness in diverse ways, such as via decomposition, nutrient cycling, nutrient acquisition, plant disease, and plant disease suppression (Garbeva et al. 2004, Singh et al. 2004, Berg and Smalla 2009, Latz et al. 2012). However, the majority of studies exploring plant–soil interactions are plant-centric, with a strong focus on measures of plant survival, productivity, and fitness, and generally give limited attention to the dynamics of soil microbial communities. As a result, we have relatively little understanding of the specific ways in which plants may impact soil microbial community composition, diversity, or function.

Plants are broadly perceived to influence soil bacterial taxa directly through the provision of carbon compounds (Bardgett and Wardle 2010). Such hypothesized plant species-specific effects may occur through variation in root exudates, mucilage, plant litter, or plant secondary metabolites, and through plant-induced changes to the abiotic soil environment (e.g., pH, soil moisture, N, P, K [Wardle et al. 2004, Bezemer et al. 2006, Boyle et al. 2008, Badri and Vivanco 2009, Herold et al. 2014]). Similarly, greater plant productivity, and thus correspondingly more carbon in soil, is suggested to have significant impacts on soil community composition (Zak et al. 2003, De Deyn et al. 2010). However, a clear predictive framework is lacking for understanding plant species, plant diversity, and species by diversity interactions on soil bacterial community composition, diversity, or function.

Most studies presume that plants are the primary selective factors for microbial community composition in soil (Garbeva et al. 2004, Marschner et al. 2004, Costa et al. 2005, Badri and Vivanco 2009). However, the assumption that plant–microbe interactions are the central force in determining microbial fitness ignores the well-established and significant role of microbial species interactions in soil community dynamics. Microbial species interactions, including competition, antagonism, syntrophy, and signaling, are critical to bacterial fitness and the assembly and dynamics of soil bacterial communities (Ryan and Dow 2008, Hibbing et

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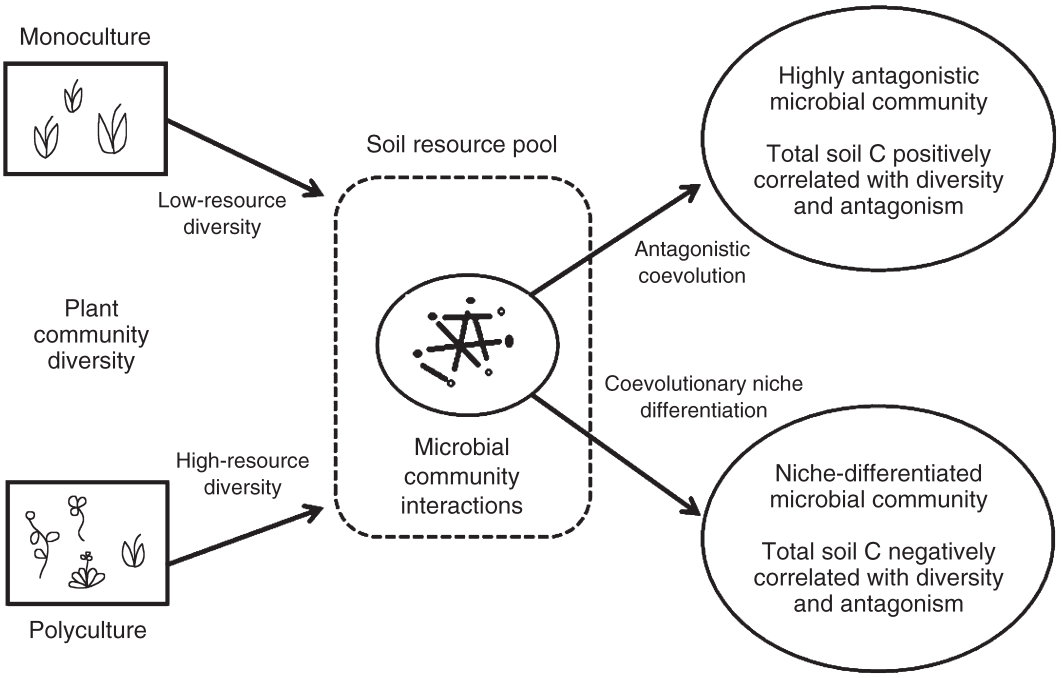

FIG. 1. Our conceptual model (modified from Kinkel et al. 2011) predicts that the impacts of plant community diversity (monocultures and polycultures) on the abundance and diversity of soil resources are a critical context in which microbial species interactions and coevolution take place. Plant monocultures that contribute a low diversity of resources to soil are expected to generate highly antagonistic soil communities that support diverse microbial communities. In contrast, plant polycultures that supply a diverse array of resources to soil may promote coevolutionary niche differentiation and favor nonantagonistic microbial communities in which antagonism plays little role in maintaining soil community diversity.

al. 2010, Becker et al. 2012, Vaz-Jauri et al. 2013). Moreover, antagonistic interactions among soil microbes have been shown to suppress diverse plant diseases, and are predicted to contribute to the maintenance of microbial diversity (Czárán et al. 2002, Kinkel et al. 2011, 2012). Thus, rather than direct plant–microbe interactions determining microbial community composition or function, plants may set the environmental context in which microbial interactions occur. Specifically, we hypothesize that plant species and diversity impact soil resources (C, N, P, K, and OM) that are important for resource competition and species interactions among soil bacteria (Fig. 1; Schlatter et al. 2009, Kinkel et al. 2011), and that microbial species interactions determine microbial community composition, diversity, and function. Consequently, we suggest that variation in plant productivity and diversity influence competitive phenotypes of soil microbes (Bakker et al. 2010, 2013*b*) and thus that plant effects on bacterial community composition and diversity may be largely indirect (Fig. 1).

Shifting the emphasis from plant–microbe interactions to microbial interactions within the context of a plant-created environment may improve our capacity to develop a generalized model for the impacts of plant communities on soil microbial community diversity and function. If microbial species interactions are fundamentally resource focused, then understanding the effects of the plant host and the plant community on soil resources will predict the effects of plant communities on soil microbial communities independent of the specific plant host under consideration. A recent model suggests that carbon quantity and carbon diversity are two primary factors that mediate microbial species interactions in soil (Kinkel et al. 2011, 2012). The model predicts that lower diversity of carbon compounds in soil, e.g., in association with plant monocultures, supports more intense resource competition and favors antagonistic phenotypes. Furthermore, because antagonistic species interactions are hypothesized to promote diversity (Czárán et al. 2002), the model predicts that more antagonistic communities will have relatively greater microbial diversity than less antagonistic communities (Kinkel et al. 2011, 2012, Bakker et al. 2014). In contrast, a greater diversity of carbon compounds, for example as predicted in high-diversity plant communities, is expected to favor microbial niche differentiation as an alternative to antagonistic coevolution, and consequently more limited soil microbial diversity. Recent work showed that more niche-differentiated populations had lower antagonistic activity, and more antagonistic populations were less niche-differentiated, suggesting such distinct coevolutionary trajectories among sympatric soil microbes (Kinkel et al. 2014). Finally, because greater productivity can enhance or accelerate coevolutionary dynamics (Lopez-Pascua et al.

2010), we expect that soil C amount will be positively correlated with both antagonistic activities and soil bacterial diversity in low-diversity plant communities undergoing antagonistic coevolution. In contrast, the amount of soil carbon is predicted to be negatively correlated with antagonistic activities and soil bacterial diversity in niche-differentiated microbial communities associated with high-diversity plant communities.

In this work we explore linkages between soil bacterial community composition, diversity, and bacterial antagonistic activities in soils from four prairie plant species growing in 1-, 4-, 8-, and 16-species plant communities. Specifically, we hypothesize that (1) plant species and plant community richness interact with each other to influence soil bacterial community composition, (2) impacts of plant species and plant community richness on soil bacterial community composition and diversity are related to differences in soil carbon and inorganic nutrients (N, P, and K), and (3) bacterial diversity is positively correlated with the prevalence of antagonistic bacteria and soil carbon in monocultures, but negatively correlated with soil carbon in high-diversity plant communities.

Materials and Methods

Experimental setup and soil sampling

Samples were collected at the University of Minnesota Cedar Creek Ecosystem Science Reserve (CCESR) in July 2009 from the long-term biodiversity experiment E120, as described in Bakker et al. (2013*b*).[3] Briefly, in 1994, plots were established with 1, 4, 8, or 16 plant species, each drawn randomly from a pool of 16 native prairie plant species (Tilman et al. 2001). In 2009, we collected soil cores (5 × 30 cm) from the base of four target plant species, two C4 grasses, *Andropogon gerardii* and *Schizachyrium scoparium*, and two legumes, *Lespedeza capitata* and *Lupinus perennis*. Within individual plots, four cores from different individuals of target plant species were collected. Soil cores from each plot were bulked for each plant species–plant richness combination, which was replicated across three plots for a total of 48 soil samples (4 species × 4 plant richness treatments × 3 plot-level replicates). Soil samples were stored at 4°C until processing. These soils are all fine sand belonging to the Zimmerman series (Bakker et al. 2013*b*).

Soil DNA extraction, PCR, and 454 pyrosequencing

DNA was extracted from soil samples using the PowerSoil DNA Kit (MO BIO, Carlsbad, California, USA) as described in Bakker et al. (2013*a*). Barcoded 454 primers with universal bacterial template-specific sequences [Primer B-27F; (27f: 5′-AGAGTTTGATCCTGGCTCAG-3′) and Primer A-MID-338R; (338R: 5′-TGCTGCCTCCCGTAGGAGT-3′)] were used to amplify bacterial 16S rRNA gene fragments from soil DNA. PCRs consisted of 45 μL Accuprime Pfx Supermix (Invitrogen, Carlsbad, California, USA), 1 μL (10 pmol/L) of each primer, 2μL (20 ng) DNA, and 1μL H_2O. PCR conditions followed the protocol of Fierer et al. (2008) with an initial denaturation step of 94°C for 3 minutes followed by 35 cycles of 94°C for 45 s, 50°C for 30 s, and 72°C for 90 s, and a final extension of 72°C for 10 minutes. PCR products were checked on a 2% agarose gel and purified using the Qiaquick PCR Purification Kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. Purified amplicons were quantified using fluorometry (Qubit dsDNA HS assay, Invitrogen) and 20 ng of each sample was combined into a single pool. Pooled DNA was run on a 1% agarose gel, re-purified from the band of expected product size with the Qiaquick kit, and quantified as described previously. The purified pool was submitted to the University of Minnesota Biomedical Genomics Center for sequencing using the 454 GS FLX+ pyrosequencing platform. Pyrosequencing data were processed using the AmpliconNoise V1.24 algorithm (Quince et al. 2011) and mothur (Schloss et al. 2009) as described in Appendix A. We obtained 476 573 high-quality sequences with an average length of 291 bp after processing. Seventy-five percent of sequences were classified to 16 known phyla (Appendix B) and when sequences were binned at 97% similarity they formed 26 153 distinct OTUs. The observed and estimated (Chao1) OTU (operational taxonomic unit) richness and the inverse Simpson's ($1/D$) index of diversity for each sample were determined and the Yue and Clayton index of community similarity (ThetaYC) was used to explore community structure among samples. Sequence data are available in the NCBI Sequence Read Archive under accession SRR786944.

Soil characteristics and culturable bacterial densities

A portion of each rhizosphere soil sample was submitted to the University of Minnesota Soil Research Analytical Lab for determination of soil pH, total organic carbon (C), total nitrogen (N), phosphorus (P), potassium (K), and percentage of organic matter (OM) according to routine procedures.[4] Briefly, pH was determined on a 1:1 (volume/volume) soil/water mixture using a Mettler Toledo Seven-Multi pH meter (Greifensee, Switzerland), total C was determined with VarioMAX C/N Analyzer (Elementar Americas, Mount Laurel, New Jersey, USA), total N was determined with the Dumas method using a LECO FP-528 Nitrogen Analyzer (LECO, St. Joseph, Michigan, USA), P concentration was determined using the Bray-1 method, K was determined with a PerkinElmer Analyst 100 spectrometer (Waltham, Massachusetts, USA), and percentage of organic matter was determined by loss-on-ignition (for detailed methods see footnote 2). Aboveground biomass and plant cover assessments for

[3] www.cedarcreek.umn.edu

[4] http://ral.cfans.umn.edu/soil-analysis-and-methods/

each plot were acquired from the CCESR database.[5] Briefly, aboveground biomass was determined by clipping narrow vegetation strips along the width of each plot. Aboveground biomass was then sorted, dried, and weighed separately for each strip. Percentage plant cover was estimated visually for each plot using CCESR protocols.

Each soil sample was evaluated for culturable bacterial densities, *Streptomyces* densities, and antagonist densities as described in Bakker et al. (2013*b*). *Streptomyces* were targeted for evaluating antagonists because they are ubiquitous in soil, produce a great diversity of antagonistic compounds, and are associated with plant disease suppression (Davelos et al. 2004*a*, Kinkel et al. 2012). Briefly, for each sample 5 g of soil was suspended in 50 mL H_2O, shaken (175 rpm for 1 hour at 4°C), and 100 uL of soil suspension was spread on 15 mL of 1% water agar. Agar plates were left to dry, overlaid with 5 mL of cooled starch-casein agar (Wiggins and Kinkel 2005), and incubated at 28°C for 3 days. Bacterial and *Streptomyces* densities were counted and each plate was covered with a second layer (10 mL) of starch-casein agar. Plates were then overlaid with each of three indicator *Streptomyces* strains having different antibiotic resistance profiles (Davelos et al. 2004*b*) that have been shown to be predictive of pathogen suppressive activity (Wiggins and Kinkel 2005). After an additional 3 days of incubation at 28°C, zones of inhibition around colonies, indicating the ability of colonies to produce antagonistic compounds, were measured for each plate. Antagonist densities and proportions were averaged across indicator strains for each soil sample.

Statistical analyses

All statistical analyses were conducted in R (R Development Core Team 2012) unless noted otherwise. The Shapiro-Wilk normality test and Bartlett's test of homogeneity were performed to check for normality and equal variance among treatments. Differences among plant species and plant richness treatments in bacterial richness and diversity were assessed using ANOVAs with Tukey's honestly significant difference tests. Significant differences in bacterial community structure (presence/absence of OTUs and their relative abundance) between plant species and plant richness treatments were assessed with AMOVA (analysis of molecular variance) in mothur (Schloss et al. 2009). Nonmetric multidimensional scaling plots were constructed in mothur and used to visualize bacterial community structure. Biplots with soil characteristics, aboveground plant biomass, and culturable bacterial community characteristics were generated in the vegan package (Oksanen et al. 2012) using 1000 permutations to determine significant relationships with nonmetric multidimensional scaling (NMDS) axes. Finally, co-occurrence networks were constructed for taxa present in at least half ($n = 24$) of all samples using custom R scripts (S. Bates, *personal communication*). Positively or negatively co-occurring taxa were defined as OTU pairs whose abundances were strongly correlated (Pearson, $R > 0.6$ or $R < -0.6$, respectively, $P < 0.05$ after correction for false discovery). The software Gephi (Bastian et al. 2009) was used to visualize networks of co-occurring taxa, and to decompose the networks into modules.[6]

Results

Plant species impacts on soil bacterial communities

Considering all plant richness treatments, the most abundant 12.9% of OTUs ($n = 2394$) were found in association with every plant species and represent predominantly taxa from Actinobacteria, Proteobacteria, and unclassified phyla (Appendix C). These ubiquitous OTUs represented 79.3% of all sequences, suggesting that a small subset of OTUs form an abundant, core community of plant-associated soil bacteria. In contrast to the most abundant OTUs, the majority of OTUs (60.6%) were found in association with only a single plant species, but these were rare (85% of these OTUs were singletons or doubletons) and represented only 5.8% of all sequences. Thus, plant-specific OTUs were relatively rare, suggesting that populations of most soil bacteria are not structured by tightly linked plant–microbe interactions.

Among all plant richness treatments, plant host species had small effects on bacterial community structure (ThetaYC community similarity). AMOVA revealed a significant influence of plant host on microbial community structure (AMOVA, $F\text{s} = 1.55$, $\text{df} = 3, 44$, $P = 0.021$), although when compared individually, only bacterial communities associated with *L. perennis* differed from those associated with *A. gerardii* and *S. scoparium* (AMOVA, $F\text{s} = 2.80$, $\text{df} = 1, 22$, $P = 0.003$ and $F\text{s} = 1.95$, $\text{df} = 1, 22$, $P = 0.032$, respectively). When considering only samples from monocultures, where we might expect to see the strongest signature of plant species, there was a marginally significant effect of plant species on bacterial community structure (AMOVA, $F\text{s} = 1.48$, $\text{df} = 3, 44$, $P = 0.07$).

Among all samples, bacterial community richness and diversity were not strongly influenced by host plant species. Specifically, bacterial community richness (observed richness and Chao estimate; ANOVA, $F = 0.422$, $\text{df} = 3, 44$, $P = 0.738$ and $F = 0.669$, $\text{df} = 3, 44$, $P = 0.576$, respectively) and diversity (ANOVA, $F = 1.127$, $\text{df} = 3, 44$, $P = 0.348$, respectively) did not vary among plant species (Fig. 1). However, when only samples from monocultures were considered, observed bacterial richness varied significantly among plant species (ANOVA,

[5] http://www.cedarcreek.umn.edu/research/data/

[6] Gephi.org

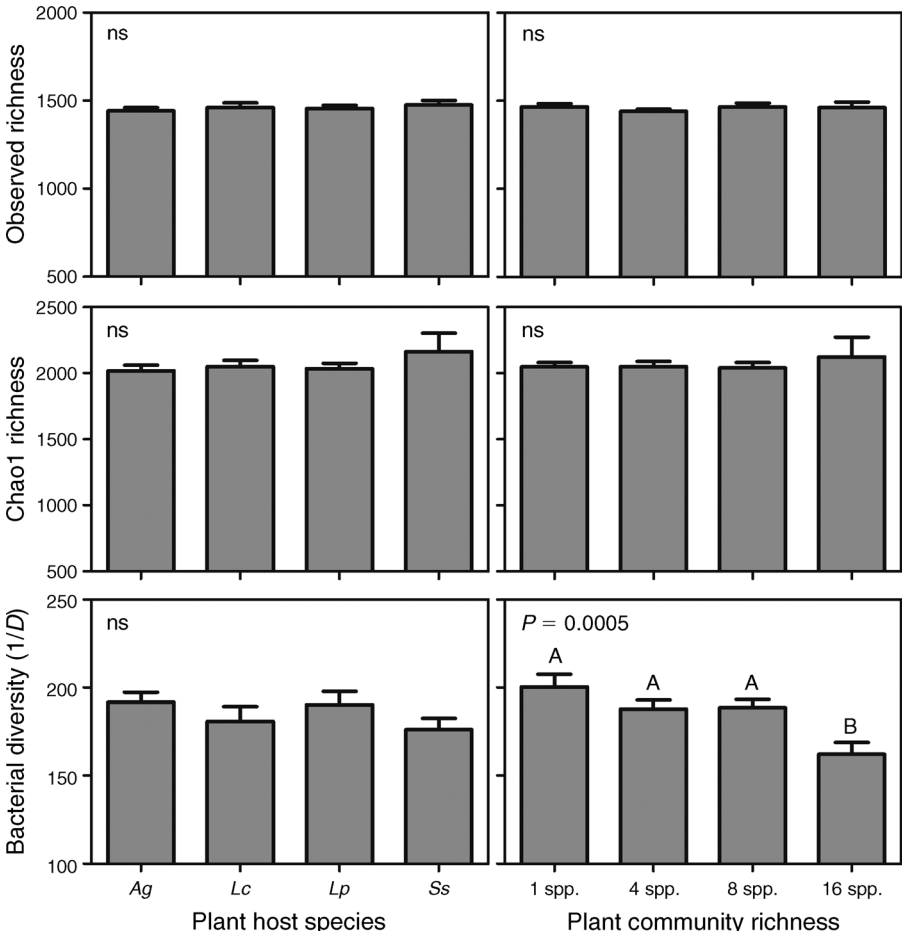

FIG. 2. Observed richness (number of observed operational taxonomic units [OTUs], top panels), estimated richness (Chao1 estimate of the number of OTUs, middle panels), and diversity ($1/D$, bottom panels) among plant host species across plant richness treatments ($n = 12$ for each plant species, left panels) and plant richness across all plant species ($n = 12$ for each plant richness treatment, right panels) treatments. Error bars represent standard errors; ns is not significant; different capital letters above bars represent significant differences among treatments (Tukey's HSD). For plant name abbreviations on the x-axis, see *Materials and methods: Experimental setup and soil sampling*.

$F = 4.47$, df = 3, 8, $P = 0.04$), but estimated richness and diversity did not (data not shown). Bacterial communities associated with *L. capitata* supported soil communities with a significantly greater number of OTUs than *A. gerardii* (average of 1547 vs. 1408 OTUs, respectively; Tukey's HSD, $P = 0.03$). Bacterial richness and diversity did not vary significantly among plant host species within 4-, 8-, or 16-species richness treatments (data not shown).

Plant richness impacts on soil bacterial communities

Across all plant species, bacterial community structure was significantly influenced by plant community richness (AMOVA; Fs = 1.85, df = P = 0.003). Comparing individual plant richness treatments, differences were significant only between monoculture and 8- and 16-species communities (AMOVA, Fs = 1.77, df = 1, 22, $P = 0.037$ and Fs = 3.98, df = 1, 22, $P < 0.001$, respectively). Plant richness treatments did not vary in observed or estimated bacterial OTU richness (Fig. 2; ANOVA, $F = 0.274$, df = 3, 44, $P = 0.844$ and $F = 0.231$, df = 3, 44, $P = 0.874$, respectively). However, there were significant differences in bacterial diversity among communities from different plant richness treatments (Fig. 2; ANOVA, $F = 7.235$, df = 3, 44, $P = 0.0005$). Specifically, 16-species plant communities harbored less diverse bacterial communities than 1-, 4-, or 8-species plant communities (Tukey's HSD, $P \leq 0.02$ for each comparison). The negative relationship between plant community richness and bacterial diversity was consistent among plant species (data not shown).

Impacts of plant species on soil bacterial communities across a plant diversity gradient

The effects of plant host species on bacterial community structure varied among plant diversity treatments for some plant species but not others. Bacterial community structure did not vary significantly among plant richness treatments for *A. gerardii*, *L. perennis*, and *S. scoparium*

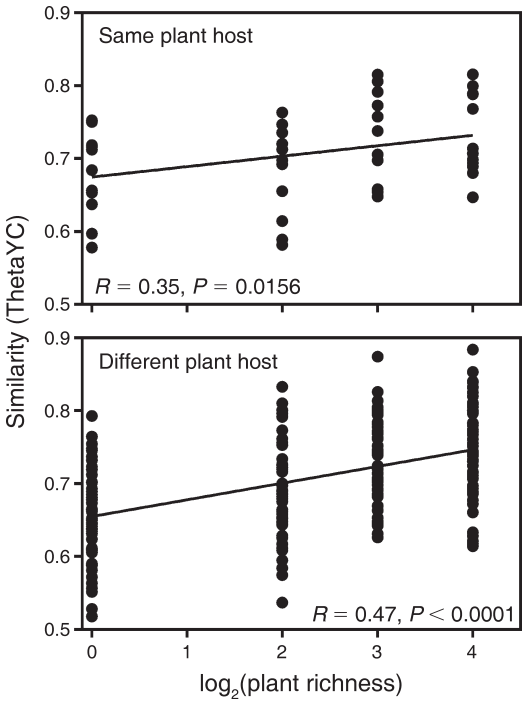

FIG. 3. Linear regression of bacterial community similarity (ThetaYC) from the same plant host (top panel) and different plant hosts (bottom panel) across plant richness treatments.

(AMOVA, $P > 0.05$), though *L. capitata*-associated communities were an exception, and the structure of these communities varied significantly among diversity treatments (AMOVA, Fs = 1.67, df = 3, 8, $P = 0.016$). Overall, when all bacterial communities from the same plant host were compared across plant richness treatments their bacterial communities became significantly more similar with increasing plant richness (Fig. 3; $R = 0.35$, $P = 0.0156$). For individual plant species, greater similarity with increasing plant richness was statistically significant only for *L. capitata* ($P = 0.01$). This trend was also present among bacterial communities from different plant species across plant richness treatments (Fig. 3). Specifically, similarity among soil communities from different plant species increased significantly with increasing plant richness ($R = 0.47$, $P < 0.0001$). This suggests that increasing plant richness has a homogenizing effect on soil bacterial communities associated with plant host species, although the sensitivity of microbial communities to this homogenizing effect varied among plant species.

Plant productivity, soil characteristics, and antagonistic bacteria as correlates of soil bacterial community composition and diversity

Plant richness, plant cover, soil pH, and K and P concentrations, bacterial diversity and density, and antagonist density were all significantly correlated with bacterial community structure along NMDS axes ($P < 0.05$; Appendices D and E). In general, soil C and OM concentrations, nutrient concentrations (K, N, and P) and plant biomass tended to be negatively correlated with bacterial richness and diversity (Table 1). However, concentrations of C, N, OM, and K were often positively correlated with one another and with plant richness (Appendix F), limiting our ability to discriminate distinct roles of soil resource concentrations and plant diversity as drivers of bacterial diversity. Relationships between soil resources and bacterial diversity differed among plant community diversity treatments (Appendix G). Among monocultures, soil K concentrations and aboveground plant biomass were positively correlated with bacterial diversity ($R = 0.70$, $P = 0.01$ and $R = 0.68$, $P = 0.015$, respectively). In contrast, among 4-, 8-, and 16- plant species treatments there were few significant relationships between bacterial diversity and both soil resource concentrations and plant biomass, although K was negatively correlated with bacterial diversity among 4-species plant communities ($R = -0.61$, $P = 0.035$). Soil resource concentrations (C, N, P, K, and OM) and aboveground biomass were not significantly different among 1-, 4-, and 8-species plots, but 16-species plots had significantly greater concentrations of soil resources (C, N, P, K, and OM) and aboveground biomass than lower-richness plots.

In addition to soil resource concentrations, antagonistic activities of soil *Streptomyces* communities were significantly correlated with bacterial community diversity. Overall, bacterial communities with greater proportions of antagonists tended to be more diverse ($R = 0.37$, $P = 0.009$, Fig. 4). However, this trend varied when considering individual plant richness treatments and was significant only among 16-species plant communities ($R = 0.68$, $P = 0.015$).

Bacterial co-occurrence networks

Across all samples, correlation network analysis identified 148 strong, positive correlations among 136 OTUs ($R > 0.6$, $P \leq 1.04 \times 10^{-11}$) and 10 strong, negative correlations among 16 OTUs ($R < -0.6$, $P \leq 1.92 \times 10^{-15}$). The network of positive correlations comprised 23 distinct modules of co-occurring taxa (Appendix H). Eight large modules with ≥7 OTUs

TABLE 1. Pearson correlations (R value) between soil edaphic characteristics, plant biomass, soil antagonists, and bacterial richness and diversity ($n = 48$).

Soil characteristic	Observed richness	Chao1	$1/D$
C (%)	−0.48**	−0.27	−0.27†
OM (%)	−0.46**	−0.22	−0.30*
N (%)	−0.43**	−0.23†	−0.20
P (ppm)	−0.05	−0.19	−0.22
K (ppm)	0.02	0.04	−0.42**
pH	0.08	−0.06	−0.01
Aboveground biomass (g/m^2)	−0.29*	−0.14	−0.14
Antagonist density (log(CFU/g))	0.09	−0.06	0.22
Proportion antagonists (%)	0.03	0.01	0.37**

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

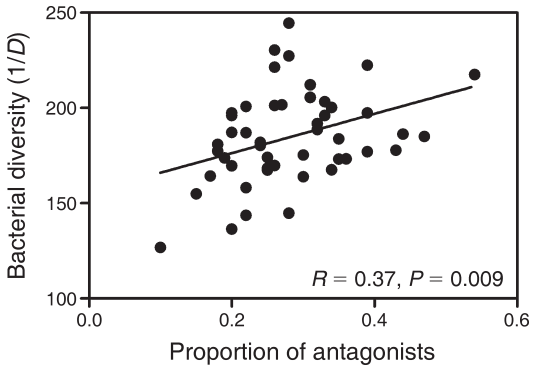

FIG. 4. Relationship between proportion of antagonists and bacterial diversity among all bacterial communities ($1/D$; Pearson $R = 0.37$, $P = 0.009$).

represented 16.5% of sequences sampled. Modules differed in their environmental preferences, suggesting that taxa belonging to the same module are ecologically similar. OTUs in some modules were consistently more common in soils that had higher quantities of C, OM, N, and K (e.g., modules 9 and 17), suggesting that these taxa are copiotrophic (Appendix H). Potentially oligotrophic modules had OTUs that were more abundant in soils with lower concentrations of C, OM, N, and K (modules 2 and 15; Appendix H). Soil pH was a consistent predictor of the abundances of OTUs in large modules, but was the best predictor among all soil characteristics only for module 13. Thus, variation in soil resources and differences in soil pH preferentially support some modules. In contrast, the abundances of some modules were more strongly correlated (negatively and positively) with antagonist population densities or proportions of antagonists (modules 20 and 22). This suggests that while some groups of co-occurring taxa respond to soil resource status, others may be highly sensitive to competitive species interactions in soil.

DISCUSSION

The concept that different plant species harbor distinct rhizosphere bacterial communities is widespread in studies of plant–soil feedbacks (Kowalchuck et al. 2002, Marschner et al. 2004, Costa et al. 2005, Garbeva et al. 2008, Berg and Smalla 2009, Lundberg et al. 2012). However, in contrast to other studies, we found only weak effects of plant species on soil bacterial communities. Specifically, we found as much variation in bacterial community structure among soils associated with the same plant hosts as in those associated with different plant hosts, although soils had been associated with perennial plant hosts maintained in monocultures for 13 years. Though most bacterial OTUs were found in soils from a single plant species, most sequences belonged to OTUs that were ubiquitous among plant species. This pattern suggests that there is an abundant, core soil bacterial microbiome commonly found in association with prairie plant species. Members of this core microbiome may play an especially important role in plant-associated soil functions, such as decomposition, nutrient cycling, and plant disease suppression. Alternatively, these ubiquitous taxa may be largely inactive and irrelevant to variation in soil functioning across the landscape. In total, the lack of a strong plant-specific signature in soil bacterial community composition or diversity suggests that plant species identity has a small impact on most members of the soil bacterial community relative to other factors that structure communities in soil, such as plant community diversity, soil characteristics, and microbial species interactions.

In contrast to plant host species, plant community richness had clear impacts on soil bacterial community structure and diversity. Although other studies have found little relationship between plant community diversity and microbial diversity in soil (Kowalchuck et al. 2002, Fierer et al. 2007, Bakker et al. 2013*a*), we found that the least diverse bacterial communities were present in plant communities with the highest species richness, and that the most diverse bacterial communities were present in monocultures. Although differences in soil resource concentrations and diversity among plant richness treatments may partially explain this trend (Wardle et al. 2004, Bakker et al. 2013*b*), the conceptual model of Kinkel et al. (2011) suggests that plant richness effects on soil resource concentrations and diversity also impact soil bacterial diversity by modulating competitive species interactions among soil bacteria. Indeed, related work in the system studied here has found that highly diverse plant communities have both substantially greater soil resource concentrations and significantly smaller proportions of antagonistic bacteria among diverse plant communities than among monocultures (Bakker et al. 2013*b*). In general, greater concentrations of soil resources (C, OM, N, K) and aboveground plant biomass were negatively correlated with soil bacterial diversity, whereas greater proportions of antagonistic bacteria were positively correlated with bacterial diversity. This pattern suggests that low-resource environments induced by plant monocultures may favor antagonistic bacteria (Bakker et al. 2013*b*). The higher frequencies of antagonistic bacteria in soil associated with monocultures may then generate more diverse bacterial communities (Czárán et al. 2002). In contrast to plant monocultures, high-diversity plant communities likely contribute greater diversities of plant-derived resources and provide more opportunity for soil microbes to specialize on distinct resources. A high diversity of resources may allow for coevolutionary niche differentiation among soil microbes and favor nonantagonistic bacteria (Kinkel et al. 2011). Thus, low frequencies of antagonistic interactions in soils associated with high-diversity plant communities may generate less diverse soil bacterial communities.

Interactive effects of plant richness and plant species on soil communities differed among plant species. The structure of bacterial communities associated with *A. gerardii*, *L. perennis*, and *S. scoparium* did not vary across plant diversity treatments, whereas communities

associated with *L. capitata* became substantially more similar as plant richness increased. Counter to the expectation that bacterial communities from different plants of the same host grown in monoculture would be most similar to one another, we found that different plants of the same host grown in monoculture frequently supported more dissimilar bacterial communities than different plants of the same species grown in polyculture. Thus, among plant monocultures the effect of plant species identity on soil bacterial communities may be especially small relative to other potential drivers, such as microbial competitive interactions. In contrast, greater similarity in bacterial communities from the same and different plant host species at high plant richness suggests that greater plant richness significantly homogenizes soil bacterial communities.

Although greater productivity in diverse plant communities may homogenize soil community composition, plants in species-rich communities may also allocate distinct resources belowground (Broz et al. 2010). As a result, plant–soil feedbacks may differ among diverse rather than simple plant communities. In particular, though plant–soil feedbacks are sometimes important for maintaining plant community diversity, the sign and strength of feedbacks can change over time (Kardol et al. 2006, Hawkes et al. 2012, van der Putten et al. 2013) and over distinct plant community contexts. Further studies linking detailed information on soil community composition with plant–soil feedbacks across a plant diversity gradient will offer insight into the specific mechanisms and organisms involved in positive and negative plant–microbe associations.

These data show that plant diversity is a significant driver of soil microbial community structure and richness, and that plant community diversity may both alter and eclipse the impacts of individual plant species on soil communities. Specifically, our findings are consistent with a model in which plants, through resource inputs to soil, set the context within which microbial competitive interactions mediate microbial community composition. Because local plant diversity can substantially alter resource abundance within the rhizosphere of an individual plant host, plant diversity can mediate microbial species interactions in ways that may alter the strength of individual plant–soil feedbacks. This work highlights the critical role of microbial species interactions in determining the composition and diversity of microbial communities in soil, and emphasizes the role of individual plants and the local plant community as determinants of the quantity and diversity of soil resources. Further studies with sampling designs that deliberately consider the complex and interactive effects of both macro- and micro-scale drivers of microbial communities, including interactions among plant species and among soil microbial populations, will be essential to understanding linkages between soil and aboveground communities.

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Supplemental Material

Ecological Archives

Appendices A–H are available online: http://esapubs.org/archive/unpublished/13-1648/