Rhizosphere bacterial communities associated with long-lived perennial prairie plants vary in diversity, composition, and structure

N. Rosenzweig, J.M. Bradeen, Z.J. Tu, S.J. McKay, and L.L. Kinkel

Abstract: The goal of this research was to investigate the variation in rhizosphere microbial community composition, diversity, and structure among individual *Andropogon gerardii* Vitman (big bluestem) and *Lespedeza capitata* Michx. (bush clover). Bacterial communities from the rhizosphere of 10 plants of each species (*n* = 20 plants total) were explored using a culture-independent pipeline. Microbial communities associated with both host plants had high bacterial diversity within individual plant rhizosphere and taxa unique to individual rhizospheres. Bacterial communities associated with the rhizosphere of *A. gerardii* were consistently more diverse than those associated with *L. capitata*, and there were significant differences between plant species in rhizosphere bacterial community composition. Differences included microbial taxa with no known functional relationship with their preferred host species, including sulfide-methylating obligate anaerobes (*Holophaga*), complete denitrifiers (*Rhodoplanes*), sludge inhabitants (*Ktedonobacter*), and nitrate oxidizers (*Nitrospira*). These results suggest the potential for plant species to have significant impacts on a broad array of ecosystem functions (e.g., cycling of carbon, nitrogen sulfur, metals, and trace elements) via their selective impacts on soil microbes. However, sequence-based community analysis and the corresponding lack of intact microbial cultures limits understanding of the potential influences of enriched microbial taxa on plant hosts and their roles in ecosystem functioning.

Key words: *Andropogon gerardii*, *Lespedeza capitata*, pyrosequencing, microbial communities, rDNA 16S gene V3 region.

Résumé : Le but de la présente recherche était de faire l'examen de la variation de la composition, de la diversité et de la structure de communautés microbiennes de rhizosphères provenant de 20 plantes de chaque espèce (*A. gerardii* Vitman et *L. capitata*) et de l'une ou l'autre de leurs hôtes végétaux. Les communautés microbienues associées à l'éspece *Andropogon gerardii* Vitman (arboretum de Gérard) et à l’espèce *Lespedeza capitata* (trèfle arbustif). Les communautés microbienues retrouvées au sein de la rhizosphère de 10 plantes de chaque espèce (*n* = 20 plants total) ont fait l’objet d’analyse composée d’une série de méthodes sans culture. Les communautés microbienues associées à l’un ou l’autre des hôtes végétaux présentaient une diversité élevée au niveau de chaque rhizosphère dans le cas du premier, et des taxons uniques dans chacune des rhizosphères dans le cas du second. Les communautés bactériennes de la rhizosphère d’A. gerardii étaient constamment plus diversifiées que celles associées à L. capitata; on a constaté des différences significatives entre les espèces végétales sur le plan de la composition de la communauté bactérienne des rhizosphères. Les différences se résumaient à des taxons microbieniens sans lien fonctionnel reconnu avec leur espèce hôte « privilégiée », à savoir des anaérobies méthylandant le sulfure (*Holophaga*), des dénitrificateurs complets (*Rhodoplanes*), des habitants de boues (*Ktedonobacter*) et des oxydants de nitrates (*Nitrospira*). Ces résultats tendent à démontrer que les espèces végétales semblent capables d’avoir une influence importante sur une large gamme de fonctions écologiques (p.ex. le cycle du carbone, de l’azote, du soufre, des métaux et des oligo-éléments) par le biais de leur impact sur les microbes du sol. Par contre, l’analyse des communautés par séquençage, qui n’implique pas de culture de microbes intacts, limite notre compréhension des incidences possibles de la surreprésentation de taxons microbieni sur les plantes hôtes et sur les fonctions écologiques des écosystèmes. [Traduit par la Rédaction]

Mots-clés : *Andropogon gerardii*, *Lespedeza capitata*, pyrosequencing, communautés microbienues, région V3 du gène de l’ADN 16S.

Introduction

Soil microbes have significant influences on plant fitness as pathogens, beneficial mutualists, decomposers, or pathogen antagonists, as well as in their capacities to produce plant hormones, influence nutrient cycling and solubilization, and induce systemic resistance responses in plants (Smalla et al. 2001). These impacts can range from highly positive to negative for the host plant. Moreover, the impacts of rhizosphere microbes on individual host plants can alter plant communities across the landscape. For example, the relative abundance of plant species in a Canadian meadow was correlated with the extent to which soil microbes associated with each plant species conferred positive impacts on host plant biomass; abundant plant species were more likely to experience positive feedbacks with their rhizosphere-associated microflora, while rare species tended to experience negative feedbacks (Klironomos 2002). Thus, soil microbes, through their positive or negative impacts on individual plants, can be key determinants of plant community diversity and composition.

Plants, in turn, have been shown to have significant influences on soil microbial communities (Bever 2002; Hart et al. 2003; Klironomos 2002; Lemanceau et al. 1995; Lucas 1998; Miethling et al. 2000; Miller et al. 1989; van der Heijden et al. 1998; Westover and Bever 2001). Plants can have differential effects on microbial community size, composition, and diversity (Bohlen et al. 2001; Klironomos 2002; Michelsen et al. 1999; Shively et al. 2001; Zak et al. 2003). In particular, root exudates can stimulate growth of fungi and bacteria in the rhizosphere (Bever 2003; Farrar et al.
The objective of this study was to characterize the composition and diversity of bacterial communities associated with 2 long-lived herbaceous perennial plant species, *Andropogon gerardii* Vitman and *Lespedeza capitata* Michx., to provide an explicit test of the hypothesis that plant species support distinct and characteristic rhizosphere bacterial communities. These 2 plant species are dominant members of the North American tall-grass prairie and have extensive natural ranges. While both establish extensive root systems, their associations with soil microbes may be expected to differ. As a legume, *L. capitata* establishes mutualistic symbioses with *Rhizobium* spp. This association contributes nitrogen to the prairie ecosystem, which can benefit warm season grasses as well as the host legume. Both plant species are known to be strong competitors in established prairies and are desirable components in both grazing lands and prairie restorations. Despite this, little is known of their relationships with the soil microbiota or, specifically, their influences on soil microbial community composition or the extent to which plant–microbe feedbacks influence host establishment or longevity.

While the rhizosphere is widely reported to support highly diverse microbial communities, there is limited understanding of the relationships between bacterial diversity within and among plants of the same and different hosts in the same or different locations. We used a spatially stratified sample design to characterize the relative influences of location and plant species on bacterial community composition and diversity in soil. We were particularly interested in characterizing the relationships among *α* diversity (diversity or taxon richness associated with individual plant rhizospheres), *β* diversity (the numbers and proportions of taxa unique to individual rhizospheres, host plants, or fields), and *γ* diversity (the total bacterial diversity) associated with these plant species at the Cedar Creek Ecosystem Science Reserve (CCESR) (East Bethel, Minnesota), part of the National Science Foundation Long Term Ecological Research (NSF-LTER) Network.
resampling without replacement approach. The Shannon (Shannon 1948) and Simpson (Simpson 1949) diversity indices of each sample were estimated using the R-statistical package. Taxonomic classification levels were determined using 97% sequence identity. PCR, GS amplicon library preparation, and pyrosequencing on a 454 Life Sciences GS-FLX machine (Roche).

**OTU-based analyses**

Operational taxonomic unit (OTU) analyses were performed using the MOTHUR software package (version 1.6.0) (Schloss et al. 2009). OTU calculations were based on the genetic distance between sequences and were used to estimate the richness and diversity of a sampled community. Nonredundant sequences were aligned to both the Greengenes alignment database (http://greengenes.lbl.gov/) and the SILVA alignment database (http://www.arb-silva.de/) using the Needleman method with a kmer size of 9 and gap open penalty of −1. Similar results were obtained from both databases and all results reported here are based on analyses of the SILVA alignment dataset. Vertical gaps were removed from the sequence alignment, and the reference database was used to determine the closest template for each candidate sequence so that the candidate sequence alignment is compatible with the original 16S rRNA gene template alignment. A column-formatted distance matrix was calculated for distances smaller than 0.10 with the multiple processor option. Sequences were clustered and assigned to OTUs at distances between 0.0 and 0.10 in 0.01 increments. The number of OTUs and the ACE (Chao 1984) and Chao1 (Chao and Lee 1992) richness estimates for the unique, 0.03, 0.05, and 0.10 OTU definitions were calculated. Single and multiple intrasample rarefaction curves were generated using a resampling without replacement approach. The Shannon (Shannon 1948) and Simpson (Simpson 1949) diversity indices of each sample were calculated for unique, 0.03, 0.05, and 0.10 OTU definitions were calculated. Subsequently, the Yue and Clayton (1994) measure of similarity between community structure and composition was determined (Yue and Clayton 2005).

**Taxon-based analyses**

A Perl script was written to sort sequencing reads by barcodes. Only bacterial sequences that were at least 90 bp long, excluding barcodes and fusion primers, were analyzed further in BLAST analysis. FASTA-formatted, trimmed sequences were compared against the National Center for Biotechnology Information (NCBI) nonredundant database (nt) using Tera-BLASTn (version 7.6.2: Active Motif Inc., Carlsbad, California) (parameters: minimum pairwise identity, 98%; minimum match length, 50 bp) (Altschul et al. 1990). MySQL databases were created on a local server and indexed, with the resulting NCBI taxonomy data files gi_taxid_nucl.dmp, names.dmp, and nodes.dmp from ftp://ftp.ncbi.nih.gov/pub/taxonomy. GenBank IDs associated with the best hits from the resulting Tera-BLASTn run were used to query the local NCBI database for taxonomic annotation. Finally, the resulting matches for each set of sequence data were summarized at various taxonomic levels and filtered to remove nonbacterial lineages. Kolmogorov-Smirnov, ANOVA (analysis of variance), and Tukey’s Honestly Significant Difference (HSD) analyses were performed on taxonomy query results. All statistical analyses were performed using the R-statistical package (version 2.10; http://cran.r-project.org).

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**Table 1.** Summary of sequencing effort; total number of sequences; mean number of sequences per plant; and total bacterial phyla, families, genera, and species classified.

<table>
<thead>
<tr>
<th>No. of plants sampled</th>
<th>Total no. of sequences</th>
<th>Mean no. of sequences per plant (range)</th>
<th>Total no. of bacterial phyla classified&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total no. of bacterial families classified&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total no. of bacterial genera classified&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total no. of bacterial species classified&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (Andropogon gerardii)</td>
<td>122 422</td>
<td>12 242 (9 358 – 16 403)</td>
<td>28</td>
<td>227</td>
<td>905</td>
<td>1683</td>
</tr>
<tr>
<td>10 (Lespedeza capitata)</td>
<td>128 892</td>
<td>12 889 (2 466 – 35 299)</td>
<td>28</td>
<td>226</td>
<td>870</td>
<td>1474</td>
</tr>
<tr>
<td>20 (Total)</td>
<td>251 314</td>
<td>12 566 (2 466 – 35 299)</td>
<td>30</td>
<td>236</td>
<td>1006</td>
<td>2206</td>
</tr>
</tbody>
</table>

<sup>a</sup>Taxonomic classification level determined at 97% sequence identity.

**Results**

A total of 251 314 high quality sequences were obtained from DNA extracted from 20 individual soil samples (Table 1). Based on the results of rarefaction analyses (Fig. 1), OTUs were defined at a conservative 10% dissimilarity, a level that provides best sample saturation. Sequences were also assigned to putative taxa using Tera-BLASTn and a best-match strategy. Finally, indicator species analyses were conducted to identify sequences that are characteristically associated with host plant species and field location. Across all samples, 27 499 distinct OTUs were observed (Supplemental Table S1). Rare OTUs, represented as singleton sequences in

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**Fig. 1.** Rarefaction analysis curve using a resampling without replacement approach across a sequenced soil sample. Rarefaction curves were generated for operational taxonomic units (OTUs) that contain unique sequences and for sequence dissimilarities that do not exceed 3%, 5%, or 10%.

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[Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjm-2012-0661.]
our data set, comprised 7145 (25%) of all OTUs, underscoring the phylogenetic richness of prairie soils. Applying a 97% sequence identity criterion, these same sequences were assigned to 30 bacterial phyla, 236 bacterial families, and 1006 bacterial genera (Table 1). Owing to the relatively short length and high sequence divergence of the V3 region, we could not confidently assign sequences to taxa below the genus level. Distinct bacterial taxa were disproportionately represented in our data set, with 10 of the 30 phyla detected comprising nearly 98% of total bacterial assemblage. Similarly, 24 of the 236 bacterial families recovered comprised nearly 66% of the total bacterial community assemblage (Fig. 2) and 26 of the 1006 genera represented 42% percent of all sequences (Fig. 3). Importantly, the commonly cultured plant-associated genera Pseudomonas and Streptomyces as well as the nitrogen-fixing Bacillus, Bradyrhizobium, and Rhizobium were among the most well-represented genera (Fig. 3).

Effects of plant species on rhizosphere bacterial communities

Of 27 499 defined OTUs, 5809 (21%) were observed on both plant species, while 11 357 (41%) and 10 333 (38%) were associated only with A. gerardii or L. capitata plants, respectively (Supplemental Table S1). Interestingly, rare taxa (represented as singletons or doubletons) comprised a substantially greater fraction of communities associated with L. capitata (6366 OTUs, 39%) than with A. gerardii (1335 OTUs, 8%).

More than 99% of sequences that could be assigned to bacterial phyla were present in the rhizosphere of both A. gerardii and L. capitata, and there were no significant differences in the relative abundance (mean number of sequences per plant) of individual bacterial phyla between host plants. However, 3 bacterial families (Acidobacteriaceae, Hyphomicrobiaceae, and Rhodocyclaceae) differed...
Similarly, bacterial diversity was greater for
had a greater relative abundance of
L. capitata
Bacterial genus

\[
0.1081 < 10^{-4} 0.0211 \text{ ns} 0.0505 \text{ ns}
\]

Bacterial species

\[
0.2180 < 10^{-15} 0.0347 \text{ ns} 0.0947 < 10^{-3}
\]

nity richness were also greater for

\[
P < 0.05: \text{Tukey's HSD}
\]

We observed significant differences in relative abundance be-

\[
A. gerardii
\]

Bacterial communities associated with
A. gerardii
and
L. capitata
differed significantly when considering bacterial genera (Table 2).

**Table 2.** Comparison of frequency distributions of bacterial sequences with the Kolmogorov–Smirnoff test and critical D statistic.

<table>
<thead>
<tr>
<th>Taxonomic level</th>
<th>Field 1a</th>
<th>Field 2b</th>
<th>Totalc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D statistic</td>
<td>P</td>
<td>D statistic</td>
</tr>
<tr>
<td>Bacterial genus</td>
<td>0.1081</td>
<td>&lt;10^{-4}</td>
<td>0.0211</td>
</tr>
<tr>
<td>Bacterial species</td>
<td>0.2180</td>
<td>&lt;10^{-15}</td>
<td>0.0347</td>
</tr>
</tbody>
</table>

Note: ns, not significant at \( \alpha = 0.05 \).

bSample size for plant species: \( n = 10 \).

cSample size for plant species: \( n = 20 \).

**Fig. 4.** Venn diagram indicating shared and unique observed operational taxonomic units (OTUs) between plant species by location. Number of OTUs based on 10% dissimilarity.

Ag1, *Andropogon gerardii* at Field 1; Lc1, *Lespedeza capitata* at Field 1; Ag2, *A. gerardii* at Field 2; and Lc2, *L. capitata* at Field 2.

significantly in relative abundance between the 2 host plants

\( P < 0.05 \): Tukey’s HSD. *Andropogon gerardii* had a greater relative abundance in mean number of sequence reads per plant of *Acidobacteriaceae* (A. gerardii: 21.2%; *L. capitata*: 18.0%) and *Hyphomicrobiaceae* (A. gerardii: 6.6%; *L. capitata*: 4.5%), while *L. capitata* had a greater relative abundance of *Rhodocyclaceae* (A. gerardii: 1.3%; *L. capitata*: 4.4%; Fig. 2).

Bacterial communities associated with *A. gerardii* and *L. capitata* differed significantly when considering bacterial genera (Table 2). We observed significant differences in relative abundance between plant species for 8 of the 24 most common bacterial genera (\( P < 0.05 \): Tukey’s HSD). Averaged across both field locations, *A. gerardii* had a significantly greater mean relative abundance of *Holophaga* (A. gerardii: 9.4%; *L. capitata*: 6.2%), *Rhodoplanes* (A. gerardii: 5.3%; *L. capitata*: 4.0%), *Ktedonobacter* (A. gerardii: 2.8%; *L. capitata*: 1.9%), *Nitrospira* (A. gerardii: 2.1%; *L. capitata*: 2.0%), and *Oscillatoria* (A. gerardii: 1.8%; *L. capitata*: 0.8%), while *L. capitata* had a greater abundance of *Acidobacterium* (A. gerardii: 8.1%; *L. capitata*: 8.9%), *Pseudononas* (A. gerardii: 2.0%; *L. capitata*: 5.5%), and *Thiorhodovibrio* (A. gerardii: 0.08%; *L. capitata*: 1.6%) \( P < 0.05 \): Tukey’s HSD).

On average, individual *A. gerardii* plants supported greater bacterial community richness than *L. capitata* plants (Fig. 4). Specifically, observed mean OTUs per plant were 3242 and 2079 for *A. gerardii* and *L. capitata*, respectively (Supplemental Table S1) and Fig. 4). Both ACE and Chao1 estimates of total bacterial community richness were also greater for *A. gerardii* than for *L. capitata*.

Similarly, bacterial diversity was greater for *A. gerardii* than for *L. capitata* (Supplemental Table S1). Within plant pairs, *A. gerardii* displayed higher diversity than *L. capitata* in 9 of 10 (Shannon index) and 10 of 10 (Simpson index) samples.

Our data reveal that host plants had consistent selective effects on rhizosphere community composition. Similarity among communities was evaluated for all possible pairwise community comparisons using the Yue and Clayton index \((\text{Yc})\) (Fig. 5). Similarity indices between bacterial communities associated with *A. gerardii* and *L. capitata* within individual fields were on average significantly different (Location 1: \( P = 0.025 \); Location 2: \( P = 0.002 \)). Moreover, despite their greater \( \alpha \) diversity (host–rhizosphere habitat), bacterial communities associated with different *A. gerardii* plants were more similar to one another than were communities associated with different *L. capitata* plants. Mean \( \text{Yc} \) values among *A. gerardii* and *L. capitata* communities were 0.0099 and 0.0022, respectively. Values ranged from 0.0004 to 0.074 across all possible pair-wise plant comparisons (Fig. 5). The low similarity in composition among communities reflects the high \( \beta \) diversity (among different host plant habitats) of bacterial communities in the rhizosphere of these prairie species and, more specifically, the many taxa unique to each community. Furthermore, removal of singleton sequences from the data set had little effect on \( \text{Yc} \) values; mean \( \text{Yc} \) values among *A. gerardii* and *L. capitata* communities were 0.0089 and 0.002 following removal of singleton sequences. Thus, the high \( \beta \) diversity and correspondingly low
bacterial genera in our data set (e.g., Holophaga, Rhodoplanes, Ktedonobacter, and Nitrospira; Fig. 3) were identified as indicator taxa for the A. gerardii rhizosphere.

Effects of field location on rhizosphere bacterial communities

Composite bacterial richness varied little between the 2 locations (Fields 1 and 2, total OTU richness 16 680 and 16 015 OTUs, respectively). In Field 1, composite OTU richness among all individual plants of each host was 10 967 and 8179 for A. gerardii and L. capitata, respectively (Supplemental Table S1). Conversely, in Field 2, total OTU richness was 8491 and 10 200 for A. gerardii and L. capitata, respectively (Supplemental Table S1). Shannon (P = 0.86; Tukey’s HSD) and Simpson (P = 0.62; Tukey’s HSD) diversity indicators or ACE (P = 0.47; Tukey’s HSD) and Chao (P = 0.60; Tukey’s HSD) richness measures were similar between locations. Additionally, overall composition was similar for host species in the 2 different fields. Mean Yue–Clayton microbial composition similarity indices were consistent for communities associated with A. gerardii in Field 1 versus Field 2 (P = 0.73) and there were no significant differences within a plant species between the 2 locations (A. gerardii: P = 0.25; L. capitata: P = 0.74). Moreover, the removal of singletons had no effect on the Yue–Clayton similarity indices for communities associated with A. gerardii or L. capitata in Field 1 versus Field 2 (P = 0.72), nor between communities within a plant species between the 2 locations (A. gerardii: P = 0.21; L. capitata: P = 0.51).

Finally, indicator taxon analyses showed only minor differences between the 2 fields. One bacterial phylum (Verrucomicrobia) was significantly more abundant in Field 1 than in Field 2 (expressed either as greater mean number of sequences per plant (Field 1: 2111 sequences; Field 2: 1344 sequences) or as relative abundance (Field 1: 20.2%; Field 2: 12.9%); P < 0.05, Tukey’s HSD). Indicator analyses identified 6 bacterial families characteristic of Field 2 and only 1 bacterial family characteristic of Field 1 (Supplemental Table S2). Only 2 of these families (Geobacteraceae and Acetobacteraceae) rank among the 24 most frequently represented families in our data set (Fig. 2). Similar to analyses at the level of bacterial family, indicator analyses also identified 11 bacterial genera that are characteristically associated with Field 2 and only 1 genus associated with Field 1. Of these, only 1, Dehalococcoides, is among the 26 most frequently represented genera in our data set (Fig. 3).

Discussion

This work reveals the presence of extraordinarily diverse bacterial communities in the rhizosphere of both the long-lived prairie grass A. gerardii and the native legume L. capitata. In addition to high a diversity (bacterial diversity within individual plant rhizosphere habitat), this work documents very high b diversity (differential variation among habitats), or taxa unique to each rhizosphere, host plant, or field. The high observed b diversity likely reflects both the enormous microbial diversity available for colonization of any single rhizosphere as well as the complex variation in the suitability of different host plants or locations to microbial colonization. Dickie 2010 assessed the effects of DNA sequence species detection errors on perceived species richness based around 2 assumptions relevant to pyrosequencing surveys of communities: DNA sequence-based species identification has a nonzero error rate; and erroneous species are likely to be singletons (Dickie 2010). Rarefaction analysis (sampling random subsets of the community) can be a robust method for comparing diversity across studies. Moreover erroneous singletons represent a very small proportion of total individuals and are essentially eliminated by subsampling the community at low or moderate effort levels in rarefaction unlike-species richness extrapolation (Dickie 2010). While it is likely that more extensive sampling and denoising (Dickie 2010) will reduce estimates of b diversity by increasing the proportion of taxa present in association with multiple rhizospheres, hosts, or locations, these data are consistent with other recent work highlighting the extraordinary diversity of plant-associated soil microbial communities (Lauber et al. 2009; Manter et al. 2010; Mendes et al. 2011; Rosenzweig et al. 2012; Sugiyama et al. 2010; Will et al. 2010). In sampling with nonzero species detection error rates, increased sampling effort may result in erroneous data dominating perceived species richness (Dickie 2010). The availability of sequencing ribosomal 16S fragments using the coded-primer (tag) approach to multiplex pyrosequencing has enhanced our approach to comprehensively characterize microbial communities in natural habitats. These approaches provide a highly efficient means of characterizing replicate microbial communities with much greater sampling depth than culturing methods while providing taxonomic information that is often unavailable using other approaches. Our data showed that the relative abundance of 3 bacterial families and 8 genera differed significantly between the 2 host-plant species. Commonly hypothesized mechanisms for plant effects on soil communities include alteration of the physical properties of the soil environment (Duda et al. 2003; Enloe et al. 2004), variation in root exudate quality or quantity (Dennis et al. 2010), input of litter with unique chemical properties (Ashton et al. 2005; Evans et al. 2004), and the release of secondary compounds (Boon and Johnstone 1997; Callaway and Aschehoug 2000). Considering A. gerardii and L. capitata, variation in total nutrient inputs, plant buffering from physical stress in the rhizosphere environment, and the specific profile of nutrient inputs into the rhizosphere seem the likely contributors to the observed differences in bacterial community diversity and composition. Andropogon gerardii and L. capitata have distinctly different root systems, which is likely to influence total nutrient availability. Andropogon gerardii is noted for its investment in an extensive thick and fibrous root system, with estimates of >80% of its biomass belowground (Craine et al. 2003). Bunchgrasses accumulate substantial food reserves in their root tissue from summer into fall, which serve as a resource for early-season growth the following spring. In contrast, L. capitata produces a primary central taproot, and reported root biomass constitutes a much smaller fraction of total plant biomass (Baer

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Table 3. Summary of indicator analysis; number of significant indicators at each taxonomic level by plant host, location, and plant host within locations.

<table>
<thead>
<tr>
<th>Taxonomic level</th>
<th>Host plant</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial family</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Bacterial genus</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>Bacterial species</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

*Table values reported for taxa found in ≥10 sequences recovered, ≥25 samples of a particular plant species; comparisons were statistically different at α = 0.05.
Roots and root exudates are the primary source of nutrients for rhizosphere microbes. The presence of a substantial and sustained nutrient-rich belowground biomass in the perennial bunchgrass *A. gerardii* may be an important contributor to the higher bacterial diversity in the rhizosphere of *A. gerardii* than *L. capitata*.

Differences in *A. gerardii* and *L. capitata* physical structure may also significantly alter the suitability of the rhizosphere for microbial colonization. As a bunchgrass, the *A. gerardii* aboveground tissue serves as a “funnel” to channel water down to the root system, while the clustered aboveground leaves shield the soil from direct sun and provide some protection from solar soil heating and evaporation (McLean and Marchand 1968). In this way, *A. gerardii* plant structure may significantly buffer the rhizosphere from physical stress. In contrast, while mature *L. capitata* generally produce multiple stems, the stems are not tightly clustered in a way that will funnel water to the roots or shade the root system. By buffering the rhizosphere from physical stress, particularly in dry, upland prairie settings, plant physical structure may be crucial to creating environments optimal for supporting both high rhizosphere bacterial community diversity and diversity. More specifically, differences in both the aboveground and belowground physical structure of *A. gerardii* and *L. capitata* may contribute to producing distinct environments for bacterial colonization and thus to the development of communities that differ significantly in bacterial community richness and diversity. Finally, in the case of other *Andropogon* and *Lespedeza* spp. it has been suggested that root exudates or litter may alter the soil chemistry, organic matter, and biology through secondary metabolites (Pradhan and Dash 1984; Yannarell et al. 2011).

In addition to differences between host plants, rhizosphere communities associated with each host differed between the 2 field locations. When considering host plant, *A. gerardii* enriched bacterial taxa at all taxonomic levels based on indicator value analyses. Moreover indicator value analyses revealed an increase in bacterial taxa in Field 2 at the genus taxonomic level. Both fields have been abandoned from pasture management for over 50 years, and are burned regularly, with the most recent burn in 2013. By buffering the rhizosphere from physical stress, particularly in dry, upland prairie settings, plant physical structure may be crucial to creating environments optimal for supporting both high rhizosphere bacterial community diversity and diversity. More specifically, differences in both the aboveground and belowground physical structure of *A. gerardii* and *L. capitata* may contribute to producing distinct environments for bacterial colonization and thus to the development of communities that differ significantly in bacterial community richness and diversity. Finally, in the case of other *Andropogon* and *Lespedeza* spp. it has been suggested that root exudates or litter may alter the soil chemistry, organic matter, and biology through secondary metabolites (Pradhan and Dash 1984; Yannarell et al. 2011).

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A multitude of bacterial taxa, including many that are unculturable to date, were detected in prairie soil from CCESR. Commonly cultured and well-known bacterial families, including *Streptomyces*, *Bacillus*, *Pseudomonas*, *Verrucomicrobiaceae*, *Bradyrhizobiaceae*, and *Acidobacteriaceae*, were present on every plant, reflecting their ubiquity and adaptation to the soil and rhizosphere environments. Though sequences representing these families, as well as the genera *Bacillus*, *Pseudomonas*, *Streptomyces*, *Bradyrhizobium*, and *Rhizobium*, were ubiquitous among samples, the sequence-based approach used here provided only partial 16S reads of approximately 196 bp and, therefore, cannot confidently resolve potential fine-scale phylogenetic variation between bacterial populations associated with different host plants or fields. Moreover, in the absence of cultural representatives of these taxa, possible phenotypic or functional variation between populations of the same taxon associated with different plant hosts or fields cannot be ruled out.

Among the commonly observed taxa, *Acidobacteriaceae* and *Hyphomicrobiaceae* were more abundant in the rhizosphere of *A. gerardii*, and *Rhodocyelaceae* was more abundant in the rhizosphere of *L. capitata*. At a more fine-scale phylogenetic resolution, *A. gerardii* and *L. capitata* also varied in the relative abundance of common bacterial genera. Specifically, *A. gerardii* had more *Holophaga*, *Rhodoplanes*, *Klebsiobacter*, *Nitrospira*, and *Oscillatoriia* (photosynthetic), while *L. capitata* had more *Acidobacterium*, *Pseudomonas*, and *Thiorhodovibrio* (purple sulfur bacterium, phototropic). These differences raise 2 key questions: Why are these microbes enriched? and What are the consequences of their enrichment to plant fitness or ecosystem functioning? Unfortunately, precise answers to these questions are constrained by the extraordinary phenotypic or functional diversity contained within most bacterial families and genera and by the lack of cultural representatives of the organisms associated with individual taxa when using metagenomic approaches. However, these findings raise questions worthy of further study. For example, *Rhodocyelaceae* includes many bacteria formerly classified as *Pseudomonadaceae*, including many aerobic denitrifying bacteria that prefer oligotrophic environments (Jie and Daping 2008). Their enrichment in the rhizosphere of *L. capitata* may reflect the availability of nitrogenous compounds to the *L. capitata* root system as a consequence of the *L. capitata—Bradyrhizobium* symbiosis. The presence of high densities of *Rhodocyelaceae* can reduce soil fertility (Song et al. 2000) and may specifically reduce the availability of nitrogen to plant species coexisting in association with *L. capitata*. In this way, high densities of *Rhodocyelaceae* have the potential to influence both the nitrogen cycling in soil and the potential impacts of competitors on *L. capitata* fitness. While further studies are needed to explore this and other hypotheses regarding the specific impacts of microbial community shifts on plant fitness and ecosystem functioning, in aggregate, the compositional differences in bacterial communities associated with different host plants shed light on possible novel microbial mechanisms by which host plants influence their own fitness, the fitness of competitors, and a broad array of distinct ecosystem functions.

This study reveals that *A. gerardii* and *L. capitata* plants in prairie fields support extraordinarily rich rhizosphere bacterial communities. Moreover we found a clear plant selective effect on the selection of rhizosphere-associated microbes. A group of “core” microbial taxa, including representatives of the families *Streptomyces*, *Bacillaceae*, *Pseudomonadaceae*, *Verrucomicrobiaceae*, *Bradyrhizobiaceae*, and *Acidobacteriaceae*, were present in the rhizosphere of every plant sampled, reflecting their ubiquity and adaptation to the rhizosphere environment. Although measures of diversity (e.g., α, β, and γ diversity) were high across samples, more extensive sampling combined with culture and functional analyses is necessary to determine (i) plant–microbe feedbacks, (ii) the influences of microbes on host fitness and community functioning, and (iii) the implications for plant fitness and ecosystem functions.

Understanding the specific factors that structure this diversity (Ettema and Wardle 2002; Torsvik and Øvreås 2002). Moreover, characterizing rhizosphere microbial composition and diversity is an important step to understanding the potential impacts of these communities on plant fitness and maintaining plant community diversity in native ecosystems.

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