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ARTICLE

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 sulfurs, 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 propres à des individus appartenant à l'espèce *Andropogon gerardii* Vitman (barbon de Gérard) et à l'espèce *Lespedeza capitata* (trèfle arbustif). Les communautés microbiennes retrouvées au sein de la rhizosphère de 10 plants de chaque espèce (n = 20 plants au total) ont fait l'objet d'une analyse composée d'une série de méthodes sans culture. Les communautés microbiennes 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 microbiens sans lien fonctionnel reconnu avec leur espèce hôte « privilégiée », à savoir des anaérobies méthylant 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 microbiens sur les plantes hôtes et sur les fonctions écologiques des écosystèmes. [Traduit par la Rédaction]

Mots-clés : Andropogon gerardii, *Lespedeza capitata*, pyroséquençage, communautés microbiennes, région V3 du gène de l'ADNr 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.

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2003; Hamilton et al. 2008; Phillips et al. 2003; Rudrappa et al. 2008). While there is clear evidence for both positive (Carvalho et al. 2010; de la Pena et al. 2010; Kueffer 2010; Shah et al. 2009; Vogelsang and Bever 2009; Zhang et al. 2010) and negative (Abawi and Widmer 2000; Ewel 2006; Lucas 1998; Lupwayi et al. 1998; Pereira et al. 2007; Perez et al. 2008) feedback between plants and soil microbes, most studies to date have taken a limited or fragmentary approach. In particular, these studies have focused on those taxa most likely to provoke notable or detectable positive or negative feedback responses (pathogens, mutualists). However, these approaches are inherently limited in their capacity to provide insight into the diverse array of interactions between plants and soil microbes. Even small volumes of soil are colonized by billions of microorganisms, most of which we know little or nothing about. Beyond the relatively few well-studied pathogenic and mutualistic taxa, there is limited information on the potential roles of less well-known microbial taxa in both responding to plant influences and in turn impacting plant fitness. In particular, the potential influences of host plants on noncultured microbes, which are believed to represent the vast majority of the soil microflora, have received little attention. However, recent advances in sequencing technology offer the opportunity to explore the composition, structure, and diversity of soil microbes associated with distinct host plants in substantially more detail. Compared with culture-based methods, sequence-based analyses provide a means for exploring plant-associated microbial communities that is more representative of their phylogenetic complexity, providing the potential for identifying novel plant-associated taxa while broadening our understanding of the diversity of plant–microbe associations.

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 microflora 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.

Materials and methods

Sample collection and DNA extraction

Soil samples were collected from the rhizosphere of *A. gerardii* and *L. capitata* plants in 2 fields (Field 1: 45°23′23.23″N, 93°09′48.49″W, elevation 280 m above sea level; Field 2: 45°23′16.24″N, 93°10′57.05″W, elevation 282 m above sea level), approximately 1000 m apart, at CCESR. These fields were restored prairie fragments with naturally established plants. Field 1 was abandoned from crop production in 1950. Prescribed burns occurred in 2000, 2002, 2005, and 2007 prior to soil collection. Field 2 was abandoned from crop production in 1955. Within each field, 5 plant pairs were identified, with the *L. capitata* and *A. gerardii* individuals in the pair both located within a 1 m^2 area. Plants of similar size were sampled. Individual plant pairs were at least 3 m apart. Two plants within a 1 m^2 area were sampled in an effort to collect soil samples with similar chemical or other soil characteristics and thus to focus on the biological differences in the microbes that develop owing to the 2 different root systems. Soil cores (including plant root material) were collected with a 25 mm soil corer (Oakfield Apparatus Co., Oakfield, Wisconsin) to a depth of 100 mm. Soil cores were placed individually into plastic bags, transported to the laboratory on ice, and stored at 4 °C until further analysis. Soil adhering to plant roots was removed, and total genomic DNA was extracted using the Soilmaster DNA Extraction kit according to the manufacturer's (Epicentre Biotechnologies Inc., Madison, Wisconsin) instructions. DNA samples were stored at –20 °C.

PCR amplification and amplicon sequencing

PCR amplification targeted the variable V3 region on the rDNA 16S gene. The V3 region was selected for this analysis because it is phylogenetically informative (variable across taxa) and the amplicon size (196 bp in *Escherichia coli*) is well suited for pyrosequencing. The previously described primer pair PRBA338F (bacterial V3 region corresponding to base pair positions 338–358 in the *Escherichia coli* reference genome (GenBank acc. No. CP000946)) (Lane et al. 1988) and PRUN518R (Universal V3 region corresponding to base pair positions 534–518 in the *E. coli* reference genome) (Muyzer et al. 1993) was modified by appending 454 Life Sciences Fusion Primer sequences (to facilitate pyrosequencing) and a 4 bp barcode unique to each DNA template (PRBA338F-454: GCCTCCCTCGCGCCATCAG*NNNN*ACTCCTACGGGAGGCAGCAG; and PRUN518R-454: GCCTTGCCAGCCCGCTCAG*NNNN*ATTACCGCGGCTGCTGG, where *NNNN* indicates the variable barcode sequences). Forward and reverse primers were identically barcoded for each PCR. Primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, Iowa). PCRs consisted of 3.0 μL (~30 ng) of template genomic DNA, 5 μL of 10× FastStart High Fidelity Reaction Buffer with 18 mmol/L $MgCl_2$ (Roche, Indianapolis, Indiana), 1 μL of 10 mmol/L dNTPs (Promega Corporation, Madison, Wisconsin), 1 μL of 25 μmol/L (each) forward and reverse primer, 1 μL of 5 U/μL FastStart High Fidelity Enzyme Blend (Roche), and 2% bovine serum albumin (New England Biolabs, Ipswich, Massachusetts), in a 50 μL reaction volume. Amplifications were performed on an Applied Biosystems GeneAmp PCR system 2700 thermocycler (Applied Biosystems, Pleasanton, California) using the following parameters: 9 min at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 55 °C, 30 s at 72°, and finally 7 min at 72 °C.

Triplicate PCRs from each DNA sample were desalted using Illustra MicroSpin S-300 HR Columns (GE Healthcare, Amersham, Bucks, UK), quantified using the PicoGreen dsDNA Assay kit (Invitrogen) and the Qubit fluorometer (Invitrogen), combined in equimolar concentrations, and diluted to 2×10^5 mol/μL in DNA nuclease-free water. For pyrosequencing, PCR pools from 20 independent samples, each associated with a different barcode, were combined in equimolar amounts and sent to the University of Minnesota Biomedical Genomics Center for emulsion

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.

No. of plants sampled	Total no. of sequences	Mean no. of sequences per plant (range)	Total no. of bacterial phyla classified[a]	Total no. of bacterial families classified[a]	Total no. of bacterial genera classified[a]	Total no. of bacterial species classified[a]
10 (*Andropogon gerardii*)	122 422	12 242 (9 358 – 16 403)	28	227	905	1683
10 (*Lespedeza capitata*)	128 892	12 889 (2 466 – 35 299)	28	226	870	1474
20 (Total)	251 314	12 566 (2 466 – 35 299)	30	236	1006	2206

[a]Taxonomic classification level determined at 97% sequence identity.

PCR, GS amplicon library preparation, and pyrosequencing on a 454 Life Sciences GS-FLX machine (Roche).

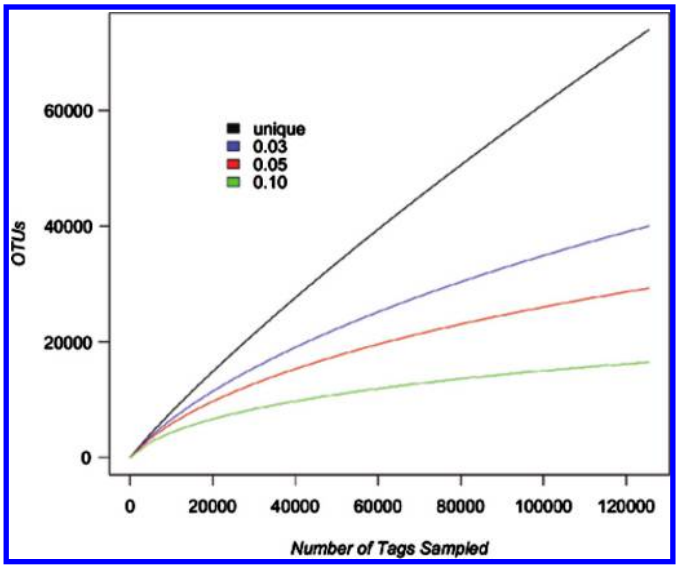
OTU-based analyses

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 data set. 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 for unique, 0.03, 0.05, and 0.10 OTU definitions were calculated. Subsequently, the Yue and Clayton (θyc) 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 pair-wise 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/).

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%.



Indicator value analyses

Indicator analysis is a composite measure that considers both prevalence and relative abundance (Dufrêne and Legendre 1997). Indicator value analysis is a method to find indicator taxa and assemblages characterizing groups of sites; it combines a taxa's relative abundance with its relative frequency of occurrence in the various groups of sites (Dufrêne and Legendre 1997). Indicator value analysis was performed on taxonomy query results. In these analyses, thresholds of 10 occurrences of a given bacterial taxon per DNA sample (i.e., a requirement that each bacterial taxon must be represented by 10 sequence reads in a given DNA sample) and of occurrences of a bacterial taxon in 50% of samples from a given plant species or field location were applied.

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[1]). Rare OTUs, represented as singleton sequences in

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjm-2012-0661.

Fig. 2. Relative sequence abundance of most frequently recovered soil bacterial families from the rhizosphere with soil samples divided into categories of plant species by location. Error bars represent standard error of the mean. **, indicates a significant difference between means for both location comparisons; ^*, indicates first location not significant and second location significantly different between means. Ag1, *Andropogon gerardii* Field 1; Lc1, *Lespedeza capitata* Field 1; Ag2, *A. gerardii* Field 2; and Lc2, *L. capitata* Field 2. Comparisons were statistically different at $\alpha = 0.05$.

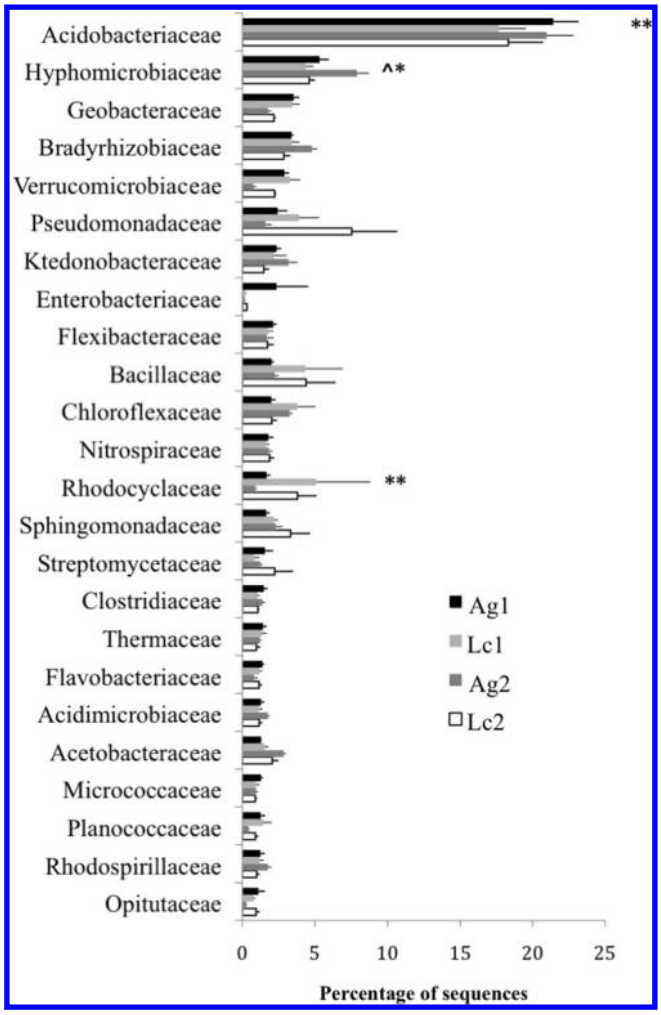
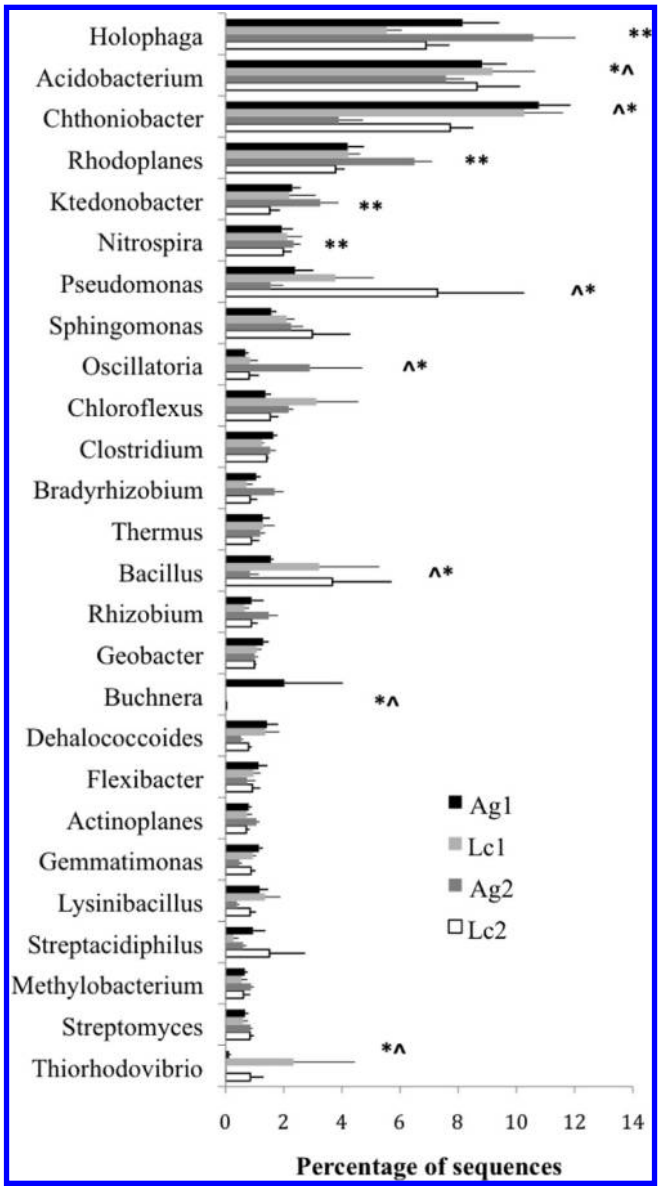


Fig. 3. Relative sequence abundance of most frequently recovered soil bacterial genera from the rhizosphere with soil samples divided into categories of plant species by location. Error bars represent standard error of the mean. **, indicates a significant difference between means for both comparisons; ^*, indicates comparison at Field 1 not significant and Field 2 significantly different between means; *^, indicates first comparison significantly different between means and second comparison not significant. Ag1, *Andropogon gerardii* Field 1; Lc1, *Lespedeza capitata* Field 1; Ag2, *A. gerardii* Field 2; and Lc2, *L. capitata* Field 2. Comparisons were statistically different at $\alpha = 0.05$.



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[1]). 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%).

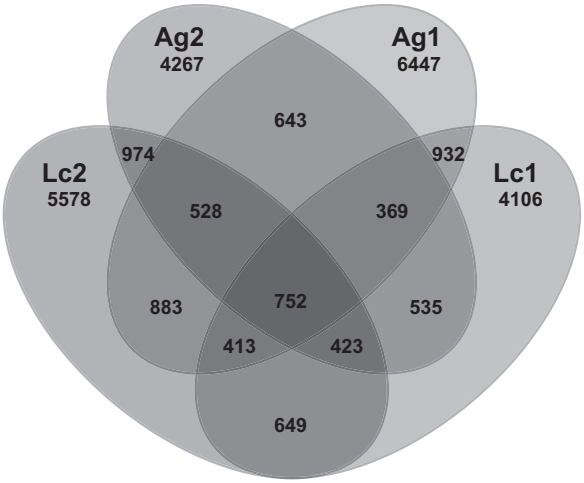
More than 99% of sequences that could be assigned to bacterial phyla were present in the rhizosphere of both *A. gerardii* and *L. capitata*, and there were no significant differences in the relative abundance (mean number of sequences per plant) of individual bacterial phyla between host plants. However,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

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

	Field 1[a]		Field 2[a]		Total[b]	
Taxonomic level	D statistic	*P*	D statistic	*P*	D statistic	*P*
Bacterial genus	0.1081	$<10^{-4}$	0.0211	ns	0.0505	ns
Bacterial species	0.2180	$<10^{-15}$	0.0347	ns	0.0947	$<10^{-3}$

Note: ns, not significant at $\alpha = 0.05$.
[a]Sample size for plant species: $n = 10$.
[b]Sample 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.

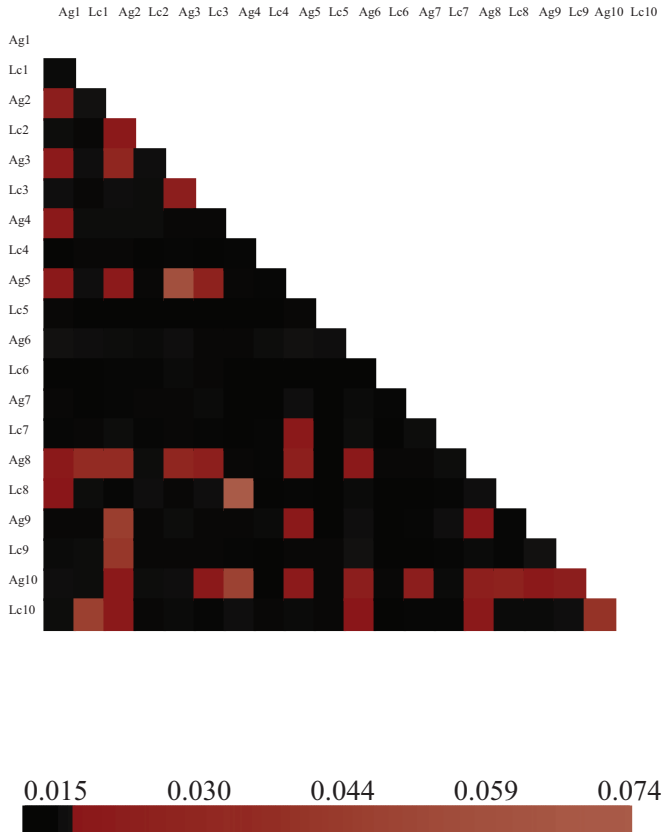


significantly in relative abundance between the 2 host plants ($P < 0.05$: Tukey's HSD). *Andropogon gerardii* had a greater relative abundance in mean number of sequence reads per plant of *Acidobacterieaceae* (*A. gerardii*: 21.2%; *L. capitata*: 18.0%) and *Hyphomicrobiaceae* (*A. gerardii*: 6.6%; *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%), *Pseudomonas* (*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[1] 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[1]). Within plant pairs, *A. gerardii* displayed higher diversity than *L. capitata* in 9 of 10 (Shannon index) and 10 of 10 (Simpson index) samples.

Fig. 5. Heatmap comparing community structure between individual plant samples based on pairwise similarity at 10% distance. Similarity among communities was evaluated for all possible pairwise community comparisons using the Yue and Clayton index (θyc). Yue and Clayton measure of similarity between the structures of 2 communities. Red intensity indicates increase in similarity between community assemblage and structure. The composition among communities represented by black intensity reflects the high similarity (among different host plant habitats) of bacterial communities. Ag1–Ag10, *Andropogon gerardii* plants 1–10; Lc1–Lc10, *Lespedeza capitata* plants 1–10. For coloured version of figure, see Web site at http://www.nrcresearchpress.com/doi/abs/10.1139/cjm-2012-0661.



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 (θyc) (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 α 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 θyc values among *A. gerardii* and *L. capitata* communities were 0.0099 and 0.0022, respectively. Values ranged from 0.00004 to 0.074 across all possible pair-wise plant comparisons (Fig. 5). The low similarity in composition among communities reflects the high β 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 θyc values; mean θyc values among *A. gerardii* and *L. capitata* communities were 0.0089 and 0.002 following removal of singleton sequences. Thus, the high β diversity and correspondingly low

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

	Total number of significant indicators[a]	
Taxonomic level	Host plant	Field
Bacterial family	16	7
Bacterial genus	27	12
Bacterial species	7	2

[a]Indicator values reported for taxa found in ≥10 sequences recovered, ≥5 samples of a particular plant species; comparisons were statistically different at $\alpha = 0.05$.

similarities in composition among communities from different plants was not simply a function of very rare taxa or sequencing errors.

Indicator analyses were performed to characterize specific differences in prevalence and relative abundance of major taxa between host plants. Using this approach, 16 bacterial families distinguished the 2 plant species (Table 3 and Supplemental Table S2[1]). Included is the family *Hyphomicrobiaceae*, the second most frequent bacterial family in our data set. Indicator analyses also identified 27 bacterial genera that distinguished the 2 plant species (Table 3 and Supplemental Table S2[1]). Interestingly, each of these bacterial genera is characteristic of the *A. gerardii* rhizosphere; indicator analysis revealed no bacterial genus characteristic of the *L. capitata* rhizosphere. Several of the best-represented 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[1]). Conversely, in Field 2, total OTU richness was 8491 and 10 200 for *A. gerardii* and *L. capitata*, respectively (Supplemental Table S1[1]). 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*or *L. capitata* 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[1]). 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 α diversity (bacterial diversity within individual plant rhizosphere habitat), this work documents very high β diversity (differentiation among habitats), or taxa unique to each rhizosphere, host plant, or field. The high observed β 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 β 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. 2001), 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

et al. 2003). 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 one 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 density 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 the spring immediately prior to the fall sampling. Differences between the 2 fields in bacterial community composition are likely to originate from a complex combination of differences in soil edaphic characteristics and prospective microbial immigrant or colonist populations as well as differences in the physical environment between fields. However, despite possible differences in soil environmental characteristics or microbial immigrant pools between the 2 fields, the consistency of differences in bacterial community composition based on similarity indices and diversity between *A. gerardii* and *L. capitata* in the 2 different fields confirms the significant selective role of the host plant in determining bacterial community composition, structure, and diversity in the rhizosphere.

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 *Streptomycetaceae*, *Bacillaceae*, *Pseudomonadaceae*, *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 *Rhodocyclaceae* 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*, *Ktedonobacter*, *Nitrospira*, and *Oscillatoria* (photosynthetic), while *L. capitata* had more *Acidobacterium*, *Pseudomonas*, and *Thiorhodovibrio* (purple sulfur bacterium, phototrophic). 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 both 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, *Rhodocyclaceae* 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 *Rhodocyclaceae* 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 *Rhodocyclaceae* 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 *Streptomycetaceae*, *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 spatial patterns and the distribution of microbial diversity may provide insight into 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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