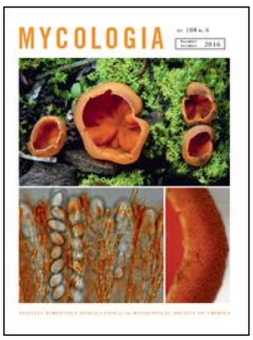

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Plant diversity and plant identity influence *Fusarium* communities in soil

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ABSTRACT

Fusarium communities play important functional roles in soil and in plants as pathogens, endophytes, and saprotrophs. This study tests how rhizosphere *Fusarium* communities may vary with plant species, changes in the diversity of the surrounding plant community, and soil physiochemical characteristics. *Fusarium* communities in soil associated with the roots of two perennial prairie plant species maintained as monocultures or growing within polyculture plant communities were characterized using targeted metagenomics. Amplicon libraries targeting the *RPB2* locus were generated from rhizosphere soil DNAs and sequenced using pyrosequencing. Sequences were clustered into operational taxonomic units (OTUs) and assigned a taxonomy using the Evolutionary Placement Algorithm. *Fusarium* community composition was differentiated between monoculture and polyculture plant communities, and by plant species in monoculture, but not in polyculture. Taxonomic classification of the *Fusarium* OTUs showed a predominance of *F. tricinctum* and *F. oxysporum* as well of the presence of a clade previously only found in the Southern Hemisphere. Total *Fusarium* richness was not affected by changes in plant community richness or correlated with soil physiochemical characteristics. However, OTU richness within two predominant phylogenetic lineages within the genus was positively or negatively correlated with soil physiochemical characteristics among samples within each lineage. This work shows that plant species, plant community richness, and soil physiochemical characteristics may all influence the composition and richness of *Fusarium* communities in soil.

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diversity; ecology; fungi; prairie; *RPB2*

INTRODUCTION

Fungi in the genus *Fusarium* show high species and genotypic diversity in soil (Balmas et al. 2010; Gordon et al. 1992) where they play many important functional roles. Plant pathogenic fungi in this genus have been suggested to be significant to both the maintenance (Liu et al. 2012) and decline of plant diversity (Mangla and Callaway 2008) in native ecosystems. Through competition for limiting resources in soil, nonpathogenic individuals have been suggested to suppress plant disease caused by plant pathogenic individuals (Alabouvette et al. 2009). The ability of these fungi to breakdown and use lignocellulose also suggests that changes in *Fusarium* community structure may influence decomposition and nutrient cycling in soil (King et al. 2011; Murase et al. 2012). However, despite the diversity and functional importance of these fungi, the factors that influence the structure and richness of *Fusarium* communities in soil are largely unknown.

Although plants are hypothesized to play significant roles in determining soil microbial community characteristics, changes in the richness or composition of microbial communities in soil may in turn influence the growth of individual plants and plant communities (Bever et al. 2012). For example, the displacement of native plant diversity by invasive plant species can be mediated by soil microbes. This displacement can occur with the accumulation of microorganisms in the rhizosphere of the invasive plant that infect or suppress the growth of the native plants (Mangla and Callaway 2008). Changes in microbial diversity or community composition in soil may also influence decomposition and soil nutrient dynamics, as microorganisms such as fungi display considerable variation in the potential for lignocellulose breakdown (Zhao et al. 2013). Hence, understanding the effects of plant diversity and identity on the diversity and structure of soil microbial communities may help to predict plant community and soil nutrient dynamics.

Although soil has served as a reservoir of diversity within the genus *Fusarium* (e.g., Backhouse et al. 2001; Balmas et al. 2010; Laurence et al. 2011), few studies

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have described or characterized correlates of the richness and composition of *Fusarium* communities in soil. For example, Yergeau et al. (2010) characterized *Fusarium* communities in soil 1 m from asparagus plants with or without symptoms of Fusarium crown rot. Using denaturing gradient gel electrophoresis targeting the genus *Fusarium*, no correlation was found between *Fusarium* community composition and plant disease. Other culture-based surveys using morphological species definitions found that the isolation frequency of particular *Fusarium* species correlated with soil physiochemical characteristics such as the concentration of organic matter or potassium (Windels and Kommedahl 1974; Bissett and Parkinson 1979). These suggested niche preferences among *Fusarium* spp. provides some evidence that changes in soil physiochemical characteristics can influence the abundance of certain species of *Fusarium* in soil, but they provide little insight into predictors of *Fusarium* community richness and composition.

Sequencing amplicon libraries generated from environmental DNAs can be an efficient method to characterize fungal communities in complex environments. Although these methods usually characterize diversity within a Kingdom by targeting multiple-copy rRNA loci (e.g., internal transcribed spacers or 28S rRNA), they can also be applied to study finer taxonomic hierarchies (e.g., Davey et al. 2013). Previously, it was shown that it is possible to amplify and sequence a portion of the single-copy *RPB2* locus (second largest subunit of the RNA polymerase) from soil DNAs (LeBlanc et al. 2015). Through primer specificity, polymerase chain reaction (PCR)-based exclusion of a large portion of the fungal kingdom was possible, allowing enrichment for sequences within the Sordariomycetes and consequently the genus *Fusarium*. The *RPB2* locus has been used to study evolution within *Fusarium* (O'Donnell et al. 2013) and for genotyping clinical isolates (O'Donnell et al. 2007), making it a good candidate to characterize communities of these fungi in soil.

The purpose of this research was to address two specific questions concerning the ecology of *Fusarium*. First, we hypothesized that *Fusarium* community richness would be greater in rhizosphere soil associated with plant species growing in richer (i.e., polyculture) plant communities than in the rhizosphere of the same plant species growing in monoculture. Although genetically different plants are known to maintain different microbial communities in the rhizosphere (Badri et al. 2009), changes in the number of different types of plant species (i.e., changes in richness) around a focal plant species may still influence the richness of microbial communities in the rhizosphere of an individual plant. For example, greater plant species richness may provide a greater number of carbon substrates in the rhizosphere, enabling a greater number of microorganisms to coexist. This is supported by the positive correlation between plant and fungal richness in soil (Peay et al. 2013; LeBlanc et al. 2015; Lange et al. 2015; but also see Bakker et al. 2013; Schlatter et al. 2015). Based on a similar rationale, our second hypothesis was that *Fusarium* community composition would differ in the rhizosphere of different plant species, but that these plant species effects would be altered with increased number of plant species (i.e., greater richness) surrounding a given plant species. To address these hypotheses, *Fusarium* communities were characterized in soil associated with the roots of the native perennial legume *Lespedeza capitata* and the native perennial grass *Andropogon gerardii*. By characterizing *Fusarium* communities associated with these two plant species maintained as monocultures or growing within richer (i.e., polyculture) experimental grassland communities, this work provides insight into the ecology of a diverse group of fungi found in soil.

MATERIALS AND METHODS

Field sampling and soil processing.—Samples were collected from the Cedar Creek Ecosystem Science Reserve, a National Science Foundation (NSF) Long-Term Ecological Research (LTER) site (https://www.cedarcreek.umn.edu/) in Minnesota, USA, in late July 2012. The samples were composed of soil associated with the roots of two different perennial plant species grown within two different plant richness treatments. The plant species were the C4 grass *Andropogon gerardii* (big bluestem, turkey's foot) and the legume *Lespedeza capitata* (round-headed bush clover). The plant diversity treatments represent contrasting levels of plant community richness (i.e., the number of different plant species within the community). The levels of plant community richness were monoculture of each plant species and polyculture plant communities in which each of the plant species grew in a community of 16 grassland plant species. These different plant community treatments have been maintained since 1994 (Tilman et al. 1997) as part of the Big Bio field experiment at Cedar Creek. The dominant soil type within the field experiment is a Nymore loamy sand. Soil from 12 individual plants was sampled, 6 samples from each plant species composed of 3 from monoculture and 3 from polyculture plant communities for each plant species. Individual sampled plants were randomly located within experimental plots.

To sample the rhizosphere, two 2.5 × 10 cm soil cores, separated by a 90° angle around the base of the plant, were taken from the base of each individual plant sampled. The two soil cores from each individual plant were pooled in the field and kept on ice until storage at −20 C. Each soil sample was homogenized by hand and passed through a 2-mm sieve to remove large plant debris. Extractable phosphorus, extractable potassium, extractable nitrate, pH, and percent organic matter of the soil were measured at the University of Minnesota Research Analytical Laboratory (ral.cfans.umn.edu).

Soil DNA extractions were performed on every soil sample, and processing order was randomized among samples. Extractions were optimized for the final adjustments to the PowerSoil (MoBio, Carlsbad, California, United States) protocol: for each individual soil sample 0.5 g of soil was used, the soil was disrupted using two 1-min pulses in a Mini-BeadBeater 8 (BioSpec, Bartlesville, Oklahoma, United States) on the highest setting, and 50 µL Tris was used for the final spin step. Extracted DNAs were diluted to 5 ng DNA/µL and stored at −20 C.

Amplicon library preparation.—Amplicon libraries were created from extracted DNAs using nested PCR. All reactions were performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany). The initial PCR was used to create an amplicon library from the soil DNAs using the RPB2-5F2 (5'-GGGGWGAYCAGAAGA AGGC-3') and RPB2-7cR (5'-CCCATRGCTTGYT TRCCCAT-3') primers (O'Donnell et al. 2007). The second PCR was used to tag the amplicons with 454 GS FLX+ (Roche Diagnostics, Basel, Switzerland) specific adaptors and sample-specific barcodes as well as to reduce the size of the amplicons from 1.2 to 0.8 kb for compatibility with 454 sequencing technology. For the second round of PCR, the RPB2-5F2 primer was paired with the primer RPB2-RP1 (5'-GGNGTCATGCARATCATNGC-3') (LeBlanc et al. 2015), which is nested within the 1.2-kb amplicon from the initial PCR.

The first round of PCR used 20-µL reactions with 2 µL Takara Ex-Taq buffer (Takara Bio, Shiga, Japan), 1.6 µL dNTPs (2.5 µM of each), 2 µL of each forward and reverse primers (10 µM), 11.3 µL water, 0.1 µL Takara Ex-Taq polymerase (5 units/µL), and 1 µL template (5 ng DNA/µL). The reactions were performed with an initial denaturing step at 95 C (5 min) followed by 24 cycles of 95 C (1 min), 60 C (1 min), and 72 C (1 min), and ended with an extension of 72 C (10 min). Three reactions were performed per sample and gel extracted at 1.2 kb using a Promega Wizard kit (Promega, Madison, Wisconsin, United States). The second round of PCR was performed using the same reaction ingredients except 0.4 µL of forward and reverse primers, 7.5 µL water, and 8 µL template (1 ng DNA/µL). The reactions were performed similarly, but with an annealing temperature of 65 C. Three reactions per sample were pooled and gel extracted using a Promega Wizard kit (Promega). The amplicon DNAs were quantified using a Hoefer fluorometer (Hoefer, Holliston, Massachusetts, United States) and pooled in equal sample concentrations. The amplicon libraries were sequenced using the Roche 454 GS FLX+ platform through the Roy J. Carver Biotechnology Center at the University of Illinois (http://www.biotech.uiuc.edu/). Demultiplexed raw sequence data are archived in the National Center for Biotechnology Information (NCBI) Sequence Read Archive accession SRX894060.

Sequence processing.—Sequences were processed in MOTHUR v.1.27.0 (Schloss et al. 2009). The MOTHUR implementation of the PyroNoise algorithm (Quince et al. 2009) was used to screen for and correct sequencing errors common to the pyrosequencing technology, such as insertion/deletions in homopolymers. Following use of the PyroNoise algorithm, sequences were clustered into operational taxonomic units (OTUs) following the recommendations highlighted in Schloss et al. (2011). Sequences were screened for the presence of sample barcodes, sequencing primer, and a minimum length of 400 nucleotides; sequences not fulfilling these criteria were culled. Following barcode and primer removal, the sequences were truncated to the first 400 (5') nucleotides. From this complete sequence set a random subset (n = 1000) was aligned using the program MUSCLE (Edgar 2004). The random subset of aligned sequences was then used as a template alignment to align the complete sequence set in MOTHUR. To further reduce the effect of sequencing and PCR error, sequences were preclustered and screened for chimeras using the UCHIME algorithm in MOTHUR (Edgar et al. 2011). Following removal of chimeras, sequences were clustered into OTUs at 0.97 identity using the average neighbor algorithm. To account for differences in sequencing depth per sample, the final OTU table was subsampled based on the sample with the fewest sequences (n = 1082). Operational taxonomic units represented by a single sequence (singletons) prior to subsampling were omitted from downstream analysis.

Classification of* Fusarium *OTUs.—A randomly selected representative sequence from each OTU was used for phylogenetic classification. First, the sequences

were aligned to a custom database of reference *RPB2* sequences (LeBlanc et al. 2015) using the default parameters of the BLASTN algorithm (Altschul et al. 1997). Sequences that showed a minimum of 0.90 identity with reference sequences from *Fusarium sensu* O'Donnell et al. (2013) were further classified using the Evolutionary Placement Algorithm (EPA) program (Berger et al. 2011).

The EPA uses a phylogeny aware method to classify short sequence reads (e.g., targeted metagenomic OTU sequences) based on alignment to a set of reference sequences and a phylogenetic tree constructed from the same reference sequences. The concatenated *RPB1* and *RPB2* alignment from the most comprehensive *Fusarium* phylogeny to date (O'Donnell et al. 2013) (TreeBASE accession: S12813) was used to construct a maximum likelihood phylogeny in the program RAXML (Stamatakis 2006) using the GTRGAMMA model of evolution with 100 bootstraps. The *Fusarium* OTU sequences were aligned to each other and then the reference alignment (the *RPB2* sequence data from the concatenated alignment used to construct the phylogeny) using MUSCLE. The EPA was accessed through a Web server at http://epa.h-its.org/raxml.

Sequences classified as *F. beomiforme* using the EPA were further classified using *RPB2* reference sequences from closely related *Fusarium* taxa. The OTU sequences were aligned with the following accessions from the NCBI nucleotide database: HQ646389, HQ646391–HQ646397, and HQ662683–HQ662689 (Laurence et al. 2011). A neighbor-joining tree based on Jukes-Cantor distance with 100 bootstraps was constructed in MEGA6 (Tamura et al. 2013).

To measure subgenus richness (the number of non-singleton OTUs) within *Fusarium*, two primary lineages were identified within the genus. These represent two major groups that have evolved within the "Gibberella" section of *Fusarium*, where the majority of the OTUs were classified using the EPA (see Fig. 1).

Phylogenies were plotted using Archaeopteryx (Han and Zmasek 2009).

Statistics.—All statistical analyses were performed in R (R Development Core Team 2012) using the vegan (Oksanen et al. 2016), ggplot2 (Wickham 2009), and base packages. Pearson's correlation coefficient was calculated and used to test for a relationship between *Fusarium* OTU abundance (log-transformed sequence number per OTU) and *Fusarium* OTU ubiquity (number of soil samples OTU was found in). Pearson's correlation coefficient was also used to test for a relationship between *Fusarium* OTU richness (number of non-singleton OTUs) and individual soil physiochemical characteristics.

Two-sided *t* tests were used to test for an effect of plant community richness (i.e., monoculture versus polyculture) on individual soil physiochemical characteristics as well as *Fusarium* OTU richness (the number of non-singleton OTUs). Two-way analysis of variance (ANOVA) was used to test for an effect of plant diversity (monoculture versus polyculture) and plant species identity (*A. gerardii* versus *L. capitata*), as well as the interaction between plant diversity and identity on *Fusarium* OTU richness. Two-way ANOVA was similarly used to test for an effect of plant diversity, plant identity, and the interaction between plant diversity and plant identity on individual soil physiochemical characteristics. Where the ANOVAs gave a significant result ($P < 0.05$), Tukey's honest significant differences (TukeyHSD) post hoc test was used to determine differences in the response variable among the different plant treatments.

Using the R package vegan, nonmetric multidimensional scaling (NMDS) was used to visualize differences in *Fusarium* community structure between individual soil samples. *Fusarium* OTU abundance data (Bray-Curtis distance) was used in NMDS analyses. Soil physiochemical characteristics were fit within the NMDS ordination space and tested as statistically significant predictors of community composition using the *envfit* function in the R package vegan.

RESULTS

Sequence output and processing. —After sequence processing, sequence numbers per sample ranged from 1082 to 2350, with a mean of 1927 ($\sigma = 414$). The processed *RPB2* sequences clustered into 497 OTUs (0.97 identity). Sequences representing 89% of the OTUs aligned to reference *RPB2* sequences in the class Sordariomycetes. Within the Sordariomycetes, sequence data representing 72 OTUs aligned to reference sequences in the genus *Fusarium*. Only the 72 *Fusarium* OTUs are included in subsequent analyses. This included sequence numbers per sample ranging from 78 to 748, with a mean of 261 ($\sigma = 222$).

Phylogenetic characterization of* Fusarium *soil communities.—Using the EPA, the 72 *Fusarium* OTUs were classified within a genus-wide reference phylogeny generated using data from O'Donnell et al. (2013). The OTUs were classified to 19 different positions within the reference phylogeny, representing seven species or species

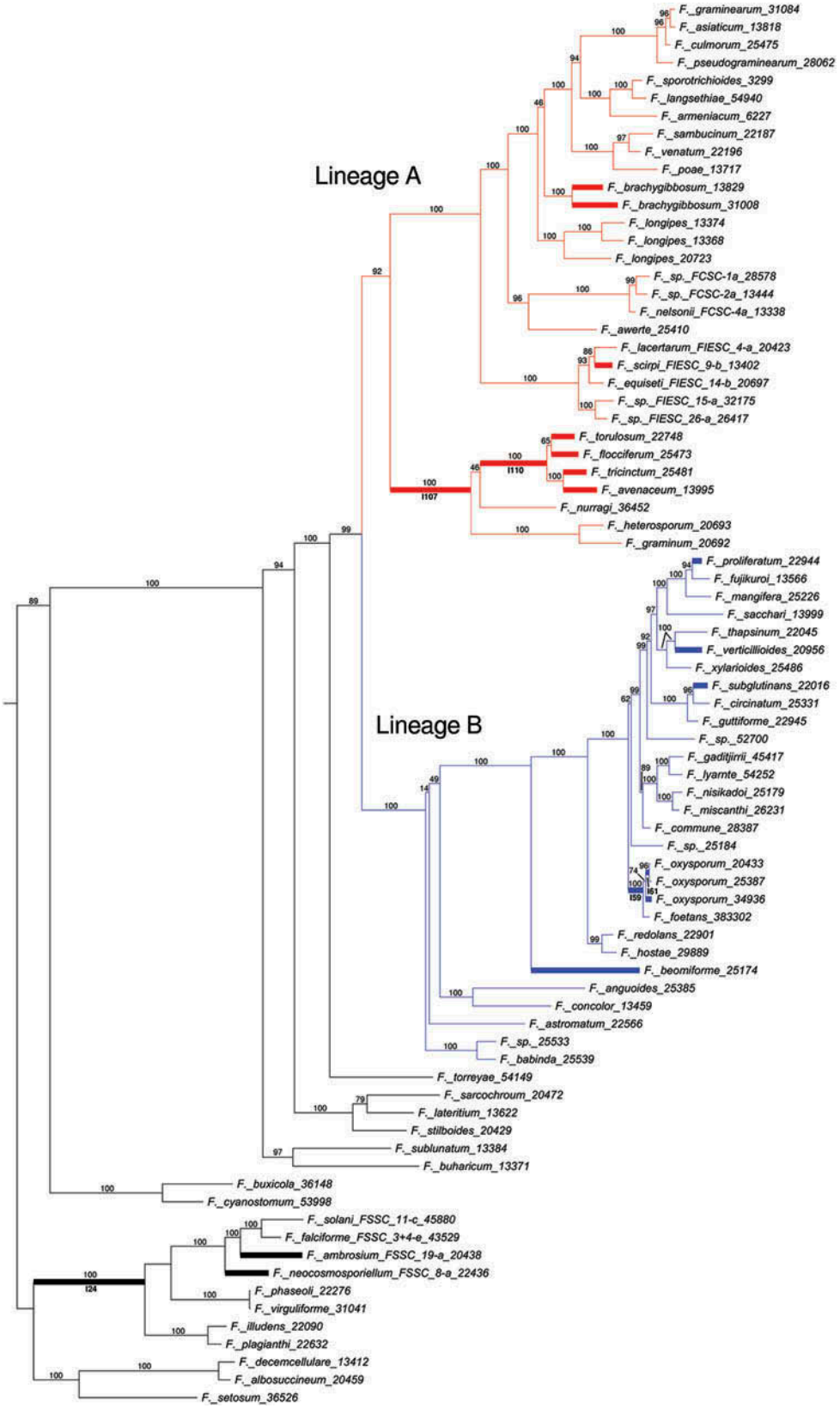

Figure 1. Classification of *Fusarium* OTUs within a genus-wide reference phylogeny. The reference phylogeny was constructed from the two-locus (*RPB1* and *RPB2*) alignment from O'Donnell et al. (2013), and sequences representing OTUs were classified using the EPA (Berger et al. 2011). The phylogeny shown is a subset of the complete reference phylogeny, showing the portion relevant to the classified OTUs. Thick branches indicate classification of an OTU to that branch in the reference phylogeny. The red subtree indicates lineage A, and the blue subtree represents lineage B. Numbers above branches are bootstrap values for the reference phylogeny, and bold text below thick branches correspond to the EPA classification shown in Table 1.

Table 1. Classification of *Fusarium* OTUs in a genus-wide reference phylogeny using the EPA (Berger et al. 2011).

Classification[a]	Clade[b]	Sequences[c]	OTUs[d]	Samples[e]
F. avenaceum 13995	*tricinctum*	1810	35	12
I61	*oxysporum*	610	6	11
F. brachygibbosum 31008	*sambucinum*	530	2	5
F. flocciferum 25473	*tricinctum*	104	3	9
F. verticillioides 20956	*fujikuroi*	30	1	5
F. beomiforme 25174	*beomiforme*	18	2	2
F. scirpi 13402	*incarnatum-equisiti*	13	1	3
F. proliferatum 22944	*fujikuroi*	13	1	2
F. neocosmosporiella 22436	*solani*	10	2	4
F. oxysporum 34936	*oxysporum*	9	5	4
F. torulosum 22748	*tricinctum*	7	2	2
F. tricinctum 25481	*tricinctum*	5	1	1
F. ambrosium 20438	*solani*	5	2	3
I59	*oxysporum/foetans*	4	3	4
I110	*tricinctum*	2	2	2
F. brachygibbosum 13829	*sambucinum*	2	1	1
I24	*solani*	2	1	1
F. subglutinans 22016	*fujikuroi*	2	1	1
I107	*tricinctum/heterosporum*	1	1	1

[a]Classification within the reference phylogeny for which at least 1 OTU was assigned. Alphanumeric codes indicate placement of an OTU deeper within the phylogeny (see Fig 1. for classifications).
[b]Phylogenetic clade(s) for the given classification according to O'Donnell et al. (2013).
[c]Total number (across OTUs) of sequences assigned to the classification in column 1.
[d]Number of OTUs assigned to the classification in column 1.
[e]Number of individual soil samples (out of 12) in which the classification in column 1 was found.

complexes: *F. sambucinum, F. incarnatum–equisiti, F. tricinctum, F. fujikuroi, F. oxysporum, F. beomiforme*, and *F. solani* (Table 1 and Fig. 1). Thirty-five of the OTUs were classified as a single reference species within the *F. tricinctum* species complex, a total of 1810 sequences (Table 1). Operational taxonomic units assigned to a single position within the *F. oxysporum* complex of the reference phylogeny were the next richest unique classification, represented by 6 OTUs and 610 sequences. The only other individual classifications represented by more than 100 sequences were within the *F. sambucinum* and another reference species in the *F. tricinctum* species complexes, represented by 2 and 3 OTUs, respectively (Table 1). The abundance (number of sequences/OTU) and ubiquity (number soil samples the OTU was found in) of all of the individual *Fusarium* OTUs was significantly positively correlated ($r = 0.892$, $P < 0.001$).

In addition to these common species or species complexes of *Fusarium*, 2 OTUs were classified, represented by a total of 18 sequences and found in two soil samples, as *F. beomiforme*. Since *F. beomiforme* had not been reported in the Northern Hemisphere (Laurence et al. 2011), classification of OTUs as the species warranted further phylogenetic analysis. As shown in Fig. 2, the sequences representing OTUs originally classified as *F. beomiforme* using the EPA fall within the closely related species complex *F. burgessii*. More specifically, these OTU sequence data form a group, supported by 94% bootstrap confidence values, with the morphotype B clade as described in Laurence et al. (2011).

***Fusarium* community composition.**—*Fusarium* community composition varied with plant community richness (Fig. 3). *Fusarium* communities in soil of monoculture plant communities were also differentiated between the two plant species in monoculture, but not in polyculture plots (Fig. 3). Fitting soil physiochemical characteristics as vectors in ordination space showed all of the measured soil physiochemical characteristics increased in soil from polyculture as compared with monoculture plant communities, the exception being nitrogen (Fig. 3). Using a permutation test, the only physiochemical characteristic that was statistically significant ($P < 0.01$) predictor of community composition was organic matter.

***Fusarium* community richness.**—*Fusarium* OTU richness (excluding singleton OTUs in a sample) did not differ in soil of monoculture versus polyculture plant communities ($t = 1.564$, $P > 0.1$). Based on a two-way ANOVA, there was no significant effect of plant community richness or plant identity, and no significant interaction between plant richness and plant identity in determining *Fusarium* OTU richness. There was also no significant correlation between *Fusarium* OTU richness with any of the soil physiochemical characteristics (data not shown).

By defining subgenus lineages, it was possible to test for additional significant plant or soil physiochemical predictors of richness within different *Fusarium* lineages. As the majority of the OTUs were classified within the *F.*

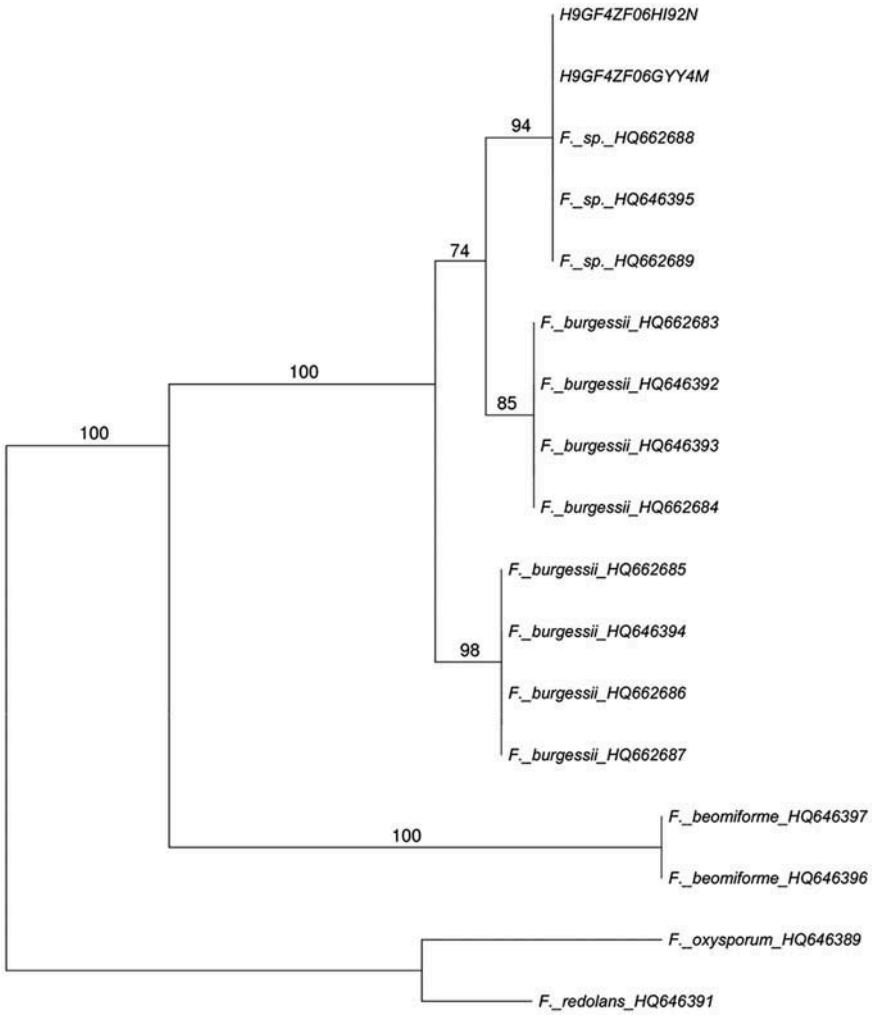

Figure. 2. A neighbor-joining tree constructed from *RPB2* reference sequences representing the *F. beomiforme* and *F. burgessii* lineages and sequences from OTUs classified as *F. beomiforme* using the EPA (see Table 1 and Fig. 1). Numbers above branches are bootstrap values, the tip labels beginning with H9GF4ZF06 are sequences from the OTUs, and the last string of digits in the remaining tip labels are NCBI accession numbers. The sequences representing the OTUs fall within the same clade as *Fusarium* sp. morphotype B as described in Laurence et al. (2011).

tricinctum or *F. oxysporum* species complexes or closely allied taxa (Fig. 1 and Table 1), we defined two lineages that circumscribe these two major groups that have evolved within the "Gibberella" section of the genus *Fusarium*. Lineage A represents OTUs classified as *F. tricinctum* or closely related taxa, and lineage B represents OTUs classified as *F. oxysporum* or closely related taxa (Fig. 1).

In contrast to total *Fusarium* richness, OTU richness within lineage A (see Fig. 1) was significantly negatively correlated with organic matter (R^2 = 0.41, P < 0.05) (Fig. 4). Plant community richness was also related to OTU richness within lineage A. Based on a two-way ANOVA, there was a significant effect of plant community richness (F = 10.458, P < 0.05), but not plant identity, and no significant interaction between plant community richness and identity in influencing richness within lineage A. Post hoc comparison between treatments showed that OTU richness within lineage A was significantly greater in soil from *L. capitata* growing in monoculture than soil from *L. capitata* or *A. gerardii* growing in polyculture (TukeyHSD, P < 0.05) (Fig. 5). Soil physiochemical characteristics were also significantly correlated with OTU richness within lineage B. Specifically, OTU richness within lineage B was positively correlated with pH (R^2 = 0.57, P < 0.01) and potassium (R^2 = 0.39, P < 0.05) (Fig. 6).

Soil physiochemical characteristics.—Across plant species, soil in polyculture plant communities had greater phosphorus (t = −3.023, P < 0.05) and organic matter (t = −3.34, P < 0.01) than soil from monoculture plant communities. Based on a two-way ANOVA, there

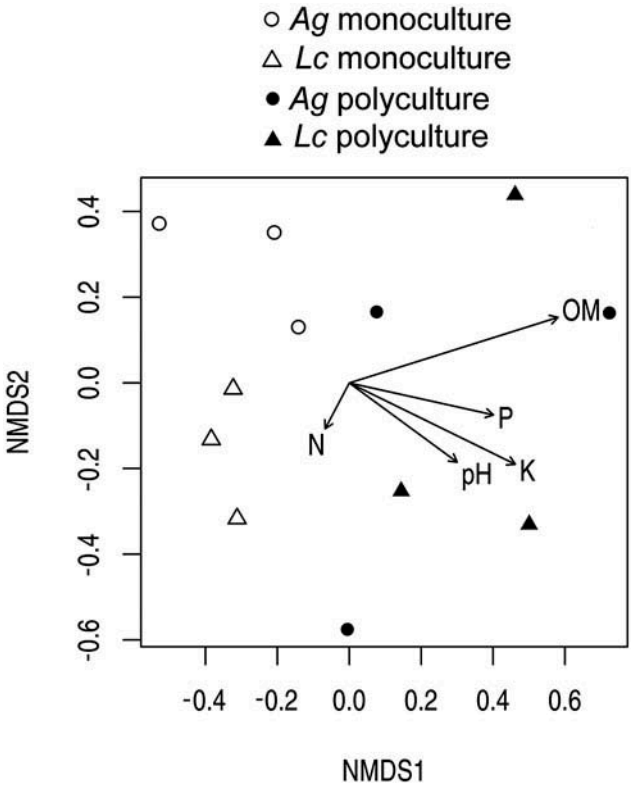

Figure. 3. Nonmetric multidimensional scaling plot showing differences in *Fusarium* community structure between soil from monoculture and polyculture plant communities and differences between the two plant species in monoculture. Vectors show soil edaphic characteristics fit within ordination space: K = potassium, N = nitrogen, OM = organic matter, P = phosphorus, pH = pH.

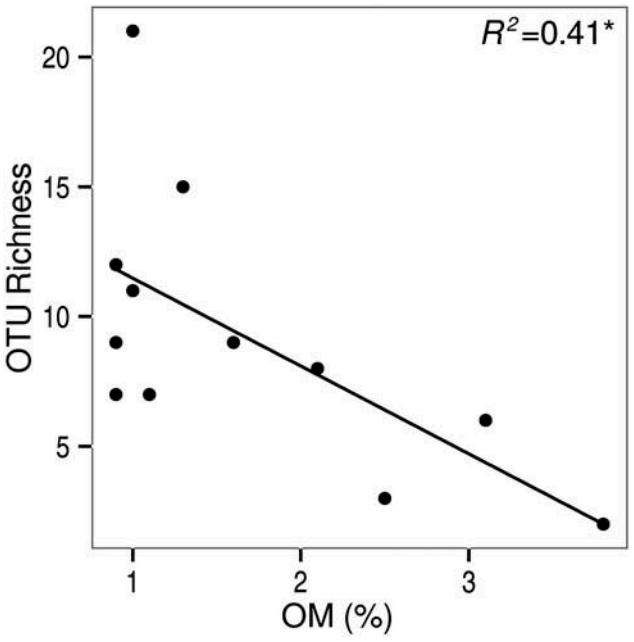

Figure. 4. Scatter plot showing the relationship of OTU richness (number of OTUs in a sample) within lineage A (see Fig. 1) with soil organic matter. Pearson correlation coefficients were used to calculate R^2 and P values. *$P < 0.05$.

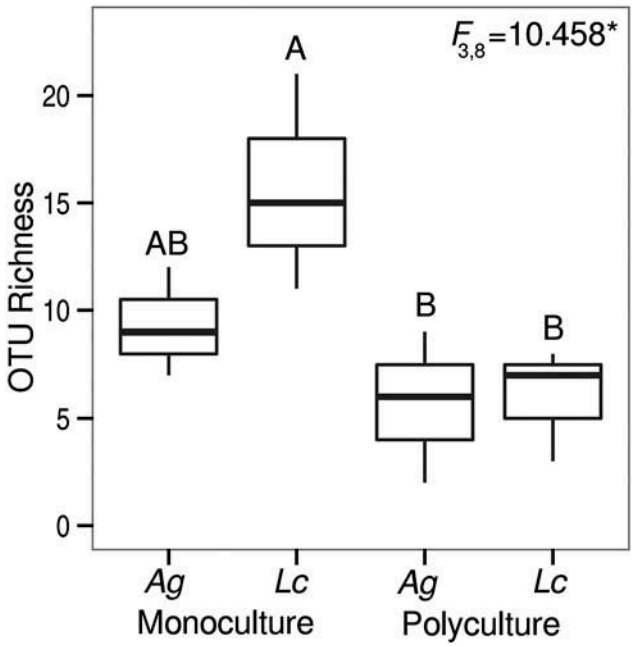

Figure. 5. Box plot showing OTU richness (number of OTUs in a sample) within lineage A (see Fig. 1) in the different plant diversity and species treatments. F statistics and P values shown are calculated based on a two-way ANOVA and highlight the significant effect of plant diversity. There was no significant effect of plant identity or the interaction between plant diversity and plant identity. *$P < 0.05$.

was a significant effect of plant community richness on phosphorus and organic matter ($F = 10.222$, $P < 0.05$ and $F = 12.36$, $P < 0.01$, respectively). Post hoc comparison between treatments showed that phosphorus and organic matter were greater in soil from *A. gerardii* growing in polyculture than soil from *A. gerardii* growing in monoculture (TukeyHSD, $P < 0.05$). Across plant richness treatments, soils from the legume *L. capitata* had greater nitrogen than rhizosphere soil from the grass *A. gerardii* ($t = -2.756$, $P < 0.05$).

DISCUSSION

This research characterized the diversity of *Fusarium* communities in the rhizosphere of native plant species to determine how changes in plant communities and soil physiochemical characteristics might influence the richness and composition of these fungi. The most common species complexes (i.e., found in the most soil samples and with the greatest number of sequences overall) were *F. tricinctum* and *F. oxysporum*. This is similar to observations made from samples from a prior year within the same experimental plots (LeBlanc et al. 2015), suggesting that these species complexes may remain dominant over time. These two species

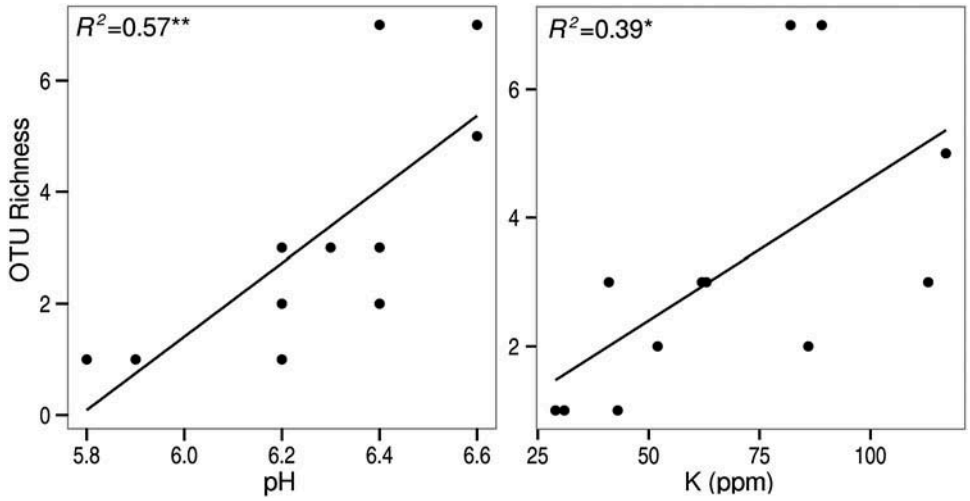

Figure. 6. Scatter plots showing the relationship of OTU richness (number of OTUs in a sample) within lineage B (see Fig. 1) with soil edaphic characteristics. Pearson correlation coefficients were used to calculate R^2 and P values. $^{**}P < 0.01$; $^{*}P < 0.05$.

complexes were also the richest, having the greatest number of OTUs among the different species complexes. The predominance of *F. oxysporum* is in agreement with other surveys of *Fusarium* taxa in soil of native grassland ecosystems (Kommedahl et al. 1975; Harrow et al. 2010). However, although *F. tricinctum* has been reported as the predominant *Fusarium* taxa in grassland ecosystems, these fungi are predominantly recovered from plants, not soil (Harrow et al. 2010). This may reflect a bias in the methods used in this work or previous culture-based studies, which will ultimately be answered through culturing *Fusarium* isolates from these same soil samples.

Of the remaining species complexes found in these soils, classification of OTUs within the *F. beomiforme* species complex was unexpected. Through further phylogenetic characterization of the OTUs classified as *F. beomiforme*, we found that these OTUs fall within the *F. burgessii* species complex (Laurence et al. 2011). As *F. burgessii* has only been found in a region of Australia (Laurence et al. 2011), the evidence of this fungus in North America suggests a more cosmopolitan distribution. Interestingly, to date these fungi have only been reported from non-agricultural soils, suggesting that they may play uncharacterized roles in native ecosystems.

Fungal community richness has been reported to be a positively correlated with plant community richness (Peay et al. 2013; Lang et al. 2015; LeBlanc et al. 2015). This motivated our initial hypothesis, that *Fusarium* community richness would be greater in more species-rich (i.e., polyculture) plant communities. Within the genus *Fusarium*, there was no significant difference in the richness (number of OTUs) in soil of polyculture versus monoculture plant communities. Additionally, although previous research has found a positive correlation between fungal community richness and soil organic matter (LeBlanc et al. 2015), none of the measured soil physiochemical characteristics were correlated with *Fusarium* richness in this study. In part, the inability to find significant correlates of *Fusarium* richness may be explained by the different patterns of richness within subgenus phylogenetic lineages.

To further differentiate *Fusarium* richness within the genus, we defined the A and B lineages. Operational taxonomic unit richness within lineage A was negatively correlated with soil organic matter and greater in soil of monoculture than polyculture plant communities, particularly in the rhizosphere of the legume *L. capitata*. The concentration of these fungi in the rhizosphere of this native legume is similar to observations by Yli-Mattila et al. (2010) who found that fungi in the *F. tricinctum* species complex were the most common fungus isolated from the roots of the the legume *Trifolium pratense*. Depending on the life-history characteristics of these fungi, greater richness in the rhizosphere of *L. capitata* growing in monoculture implicates them in plant soil feedbacks (Bever et al. 2012). For example, if these fungi are pathogens of *L. capitata*, they could constrain the proliferation of this plant and help maintain native plant diversity (e.g., Liu et al. 2012).

The greater richness of fungi in lineage A in monoculture soil is contrary to our initial hypothesis. Although unexpected, this observation is similar to the greater microbial diversity in soil of monoculture, low carbon plant communities found by Schlatter et al. (2015). As one of the potential mechanisms for this

relationship, Schlatter et al. (2015) hypothesized that decreased carbon in monoculture soil leads to greater antagonistic interactions between microorganisms. As increased antagonism has been shown to have a positive effect on microbial diversity (Czàran et al. 2002), this offers a compelling mechanism for the observed patterns in richness observed in this work. It also suggests that antagonistic interactions between fungi in lineage A (members and relatives of the *F. tricinctum* species complex) would be greater in soil of monoculture plant communities. Interestingly, genomic data from *F. tricintum* and closely related species have shown that the genomes of these fungi are enriched for genes predicted to produce secondary metabolites (Lysøe et al. 2014), pointing to biochemical mechanisms underlying potential antagonistic interactions.

Richness of the second predominant phylogenetic lineage (lineage B) in these soils was positively correlated with pH and potassium. Although these soil physiochemical characteristics may be directly influencing the richness of fungi in this lineage, interactions with other soil microorganisms in the rhizosphere may also be responsible. As demonstrated by Czàran et al. (2002), antagonistic interactions can promote diversity in microbial communities, and within these same soils Bakker et al. (2013) found the density of antagonistic bacteria in the genus *Streptomyces* increased with soil pH. Although these correlations should be treated with caution, the potential for antagonistic interactions with other microbial taxa to structure the diversity within *Fusarium* communities provides a testable hypothesis.

By characterizing *Fusarium* communities in soil associated with the roots of two different plant species maintained as monocultures or growing within polyculture plant communities, it was possible to test the effect of plant community richness and plant identity on *Fusarium* community composition. Root exudates are known to influence the structure of microbial communities in soil (Broeckling et al. 2008; Badri et al. 2006), motivating calls for efforts to breed for beneficial rhizosphere microbial communities in agricultural ecosystems (Bakker et al. 2012). However, the identity of plants surrounding a focal plant (Broz et al. 2010) as well as changes in plant diversity (Scherling et al. 2010) can influence the metabolism of focal plants, with likely consequences on root exudates in the rhizosphere. Considering these plant-mediated mechanisms as well as previous observations of fungal communities in soil (LeBlanc et al. 2015), we hypothesized that any effect of plant species in monoculture on *Fusarium* community composition would be reduced when that same plant species was grown in polyculture plant communities. As shown in these data, the clustering of *Fusarium* communities by plant species in monocultures was not observed for the same plant species growing in polyculture. Depending on the distribution of functionally relevant traits across the genus, changes in the composition of *Fusarium* communities in soil may have important functional consequences. For example, variation in the ability to produce different secondary metabolites is correlated with phylogeny among *Fusarium* taxa for which genomic data are available (Hansen et al. 2015). As many secondary metabolites produced by fungi in the genus *Fusarium* are biologically active towards other soil microorganisms (e.g., Van Rij et al. 2005) or plants (e.g., Brian et al. 1954), the presence or absence of certain taxa, as reflected in changes in *Fusarium* community composition, likely will have downstream functional consequences.

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