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Phylogeny, Plant Species, and Plant Diversity Influence Carbon Use Phenotypes Among *Fusarium* Populations in the Rhizosphere Microbiome

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ABSTRACT

Carbon use by microorganisms in the rhizosphere microbiome has been linked to plant pathogen suppression and increased mineralization of soil nutrients for plant uptake; however, factors that influence carbon use traits are poorly understood for most microbial groups. This work characterized the relationships of phylogeny, plant species, and plant diversity with carbon use among fungi in the genus *Fusarium* from rhizosphere soil. Eighty-four randomly collected *Fusarium* isolates were cultured from the rhizosphere of the perennial plants *Lespedeza capitata* and *Andropogon gerardii*, maintained as long-term monocultures or growing in 16-plant species polycultures. For each isolate, a portion of the *RPB2* locus was sequenced for phylogenetic analyses and growth on 95 carbon substrates was measured using Biolog SF-P2 plates. Similarity in carbon use among isolates decreased with increasing genetic distance and there were differences in niche width (i.e., number of carbon substrates used) and growth on preferred substrates (i.e., mean growth on the five carbon substrates supporting the greatest growth) among isolates

within two predominant phylogenetic clades. Carbon use also varied with plant species and the diversity of the surrounding plant community. Within each of the two predominant clades, niche width was greater among *Fusarium* isolates from the rhizosphere of *L. capitata* than *A. gerardii*. The correspondence of phylogeny with carbon use suggests changes in *Fusarium* community composition may lead to the differential use of carbon substrates in the rhizosphere, while the effects of plant species and diversity suggest variation in plants communities may also correspond to variation in carbon use by these fungi. In addition, the consistent effect of plant species on niche width within different clades provides evidence that the rhizosphere environment of the two plants selects for particular traits, rather than promoting the presence of clades with those traits. Overall, this research shows the dynamics of plant and fungal communities are likely to influence carbon use in the rhizosphere and consequently processes related to this phenotype, such as soil nutrient cycling and competition for carbon among soil microbes.

Soil microbe-mediated feedbacks on plant growth and fitness are recognized as key components of native and agricultural ecosystems (Bever et al. 2010, 2012; Van Nuland et al. 2016). Traditionally, interactions between plants and soil microorganisms (plant-soil feedbacks) have been studied in the context of highly coevolved

symbionts that have clear negative (e.g., pathogens [Liu et al. 2012; Mills and Bever 1998]) or positive feedbacks (e.g., mycorrhizal fungi or rhizobial bacteria [Revillini et al. 2016]) on plant growth. However, the focus on highly coevolved or specific plant-microbe symbiotic interactions ignores the significance of indirect effects of microorganisms on plant growth. Such indirect effects include the ability of free-living microorganisms in the soil or rhizosphere microbiome to change the availability of plant nutrients through decomposition and soil mineralization (Weidner et al. 2015) or suppress the activity of soilborne plant pathogens (Kinkel et al. 2011).

Though many eukaryotic and prokaryotic microorganisms influence soil nutrient cycling, fungi in the genus *Fusarium* are distinctive considering their high capacity to break down and use a wide variety

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of carbon substrates (King et al. 2011; Li et al. 2008; Regalado et al. 1997). In particular, comparison of *Fusarium* genomes with genomes of more than 90 other fungi shows an enrichment for genes predicted to produce lignocellulosic enzymes (i.e., CAZymes) in *Fusarium* genomes (Zhao et al. 2013). These phenotypic and genomic capacities coupled with the cosmopolitan distribution of *Fusarium* (Burgess 1982) implicates these fungi in the global decomposition and transformation of carbon substrates in soil, potentially increasing mineralization of plant nutrients. In addition to their possible influence on soil carbon and nutrient dynamics, nonpathogenic *Fusarium* have been suggested to suppress soilborne plant pathogens in the same genus through competition for growth-limiting carbon in soil (Alabouvette et al. 2009; Couteaudier and Alabouvette 1990). These examples highlight two indirect mechanisms through which carbon use by *Fusarium* may affect plant health and productivity. Determining the factors that influence variation in carbon use among *Fusarium* communities and populations in the soil and rhizosphere microbiome will refine understanding of the potential impacts of these fungi on plants in agricultural and native ecosystems.

Variation in root exudates among plant species and genotypes is hypothesized to contribute to variation in soil nutrients and the composition of microbial communities in soil (Badri et al. 2009; Broeckling et al. 2008). This variation in plant-derived nutrients may also influence carbon use among heterotrophic soil microorganisms by selection within the rhizosphere. For example, high concentrations of homoserine in the rhizosphere of pea (*Pisum sativum*) results in selective enrichment of fungi that are able to use this substrate (Rodriguez-Carres et al. 2008). Similarly, Gram-positive bacteria in the genus *Streptomyces* isolated from the rhizosphere of different plant species exhibit differences in carbon use phenotypes (Essarioui et al. 2016). Variation in the types and diversity of carbon substrates in the rhizosphere of different plant species may also influence carbon use by rhizosphere microorganisms. This is suggested by research showing treatment of microbial populations with greater numbers of carbon substrates selects for an increased capacity to use more substrates by those populations (Gravel et al. 2011).

Plant diversity has varied effects on microbial communities in soil and consistently increases carbon in soil (Lange et al. 2014; Tilman et al. 1997). Increased plant diversity has been linked to greater fungal diversity (LeBlanc et al. 2015; Peay et al. 2013). In contrast, the diversity of prokaryotic communities in soil has shown negative responses (Schlatter et al. 2015) as well as no response (Dassen et al. 2017). Differences in rhizosphere carbon composition among different types of plants (Badri et al. 2009) may also lead to a greater diversity of carbon substrates in soil of diverse polyculture plant communities compared with monoculture plant communities.

The goal of this research was to characterize carbon use among *Fusarium* isolates from rhizosphere soil to identify potential correlates of variation in carbon use phenotypes. Specifically, we propose to investigate the relationships among plant host, plant community diversity, *Fusarium* phylogeny, and carbon use phenotypes among *Fusarium* isolates from the rhizosphere microbiome. Carbon use phenotypes provide fundamental insight into the ecological roles of fungi in phytobiomes and are related to capacities to suppress plant pathogens (Alabouvette et al. 2009; Essarioui et al. 2017) and to nutrient cycling dynamics in soil. We pose three hypotheses in our work. First, we hypothesize that *Fusarium* isolates from the rhizosphere of different plant species and plant diversity treatments will differ in carbon use phenotypes (niche width, growth efficiency, and growth on preferred substrates). Second, we hypothesize that more closely related *Fusarium* isolates will display greater similarity in carbon use phenotypes than distantly related fungi. Finally, we hypothesize that, within phylogenetic clades, *Fusarium* isolates from the same plant species or diversity treatment will have more similar carbon

use phenotypes than isolates from different plant species or diversity. Overall, this work provides novel insights into the relationships between *Fusarium* phylogeny and carbon use, the influences of plant communities on *Fusarium* carbon use phenotypes, and the potential ecological roles of these fungi as mediators of soil nutrient cycling and or as competitors for growth limiting carbon in the phytobiome.

MATERIALS AND METHODS

Field sampling and soil sample processing. Soil was sampled from the Cedar Creek Ecosystem Science Reserve, a National Science Foundation LTER site in east-central Minnesota in July 2012. Two soil cores, separated by a 90° angle around the base of the sampled plant, were taken from the base of individual plants growing in monoculture or 16-species (i.e., polyculture) plant communities as part of replicated experimental plots established and maintained since 1994 (Tilman et al. 1997). The individual plants sampled were the perennial grass *Andropogon gerardii* and the perennial legume *Lespedeza capitata*, identified by inflorescence morphology. The two plant species were selected due to their predominance in prairie ecosystems and evolutionary relationship with common cultivated grasses (e.g., *Triticum aestivum*) and legumes (e.g., *Glycine max*) in the region. Two soil cores per individual plant sampled, from a total of 12 plants, were pooled in the field and kept on ice until storage at −20°C. Extractable phosphorus, extractable potassium, extractable nitrate, pH, and organic matter of the soil samples were measured at the University of Minnesota Research Analytical Laboratory (ral.cfans.umn.edu), as previously reported (Essarioui et al. 2016).

Culturing of *Fusarium* isolates from soil. To culture *Fusarium* isolates from the soil samples, *Fusarium*-selective Komada and Nash-Snyder media (Leslie and Summerell 2006) were inoculated with soil suspensions, as previously reported (Essarioui et al. 2017). Soil suspensions were made by diluting 1 g of air-dried soil in sterile water for a final concentration of 1×10^{-3} g of soil/ml of water. For each individual soil sample, 1 ml of the diluted soil suspension was inoculated and spread onto two plates of each type of media. The inoculated plates were incubated at 28°C for 5 days. Individual colonies for subculturing were selected randomly using a grid and randomized coordinates. Colonies were subcultured onto 1/2-strength potato dextrose agar (1/2-PDA: 12 g of potato dextrose broth (Difco) + 15 g of agar/liter) and grown under a 12 h photoperiod at 25°C for 5 to 7 days. A culture was classified as within the genus *Fusarium* based on the production of fusiform-shaped (banana or canoe) spores. For individual soil samples failing to produce more than five *Fusarium* isolates after the initial soil suspension, repeated soil suspensions were performed. Single-spore isolates were generated from the cultured *Fusarium* isolates and stored in 50% glycerol at −70°C.

Carbon use phenotyping. Carbon use phenotypes were measured based on the ability of the cultured *Fusarium* isolates to grow on 95 different carbon substrates using Biolog SF-P2 plates (Biolog, Hayward, CA). A portion of these data have previously been reported and the methods used to standardize fungal material and inoculate the Biolog SF-P2 plates have been described in Essarioui et al. (2017). Briefly, individual isolates were grown for 5 days on 1/2-PDA at 25°C under a 12 h photoperiod. Spores from each isolate were harvested from the media using 0.2% carrageenan in sterile water and spore suspensions were adjusted to an optical density (OD) of 0.22 at 590 nm. One and a half milliliter of the adjusted spore suspensions was dispensed into 13.5 ml of sterile water. The spore suspensions for each isolate were then inoculated into individual Biolog SF-P2 plates (i.e., one isolate per plate). Optical densities within the 96 wells were measured 96 h (4 days) postinoculation using a Synergy H1 Reader (BioTek Instruments, Winooski, VT) at 590 nm.

Processing of the Biolog data and focus on specific of carbon use traits followed a similar rationale outlined by previous work using similar methods (Essarioui et al. 2016; Schlatter et al. 2013). Optical densities measured from the water control well of each plate were subtracted from OD of all the 95 remaining wells, after which individual wells with $OD \leq 0.005$ were set to 0. Three main carbon use traits were calculated from the processed OD values representing growth of individual isolates on the 95 carbon substrates. The three traits were represented the number of substrates supporting growth (niche width), the capacity to produce biomass across the used substrates (growth efficiency), and the capacity to produce biomass on the preferred substrates (growth on preferred substrates). Niche width was calculated as the number of carbon substrates yielding positive OD values greater than 0.005 for a given isolate. Growth efficiency was calculated as the mean OD across the 95 carbon substrates for a given isolate. Growth on preferred substrates was calculated as the mean OD across the five substrates with the highest OD values.

PCR amplification and Sanger sequencing. A portion of the *RPB2* (DNA directed RNA polymerase second largest subunit) gene was amplified and sequenced from each *Fusarium* isolate to measure the phylogenetic relatedness among and genetic distance between the isolates. These data were also used for preliminary taxonomic classification. Deoxyribonucleic acids were extracted using a cetrimonium bromide (CTAB)/chloroform-based protocol as described in Gale et al. (2011) and diluted to 10 ng/ μ l. A portion of the *RPB2* gene was amplified from the extracted DNA using the RPB2-5F2 and RPB2-7cR primers (O'Donnell et al. 2007). Reactions were performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using 20- μ l reactions with 2 μ l of Ex-Taq buffer, 1.6 μ l of dNTPs (2.5 μ M of each), 2 μ l of each forward and reverse primers (10 μ M), 11.3 μ l of water, 0.1 μ l of Takara Ex-Taq (Takara Bio Inc., Shiga, Japan) polymerase (5 units/ μ l), and 1 μ l template (10 ng of DNA/ μ l). The PCR reactions were performed with an initial denaturing step at 95°C (5 min), followed by 24 cycles at 95°C (1 min), 57°C (1 min), 72°C (1 min), and ended with a final extension of 72°C (10 min). Amplicons were purified using a Promega Wizard kit (Promega, Madison, WI) and sequenced with the RPB2-5F2 primer using Sanger technology at the Biomedical Genomics Center at the University of Minnesota (<http://genomics.umn.edu>). Five prime and 3' low quality sequence data were trimmed from the sequences using 4Peaks (<http://nucleobytes.com/4peaks/>). Processed sequence data were aligned using MUSCLE (Edgar 2004). Sequences have been deposited in as GenBank accession numbers KY354597 to KY354680.

Phylogenetics and taxonomy. To determine the phylogenetic relatedness among the cultured *Fusarium* isolates, the aligned *RPB2* sequences were used to construct a phylogenetic tree using the neighbor-joining method implemented in MEGA6 (Tamura et al. 2013). Clade support was measured based on nonparametric bootstrap values, with 100 replications. For taxonomic identification of the isolates, the same sequence data were aligned to culture-backed *RPB2* reference sequences in the *Fusarium* ID database (Geiser et al. 2004) using BLASTN (Altschul et al. 1997).

Statistics. All statistics were performed in the R environment (R Development Core Team 2015), using the corplot (Wei 2013), ggplot2 (Wickham 2009), seqinr (Charif and Lobry 2007), and vegan (Oksanen et al. 2016) packages.

A Mantel test was used to test for a relationship between genetic and carbon use distance. To perform the Mantel test, a genetic distance (square root of the pairwise sequence identity) matrix was calculated from the aligned *RPB2* sequence data using the seqinr package and a carbon use distance (Euclidean) matrix was calculated from OD values from growth of individual isolates on the 95 distinct

carbon substrates. The Mantel test was performed with the vegan package using the Pearson correlation method with 999 permutations.

To visualize pairwise correlation in growth among *Fusarium* isolates across the 95 carbon substrates, a heat-map was generated with the corplot package (Wei 2013). Pearson correlation coefficients were calculated from OD values from growth of individual isolates on the 95 carbon substrates for a given comparison between pairwise isolates. The isolates represented in the heat-map were reordered to match the order of the isolates in the phylogeny constructed from the *RPB2* sequence data (see above).

Two-sided *t* tests were used to test for differences in the three carbon use traits (described above) between two main phylogenetic clades as well as for differences between isolates from *A. gerardii* versus *L. capitata* or monoculture versus polyculture, within the clades. Two-sided *t* tests with false discover rate (FDR) (Benjamini and Hochberg 1995) correction for multiple comparisons were used to test for differences in growth on the 95 individual substrates between isolates from different phylogenetic clades, different plant species, and different levels of plant diversity. Two-way analysis of variance tests (two-way ANOVA) were used to test the effect of plant species, plant diversity, and the interaction between plant species and diversity on the three carbon use traits. Finally, Spearman rank correlation coefficients (ρ) were used to test for relationships between niche width, growth efficiency, and growth on preferred substrates with individual soil edaphic characteristics, including organic matter, potassium, pH, phosphorus, and nitrogen.

RESULTS

Variation in recovered isolates and carbon use. A total of 84 *Fusarium* isolates were cultured from the 12 individual soil samples. There was variation in the number of isolates cultured from the individual soil samples. Within soil from the different plant species, a mean of 4.33 ($\sigma = 2.944$, min = 2, max = 10) isolates were cultured from the rhizosphere of individual *A. gerardii* plants and a mean of 9.667 ($\sigma = 0.816$, min = 8, max = 10) isolates were cultured from the rhizosphere of each *L. capitata* plant. Within soil from the different plant diversity treatments, a mean of 6.333 ($\sigma = 3.445$, min = 2, max = 10) isolates were cultured from monoculture and a mean of 7.667 ($\sigma = 3.67$, min = 2, max = 10) isolates were cultured from polyculture plots.

All the 95 carbon substrates were used by at least one *Fusarium* isolate. The mean number of substrates used by each isolate was 91.31 ($\sigma = 3.818$, min = 74, max = 95). Among the 84 *Fusarium* isolates there were 56 unique carbon use phenotypes (i.e., combinations of carbon substrates used by individual isolates). Fifty of these patterns were unique to a single isolate, while the most common carbon use phenotype (use of all 95 carbon substrates) was observed for 14 isolates. The five substrates used by all of the isolates and supporting the greatest growth (i.e., highest mean OD among isolates) were turanose, *N*-acetyl-D-glucosamine, polyoxyethylene (20) sorbitan monopalmitate (Tween 40), D-trehalose, and polyoxyethylene (20) sorbitan monooleate (Tween 80). The five substrates used the least frequently and also supporting the least growth were 3'-methyl-D-glucose, adenosine, adenosine-5'-monophosphate, D-fructose-6-phosphate, and D-L- α -glycerol phosphate.

Taxonomy and phylogeny of *Fusarium* isolates. Taxonomic classification of the isolates was based on alignment and similarity of *RPB2* sequence data from the isolates with *RPB2* reference sequences in the *Fusarium* ID database (Geiser et al. 2004). Based on the alignments, the *RPB2* sequences from the 84 isolates were similar to seven different culture-backed reference sequences, representing six different phylogenetic species: *F. oxysporum*, *F. foetens*, *F. commune*, *F. brachygybosum*, *F. incarnatum-equiseti*, and *F. solani* (Table 1).

A neighbor-joining phylogeny was constructed using *RPB2* sequence data to assess the phylogenetic relatedness among the isolates and the presence of different phylogenetic clades. The *Fusarium*

isolates formed four different phylogenetic clades supported by bootstrap values of at least 97% (Fig. 1A). The previous taxonomic classification of the isolates showed that the largest clade (59 of 84 isolates) represented isolates showing high *RPB2* sequence similarity to *F. oxysporum* reference sequences and the closely related phylogenetic species *F. foetens*. Because distinguishing between *F. oxysporum* and *F. foetens* based on the length of the isolate *RPB2* sequence data are not feasible, this clade will be referred to as *F. oxysporum*, hereafter. The second largest clade (17 of 84 isolates) represented isolates showing high sequence similarity to *F. solani* reference sequences (referred to as the *F. solani* clade, hereafter) (Fig. 1A).

Phylogeny and carbon use of the *Fusarium* isolates. Similarity in carbon use among the *Fusarium* isolates was greater within than between phylogenetic clades. As shown in the heat-map, Pearson correlation coefficients were generally higher for isolate pairs within phylogenetic clades than between phylogenetic clades (Fig. 1A and B). There was also a significant positive relationship between genetic distance and carbon use distance (Mantel correlation = 0.294, $P < 0.01$).

Carbon use traits also differed between the two largest phylogenetic clades, isolates closely related to *F. oxysporum* and *F. solani* (Table 1; Fig. 1A). Niche width (the number of substrates used) was greater for isolates in the *F. oxysporum* clade compared with isolates in the *F. solani* clade (two-sided t test, $P < 0.01$). In contrast, growth on preferred substrates (mean OD across the five substrates with the highest OD for every isolate) was greater for

TABLE 1 Taxonomic classification of the <i>Fusarium</i> isolates		
Reference ^a	Isolates ^b	Sequence identity ^c
<i>F. oxysporum</i> NRRL34936	35	0.995
<i>F. foetens</i> NRRL38302	24	0.99
<i>F. commune</i> NRRL28387	6	0.979
<i>F. brachygibbosum</i> NRRL31008	1	1
<i>F. incarnatum-equiseti</i> 14-b NRRL20697	1	0.985
<i>F. solani</i> 5-d NRRL43681	14	1
<i>F. solani</i> 5-l NRRL32791	3	1

^a Species and strain designation of the references in *Fusarium* ID (Geiser et al. 2004) that were most similar to the *RPB2* sequences from the *Fusarium* isolates.

^b Total number of *Fusarium* isolates for which *RPB2* sequence data aligned (BLASTN) to references shown in column 1.

^c Mean identity between *RPB2* sequences of the isolates and *RPB2* sequences of the references shown in column 1.

Fig. 1. A, A neighbor-joining phylogeny representing phylogenetic relationships between *Fusarium* isolates and **B,** a heat-map representing similarity in carbon use among isolates. The phylogeny was constructed from *RPB2* sequence data from the isolates and numbers above branches represent nonparametric bootstrap values. Taxonomy is based on alignment of *RPB2* sequence data to *Fusarium* ID (Geiser et al. 2004) (Table 1). *F. oxysporum* and *F. solani* clades represent the two clades used in additional analyses. The heat-map represents Pearson correlation coefficients calculated from optical density (OD) values (growth) on the 95 carbon substrates for a given comparison between pairwise isolates. The isolates represented in the heat-map were reordered to match the order of the isolates represented in the phylogeny.

isolates in the *F. solani* clade than isolates in the *F. oxysporum* clade (two-sided *t* test, $P < 0.01$). Growth on individual substrates also differed between these two clades. Following two-sided *t* tests and FDR based correction for multiple comparisons, 59 of the 95 substrates supported differential growth of isolates from the two different clades. Members of the *F. oxysporum* clade grew more on 28 of the 59 differentially used substrates (Supplementary Table S1).

Effect of plant species and plant diversity on *Fusarium* carbon use. Carbon use differed between *Fusarium* isolates from the rhizosphere of *L. capitata* and *A. gerardii*, though the differences were dependent on the diversity of the surrounding plant community. *Fusarium* niche width was significantly affected by plant species (two-way ANOVA, $P < 0.001$); however, there was also a significant interaction between plant species and plant diversity (two-way ANOVA, $P = 0.016$). Posthoc comparison of niche width between the plots showed *Fusarium* isolates from the rhizosphere of *A. gerardii* in polyculture had reduced niche width compared isolates from *A. gerardii* in monoculture or from *L. capitata* growing in monoculture or polyculture (Tukey's honest significant difference test, $P < 0.05$) (Fig. 2). Though growth efficiency was not influenced by plant diversity or plant species (data not shown), growth on preferred substrates was. Overall there was a significant effect of plant species (two-way ANOVA, $P = 0.013$) and plant diversity (two-way ANOVA, $P = 0.048$) on growth on preferred substrates of the *Fusarium* isolates. Posthoc comparison showed isolates from the rhizosphere of *A. gerardii* growing in polyculture had significantly greater growth on preferred substrates compared with isolates from the rhizosphere of *L. capitata* growing in monoculture (Tukey's honest significant difference test, $P < 0.05$) (Fig. 3).

Fusarium isolates from the rhizosphere of *L. capitata* and *A. gerardii*, across plant diversity treatments, displayed growth differences on individual carbon substrates. Following correction

for multiple comparisons, 15 of the 95 substrates supported differential growth of isolates from the two plant species. Isolates from *A. gerardii* grew more on 4 of the 15 differentially used substrates. There were no differences in growth on individual substrates for isolates from monoculture or polyculture plant communities evaluated across plant species (data not shown).

Within the two predominant phylogenetic clades, plant species had a significant effect on the niche width of the *Fusarium* isolates. Niche width was greater for isolates from the rhizosphere of *L. capitata* than *A. gerardii* for both the *F. oxysporum* (two-sided *t* test, $P < 0.01$; (Fig. 4A) and *F. solani* (two-sided *t* test, $P < 0.001$) (Fig. 4B) clades (ANOVA tests used above were not feasible do to the reduced sample sizes within the two clades). Though carbon use differed between these clades (see above), these results show different plant species can have a similar effect on carbon use phenotypes within different phylogenetic clades.

Soil edaphic characteristics and carbon use. Differences in soil edaphic characteristics between soil from the two plant species and levels of plant diversity have been reported previously (Essarioui et al. 2016). As shown in Table 2, save for the positive correlation between *Fusarium* niche width and soil nitrogen, increases in soil edaphic characteristics corresponded to decreases in niche width, growth efficiency, and growth on preferred substrates. This highlights the potential for variation in the abiotic component of soil to explain variation in *Fusarium* carbon use.

DISCUSSION

Carbon use by fungi in the genus *Fusarium* has implications for their role in decomposition and soil nutrient cycling as well as their ability to suppress soilborne plant pathogens through competition for nutrients. This research characterized the phylogenetic diversity

Fig. 2. Box-plots and data representing niche width (number of substrates used) of *Fusarium* isolates from the rhizosphere of the plant species *Andropogon gerardii* and *Lespedeza capitata*, maintained as monocultures (mono.) or growing in polyculture (poly.) plant communities. Different numbers above the box-plots indicate significant ($P < 0.05$) differences between the groups, based on Tukey's honest significant difference posthoc comparisons.

Fig. 3. Box-plots and data representing growth on preferred substrates (mean optical density [OD] across the five substrates with the highest OD) of *Fusarium* isolates from the rhizosphere of the plant species *Andropogon gerardii* and *Lespedeza capitata*, maintained as monocultures (mono.) or growing in polyculture (poly.) plant communities. Different numbers above the box-plots indicate significant ($P < 0.05$) differences between the groups, based on Tukey's honest significant difference posthoc comparisons.

and carbon use of *Fusarium* isolates from the rhizosphere of two plant species growing as long-term monocultures or within polyculture plant communities. Integrating phylogenetic data with measurement of *Fusarium* carbon use under the influence of different plant species or different levels of plant diversity allowed us to determine the importance of evolutionary constraints and ecological context in determining this functionally relevant phenotype. We found *Fusarium* isolates within phylogenetic clades displayed greater similarity in carbon use than between clades, independent of plant species or diversity. Carbon use differed between isolates from *A. gerardii* and *L. capitata*, depending on the diversity of the surrounding plant community. Within different phylogenetic clades, isolates from *A. gerardii* had reduced niche width compared with isolates from *L. capitata*. Overall, this work suggests changes in the composition of *Fusarium* and plant communities may change *Fusarium* mediated soil nutrient availability with potential feedbacks on plant growth and the suppression of soilborne plant pathogens.

The cultured *Fusarium* isolates displayed a high capacity to grow on the 95 assayed carbon substrates; the most common carbon use phenotype was growth on all the 95 carbon sources. Growth across the 95 substrates was very similar among the individual *Fusarium* isolates (i.e., high positive correlation coefficients). Despite this similarity in carbon use among isolates, there was significant phylogenetic signal in carbon use. With increasing genetic distance among the isolates there was also an increasing dissimilarity in growth on the 95 carbon substrates. Additionally, carbon use was significantly different between isolates within the predominant *F. oxysporum* and *F. solani* clades. This supports our initial hypothesis that carbon use would vary with phylogeny, providing a tentative link between *Fusarium* community composition and function. Based on empirical study, there is a link between carbon use and the outcome of competition between fungi in soil (Couteaudier and Alabouvette 1990; Rodriguez-Carres et al. 2008). This suggests the variation in the presence or abundance of fungi in these different clades, with different carbon use phenotypes, may influence competitive interactions between *Fusarium* and other heterotrophic microorganisms. Ultimately, the consequences of differences in *Fusarium* carbon use exhibited between different phylogenetic clades will be understood by linking specific phenotypes to effects on carbon and nutrient concentrations in soil.

The differences in niche width and preferred growth of isolates from the rhizosphere of the two plant species growing in different plant diversity treatments provides some support for our initial hypothesis of an effect of plant species and diversity on *Fusarium* carbon use. However, the effects of plant diversity on niche width was not consistent between plant species. Specifically, *Fusarium* niche width was greater in the rhizosphere of *A. gerardii* growing in monoculture than the same species in diverse plant communities, while there was no difference in *Fusarium* niche width in the rhizosphere of *L. capitata* between the two plant diversity treatments. This makes it difficult to generalize the effects of plant species and diversity on *Fusarium* carbon use in the rhizosphere microbiome. However, these results share similarities with research showing individual plant species do not display consistent changes

TABLE 2

Correlation between *Fusarium* carbon use traits and soil edaphic characteristics

Carbon use trait ^a	Soil edaphic characteristic ^b	Spearman rho (P value) ^c
Niche width	Nitrogen	0.312 (0.016)
Niche width	pH	−0.509 (<0.001)
Niche width	Potassium	−0.413 (<0.01)
Niche width	Organic matter	−0.366 (<0.01)
Growth efficiency	pH	−0.368 (<0.01)
Growth efficiency	Potassium	−0.385 (<0.01)
Growth on preferred substrates	pH	−0.266 (0.041)
Growth on preferred substrates	Potassium	−0.284 (0.029)

^a Carbon use trait of *Fusarium* isolates. See Carbon use phenotyping section for definitions.

^b Edaphic characteristic of soil sample from which the *Fusarium* isolates were cultured.

^c Spearman rank correlation coefficients and associated P value testing the correlation between *Fusarium* carbon use trait in column 1 and soil edaphic characteristics in column 2.

Fig. 4. Box-plots and data representing niche width (number of substrates used) of *Fusarium* isolates from the rhizosphere of the plant species *Andropogon gerardii* and *Lespedeza capitata*, across plant diversity treatments. Based on two-sided *t* tests, in both the *F. oxysporum* (A, *P* < 0.01) and *F. solani* (B, *P* < 0.001) clades from *L. capitata* have greater niche width than isolates from *A. gerardii*.

in growth (Marquard et al. 2009) or metabolism (Scherling et al. 2010) when diversity of the surrounding plant community is altered. Regardless of the context-specific nature of these results, they do provide a link between the dynamics of plant communities and functional traits within a ubiquitous group of fungi.

With evidence that *Fusarium* carbon use is influenced by phylogeny the effects of plant species and diversity could simply reflect environmental filtering (Saunders et al. 2010), with these treatments selecting for different carbon use traits restricted to different phylogenetic clades. However, we found a consistent effect of plant species within the rhizosphere of the two plant species. *Fusarium* isolates within the predominant *F. oxysporum* and *F. solani* clades both displayed greater niche widths in the rhizosphere of *L. capitata* than *A. gerardii*. This shows that differences between the two plant species directly influence carbon use of *Fusarium* in the rhizosphere, rather than simply excluding certain clades based on clade-specific traits (i.e., environmental filtering). Interestingly, parallel research found carbon use niche overlap of these *Fusarium* isolates with *Streptomyces* bacteria in the same soil samples was greater in the rhizosphere of *L. capitata* than *A. gerardii*. While this does not provide a mechanism responsible for the increased niche width of the *Fusarium* isolates, it does suggest competition for carbon between these two microbial groups will be greater in the rhizosphere of *L. capitata* than *A. gerardii* (Essarioui et al. 2017).

This work showed that the use of carbon in the rhizosphere microbiome by *Fusarium* is determined by the phylogenetic history of the fungi as well as the composition and diversity of the plant communities they are associated with. Linking carbon use and phylogenetics of these fungi suggests that changes in *Fusarium* community composition may have functional consequences for nutrient mineralization and the growth of other heterotrophic microorganisms, with implications for plant growth. Similarly, the effect of plant species and plant diversity on *Fusarium* carbon use may also have functional consequences for nutrient cycling dynamics in soil. Future research on the effect of varying carbon use on nutrient cycling and microbiome dynamics will enable the potential for management of *Fusarium* communities to promote a healthy phytobiome. Finally, though the genus *Fusarium* is an important soil-borne plant pathogen (Blomme et al. 2013; Michielse and Rep 2009), the predominance of nonpathogenic forms in the rhizosphere of healthy plants (Demers et al. 2015; Edel et al. 1997; Nel et al. 2006; Yli-Mattila et al. 2010) raises question as to the broader relevance of these fungi to plant fitness (Steinberg et al. 2016). In addition to their role as saprotrophs explored here, there is growing evidence that the production of secondary metabolites, commonly studied as mycotoxins, can also influence the growth of other soil microbes (van Rij et al. 2005) and plants themselves (Tsavkelova et al. 2012). A broader understanding of these fungi is needed to shed light on their relevance to plant growth in native and agricultural ecosystems, including their roles in nutrient cycling, plant growth promotion, and pathogen suppression as a counterweight to the historical emphasis on plant pathogenicity within this genus.

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