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Resource availability underlies the plant-fungal diversity relationship in a grassland ecosystem

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Abstract. It is commonly assumed that microbial communities are structured by "bottom-up" ecological forces, although few experimental manipulations have rigorously tested the mechanisms by which resources structure soil communities. We investigated how plant substrate availability might structure fungal communities and belowground processes along an experimental plant richness gradient in a grassland ecosystem. We hypothesized that variation in total plant-derived substrate inputs, plant functional group diversity, as well as the relative abundance of C_4 grasses and legumes would modulate fungal α- and β-diversity and their rates of soil carbon (C) and nitrogen (N) cycling. To test these predictions, we molecularly characterized fungal communities, as well as potential extracellular enzyme activity, net N mineralization, and soil organic matter respiration. We found higher fungal richness was associated with increasing aboveground plant biomass; whereas, fungal β-diversity was explained by contributions from C_4 grass and legume relative dominance, plant functional group diversity, as well as plant biomass. Furthermore, aboveground plant biomass consistently shaped the richness and composition of individual fungal trophic modes (i.e., saprotrophs, symbiotrophs, pathotrophs). Finally, variation in extracellular enzyme activity, net N mineralization rates, and soil organic matter respiration was significantly explained by fungal β-diversity when fungi were functionally classified. Via changes in the supply and composition of organic substrates entering soil, our study demonstrates that changes in the plant species richness and functional composition collectively influence fungal communities and rates of soil C and N cycling.

Key words: belowground diversity; community assembly; decomposition; extracellular enzymes; fungi; mineralization; plant species richness; resource availability.

Introduction

Soil fungi are important determinants of primary productivity through the direct and indirect transfer of growth-limiting nutrients from soil to plants (de Boer et al. 2005, Berg and McClaugherty 2008, Smith and Read 2010). As mediators of soil nutrient availability, understanding the ecological factors that structure soil fungal communities is of ecosystem-scale importance. However, we have historically lacked a conceptual framework for the study of microbial community assembly (Nemergut et al. 2013). Resource availability is a key factor regulating biodiversity in plant and animal communities (Tilman et al. 1982, Chesson 2000, Chase and Leibold 2003). It is commonly assumed that microbial communities are similarly structured by "bottom-up" ecological forces (Kowalchuk et al. 2002, Zak et al. 2003). However, ecological theory encompassing microorganisms and rigorous experimental manipulations provide limited evidence for this assumption. The expanding use of DNA sequencing provides new opportunities to examine plant controls on fungal diversity via resource availability.

Plants regulate fungal resource availability in the form of organic substrate inputs. Saprotrophic fungi are limited by the energy that can be obtained from the decay of plant detritus and other soil organic materials. In contrast, symbiotic mycorrhizal fungi receive photosynthates directly from plant root tips; therefore, their energy supply is largely limited by plant photosynthetic rates and C allocation to symbionts (Berg and McClaugherty 2008, Smith and Read 2010). Individual fungal taxa also vary in their relative capacities to extract energy from plant resources (Frankland 1998, Schneider et al. 2012). For example, a subset of saprotrophic fungi produce extracellular enzymes that degrade lignin, a major component of the plant cell wall (i.e., members of the phylum Basidiomycota; Floudas et al. 2012); whereas, mycorrhizal fungi are constrained by their capacity to form associations with plant hosts (Verbruggen et al. 2012). Thus, plant communities may serve as a strong selective force for soil fungi due to their

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reliance on plants for organic substrates and varied metabolic capacities.

Specifically, plant species richness and composition may influence soil fungal communities and their biogeochemical processes as a result of three potential influences on fungal substrate availability. First, plant species richness has been associated with increased plant biomass (Tilman et al. 2001), leading to corresponding increases in total soil C and N inputs available to fungi (Fornara and Tilman 2008). Second, due to variation in biomass, biochemistry, and root exudation by plant species (Grayston et al. 1998, Craine et al. 2002), increased plant species richness may increase the richness of substrates entering soils, thereby increasing the niches available to soil fungi (Grayston et al. 1998, Hooper et al. 2000, Waldrop et al. 2006). Third, a change in the composition of plant functional groups could alter the relative abundance of organic substrates entering soil (Hooper and Vitousek 1997) to favor fungi with particular metabolic capacities (Wardle et al. 2004, Bardgett et al. 2005). For example, due to the high lignin:N ratios of C_4 grass tissues (Craine et al. 2001, Knops et al. 2001, Fornara et al. 2009), plant communities dominated by C_4 grasses may correspond with a higher abundance of fungi with lignolytic capacities (Cline and Zak 2015).

Observational, experimental, and theoretical work describing the patterns and mechanisms by which resource availability structures plant α- and β-diversity provides a potentially useful framework to generate hypotheses regarding the relationship between fungal communities and resource supply. For example, the relationship between soil nutrient availability and plant richness has been observed to be unimodal (Inouye et al. 1987, Theodose and Bowman 1997, Fraser et al. 2015), although not this is not always the case (Adler et al. 2011, Willig 2011). At low initial nutrient availability, plant richness increases with soil nutrients as more species are able to meet their minimum resource requirements (i.e., resource-ratio hypothesis; Tilman et al. 1982), and potentially leading to species co-existence via "trade-off based mechanisms," including interspecific complementarity and greater use of limiting resources (Tilman et al. 2014). At high nutrient availability, plant diversity may decrease as a result of fewer belowground limiting factors (i.e., reduced niche dimensionality) and increasing light limitation (Goldberg and Miller 1990, Harpole et al. 2016). We might observe a similar unimodal relationship in fungal richness along a plant diversity gradient (Waldrop et al. 2006, Langenheder and Prosser 2008), if increased fungal access to organic substrates (a consequence of increased plant diversity) increases fungal richness until competition for mineral nutrients leads to declining fungal richness (Fig. 1a). Alternatively, the combined biochemical complexity of organic substrates (e.g., cellulose, hemicellulose, lignin, tannins) and varied metabolic capacities of fungi (i.e., extracellular enzyme synthesis), may lead to linear increases in fungal OTU richness along a resource gradient (Cline and Zak 2015; Fig. 1a) as substrate diversity subsequently increases the number of "niches" available to fungi (Grayston et al. 1998).

Observations of increasingly distinct plant communities along a nutrient gradient (Chapin 1980, Bedford

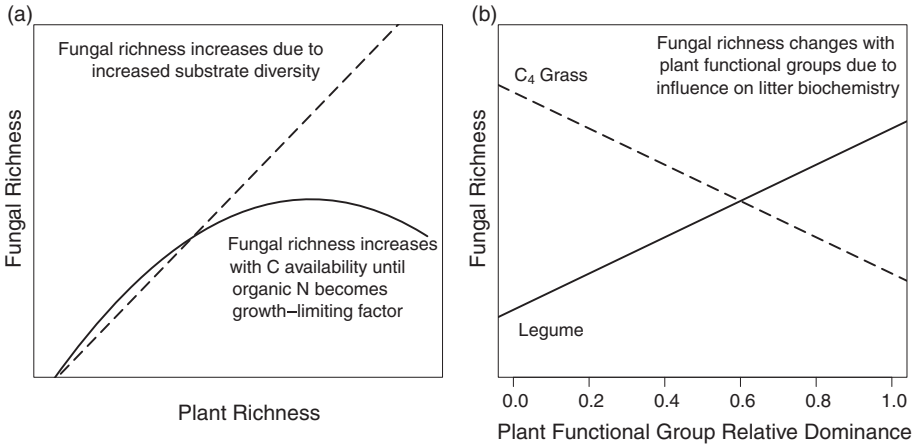

FIG. 1. Hypothesized relationships between substrate availability and fungal richness along a plant diversity gradient. Plant species richness may modulate fungal richness as a result of total inputs or the diversity of substrates (a), or plant composition may shape fungal richness as a result of its influence on functional groups and resulting litter biochemistry (b). In the grassland diversity experiment at Cedar Creek Ecosystem Science Reserve, plant biomass and substrate diversity increase with plant species richness, due to increasing productivity and representation of plant functional groups. We may observe a unimodal relationship in fungal richness along a plant diversity gradient, if increased fungal access to organic substrates increases fungal richness until competition for mineral nutrients leads to declining fungal richness (solid line; a). Alternatively, increasing plant richness may lead to linear increases in fungal richness, if substrate diversity increases the number of "niches" available to fungi (dashed line; a). If the plant litter biochemistry is a primary governor of fungal diversity, we expect fungal richness increases with the relative dominance of legumes and/or decrease with C_4 grass dominance (b).

et al. 1999) can inform the generation of hypotheses about whether organic substrate availability may similarly structure fungal β-diversity. For example, low nutrient habitats favor plant species with lower maximal growth and lower nutrient uptake rates (Chapin 1980); whereas, plant species with rapid resource capture and high dispersal dominate soils with high nutrient availability (Aerts et al. 1990). If analogous life history tradeoffs occur between fungal resource uptake and growth, we expect that fungal communities will become increasingly dissimilar along a resource gradient (Johnson et al. 2003, Peay et al. 2011, Cline and Zak 2015). Further, if plant litter biochemistry governs fungal β-diversity as a result of varied metabolic capacities among fungal taxa, we expect that the relative dominance of individual plant functional groups (i.e., C_4 grasses and legumes) will shape fungal communities, independent of total substrate entering the system (Fig. 1b; Meier and Bowman 2008). Alternatively, the potential influence of stochastic spore dispersal and priority effects in fungal community assembly may override the influence of resource supply on fungal communities (Fukami et al. 2010, Peay and Bruns 2014).

Through its effects on the richness and composition of the fungal community, substrate availability may further influence soil biogeochemical cycling. It is well established that increases in belowground biomass lead to more rapid C and N cycling as a result of increased respiration, enzyme activity, and mineralization rates (Zak et al. 1990). Independent of fungal biomass, soil process rates may be expected to increase with fungal richness due to varying metabolic capacities of individual fungi (Gessner et al. 2010, Hättenschwiler et al. 2011). Furthermore, if soil fungal communities exhibit some degree of functional equivalence (Talbot et al. 2014), we expect that the turnover of functional groupings of fungi across experimental plots (i.e., fungal guilds) will account for more variation in soil function than taxonomic considerations alone (Hättenschwiler et al. 2011).

To explore the potential mechanisms by which plant diversity might structure fungal α- and β-diversity, via controls on resources, we characterized fungal communities and belowground processes along an experimental plant richness gradient in a grassland ecosystem. We measured above and belowground biomass, root chemistry, substrate diversity (i.e., the diversity of aboveground biomass when classified by plant functional group) and plant functional group composition. We also quantified potential extracellular enzyme activity, net N mineralization and soil organic matter respiration in order to understand whether variation in fungal communities along a plant diversity gradient have consequences for belowground processes. Furthermore, fungal sequences were classified according to "species" level classifications (i.e., operational taxonomic units; OTUs) as well as trophic modes (i.e., saprotrophs, symbionts, pathogens) to understand whether plant-allocated resources vary with fungal mode of resource capture.

Methods

Experimental design and sample harvest

Our study was conducted at Cedar Creek Ecosystem Science Reserve (East Bethel, Minnesota), where a grassland biodiversity experiment was established in 1994 (Tilman et al. 2001). One, 2, 4, 8, or 16 perennial grassland species were planted from a pool of 18 species (four species, each, of C_4 grasses, C_3 grasses, legumes, non-legume forbs; two species of woody plants) in 9 × 9 m plots (n = 168). Soil was sampled to a depth of 10 cm (2 cm diameter) from 35 plots in July 2014 to quantify soil edaphic properties, soil microbial biomass, microbial respiration, net N mineralization, and extracellular enzyme activity. In July 2015, soil from 125 plots (including the 35 plots from the 2014 sampling) was similarly sampled for fungal molecular characterization. Five soil cores were sampled from each plot and composited into a single sample. Roots were removed from soil samples using a 2 × 2 mm sieve and dried at 60°C for 48 h. We measured soil pH using a 1:1 (v/v) soil:water mixture. Sieved soils dried at 60°C for 48 h were used to measure total soil % C and % N by combustion using a Costech ECS4010 element analyzer (Valencia, California, USA) and root carbon fractions (cell solubles, cellulose, hemicellulose + bound proteins, nonhydrolyzable) using an ANKOM forage analyzer (Macedon, New York, USA). Sieved 2015 soils were transported on ice and stored at −80°C until fungal molecular characterization was initiated.

Plant community analysis

To quantify plot productivity and the relative dominance of plant functional groups, we collected aboveground plant biomass for each plot (n = 125) in a 9 m × 6 cm strip clip in July 2014 and also July 2015. All aboveground biomass was classified by plant species, dried at 60°C for 48 h and weighed. Aboveground biomass was assigned to 79 plant species and 5 plant functional groups, including C_3 grasses, C_4 grasses, forbs, legumes, and sedges. We quantified total aboveground biomass of each plot, as well as the relative proportion of biomass (i.e., relative dominance) made up by individual plant functional groups. Plant functional group β-diversity was calculated using the Bray-Curtis distance metric, when the biomass of all five plant functional groups was considered.

Molecular characterization of fungi

DNA was extracted from 0.5 g soil using the FastDNA SPIN Kit (MP Biomedical, Solon, Ohio, USA). PCR amplification of the ITS1 gene region was conducted using primers ITS1F and ITS2 (Smith and Peay 2014). See Appendix S1 for details of PCR conditions, amplicon clean-up, and normalization. Sequencing was performed on the MiSeq platform (Illumina, San Diego,

California, USA) with 250 paired-end reads at West Virginia University's Genomic Core Facility. Low quality reads were removed bioinformatically using the FAST pipeline (https://github.com/ZeweiSong/FAST; see Appendix S1). Taxonomy was assigned using the BLAST algorithm (Altschul et al. 1990) against the UNITE database (v.7.0.; Kõljalg et al. 2013), and functional assignments (i.e., trophic mode and guild) were made in FunGuild using taxonomic assignments (Nguyen et al. 2016). Raw sequences were uploaded to the NCBI Sequence Read Archive (SRP108802).

Microbial biomass

Using samples collected in 2014 (n = 35) as well as 2015 (n = 125), we analyzed microbial biomass C using a chloroform fumigation extraction procedure (Appendix S1; Brookes et al. 1985). As in Riggs and Hobbie (2016), soil microbial biomass C was calculated as the difference between extractable C in the fumigated and unfumigated samples divided by an extraction efficiency coefficient (0.45). Values ranged between 0.006 and 0.18 mg C/g soil.

Soil process rates

Also from plots sampled in 2014 (n = 35), we calculated microbial respiration, extracellular enzyme potential activity and *in situ* net N mineralization rates. We quantified microbial respiration during a year-long laboratory incubation. Microbial respiration rate (mg CO_2-C·[g soil]$^{-1}$·d^{-1}) was quantified from a 50 g subsample of fresh, sieved soil by measuring the accumulation of CO_2 in airtight 1 L Mason jars during 24–48 h intervals on days 5, 7, 9, 12, 19, 26, 54, 84, 117, 178, 207, 237, 269, 301, 325, 355 of the incubation (Appendix S1). We calculated cumulative C respired (mg CO_2-C/g soil) as the sum of the average respiration rate between adjacent measurement dates multiplied by the time interval between measurements, ranging from 0.23 to 1.25 mg CO_2-C/g soil. We estimated the hydrolytic, chitinolytic, and lignolytic enzyme activity of soil communities using extracellular enzyme assays (Appendix S1). To measure activity of α-glucosidase (AG; EC 3.2.1.20), β-1,4-glucosidase (BG; EC 3.2.1.21), cellobiohydrolase (CBH; EC 3.2.1.91), β-1,4-xylosidase (BX; EC 3.2.1.37), and *N*-acetyl-β-glucosaminidase (NAG; EC 3.1.6.1), we used 200 μmol/L methylumbellyferyl-linked substrates (German et al. 2011). A 25-mM L-dihydroxy-phenylalanine substrate was used to assay phenol oxidase (PO; EC 1.10.3.2) and peroxidase (PX; EC 1.11.1.7) activity. Chitinolytic enzyme potential was calculated as NAG activity (ranging from 14.1 to 215.8 μmol·g^{-1}·h^{-1} across experimental plots). Hydrolytic enzyme potential represented the summed activities of AG, BG, BX, and CBH activity (95.3–637.1 μmol·g^{-1}·h^{-1}), and lignolytic activity was calculated as the sum of PX and PO (0.089–0.48 μmol·g^{-1}·h^{-1}). Mid-growing season in situ net N mineralization rates were estimated using 1.9 cm diameter plastic tubes (PVC) sunk to a depth of 10 cm at two different locations within each plot and covered with caps to prevent leaching losses. NH_4^+ and NO_3^- was measured initially in soil subsamples, as well as following 30 d of soil incubation within each PVC. We calculated net N mineralization rates (μg N·[g soil]$^{-1}$·d^{-1}) as the difference between initial and final extractable concentrations of NH_4^+ and NO_3^- (Doane and Horwath 2003) using a BioTek Synergy H1 microplate reader (Winooski, Vermont, USA).

Statistical analysis

Univariate and multivariate statistics tested whether plant diversity, via changes in plant biomass, substrate diversity (i.e., plant functional group diversity) and functional group composition, significantly predicted fungal community composition and soil process rates. To understand how plant richness altered microbial substrate availability, linear regression quantified the relationship between planted species richness and plant biomass as well as plant functional group diversity, calculated as Shannon diversity index according to aboveground biomass of the five plant functional groups. Permutational multivariate analysis of variance (PERMANOVA) and dispersion analysis assessed whether plant species richness contributed to significant differences in plant functional group β-diversity. To understand whether C_4 grasses and legumes influenced root chemistry, we performed simple linear regression to quantify the relationship between root carbon fractions and predictors, including C_4 grass and legume relative dominance. To investigate potential ways by which plant communities shape fungal communities, linear regression tested the relationship between fungal response variables (i.e., fungal richness and microbial biomass) and four plant explanatory variables (i.e., aboveground plant biomass, plant functional group diversity, C_4 grass relative dominance, and legume relative dominance). Using this same set of predictors, we performed distance-based redundancy analysis (db-RDA; Legendre and Anderson 2006) to quantify whether plant metrics accounted for variation fungal β-diversity. Using Hellinger-transformed abundances, fungal OTU β-diversity (all fungi, saprotrophs, symbiotrophs, pathotrophs) and guild β-diversity was calculated by the Bray-Curtis dissimilarity metric (Legendre and Gallagher 2001). To further assess which combination of plant substrate metrics accounted for the largest amount of variation in fungal richness and composition, all four explanatory variables were included into a single linear regression model (fungal richness as response variable) and a single db-RDA model (fungal β-diversity as response variable), followed stepwise model selection (forward and backward) based on Akaike's information criterion (AIC) of variables with a variance inflation factor <2. To investigate whether substrate availability influenced individual taxa, we conducted spearman correlations between the 176

OTUs representing >0.1% of the community and aboveground plant biomass, plant functional group diversity, and C_4 grass and legume relative dominance. *P*-values were adjusted for multiple comparisons (Benjamini and Hochberg 1995). To test whether soil edaphic factors also contributed to fungal β-diversity, we calculated a Euclidean distance matrix from soil moisture, pH, soil C, and soil N of the 2014 field subset, and conducted a Mantel correlated test with fungal OTU β-diversity. To understand whether variation in soil process rates correlated with fungal richness and fungal β-diversity, we expressed rates per unit microbial biomass C and conducted linear regression (fungal richness as response variable) and db-RDA (fungal β-diversity as response variable) models using soil progress rates as predictors and adjusting *P*-values for multiple comparisons. Analyses were performed in the R environment (http://www.R-project.org) using stats (Version 3.01; R Code Team 2016), vegan (Oksanen et al. 2016) packages.

Results

Plant species richness and measures of fungal substrate availability

Planted species richness was positively associated with aboveground plant biomass ($F_{1,107} = 74.3$; $r^2 = 0.41$; $P < 0.001$) and had a significant log-linear relationship with belowground biomass (Fig. 2a; $F_{1,28} = 22.6$; $r^2 = 0.45$; $P < 0.001$). Plant functional group diversity was positively associated with log-transformed planted species richness (Fig. 2b; $F_{1,107} = 78.0$; $r^2 = 0.42$; $P < 0.001$). Plant functional group composition was also significantly different between plant richness treatments (PERMANOVA; $F_{1,107} = 9.33$; $P < 0.001$), as indicated by a positive linear relationship between plant richness and C_4 grass biomass (Fig. 2c; $F_{1,\ 107} = 31.6$; $r^2 = 0.23$; $P < 0.001$) as well as legume biomass ($F_{1,\ 107} = 8.7$; $r^2 = 0.075$; $P = 0.004$). Furthermore, variation in plant functional group β-diversity was significantly greater in species-rich plots, as indicated by an inverse relationship between average distance to centroid and richness treatment (dispersion analysis; $F_{1,3} = 60.3$; $r^2 = 0.95$; $P = 0.004$). C_4 grass relative dominance was positively related to root hemicellulose content (Appendix S2: Fig. S1; $F_{1,25} = 4.3$; $r^2 = 0.21$; $P = 0.008$) and negatively related to the soluble root components ($F_{1,25} = 8.1$; $r^2 = 0.30$; $P = 0.001$). No other root C fractions varied with C_4 grass or legume relative dominance ($P = 0.19$–0.93).

Fungal community characterization

Taxonomy and guild assignments.—Following removal of fungal sequences of poor quality, 57% were classified in the phylum Ascomycota, 19% Basidiomycota, 4% Zygomycota, and 20% unidentified at the phylum level. One hundred and seventy-six OTUs and 10 fungal guilds, including undefined saprotoph, plant pathogen, arbuscular mycorrhizal fungi, comprised at least 0.1% of the fungal sequences across 2015 experimental plots ($n = 125$). Dominant guilds were classified in the trophic modes saprotrophs, pathotrophs, and symbiotrophs (Appendix S2: Fig. S2).

OTU richness.—In simple regression models, fungal OTU richness significantly increased with aboveground plant biomass and plant functional group diversity (Fig. 3a, b), but was not related to C_4 grass or legume relative dominance (Fig. 3c; $P = 0.49$–0.63). Fungal richness was best explained by a multiple regression

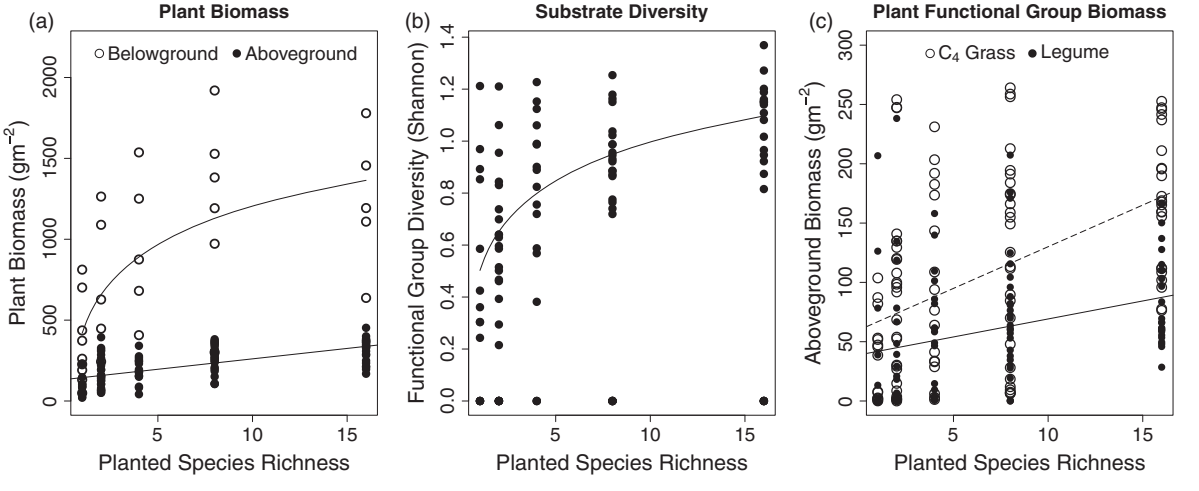

FIG. 2. Planted species richness is positively related to aboveground and belowground plant biomass (a), plant functional group diversity (b), and C_4 grass and legume biomass (c). Belowground biomass was collected for a subset of plots ($n = 35$). Plant functional group diversity (a proxy for substrate diversity) was calculated from the Shannon index at each plot, according to the biomass of C_3 grasses, C_4 grasses, forbs, legumes and sedges. In panel (c), dashed regression line represents the significant increase in C_4 grass biomass; whereas, solid line represents the significant increase in legume biomass. Significance was determined at $\alpha < 0.05$.

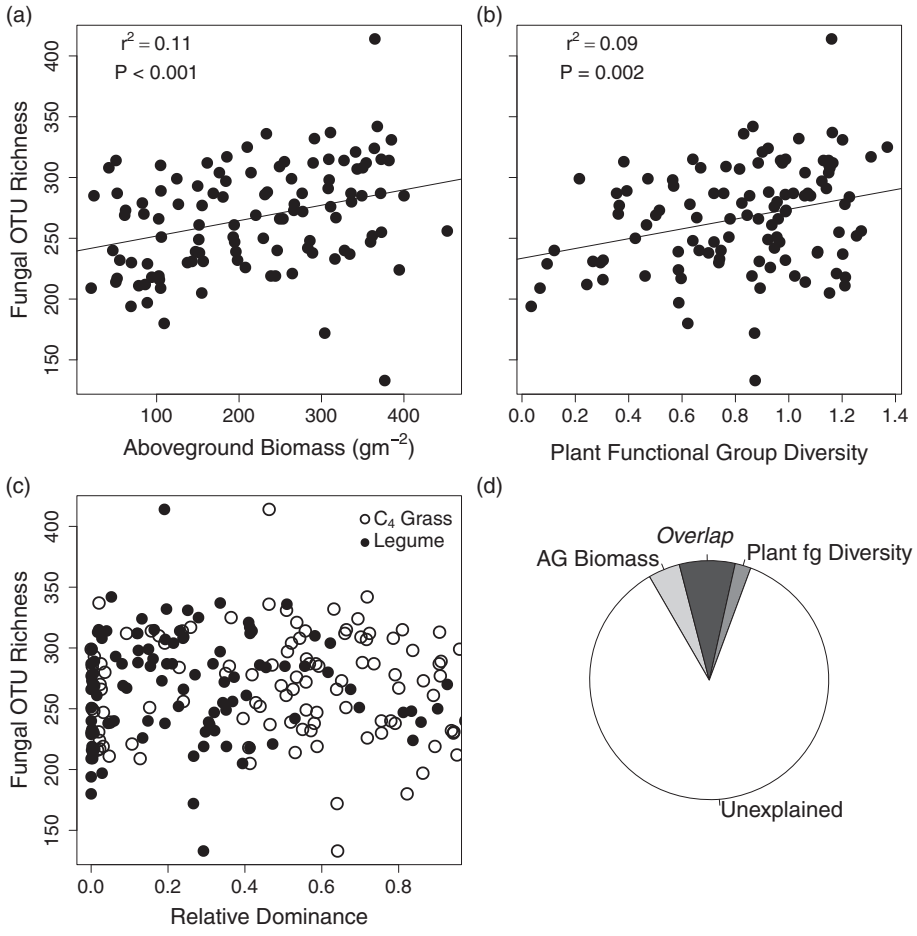

FIG. 3. Simple linear regression between fungal OTU richness and aboveground plant biomass (a), plant functional group diversity (b), C_4 grass and legume relative dominance (c), as well as the proportion of variance accounted for by significant variables in a multiple regression model (d). Plant functional group diversity was calculated for each plot from the Shannon index, according to the biomass of 5 plant functional groups, including C_3 grasses, C_4 grasses, forbs, legumes, and sedges. Significance was determined at $\alpha < 0.05$.

model containing both aboveground plant biomass and functional group diversity ($F_{2,83} = 5.8$; $R^2 = 0.13$; $P = 0.004$). In this two-factor model of fungal richness, total biomass remained significant ($\beta = 0.1$; $P = 0.02$); whereas, plant functional group diversity was marginally significant ($\beta = 27.9$; $P = 0.07$), and accounted for only an additional 2% of variation (Fig. 3d). When we modeled the richness of saprotrophs, pathogens and symbiotrophs separately, saprotrophic richness was best modeled by aboveground plant biomass and plant functional group diversity; whereas, aboveground biomass was selected to model pathogenic richness, and the best model of symbiotrophic richness consisted of aboveground plant biomass and legume relative dominance (Appendix S2: Table S1).

Fungal β-diversity.—Principal coordinates analysis illustrated that fungal OTU β-diversity varied along the plant diversity gradient (Fig. 4a), co-varying with plant aboveground biomass, plant functional group diversity, and the relative dominance of C_4 grasses and legumes. All four variables were selected to model fungal OTU β-diversity (stepwise db-RDA; $F_{4,\ 104} = 2.8$; $P < 0.001$), cumulatively accounting for 9.7% of the total variation across plots (Fig. 4b). Furthermore, collective variation in soil edaphic factors (i.e., pH, moisture, C and N) did not significantly explain fungal β-diversity (Mantel; $R = -0.003$; $P = 0.50$). Considering individual trophic modes, aboveground plant biomass and the relative dominance of C_4 grasses accounted for variation in saprotroph β-diversity (stepwise db-RDA; $F_{3,105} = 3.5$; $P < 0.001$; $R^2 = 0.091$). Similarly, symbiotroph β-diversity was best modeled by aboveground plant biomass and C_4 grass relative dominance ($F_{2,106} = 2.2$; $P < 0.001$; $R^2 = 0.044$). Aboveground plant biomass and legume relative dominance were included in the best model of pathogen β-diversity ($F_{2,106} = 5.8$; $P < 0.001$; $R^2 = 0.098$).

Taxa-specific responses.—Of the 176 dominant OTUs, 26% were significantly correlated to aboveground plant

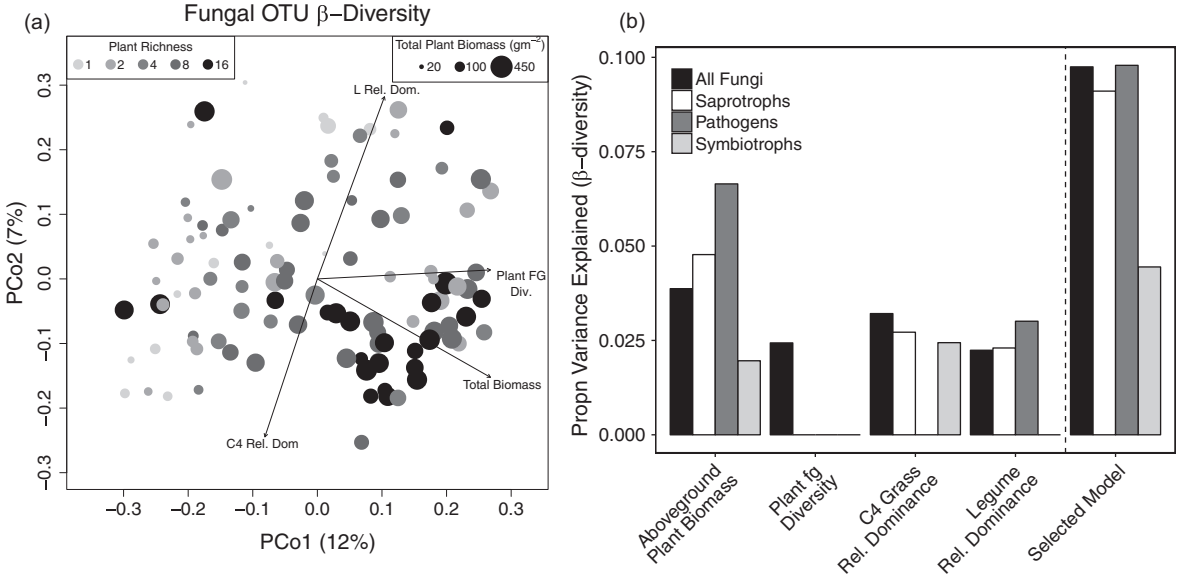

FIG. 4. Visualization of fungal OTU β-diversity along a plant diversity gradient (a; principal coordinates analysis), and the variation explained by aboveground plant biomass, plant functional group (fg) diversity, and relative dominance of C_4 grasses and legumes in a distance-based redundancy analysis (b). In panel (a), arrows represent overlaid vectors of plant aboveground biomass, plant functional group diversity, and C_4 grass relative dominance using function envfit in R. In panel (b), the vertical dashed line separates model selected following stepwise regression (right) from individual factors incorporated in the selected model (left). Fungal β-diversity was calculated from Bray-Curtis distances of Hellinger-transformed abundances of all fungal OTUs, as well those classified as saprotrophs, symbiotrophs and pathogens in FunGuild. Above-ground biomass was calculated per plot as g/m^2. C_4 grass and legume relative dominance was calculated as the relative proportion of total aboveground plant biomass.

biomass (45 OTUs), 19% were significantly related to C_4 grass relative dominance (34 OTUs), 15% were correlated with legume relative dominance (27 OTUs), and 4.5% (8 OTUs) correlated with plant functional group diversity (Fig. 5; Spearman; $-0.50 > \rho < 0.60$). Significant OTU correlations occurred within 24 fungal genera, most of which are classified as Ascomycetes (79%). Of OTUs with trophic mode classifications, the largest number of OTU correlations was observed within saprotrophs (32 OTUs). While significant OTU-aboveground plant biomass correlations were a mixture of positive and negative relationships (19 and 26, respectively), we observed largely positive OTU correlations with legume relative dominance (20 of 27 total) and plant functional group diversity (6 of 8) and mostly negative OTU correlations with C_4 grass relative dominance (21 of 34). Within each trophic mode, the direction of correlations between OTU abundances and plant variables was generally mixed. Saprotrophic OTUs were positively and negatively correlated to aboveground plant biomass and C_4 grass relative dominance. Interestingly, only positive correlations occurred between saprotrophic OTUs and legume relative dominance, as well as plant functional group diversity.

Microbial biomass C.—Microbial biomass C increased with aboveground plant biomass (Appendix S2: Fig. S3; $F_{1,33} = 11.7$; $r^2 = 0.26$; $P = 0.002$), but was not associated with plant functional group diversity ($P = 0.47$) or the relative dominance of C_4 grasses ($P = 0.32$) and legumes ($P = 0.35$). Furthermore, increases in microbial biomass C along the plant diversity gradient were independent of changes in fungal richness ($P = 0.22$). Microbial biomass C was positively related to cumulative respiration ($F_{1,33} = 29.1$; $r^2 = 0.47$; $P_{adjust} < 0.001$), as well as hydrolytic ($F_{1,33} = 7.2$; $r^2 = 0.18$; $P_{adjust} = 0.022$) and chitinolytic ($F_{1,33} = 20.4$; $r^2 = 0.38$; $P_{adjust} < 0.001$) potential enzyme activity. However, no relationship was observed between microbial biomass C and lignolytic enzyme potential or net N mineralization ($P_{adjust} > 0.44$).

Links between fungal communities and soil processes

While fungal richness did not account for variation in soil process rates, fungal guild composition was a significant predictor. Specifically, no significant relationships occurred between fungal OTU richness and cumulative C respired per unit microbial biomass (mg CO_2-C/g soil C; linear regression; $P_{adjust} = 0.50$), enzyme activity (hydrolytic, chitinolytic, and lignolytic) per gram microbial biomass C (μmol substrate·[g microbial biomass C]$^{-1}$·h^{-1}; $P_{adjust} = 0.41$–0.70), or N mineralization rates (μg N·[g soil]$^{-1}$·[g microbial biomass C]$^{-1}$·d^{-1}; $P_{adjust} = 0.41$–0.60), indicating that fungal richness did not contribute to variation in soil process rates (after considering microbial biomass C). We also tested the hypothesis that fungal community composition accounted for variation in soil biogeochemical processes and found no relationship between fungal OTU β-diversity and any measured soil

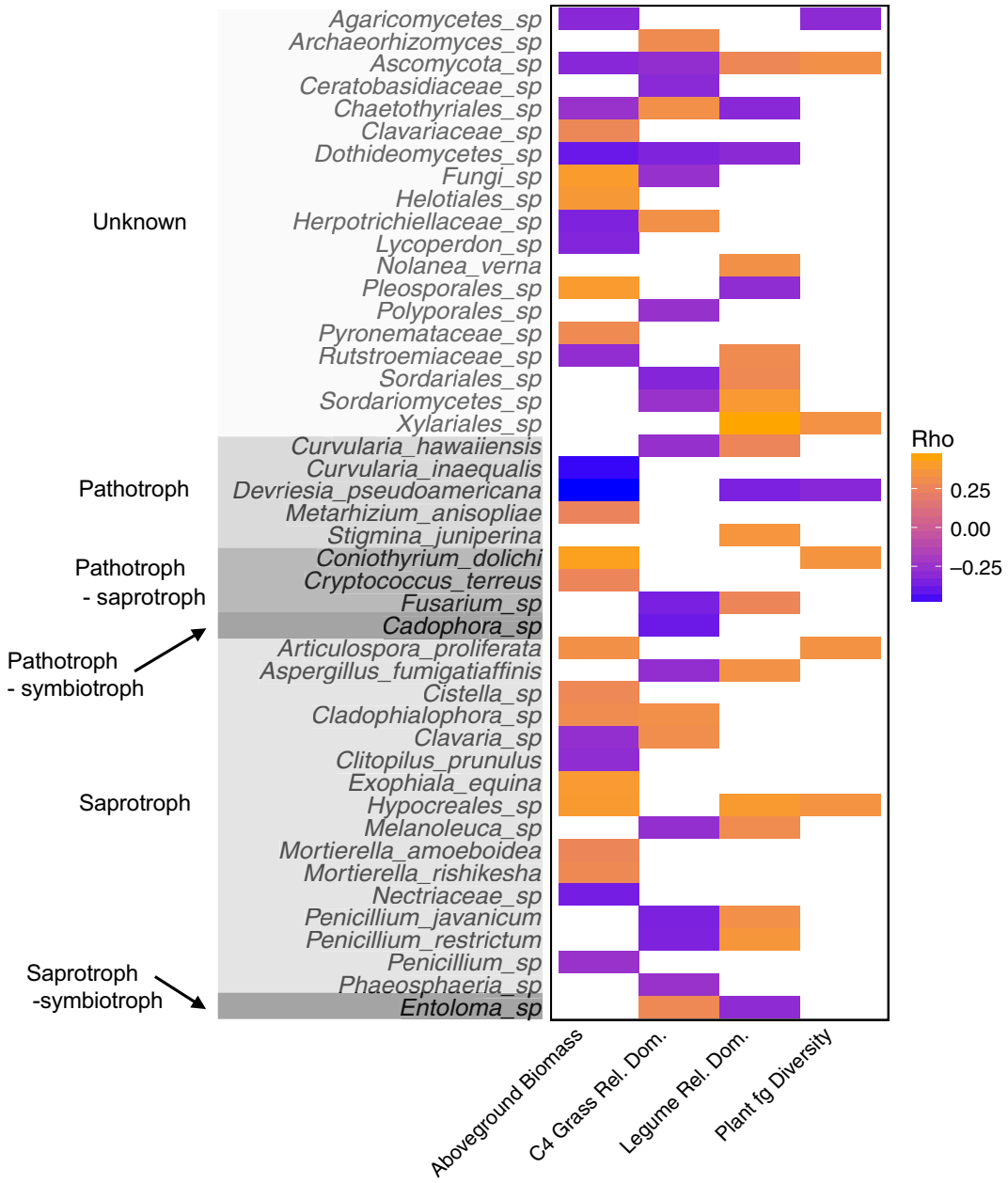

FIG. 5. Significant spearman correlations between dominant OTUs and plant-associated predictors of varied substrate availability, including aboveground plant biomass, C_4 grass relative dominance, legume relative dominance and plant functional group (fg) diversity. Species labels are organized by trophic mode. Significance $\alpha < 0.05$ following Benjamini-Hochberg *P*-value corrections. Dominant OTUs represented at least 0.1% of the fungal community across the plant diversity gradient.

process rate per gram microbial biomass C (db-RDA; P_{adjust} = 0.20–0.28). However, when β-diversity was calculated from the relative abundance of fungal guilds across experimental plots, guild β-diversity was significantly related to cumulative respiration/(g microbial biomass C), hydrolytic, chitinolytic, and lignolytic enzyme activities/(g microbial biomass C), as well as in situ net N mineralization/(g microbial biomass C), accounting for between 9% and 14% of process rates (Table 1).

Of the guilds representing at least 0.1% of the overall fungal community (Appendix S2: Fig. S2), cumulative respiration (per g microbial biomass C) and potential hydrolytic enzyme activity (per g microbial biomass C) increased with the abundance of taxa classified as soil and wood saprotrophs, as well as plant pathogens (Spearmen ρ = 0.55–0.58; P_{adjust} = 0.008–0.017). No individual guilds were significantly correlated with chitinolytic or lignolytic enzyme potential or net N

TABLE 1. Significant relationships between guild β-diversity and soil process rates standardized per unit microbial biomass C, as revealed by distance-based redundancy analysis.

Factor	F	R^2	P_{adjust}
Cumulative respiration	3.32	0.11	0.006
Chinitolytic potential	3.32	0.11	0.008
Hydrolytic potential	4.31	0.14	0.006
Lignolytic potential	3.51	0.12	0.006
Net N mineralization (*in situ*)	2.54	0.09	0.022

Notes: Cumulative respiration expressed as mg CO_2-C·[g soil C]$^{-1}$·[g microbial biomass C]$^{-1}$·d^{-1}, potential enzyme activities calculated as mmol substrate·[g microbial biomass C]$^{-1}$·h^{-1}; and net N mineralization mg N·[g soil microbial biomass C]$^{-1}$·d^{-1}. *P*-values were corrected for multiple comparisons using the Benjamini-Hochberg correction (P_{adjust}). Guild β-diversity was calculated from guilds representing >0.1% of sequence abundance across the plant diversity gradient, including (in descending order of abundance) undefined saprotroph, taxa dually classified as undefined saprotroph/symbiotroph, plant pathogen/soil and wood saprotroph, as well as plant pathogen, plant pathogen/wood saprotroph, dung-undefined-wood saprotroph, soil saprotroph, undefined endophyte, wood saprotroph, and arbuscular mycorrhizae (Appendix S2: Fig. S2).

mineralization following *P*-value corrections for multiple comparisons.

Discussion

Despite rigorous theory and empirical tests relating resource availability to plant diversity and productivity, we lack an analogous mechanistic understanding of the role that plant diversity structures soil fungal diversity, via changes in substrate supply and composition. Here, we show that changes in plant diversity influence the richness and composition of soil fungal communities by altering the quantity of organic substrates, as well as plant functional group diversity and composition, in ways that have functional consequences for soil C cycling. First, we consistently found higher fungal richness where plant biomass was high. Second, increasing substrate diversity and the relative dominance of individual plant functional types also had significant influences on fungal α- and β- diversity, although the relative importance of these factors varied with fungal trophic mode (i.e., saprotroph, pathotroph, symbiotroph). Finally, respective changes in fungal guild abundance across the plant diversity experiment had significant consequences for rates of soil C and N cycling, indicating the functional significance of different modes of fungal resource capture.

Plant diversity appeared to influence fungal richness largely through the positive relationship between plant diversity and plant biomass across the grassland biodiversity manipulation (Fig. 3), suggesting that access to organic substrates increased the fungal taxa able to meet their minimum resource requirements (Waldrop et al. 2006, Cline and Zak 2015). The relationship between plant biomass and fungal richness was linear, not unimodal (as reported by Waldrop et al. 2006, Langenheder and Prosser 2008). Thus, it appears that experimental plots with high plant richness did not increase organic substrate availability to the point that belowground competition for mineral nutrients began limiting fungal richness (i.e., reduced niche dimensionality; Harpole et al. 2016). While plant richness appears to structure belowground richness at local or regional scales (Mitchell et al. 2010, Hiiesalu et al. 2014, LeBlanc et al. 2015, Chen et al. 2017), there is less evidence of a global relationship between plant and fungal diversity. Fungal richness exhibited the latitudinal diversity gradient commonly observed in plants, although this global pattern appeared to be best explained by parallel responses to climatic and edaphic variables (Tedersoo et al. 2014). Furthermore, grassland fungal communities across continents were more strongly shaped by variation in plant community composition relative to plant species richness (Prober et al. 2015). Because plant productivity does not appear to predict plant species richness at the global scale (Adler et al. 2011), global investigations of plant-microbial richness patterns may fail to target changes in microbial substrate availability. Alternatively, weak relationships between plant and fungal diversity at these broad spatial scales may be the result of large variability in abiotic factors combined with the low sampling density, relative to studies across smaller spatial scales, required to span the globe.

Unlike the strong relationship observed between fungal richness and aboveground plant biomass, fungal community composition (i.e., β-diversity) was explained by comparable –albeit small– contributions from C_4 grass and legume relative dominance, plant functional group diversity, and plant biomass (Fig. 4). Thus, the abundance of individual fungal taxa appeared sensitive to particular plant functional groups, plausibly through an influence on litter biochemistry and available plant hosts (Spehn et al. 2000, Fornara et al. 2009). For example, the relative dominance of C_4 grasses likely suppressed the abundance of saprotrophic taxa in *Penicillium* and *Aspergillus* (Fig. 5), due to increasingly recalcitrant litter chemistry (Appendix S2: Fig. S1; Knops and Tilman 2000, Cline and Zak 2015). As cellulolytic Ascmoycetes dominate the early stages of litter decay (Frankland 1998, Voříšková and Baldrian 2013), it is plausible these genera were outcompeted by fungi with hemicellulolytic and lignolytic capacities when C_4 grasses dominated. In contrast, many significant correlations between fungal taxa and legume relative dominance were positive, suggesting that the relatively N-rich litter of legumes favored particular saprotrophic and pathotrophic taxa.

Individual fungal trophic modes responded to different metrics of plant substrate availability along the plant diversity gradient. Specifically, while the richness of saprotrophs, symbiotrophs, and pathogens consistently increased with aboveground plant biomass, saprotrophic richness was positively related to plant functional group

diversity. This pattern could be explained by the varied metabolic capacities of saprotrophic fungi (de Boer et al. 2005, Baldrian 2006, McGuire et al. 2010) in conjunction with the increasing number of niches introduced by biochemically diverse plant litter (Grayston et al. 1998, Loreau 1998). Along the same lines, only symbiotroph richness (primarily AMF; Appendix S2: Fig. S2) increased with legume relative abundance, a plant functional group associated with increasing soil N availability (Knops and Tilman 2000, Fornara and Tilman 2008). At the low end of soil N availability, greater soil N may increase the opportunity for symbiotic fungi to transfer soil N to host plants (George et al. 1995, Fellbaum et al. 2012). Thus, soil N added by legume N-fixation in the sandy OM-poor soils of our experiment could plausibly stimulate symbiotroph richness.

Although organic substrate availability significantly shaped fungal richness and composition, we accounted for a relatively small amount of the total variation in fungal communities across the plant diversity gradient. One potential explanation of the unexplained variation in fungal α- and β-diversity is that by measuring root biomass and chemistry in a subset of plots, we failed to adequately characterize the belowground C inputs (i.e., roots and microbial detritus), which are increasingly realized as critical to soil metabolism and the long-term storage of soil C (Clemmensen et al. 2013, Cotrufo et al. 2013). Additionally, it is plausible that soil fauna mediate plant diversity effects on fungal communities by changing the physical and chemical structure of plant detrital inputs (Carrillo et al. 2011) and altering fungal competitive dynamics during fungal herbivory (Crowther et al. 2011). Lastly, land use history may have limited the importance of the plant richness gradient in structuring soil fungal communities, due to either a lasting influence on organic substrates present in soil (Jangid et al. 2011) or historical contingencies that were introduced by fungal priority effects (Fukami et al. 2010).

Independent of the predominant community assembly mechanism at work, the varied composition of fungi across the plant diversity gradient significantly accounted for rates of soil C and N cycling when taxa were functionally classified (e.g., soil saprotroph, arbuscular mycorrhizae, plant pathogen; Table 1). The significance of guild β-diversity in accounting for soil process rates supports mounting evidence that method of resource capture influences the metabolic capacities and substrate-use efficiencies of fungi to break down organic substrates (Brzostek et al. 2015, Tunlid 2015, Bödeker et al. 2016). Whereas, the lack of significant relationship between OTU β-diversity (i.e., species-level classification) and soil respiration, potential enzyme activity or net N mineralization indicates fungal taxa within guilds have overlapping metabolic capabilities. While soil process rates were quantified a year earlier than fungal community characteristics, the consistent plant inputs and microbial biomass C quantified between years (Appendix S2: Figs. S4, S5) suggests that treatment effects on the fungal community and soil process rates were likely preserved between years. Furthermore, soils were collected in July across both years, constraining any potential variation that would be associated with seasonality. Convergent functions across distinct fungal communities have been described as functional redundancies that likely occur due to common selection pressures (Talbot et al. 2014). However, it is also possible that functional equivalencies depend on environmental context (Fetzer et al. 2015), such that specific environmental conditions result in similar efficiencies between enzymes that mediate the same biological process. Thus, characterizing fungi by fungal traits may be an increasingly promising approach to explain variations in biogeochemical cycling (Crowther et al. 2014, Aguilar-Trigueros et al. 2015).

Conclusions

Across a grassland biodiversity manipulation, our study demonstrates that plant species richness and functional composition collectively structured fungal communities and soil processes. While these findings support previous observations of the relationship between plants and fungi (Waldrop et al. 2006, LeBlanc et al. 2015), our manipulation of plant species richness and functional group composition provides a mechanistic framework to begin to understand how plant-mediated changes in organic substrate availability contribute to the composition and functioning of soil communities. Similar to observed plant richness patterns along nutrient gradients (Theodose and Bowman 1997), we found that fungal richness increased with total substrate availability along the plant diversity gradient. We also provided evidence that analogous life history tradeoffs occurred between plants and fungi along a resource gradient, due to increasingly distinct plant and fungal communities along their respective resource gradients (i.e., soil N and organic substrates). Despite these parallels, the relatively large amount of unexplained variation in fungal communities could indicate the potential importance of fine-scale variation in belowground inputs (i.e., root or microbial detritus), soil fauna, or priority effects acting as additional governors of fungal community assembly. To ultimately elucidate the factors that govern soil biogeochemical cycling, these insights highlight the importance of integrating microbial community studies into a unified conceptual framework (Vellend 2010, Nemergut et al. 2013) as well as the need to understand how community assembly mechanisms influence fungal traits.

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Literature Cited

Adler, P. B., et al. 2011. Productivity is a poor predictor of plant species richness. Science 333:1750–1752.

Aerts, R., F. Berendse, H. DeCaluwe, and M. Scmitz. 1990. Competition in heathland along an experimental gradient of nutrient availability. Oikos 57:168–172.

Aguilar-Trigueros, C. A., et al. 2015. Branching out: towards a trait-based understanding of fungal ecology. Fungal Biology Reviews 29:34–41.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Baldrian, P. 2006. Fungal laccases – occurrence and properties. FEMS Microbiology Reviews 30:215–242.

Bardgett, R. D., W. D. Bowman, R. Kaufmann, and S. K. Schmidt. 2005. A temporal approach to linking aboveground and belowground ecology. Trends in Ecology & Evolution 20:634–641.

Bedford, B. L., M. R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. Ecology 80:2151–2169.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological) 57:289–300.

Berg, B., and C. McClaugherty. 2008. Plant litter. Decomposition, humus formation, carbon sequestration. Second edition. Springer, Berlin, Germany.

Bödeker, I. T., B. D. Lindahl, A. Olson, and K. E. Clemmensen. 2016. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. Functional Ecology 30:1967–1978.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17:837–842.

Brzostek, E. R., D. Dragoni, Z. A. Brown, and R. P. Phillips. 2015. Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. New Phytologist 206:1274–1282.

Carrillo, Y., B. A. Ball, M. A. Bradford, C. F. Jordan, and M. Molina. 2011. Soil fauna alter the effects of litter composition on nitrogen cycling in a mineral soil. Soil Biology and Biochemistry 43:1440–1449.

Chapin, F. S. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11:233–260.

Chase, J. M., and M. A. Leibold. 2003. Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago, Illinois, USA.

Chen, Y.-L., T.-L. Xu, S. D. Veresoglou, H.-W. Hu, Z.-P. Hao, Y.-J. Hu, L. Liu, Y. Deng, M. C. Rillig, and B.-D. Chen. 2017. Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. Soil Biology and Biochemistry 110:12–21.

Chesson, P. L. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–358.

Clemmensen, K. E., A. Bahr, O. Ovaskainen, A. Dahlberg, A. Ekblad, H. Wallander, J. Stenlid, R. D. Finlay, D. A. Wardle, and B. D. Lindahl. 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339:1615–1618.

Cline, L. C., and D. R. Zak. 2015. Soil microbial communities are shaped by plant-driven changes in resource availability during secondary succession. Ecology 96:3374–3385.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19: 988–995.

Craine, J. M., J. Froehle, D. G. Tilman, D. A. Wedin, and F. S. Chapin. 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. Oikos 93:274–285.

Craine, J. M., D. Tilman, D. Wedin, P. Reich, M. Tjoelker, and J. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Functional Ecology 16:563–574.

Crowther, T. W., L. Boddy, and T. H. Jones. 2011. Outcomes of fungal interactions are determined by soil invertebrate grazers. Ecology Letters 14:1134–1142.

Crowther, T. W., D. S. Maynard, T. R. Crowther, J. Peccia, J. R. Smith, and M. A. Bradford. 2014. Untangling the fungal niche: the trait-based approach. Frontiers in Microbiology 5:579.

de Boer, W., L. B. Folman, R. C. Summerbell, and L. Boddy. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29: 795–811.

Doane, T. A., and W. R. Horwath. 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical Letters 36:2713–2722.

Fellbaum, C. R., E. W. Gachomo, Y. Beesetty, S. Choudhari, G. D. Strahan, P. E. Pfeffer, E. T. Kiers, and H. Bucking. 2012. Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences USA 109:2666–2671.

Fetzer, I., K. Johst, R. Schawe, T. Banitz, H. Harms, and A. Chatzinotas. 2015. The extent of functional redundancy changes as species' roles shift in different environments. Proceedings of the National Academy of Sciences USA 112: 14888–14893.

Floudas, D., et al. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719.

Fornara, D. A., and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96:314–322.

Fornara, D. A., D. Tilman, and S. E. Hobbie. 2009. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. Journal of Ecology 97:48–56.

Frankland, J. C. 1998. Fungal succession – unravelling the unpredictable. Mycological Research 102:1–15.

Fraser, L. H., J. Pither, A. Jentsch, M. Sternberg, M. Zobel, D. Askarizadeh, S. Bartha, C. Beierkuhnlein, and J. A. Bennett. 2015. Worldwide evidence of a unimodal relationship between productivity and plant species richness. Science 349:302–306.

Fukami, T., I. A. Dickie, J. Paula Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan, and R. B. Allen. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. Ecology Letters 13:675–684.

George, E., H. Marshner, and I. Jakobsen. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Critical Reviews in Biotechnology 15:257–270.

German, D. P., M. N. Weintraub, A. S. Grandy, C. L. Lauber, Z. L. Rinkes, and S. D. Allison. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43:1387–1397.

Gessner, M. O., C. M. Swan, C. K. Dang, B. G. McKie, R. D. Bardgett, D. H. Wall, and S. Hättenschwiler. 2010. Diversity

meets decomposition. Trends in Ecology & Evolution 25:372–380.
Goldberg, D. E., and T. E. Miller. 1990. Effects of different resource additions of species diversity in an annual plant community. Ecology 71:213–225.
Grayston, S. J., S. Wan, C. D. Campbell, and A. C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry 39:369–378.
Harpole, W. S., et al. 2016. Addition of multiple limiting resources reduces grassland diversity. Nature 537:93–96.
Hättenschwiler, S., N. Fromin, and S. Barantal. 2011. Functional diversity of terrestrial microbial decomposers and their substrates. Comptes Rendus Biologies 334:393–402.
Hiiesalu, I., P. Meelis, J. Davison, P. Gerhold, M. Metsis, M. Moora, M. Opik, M. Vasar, M. Zobel, and S. D. Wilson. 2014. Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. New Phytologist 203:233–244.
Hooper, D. U., and P. M. Vitousek. 1997. The effects of plant composition and diversity on ecosystem processes. Science 277:1302–1305.
Hooper, D. U., et al. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. BioScience 50:1049.
Inouye, R. S., N. J. Huntly, D. Tiiman, and J. R. Tester. 1987. Pocket gophers, vegetation and soil nitrogen along a successional sere in east central Minnesota. Oecologia 72:178–184.
Jangid, K., M. A. Williams, A. J. Franzluebbers, T. M. Schmidt, D. C. Coleman, and W. B. Whitman. 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43:2184–2193.
Johnson, N. C., D. L. Rowland, L. Corkidi, L. M. Egerton-Warburton, and E. B. Allen. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84:1895–1908.
Knops, J. M. H., and D. Tilman. 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. Ecology 81:88–98.
Knops, J. M. H., D. Wedin, and D. Tilman. 2001. Biodiversity and decomposition in experimental grassland ecosystems. Oecologia 126:429–433.
Kõljalg, U., et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22:5271–5277.
Kowalchuk, G. A., D. S. Buma, W. de Boer, P. G. L. Klinkhamer, and J. A. van Veen. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie van Leeuwenhoek 81:509–520.
Langenheder, S., and J. I. Prosser. 2008. Resource availability influences the diversity of a functional group of heterotrophic soil bacteria. Environmental Microbiology 10:2245–2256.
LeBlanc, N., L. L. Kinkel, and H. C. Kistler. 2015. Soil fungal communities respond to grassland plant community richness and soil edaphics. Microbial Ecology 70:188–195.
Legendre, P., and M. J. Anderson. 2006. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecological Monographs 69:1–24.
Legendre, P., and E. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280.
Loreau, M. 1998. Biodiversity and ecosystem functioning: a mechanistic model. Proceedings of the National Academy of Sciences USA 95:5632–5636.
McGuire, K. L., E. Bent, J. Borneman, A. Majumder, S. D. Allison, and K. K. Treseder. 2010. Functional diversity in resource use by fungi. Ecology 91:2324–2332.
Meier, C. L., and W. D. Bowman. 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proceedings of the National Academy of Sciences USA 105:19780–19785.
Mitchell, R. J., A. J. Hester, C. D. Campbell, S. J. Chapman, C. M. Cameron, R. L. Hewison, and J. M. Potts. 2010. Is vegetation composition or soil chemistry the best predictor of the soil microbial community? Plant and Soil 333:417–430.
Nemergut, D. R., et al. 2013. Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews 77:342–356.
Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241–248.
Oksanen, J., et al. 2016. vegan: community ecology package. R package version 2.3.4. https://CRAN.R-project.org/package=vegan
Peay, K. G., and T. D. Bruns. 2014. Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant-fungal interactions. New Phytologist 204:180–191.
Peay, K. G., P. G. Kennedy, and T. D. Bruns. 2011. Rethinking ectomycorrhizal succession: Are root density and hyphal exploration types drivers of spatial and temporal zonation? Fungal Ecology 4:233–240.
Prober, S. M., et al. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology Letters 18:85–95.
R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Riggs, C. E., and S. E. Hobbie. 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biology and Biochemistry 99:54–65.
Schneider, T., K. M. Keiblinger, E. Schmid, K. Sterflinger-Gleixner, G. Ellersdorfer, B. Roschitzki, A. Richter, L. Eberl, S. Zechmeister-Boltenstern, and K. Riedel. 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. ISME Journal 6:1749–1762.
Smith, D. P., and K. G. Peay. 2014. Sequence depth, Not PCR replication, improves ecological inference from next generation DNA sequencing. PLoS ONE 9:e90234.
Smith, S. E., and D. J. Read. 2010. Mycorrhizal Symbiosis. Academic Press, Cambridge, MA, USA.
Spehn, E. M., J. Joshi, B. Schmid, J. Alphei, and C. Körner. 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant and Soil 224:217–230.
Talbot, J. M., et al. 2014. Endemism and functional convergence across the North American soil mycobiome. Proceedings of the National Academy of Sciences USA 111:6341–6346.
Tedersoo, L., et al. 2014. Global diversity and geography of soil fungi. Science 346:1256688.
Theodose, T. A., and W. D. Bowman. 1997. Nutrient availability, plant abundance, and species diversity in two alpine tundra communities. Ecology 78:1861–1872.
Tilman, D., F. Isbell, and J. M. Cowles. 2014. Biodiversity and ecosystem functioning. Annual Review of Ecology, Evolution, and Systematics 45:471–493.

Tilman, D., S. S. Kilham, and P. Kilham. 1982. Phytoplankton community ecology: the role of limiting nutrients. Ecology 13:349–372.

Tilman, D., P. B. Reich, J. M. H. Knops, D. A. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science 294:843–845.

Tunlid, A. 2015. Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. New Phyologist 205:1443–1447.

Vellend, M. 2010. Conceptual synthesis in community ecology. Quarterly Review of Biology 85:183–206.

Verbruggen, E., M. G. Van Der Heijden, J. T. Weedon, G. A. Kowalchuk, and W. F. M. Rö-Ling. 2012. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. Molecular Ecology 21:2341–2353.

Voříšková, J., and P. Baldrian. 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME Journal 7:477–486.

Waldrop, M. P., D. R. Zak, C. B. Blackwood, C. D. Curtis, and D. Tilman. 2006. Resource availability controls fungal diversity across a plant diversity gradient. Ecology Letters 9: 1127–1135.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. Van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Willig, M. R. 2011. Biodiversity and productivity. Science 333:1709–1710.

Zak, D. R., D. F. Grigal, S. Gleeson, and D. Tilman. 1990. Carbon and nitrogen cycling during old-field succession: constraints on plant and microbial biomass. Biogeochemistry 11:111–129.

Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock, and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology 84: 2042–2050.

Supporting Information

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