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RESEARCH ARTICLE

Soil moisture and chemistry influence diversity of ectomycorrhizal fungal communities associating with willow along an hydrologic gradient

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One sentence summary: Soil moisture, pH and organic matter alter the ectomycorrhizal fungal species present in communities regardless of host plant identity.

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ABSTRACT

Influences of soil environment and willow host species on ectomycorrhizal fungi communities was studied across an hydrologic gradient in temperate North America. Soil moisture, organic matter and pH strongly predicted changes in fungal community composition. In contrast, increased fungal richness strongly correlated with higher plant-available phosphorus. The 93 willow trees sampled for ectomycorrhizal fungi included seven willow species. Host identity did not influence fungal richness or community composition, nor was there strong evidence of willow host preference for fungal species. Network analysis suggests that these mutualist interaction networks are not significantly nested or modular. Across a strong environmental gradient, fungal abiotic niche determined the fungal species available to associate with host plants within a habitat.

Keywords: Cedar Creek; *Salix*; niche; host preference; ectomycorrhizae; mutualism network

INTRODUCTION

The consequences of mutualisms for community assembly, composition and diversity remain underexplored (Morin 2011). Mutualisms can directly influence community structure by expanding the range of a facilitated species via reducing environmental or biotic stresses or indirectly via feedback mechanisms (Hacker and Gaines 1997; Stachowicz 2001). Many plants and microbes form functionally obligate relationships, such as between mycorrhizal fungi and plants, that shape the community assembly, diversity and function of both symbionts (Jonsson *et al.* 2001; Maherali and Klironomos 2007; Bever *et al.* 2010). Plants may influence ectomycorrhizal community assembly through direct mechanisms, such as host preference (Tedersoo *et al.* 2013) and potentially by differential resource allocation to more beneficial fungal partners (Kiers *et al.* 2011), as well as indirect mechanisms, such as modifying communities through litter inputs (Becklin, Pallo and Galen 2012). As an interface between soil and plant roots, ectomycorrhizal fungi may influence plant communities by enabling access to new nutrient pools (resource partitioning) and by shaping plasticity in plant physiology or traits (Reynolds *et al.* 2003; Bever *et al.* 2010; Friesen *et al.* 2011).

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Outcomes of plant–fungal interactions depend in part on environmental context due to niche requirements and traits of either partner—a fungus that is beneficial in one context, may not be beneficial in another context (Johnson, Graham and Smith 1997; Jonsson *et al.* 2001). For example, soil moisture can determine whether or not a specific fungus promotes plant growth (Kennedy and Peay 2007). This context dependence is likely a result of the physiological attributes of the fungus that influence responses to soil moisture (Mexal and Reid 1973; Cavender-Bares *et al.* 2009; Lennon *et al.* 2012) and changes in plant nutrient or growth requirements under different environmental conditions. Understanding the relationship between fungal and plant niches will help predict outcomes of plant–fungal interactions. This is particularly important in multiparty mutualisms where the identity of the interacting symbionts is highly variable and these changes may impact partner fitness. For example, ectomycorrhizal species differ in their functional traits, such as the ability to utilize organic and mineral substrates (Lilleskov *et al.* 2002; Lilleskov, Hobbie and Fahey 2002; Courty, Franc and Garbaye 2010), and differentially influence plant physiological and growth attributes, such as leaf chemistry and resource allocation (Baum *et al.* 2009; Fransson *et al.* 2013). On the other hand, plant host species may select different fungi depending on environmental context (Cavender-Bares *et al.* 2009) and host phylogenetic distance influences fungal community richness and composition (Tedersoo *et al.* 2013). Host preference between fungi and plants is likely an outcome of individual niche requirements of both partners—a dimension of the realized niche (Dickie 2007). As a result, partner niche requirements may play an important role in determining local fitness of each partner.

Examining plant fungal communities along an environmental gradient can reveal the niche requirements of both partners along the natural range of environmental contexts where the mutualism functions (Whittaker 1967; Bronstein 1989). Ectomycorrhizal fungi community responses to nutrient gradients are relatively well characterized (Toljander *et al.* 2006; Moeller, Peay and Fukami 2013) given early recognition of the nutrient benefit ectomycorrhizal fungi provide to plants. However, dimensions of fungal niches beyond nutrients are important in determining fungal community structure (Crowther *et al.* 2014)—fungi have their own physiological limits in addition to negotiating host relations (Allen *et al.* 1995).

Moisture is one of the most important environmental variables to life on Earth and the handful of studies on ectomycorrhizal community response to moisture suggest ectomycorrhizae are not exception. From the global to the root level, moisture alters fungal distibutions, community composition and colonization of roots (Lodge 1989; Robertson *et al.* 2006; Cavender-Bares *et al.* 2009; Tedersoo *et al.* 2013). At a landscape scale, precipitation, as well as temperature and soil texture, correlate with changes in ectomycorrhizal composition and richness across the earth (Tedersoo *et al.* 2012). Pot and culture-based experiments also show that moisture is an important niche axis for ectomycorrhizal fungi. For example, ectomycorrhizal fungal species vary in tolerance to flooding of soil (Stenstrom 1991) and in growth response to osmotic potential of the culturing media (Mexal and Reid 1973; Coleman, Bledsoe and Lopushinsky 1989; Lennon *et al.* 2012). Biotic interactions are also altered by moisture. Willow associate with both arbuscular and ectomycorrhizal fungi, which exclude each other across soil moisture gradients (Lodge 1989 and Gehring, Mueller and Whitham 2006).

Distinguishing the relative contribution of abiotic and biotic factors to ectomycorrhizal fungal community structure is often difficult in the field because plant host communities typically turn over across environmental gradients (Toljander *et al.* 2006). One way to study interaction between biotic and abiotic factors in the field is to compare fungal communities associated with the same host species, in the same genus, that span the same environmental gradient, bringing further insight to the fungal niche (Robertson *et al.* 2006; Moeller, Peay and Fukami 2013). Such work is an essential step for understanding relationships between fungal niche, community structure, and how both may influence outcomes of plant–fungal mutualisms.

To study the consequences of changes in soil moisture and other abiotic and biotic factors for fungal community structure, we characterized ectomycorrhizal fungal communities associated with willow (genus *Salix*) species growing across a hydrologic gradient at Cedar Creek Ecosystem Science Reserve in southwest Minnesota, USA. Cedar Creek presents an ideal system to examine variations in hydrologic conditions at small spatial scales with replication across a landscape. Willow species segregate along this hydrologic gradient (Savage and Cavender-Bares 2012), partially based on their drought tolerance (Savage, Cavender-Bares and Verhoeven 2009; Savage and Cavender-Bares 2011). As a result of differences in physiological trade-offs, several willow species are habitat specialists in wetlands or uplands and other species are habitat generalists (Table 1). Nothing is known about the

Table 1. Willow species sampled, hydrology, soil data and habitat for each plot.

Plot	Willow species sampled	Mean depth to water table (m)	pH	Phosphorus (ppm)	Potassium (ppm)	Nitrate (ppm)	Organic matter (%)	Habitat type
1	*S. bebbiana*, *S. humilius*[a], *S. petiolaris*	−1.3	5.75	8.5	14	1.4	2.45	Sand Prairie
2	*S. discolor S. humilis*[a], *S. petiolaris*	−1	5.25	9.5	28.5	1.25	3.1	Sand Prairie
3	*S. bebbiana*, *S. humilis*[a], *S. petiolaris*	−0.9	5.4	8.5	77.5	1.65	4.2	Sand Prairie
4	*S. bebbiana*, *S. discolor*, *S. petiolaris*	−0.4	5.65	31.5	29	1.65	4.85	Wet Meadow
5	*S. bebbiana*, *S. discolor*	−0.4	5.75	6.5	12	2.05	0.9	Emergent Marsh
6	*S. bebbiana*, *S. discolor*, *S. petiolaris*	−0.3	6.25	28	24	1.3	1.05	Emergent Marsh
7	*S. candida*[b], *S. petiolaris*, *S. serissima*[b]	−0.1	6.275	10	19.75	1.3	76.92	Marsh
8	*S. discolor*, *S. pedicellaris*[b], *S. petiolaris*	−0.1	6.05	7.5	14.5	1.85	73.8	Marsh
9	*S. bebbiana*, *S. discolor*, *S. petiolaris*	0	6.3	25.5	32	1.55	19.35	Swamp
10	*S. candida*[b], *S. pedicellaris*[b], *S. petiolaris*	0	8	20	23	1.425	72.1	Marsh
11	*S. candida*[b], *S. petiolaris*, *S. serissima*[b]	0	6.15	9.5	8.5	1.7	73.1	Marsh
12[c]	*S. discolor*, *S. pedicellaris*[b], *S. petiolaris*	0	NA	NA	NA	NA	NA	Peatland

[a]upland specialist, all other species are generalists, [b]wetland specialist, based on habitat distribution (Savage 2010).
[c]Plot 12, a peat bog, consisted of sphagnum and soil data within the top meter was not available.

mycorrhizal fungi associated with the willow species in this system.

In this system we ask (i) Do ectomycorrhizal fungal communities associated with willow species change along a hydrological gradient? (ii) What abiotic factors explain changes in ectomycorrhizal community structure? (iii) Does willow species, ie. host identity, influence ectomycorrhizal community structure across the gradient? We expected fungal community composition to strongly respond to the hydrologic gradient and expected willow host identity to explain relatively less of the changes in fungal community structure.

METHODS

Study site, willow species and field sampling

Cedar Creek Ecosystem Science Reserve (Cedar Creek) and the Helen Allison Savannah Scientific and Natural Area (HAS) are located on the Anoka Sand Plain in southeastern Minnesota (45°25′ N, 93°12′ W). Southeastern Minnesota has a continental climate and the monthly mean temperature ranges from 22°C in July to −11.5°C in January.

This study focused on seven willow species of varied ecological amplitude naturally distributed across Cedar Creek and HAS (Savage 2010, see Table 1). Between May and August 2012, plots were established using GPS points for 12 plots from a previous willow study (Savage and Cavender-Bares 2012, see map in Fig. S1, Supporting Information). The depth of the water table from the soil surface varied among the 12 selected plots resulting in a hydrologic gradient, from inundated to dry soil. Each plot included at least three willow species and at least one habitat specialist co-occurred with habitat generalists at multiple plots. Study plots were of six habitat types (Table 1). Willow species dominate the study plots and various ectomycorrhizal hosts (for example, *Populus*, *Alnus*, *Larix*, *Quercus*, *Pinus*) grow nearby.

To sample ectomycorrhizal species, fine roots were traced and collected from nine willow plants at each plot; three willow species per plot and three individuals of each species. Distance was maximized between plants, but confined to 10 m from plot center. At four plots, only two individuals were found of these species: plot 5, *Salix petiolaris*; plot 7, *S. serissima*; plot 12, *S. discolor*; plot 11, *S. petiolaris* and *S. serissima*. A total of 93 individual willow plants were sampled. Tracing roots from the base of the willow stem ensured identity of species collected. Approximately 15 cm of secondary root length was delicately detached from main root and disentangled from soil—keeping fine roots attached. Root samples were sealed in sterile plastic bags, transported on ice, and then refrigerated at 4°C until processed within 4 days of collection. Each plot was sampled once between June and July.

To select a subset of root tips for identification gently washed fine roots were laid evenly on a square gridded dish and examined under a dissecting scope at ×100. A total of 12 ectomycorrhizal root tips per plant were randomly sampled by picking the ectomycorrhizal root tip nearest to a randomly generated coordinate on the gridded dish. Ectomycorrhizal status was verified for a subsample of roots at ×400 magnification by cross section and searching for Hartig net and mantel.

Hydrology and soil chemistry

Hydrologic status was quantified in all plots by measuring the depth from soil surface to the water table at each plot center. Briefly, 1.5 m PVC pipes were buried at plot centers and depth to water table was measured during the growing season (May–Sept) for two years, 2007–09 and averaged over that time (Savage and Cavender-Bares, 2011, 2012). For example, the water table is 1 m below the soil surface at plot 1 on average over the growing season; at the other end of the gradient, plot 12 is flooded on average over the growing season. Soil was collected once for chemical and nutrient analysis in June 2008 by Savage (2010). A total of 15 18.5 cm by 2.5 cm soil cores per plot were homogenized and analyzed at the University of Minnesota Analytical Lab for extractable phosphorus (Bray-1 method), potassium, nitrate, pH and percent organic matter following protocols from the NCR-13 Committee (1998) (Table 1).

Molecular identification of ectomycorrhizal fungi

Colonized root tip genomic DNA was extracted with the XNAT2 Extract-N-Amp Tissue Kit (Sigma-Aldrich, St Louis, Missouri, USA). The internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA genes were amplified using fungal-specific primers ITS1f (Gardes and Bruns 1993) and ITS4 (White *et al.* 1990). PCR was carried out under the following conditions: 95°C for 1 min initial step; 94°C for 30 s, 52°C for 30 s, 68°C for 1 min for 35 cycles; and a final elongation step at 68°C for 5 min. PCR products were run on 1.5% agarose gels in 1X TBE buffer pH 8.3 and visualized with GelRed nucleotide stain (Phenix Research Products, North Carolina, USA). For root tip samples with unsuccessful amplification, extracted DNA was diluted 10:1 or 20:1 and re-amplified. PCR products visualizing multiple bands were re-amplified using ITS1f and basidiomycete-specific primer ITS4b (Gardes and Bruns 1993). Sanger sequencing of PCR amplicons was done at Beckman Coulter Genomics, Massachusetts, USA.

To assign taxonomic rank to sequencing reads obtained from root tips, reads were manually processed and queried against ITS databases. Nucleotide base calls with an error probability greater than 5% were trimmed from read ends to improve read quality. Next, reads were assembled into contigs at 90% base pair similarity (68 contigs, 106 unassembled reads) and reads were individually inspected for base call quality. Contigs were disassembled and a final assembly was made at 97% base similarity to generate operational taxonomic units (OTUs). These read processing steps were completed in Geneious version 6.0 (http://www.geneious.com, Kearse *et al.* 2012). One read from each contig was searched against the NCBI and Unite databases to assign taxonomic names (Kõljalg *et al.* 2013). Ectomycorrhizal status of OTUs were determined according to Tedersoo, May and Smith (2010). Representative reads from each OTU were submitted to GenBank under the accession numbers KT275603 - KT275682 (Table S2, Supporting Information).

Data analysis

All analyses, except modularity, were completed in R version 3.1.2 (R Core Development Team 2013), also using the packages vegan (Oksanen *et al.* 2013) and ggplot2 (Wickham 2009). To summarize depth to water table for analyses, the mean depth to water table was calculated for May–August of 2007–09. Pearson's correlation was calculated between all soil chemistry and depth to water table variables.

Ectomycorrhizal richness estimates were calculated for plots and the willow species using the abundance-based coverage estimator (ACE) and Chao1 in EstimateS (Cowell 2013). To compare richness among plots and willows (since there is variation in sample size), species accumulation curves were also extrapolated to the largest sample size (Colwell *et al.* 2012). Analysis

of variance (ANOVA) was used to test for differences in richness among plots and willow species. Relationships between fungal richness, hydrology and soil chemistry were tested using linear regression; predictor variables were regressed untransformed as well as log-transformed. Stepwise regression with Akaike's Information Criterion was used to find the subset of variable(s) that best explained richness then the model was run with the best subset of variable(s).

To test if hydrology and soil chemistry explain variation in ectomycorrhizal community composition, we used redundancy analysis (RDA). RDA uses principle coordinates analysis and regression to test the relationship between two multivariate datasets. It is a nonparametric multivariate linear regression (McArdle and Anderson 2001). Raw ectomycorrhizal OTU counts were regressed against principle components of scaled hydrology and soil chemistry, plot 12 was excluded due to missing soil chemistry data. A permutation test was used to test the significance of environmental variable RDA axes in explaining community composition.

To visualize and test interaction patterns between willow and ectomycorrhizal species, we analyzed two network properties, nestedness and modularity (Bascompte and Jordano 2007; Olesen *et al*. 2007). In a perfectly nested species interaction network-matrix, a specialist plant would interact with a proper subset of fungal species from a more generalist plant, and vice versa for fungi (Bascompte *et al*. 2003; Ulrich, Almeida-Neto and Gotelli 2009). The nestedness metric based on overlap and decreasing fill (NODF) quantifies a matrix's degree of deviation from a perfectly nested matrix of the same size (Almeida-Neto *et al*. 2008). We calculated NODF for a maximally packed willow–ectomycorrhizal fungi interaction matrix, where 'packed' means the upper-left corner of the matrix is maximally filled. A conservative 'fixed-fixed' null model was used to generate 1000 randomized matrices to calculate a null NODF mean and 95% confidence intervals. This null model maintains row and column marginal totals when generating randomized matrices (Miklós and Podani 2004).

Modularity is a statistic that quantifies closely knit groups in a community (Newman 2006). To look for willow–fungal modules—groups that form more closely interacting communities within the entire network—we used the netcarto program (Guimera and Amaral 2005a,b). Netcarto uses a simulated annealing algorithm to find a partition of a network that maximizes the modularity statistic. Larger values of modularity (closer to 1) indicate highly intraconnected modules that are less interconnected with other modules. We used recommended, default settings in Netcarto and a null modularity statistic was constructed by calculating average modularity and standard deviation of 1000 randomized networks (Guimera, Sales-Pardo and Amaral 2004).

RESULTS

Overall, ectomycorrhizal root tip identification rate was 56% (694 reads out of 1236 picked tips). We identified 81 ectomycorrhizal taxa (OTUs) within 24 genera (Fig. 1); few taxa were highly abundant, while many taxa were rare (Table S1, Supporting Information). *Tomentella* was the richest, most frequent genus with 25 taxa and comprised 36% of the OTUs. *Tomentella ellisii* alone accounted for 13% of OTUs. *Sebacina* and *Inocybe* were the next most taxa-rich genera with eight and six OTUs, respectively, followed by *Russula* and *Peziza* with 9.8% and 9.3% of the OTUs. The species accumulation curves for plots and willows did not reach an asymptote, thus more sampling would uncover additional unique species (Figs S2 and S3, Supporting Information).

Percent organic matter and pH were positively correlated with depth to water table (Pearson's $r = 0.64$, $P = 0.03$). Higher pH and percent organic matter tended to occur at plots with higher water tables. Other soil variables were not significantly correlated.

The ACE and Chao1 provided similar, consistent estimates. The observed OTUs, ACE and estimated percentage of the community captured are reported for each plot and willow species

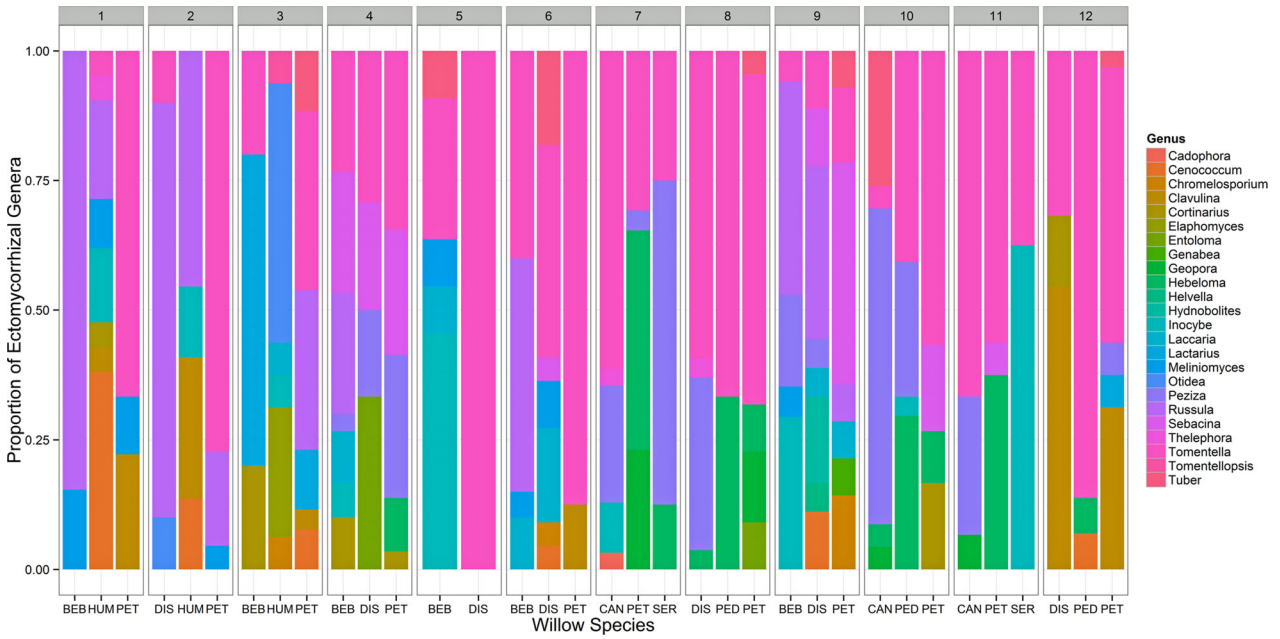

Figure 1. Relative abundance of ectomycorrhizal genera on willow species in each plot. The three individuals of each willow species per plot have been pooled. Each bar represents the proportion of ectomycorrhizal genera present at a plot scaled to 100 percent. Plots on x-axis are ordered from low to high water table (dry to saturated soils). Willow species abbreviations correspond to these species: BEB, S. bebbiana; CAN, S. candida; DIS, S. discolor; HUM, S. humilis; PED, S. pedicellaris; PET, S.petiolaris; SER, S. serissima.

Table 2. Ectomycorrhizal root tips sample sizes (n) and community richness per plot.

Plot	n	Observed taxa	ACE	% community captured (Obs/ACE*100)
1	43	10	15	67%
2	54	9	11	82%
3	47	13	20	65%
4	83	19	19	100%
5	17	5	7	71%
6	50	19	30	63%
7	73	12	17	71%
8	76	12	16	75%
9	49	21	23	91%
10	80	12	12	100%
11	39	13	14	93%
12	83	14	15	93%

Table 3. Ectomycorrhizal root tips sample sizes (n) and community richness tabulated for each willow species across all plots.

Salix spp.	n	Observed taxa	ACE	% community captured (Obs/ACE*100)
S. bebbiana	96	21	21	100%
S. candida	69	12	14	86%
S. discolor	129	37	51	73%
S. humilis	59	18	35	51%
S. pedicellaris	83	11	12	92%
S. petiolaris	234	41	43	95%
S. serissima	24	7	7	100%

(Tables 2 and 3). Phosphorus concentration was the single best linear model fit for OTU richness across plots and was significantly positively correlated with richness (Fig. 2). There was not a significant trend between willow host identity and fungal taxa richness (Fig. 3).

Ectomycorrhizal community composition across plots was significantly explained by the first RDA axis ($P = 0.025$) and the individual soil variables of depth to water table ($P = 0.004$) and organic matter ($P = 0.045$). The entire RDA model was significant ($P = 0.045$) (Fig. 4).

The nestedness of the willow–fungal network, NODF = 42.27, was not different from the null model, NODF = 42.77 (41.35, 43.74). The netcarto modularity algorithm identified five modules: three groups of fungi that interact with habitat generalist willows (*S. bebbiana, S. discolor, S. petiolaris*), one group that included all wetland specialist willows (*S. candida, S. pedicellaris, S. serissima*), and the upland willow (*S. humilis*) with its own fungal group (Fig. S4, Supporting Information). The modularity of this willow–fungal network (0.3980) was not significantly different than the null modularity statistic (0.4055 ± 0.009).

DISCUSSION

Our data show that ectomycorrhizal community structure changes along multiple, co-varying abiotic gradients in southeastern Minnesota. Two aspects of community structure are influenced by separate abiotic variables, while willow host species identity does not appear to structure communities. Fungal taxa present in a community are associated with soil organic matter and hydrology, while taxa richness is influenced by soil nutrient status, primarily phosphorus concentration. Given turnover in ectomycorrhizal community structure and evidence for distinct groups of plant–fungal interactions along the hydrologic gradient, willow plants must associate with different suites of fungi as soil abiotic characteristics change.

Fungal richness

We found that ectomycorrhizal richness positively correlates with phosphorus availability. This positive correlation in contrast to typical nutrient-taxa richness relationships, where higher nutrient status of soil is often negatively correlated with ectomycorrhizal richness (Lilleskov, Hobbie and Fahey 2002;

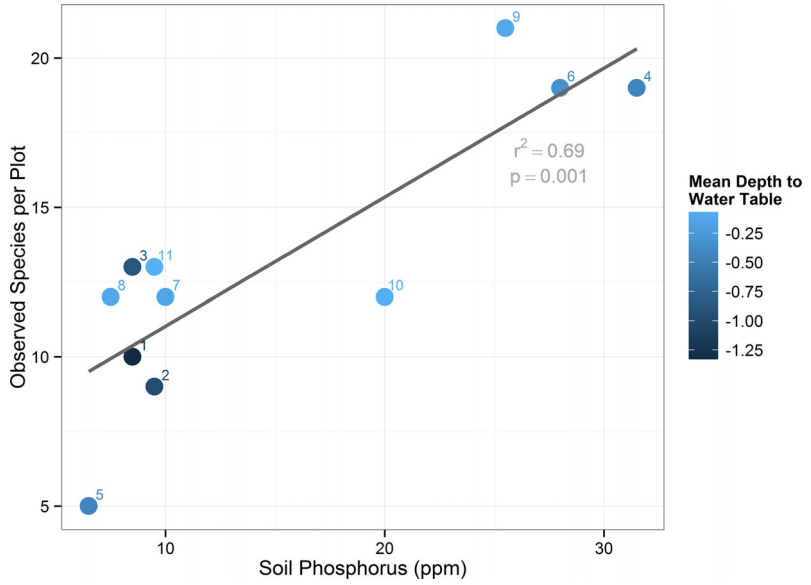

Figure 2. Observed ectomycorrhizal richness is significantly positively correlated with level of phosphorus per plot ($P = 0.001$). Darker shaded points indicate low water table at the plot (dry), lighter points indicate high water table at the plot (wet). The legend key indicates how color corresponds to the depth from the soil surface to the water table in meters; 0 = the plot is flooded, −1 = water table is 1 m below soil surface. Plot number is indicated next to each point.

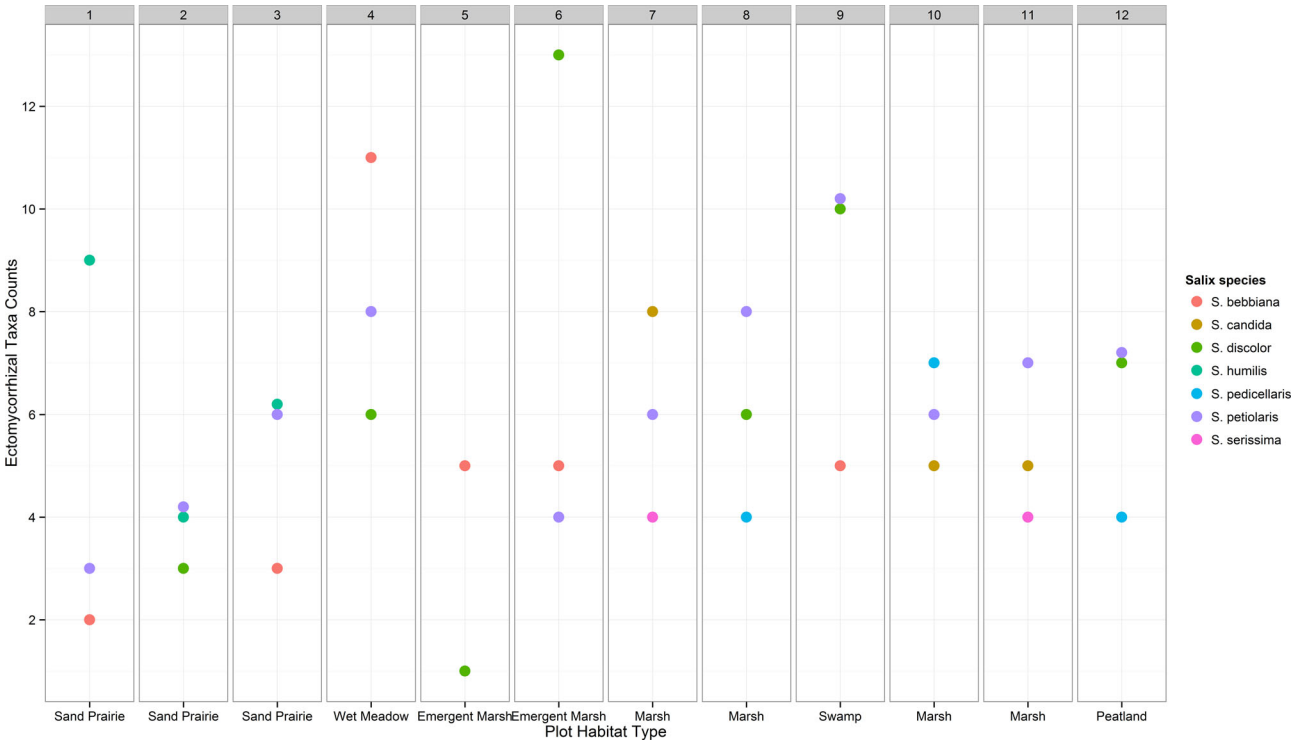

Figure 3. Ectomycorrhizal taxa counts on willow species per plot (individuals per species are pooled at each plot). Plot number is indicated at the top of each panel; habitat association is indicated on the *x*-axis. Point color indicates willow (*Salix*) species.

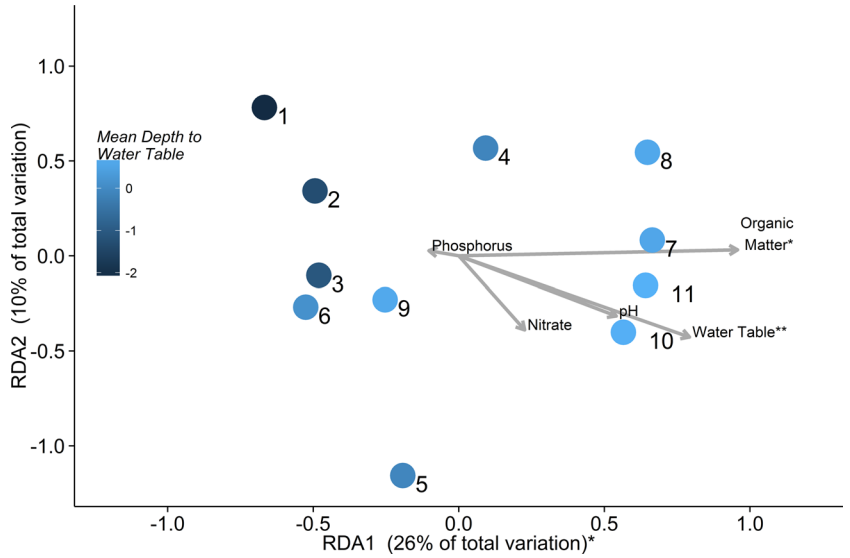

Figure 4. RDA of soil variables and ectomycorrhizal community composition. Darker shaded points indicate plots with low water table (dry, well-drained soils), lighter points indicate plots with high water table (inundated soils). RDA1, organic matter, and depth to water table significantly explained variation in ectomycorrhizal communities; significance level is indicated by $^{*}(P > 0.05)$ or $^{**}(P > 0.01)$. Arrows indicate direction and strength of abiotic variables in explaining ectomycorrhizal community composition.

Parrent, Morris and Vilgalys 2006; Cox *et al.* 2010). In this system, plots with high phosphorus availability and high ectomycorrhizal richness have nitrate concentrations similar to low richness plots, which may suggest that the positive correlation could be due to increased niche space for ectomycorrhizal fungi to scavange nitrogen for plants, if the plants have high nitrogen demand as a result of greater phosphorus availability. Additionally, abiotic factors that co-vary may interact to limit nutrients (nitrate or phosphorus) available to plants and fungi, for example soil moisture and pH control availability of mineral nutrients to plants (Barrow 1984; Garnett and Smethurst 1999; Hinsinger 2001).

Biotic factors could also contribute to this richness pattern. Willow associate with both ectomycorrhizal and arbuscular mycorrhizal fungi. Phosphorus is exchanged in both arbuscular and ectomycorrhizal symbiosis, but arbuscular mycorrhizae are

more effective at transporting phosphorous, while nitrogen is the primary currency for ectomycorrhizae (Smith, Smith and Jakobsen 2003). While we do not have arbuscular mycorrhizal colonization data for these roots, studies that have focused on dual colonization of willows find that ectomycorrhizal and arbuscular mycorrhizal fungi exclude each other along soil moisture gradients (Lodge 1989; Gehring, Mueller and Whitham 2006) and across ecotones (Becklin, Pallo and Galen 2012). The positive correlation between ectomycorrhizal richness and phosphorus could be explained by a hand-off from colonization by arbuscular mycorrhizae at low phosphorus to ectomycorrhizae at high phosphorus concentrations. Lastly, we could not effectively compare ectomycorrhizal richness of willow host species due to uneven sampling, but, in other willow systems, host phylogenetic distance has explained differences in ectomycorrhizal richness (Tedersoo *et al.* 2013).

Ectomycorrhizal community composition

This study system allowed us to examine the effects of different abiotic variables on ectomycorrhizal community composition. Composition clearly shifts in response to a complex soil gradient—particularly in percent organic matter and water availability—that characterizes the wetland to upland transition at Cedar Creek in MN. Willow species sort along the same gradient, influenced by both hydrology and soil organic matter (Savage 2010; Savage and Cavender-Bares 2012).

Changes in fungal community composition across the gradient suggest that fungi are limited by niche requirements or tolerances along this gradient and that different fungal taxa are available to plants depending on soil moisture and chemistry. These field results reflect lab experiments that show the growth of ectomycorrhizal fungi is reduced at low water potentials and tolerance to water stress varies among species (Mexal and Reid 1973; Coleman, Bledsoe and Lopushinsky 1989; Stenstrom 1991).

The correlation between ectomycorrhizal community turnover with increasing organic matter parallels differences found in utilization of organic matter by fungi; ectomycorrhizal species vary in ability to produce proteolytic enzymes allowing utilization of organic nitrogen (Lilleskov *et al.* 2002; Lilleskov, Hobbie and Fahey 2002; Courty, Franc and Garbaye 2010). Environment explained about forty percent of the variation in community composition; other willow–ectomycorrhizal studies suggest that willow and site age (Parádi and Baar 2006) and willow host phylogenetic distance (Tedersoo *et al.* 2013) might further explain composition differences. Overall, the ectomycorrhizal taxa identified in this study were similar to other willow-associated ectomycorrhizal communities (Nara 2006; Parádi and Baar 2006; Hrynkiewicz *et al.* 2012).

Ectomycorrhizal–willow network

We used network analysis to map patterns of species interactions, to evaluate evidence for host preference and ectomycorrhizal niche overlap. Willow–fungal interactions in this study did not have a strong network structure (were not significantly nested or modular). Growing literature on symbiotic fungal networks suggests that their structures differ from other mutualists, such as pollinators, and ectomycorrhizal–plant networks tend to be less nested or non-nested and modular than expected in a random model; potentially due to changing biotic and abiotic conditions or insufficient sampling (Chagnon, Bradley and Klironomos 2012; Montesinos-Navarro *et al.* 2012; Bahram, Harend and Tedersoo 2014; Toju *et al.* 2014). Results of this study are in line with both hypotheses: abiotic variables had a strong effect on ectomycorrhizal communities suggesting fungi occupy contrasting abiotic niches, while, evidence for host preference is lacking and species accumulation curves did not reach an asymptote. Although the three generalist willows, the upland specialist willow and the wetland specialist willows were found to interact closely with different groups of fungi, these groups were not significant compared to the mean modularity of randomly constructed networks of the same size. The lack of significant host preference also shows that willow plants must associate with fungal symbionts from the local species pool that tolerate the environmental conditions. Other studies find both that host preference and environment structure ectomycorrhizal communities (Ishida, Nara and Hogetsu 2007; Tedersoo *et al.* 2013) and that environment structures ectomycorrhizal communities, with no evidence for host preference (Peay *et al.* 2015).

Overall, our results suggest that abiotic factors outweight host preference in driving ectomycorrhizal richness and distributions across hydrological gradients in this willow-dominated system. Due to the compound nature of these environmental gradients and the complexity of mutualisms—partners respond individually and interactively to varied environmental contexts—futher experiments are necessary to fully explain the direct mechanism for these patterns in ectomycorrhizal communities of willows. Future studies should be designed to tease apart the influence of mycorrhizal exclusion, fungal traits, soil moisture and chemistry on ectomycorrhizal and host distributions.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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