

[This document contains the author's accepted manuscript. For the publisher's version, see the link in the header of this document.]

**Paper citation:**

Becklin KM, Hertweck KL, and Jumpponen A. 2012. Host identity impacts rhizosphere fungal communities associated with three alpine plant species. *Microbial Ecology* 63:682-693.

KU ScholarWorks: <http://hdl.handle.net/1808/8740>

DOI: 10.1007/s00248-011-9968-7

**Abstract:**

Fungal diversity and composition are still relatively unknown in many ecosystems; however, host identity and environmental conditions are hypothesized to influence fungal community assembly. To test these hypotheses we characterized the richness, diversity, and composition of rhizosphere fungi colonizing three alpine plant species, *Taraxacum ceratophorum*, *Taraxacum officinale*, and *Polemonium viscosum*. Roots were collected from open meadow and willow understory habitats at treeline on Pennsylvania Mountain, Colorado, USA. Fungal small subunit ribosomal DNA was sequenced using fungal-specific primers, sample-specific DNA tags, and 454 pyrosequencing. We classified operational taxonomic units (OTUs) as arbuscular mycorrhizal (AMF) or non-arbuscular mycorrhizal (non-AMF) fungi, then tested whether habitat or host identity influenced these fungal communities. Approximately 14% of the sequences represented AMF taxa (44 OTUs) with the majority belonging to *Glomus* group A and B. NON-AMF sequences represented 186 OTUs belonging to Ascomycota (58%), Basidiomycota (26%), Zygomycota (14%), and Chytridiomycota (2%) phyla. Total AMF and non-AMF richness were similar between habitats, but varied among host species. AMF richness and diversity per root sample also varied among host species and were highest in *T. ceratophorum* compared to *T. officinale* and *P. viscosum*. In contrast, non-AMF richness and diversity per root sample were similar among host species except in the willow understory where diversity was reduced in *T. officinale*. Fungal community composition was influenced by host identity, but not habitat. Specifically, *T. officinale* hosted a different AMF community than *T. ceratophorum* and *P. viscosum*, while *P. viscosum* hosted a different non-AMF community than *T. ceratophorum* and *T. officinale*. Our results suggest that host identity has a stronger effect on rhizosphere fungi than habitat. Furthermore, although host identity influenced both AMF and non-AMF this effect was stronger for the mutualistic AMF community.

**Text of paper:**

**Host identity impacts rhizosphere fungal communities associated with three alpine plant species**

Katie M. Becklin<sup>1,3</sup>, Kate L. Hertweck<sup>1</sup>, and Ari Jumpponen<sup>2</sup>

<sup>1</sup>Division of Biological Sciences, University of Missouri, Columbia, MO 65211

<sup>2</sup>Division of Biological Sciences, Kansas State University, Manhattan, KS 66506

<sup>3</sup>Current Address: Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045

Author for Correspondence:

Katie M. Becklin

Address: 1200 Sunnyside Avenue, 5032 Haworth Hall, Lawrence, KS 66045

Tel: +01 785 864 3848 Fax: +01 785 864 5860

Email: [kbecklin@ku.edu](mailto:kbecklin@ku.edu)

Date Submitted: June 24, 2011

## Introduction

Fungi are ubiquitous and play a key role in plant communities as pathogens, saprobes, and symbionts. These organisms can influence plant growth and fitness [1], community structure and composition [2], and ecosystem function [3]. A growing body of evidence indicates that many of these effects depend on fungal identity [4, 5]; however, only a small fraction of the estimated 1.5 million fungal species has been characterized [6]. This is particularly true for soil microbial communities where diversity is thought to be especially high [7, 8], yet technical issues hamper species isolation and identification. The lack of information regarding soil fungi poses a problem in terms of fully understanding the importance of fungal diversity and predicting how changes in the fungal community will impact ecosystem processes [9].

Partner identity and environmental conditions have been shown to influence the strength of plant-fungal interactions and subsequent feedbacks [4, 10, 11]. Even fungi capable of colonizing numerous host species (e.g., arbuscular mycorrhizal fungi) often exhibit some degree of host specificity [10, 12-14], which can generate species-specific feedbacks that influence both above- and belowground community composition [2]. Since abiotic conditions can alter these plant-fungal feedbacks [11], rhizosphere communities also likely vary along environmental and habitat gradients [e.g., 15]. Characterizing the rhizosphere fungi associated with widespread hosts that co-occur along such gradients may provide insight into the relative importance of host identity and habitat in structuring these fungal communities. Alpine ecosystems are particularly well suited to testing these questions since the heterogeneous nature of alpine soil, frequency of disturbance, and sharp environmental boundaries [16] could generate significant variation in fungal communities across relatively small spatial scales. At the same time, many alpine plant species are distributed across fairly wide environmental gradients at and above treeline (e.g., *Polemonium viscosum* [17]). In this study we take advantage of significant microhabitat heterogeneity in treeline communities to test the relative importance of habitat and host identity on rhizosphere fungi.

Most fungal phyla and families are functionally diverse, containing pathogens, saprobes, and mutualistic symbionts, which makes it difficult to assign a specific function to a group of even closely related fungi. In contrast, arbuscular mycorrhizal fungi (AMF) comprise the phylum Glomeromycota and are functionally similar to each other. AMF are generally viewed as mutualistic symbionts, providing their host plants with soil resources in exchange for photosynthate [18]. In this study, we focus on AMF as an identifiable functional group within the overall rhizosphere community. Studies employing molecular methods to characterize AMF diversity indicate that a single host species can associate with as many as 38 AMF taxa [14]. However, many AMF appear to have limited geographic distributions [19, 20] and exhibit varying degrees of host specificity [12, 13]. Consequently, both habitat and host identity may be important determinants of AMF diversity and composition.

Over a decade ago Gardes and Dahlberg [21] highlighted the general lack of information about mycorrhizal associations in arctic and alpine ecosystems. Since that time relatively few studies have explored or characterized fungal diversity in these ecosystems. Mycorrhizal fungal diversity is predicted to be lower in arctic and alpine regions due to environmental constraints, dispersal barriers, and an increased number of facultative or nonmycorrhizal host species [22, 23]. Support for this hypothesis based on studies conducted across latitudinal and elevational gradients is mixed [15, 20, 24, 25]. Thus, additional surveys spanning a greater number of sites and host species is needed to fully address the question of fungal diversity in arctic and alpine ecosystems. In this study we expand the information available for alpine ecosystems by assessing fungal diversity in three host species and two treeline habitats.

Using a combination of sample-specific DNA tags and direct 454 pyrosequencing we tested whether host identity or habitat affected the richness, diversity, and composition of arbuscular mycorrhizal (AMF) and non-arbuscular mycorrhizal (non-AMF) fungi associated with *Taraxacum ceratophorum*, *Taraxacum officinale*, and *Polemonium viscosum* plants growing in open meadow and willow understory habitats at treeline on Pennsylvania Mountain, Colorado, USA. Based on the importance of species-specific feedbacks involving mycorrhizal and nonmycorrhizal fungi in other study systems [26, 27] we hypothesized that rhizosphere fungal communities vary among *T. ceratophorum*, *T. officinale*, and *P. viscosum*. Given the patchy distribution of fungi in alpine ecosystems [24, 28, 29] we also hypothesized that unique fungal communities persist in open meadow and willow understory habitats. We evaluated these hypotheses for both AMF and non-AMF to determine if the mutualistic AMF community and functionally diverse non-AMF community respond similarly to host and habitat characteristics.

## Methods

### *Study system and sampling*

Root samples were collected from the treeline region on Pennsylvania Mountain (Park County, CO, USA; 39° 15'N, 106° 07'W, 3590–3630 m a.s.l.) in early August of 2008. This site is located east of the continental divide, and as such, has a relatively dry climate with an average precipitation of 3.7 cm per month and an average temperature of 12 °C during June–August (NOAA National Climate Data Center 2002, <http://hurricane.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl>). At this location treeline is a mosaic of willow shrub and open meadow habitats, and environmental conditions vary across this willow-meadow ecotone. Specifically, temperature, light availability, and wind exposure are higher in the open meadow whereas leaf litter is more abundant in the willow understory [30, 31]. Many plant species occur in both habitats, but at this site overall plant diversity and density is greater in the open meadow than the willow understory [32]. Likewise, on Pennsylvania Mountain colonization by AMF is higher in the open meadow, while ectomycorrhizal fungi are more abundant in the willow understory [33]. Other microbes, including salicylate-mineralizing fungi, have also been shown to associate with alpine willows at other locations in the Rocky Mountains [28]. Sampling across the willow-meadow ecotone allowed us to evaluate whether microhabitat affects the diversity and composition of rhizosphere fungi.

To characterize the rhizosphere fungal community in alpine willow and meadow habitats we sampled roots from three widely distributed herbaceous plant species. *Taraxacum ceratophorum* (Asteraceae) and *Polemonium viscosum* (Polemoniaceae) are native alpine plants, whereas *Taraxacum officinale* (Asteraceae) is an exotic species in North America. All three plant species co-occur in open meadow and willow understory habitats on Pennsylvania Mountain. Previous research shows that *T. ceratophorum* is more heavily colonized by AMF than either *T. officinale* or *P. viscosum* [33], and more responsive to colonization than *T. officinale* [32]. Sampling these three host species allowed us to evaluate whether AMF and non-AMF exhibit host specificity.

We sampled roots from 10 plants per species per habitat for a total of 60 individuals distributed across a 0.7 km distance (sampling area = 3350 m<sup>2</sup>). Plants sampled from the open meadow were at least 3 m from the nearest willow. Replicate plants in either habitat were at least 3 m from each other. From each plant we collected multiple fine root fragments from the upper 10 cm of soil, totaling approximately 15 cm of root length per plant. The roots were rinsed with water to remove soil particles, surface sterilized for 15 minutes with 10% bleach, and preserved in 2× CTAB buffer until DNA extraction.

### *DNA extraction and sequencing*

Total DNA was extracted from each root sample and eluted in 100  $\mu\text{l}$  of the EB elution buffer using Qiagen Plant DNeasy kits (Qiagen, Valencia, CA, USA). We quantified the resulting DNA concentrations using a ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and adjusted each template to 2.0  $\text{ng } \mu\text{l}^{-1}$ . Fungal small subunit (SSU) ribosomal DNA sequences were amplified using primer constructs that combined the A and B primers for 454 pyrosequencing with the fungal-specific SSU primers nu-SSU-0817-5' and nu-SSU-1536-3' [34]. A 5 base pair (bp) DNA tag was inserted between the A and nu-SSU-1536-3' primers for post-sequencing sample identification. These SSU primers amplify DNA from fungi in all the major taxonomic groups [34]. The resulting 762 bp amplicon includes the V4 (partial), V5, V7 and V8 (partial) variable regions. SSU genes have been shown to be more informative than ITS genes for AMF [35]; however, these genes may lack resolution for some NON-AMF taxa [36, 37]. Each template was PCR-amplified in 20  $\mu\text{l}$  reactions containing 10 ng of the DNA template, 200  $\mu\text{M}$  of each dNTP, 200 nM of each primer, 2.5 mM of  $\text{MgCl}_2$ , 1 U of GoTaq DNA polymerase (Promega Corporation, Madison, WI, USA), and 4  $\mu\text{l}$  of the supplied PCR buffer. The PCR reactions were run under the following conditions: initial denaturation at 94  $^{\circ}\text{C}$  for 2 min, followed by 30 cycles of 94  $^{\circ}\text{C}$  for 1 min, 58  $^{\circ}\text{C}$  for 1 min, and 72  $^{\circ}\text{C}$  for 2 min, followed by final extension at 72  $^{\circ}\text{C}$  for 8 min. Each sample was amplified in three separate reactions; 10  $\mu\text{l}$  of each amplicon was pooled, resulting in a total of 30  $\mu\text{l}$  per sample. The pooled amplicons were purified using Agencourt AMPure PCR purification kits (AgenCourt Bioscience Corporation, Beverly, MA, USA). Equal amounts of the purified amplicons were combined to create three sequencing pools consisting of 20 randomly selected samples per pool. All six combinations of habitat and host species were represented in each pool. The sequencing pools were adjusted to a concentration of 10  $\text{ng } \mu\text{l}^{-1}$  using a RapidVap vacuum evaporation system, and then sequenced from the A-primer construct in a one sixteenth region of a 454 reaction using a GS FLX sequencer (454 Life Sciences, Branford, CT, USA) at the University of Florida's Interdisciplinary Center for Biotechnology Research (Gainesville, FL, USA). Raw 454 pyrosequencing files are deposited in the Sequence Read Archive (accession number: SRA023882.1).

### *Bioinformatics and OTU designation*

Sequences without a correct DNA tag or primer sequence, shorter than 195 bp, or with more than one ambiguous base were omitted from the dataset. DNA tags on the remaining sequences were replaced with a root sample designation, aligned using CAP3 [38], and assigned to operational taxonomic units (OTUs) at 90, 95, 97 and 99% sequence similarity using a minimum overlap of 100 bp. To facilitate comparisons with other studies we focus on the results for 97% sequence similarity; patterns in fungal richness, diversity, and evenness were similar at all four levels of sequence similarity unless otherwise noted. Other CAP3 parameters (e.g., matches, mismatches, and gap penalties) were left at the default settings. Root samples that did not meet a minimum sequencing depth of 50 sequences were omitted from the analysis, which reduced the total sample size to 36 individual root samples.

Sequencing errors (e.g., insertion and deletion errors, chimeric sequences, low-quality reads) may inflate richness and diversity estimates based on 454 pyrosequencing data [39, 40]. Some authors suggest that the majority of singletons, sequences that occur only once in the dataset and can comprise >60% of taxa detected through pyrosequencing, are the result of such errors [37, 41]. Consequently, we removed all singletons from the dataset to reduce the likelihood that sequencing errors might inflate fungal richness and diversity estimates.

One randomly selected sequence per OTU was assigned to a phylogenetic group (AMF or non-AMF) using two methods. First, we conducted a BLASTN search to identify the reference taxon to which the query had the highest sequence similarity, which we assume corresponds to taxonomic relationships and phylogenetic distance (Supplementary Information, Table S1). Second, we used ClustalX (version 2.0) to perform a “full/slow” multiple sequence alignment of OTU and reference sequences from the BLASTN search followed by neighbor-joining tree construction. Reference sequences from GenBank were retained in their full length to assist in supporting the backbone of the tree, although this increased the overall length of the alignment by including additional portions of the ribosomal region. Nucleotide positions containing gaps were included in the analysis. Overall, the phylogenetic analysis enabled us to verify phylogenetic assignments and account for potential issues with the BLASTN search due to lack of coverage and misidentified sequences in GenBank. We removed two OTUs from the dataset because ambiguous BLASTN and phylogenetic distance results prevented definitive assignment to a phylogenetic group. Sequences of each OTU used in the BLASTN and phylogenetic analyses are deposited in GenBank (accession numbers are listed in the Supplementary Information, Table S1; alignment and tree files are available in Appendix A).

We conducted a more thorough neighbor-joining analysis of the AMF taxa to evaluate confidence in our phylogenetic group assignments and to identify families represented in the dataset. All non-AMF sequences were removed from the alignment. To improve our ability to separate clades of AMF we added high-quality reference sequences comprising 69 unrepresented Glomeromycota taxa and six basidiomycete and ascomycete outgroup taxa [42]. Next, we conducted 1000 neighbor-joining bootstrap replicates to assess the confidence of tree topology. Finally, we trimmed the edges of the matrix, realigned the sequences using MUSCLE [43], and recalculated the neighbor-joining tree with 1000 bootstrap replicates (alignment and tree files are available in Appendix B).

### *Richness and diversity indices*

OTU frequencies were determined for each root sample, and these data were used to calculate OTU richness and diversity (Supplementary Information, Table S2). Overall OTU richness ( $S$ ) was calculated by summing the number of OTUs within each root sample. Shannon's diversity index ( $H' = -\sum p_i(\log_e(p_i))$ ), where  $p_i$  is the relative abundance of each OTU, was calculated for each root sample using ESTIMATES (version 8.2.0, [44]). OTU evenness was calculated as the ratio of Shannon's diversity index and OTU richness ( $H'/\ln S$ ). The above richness and diversity indices were calculated separately for AMF and non-AMF datasets at all four levels of sequence similarity.

### *Statistical analyses*

Each root sample represented an experimental unit, which allowed us to statistically evaluate differences in OTU richness, diversity, and evenness due to host species and habitat. For each response variable (richness, diversity, and evenness) we evaluated the effects of host identity and habitat on the rhizosphere fungal community using analysis of variance (ANOVA). To characterize differences in the response of AMF and non-AMF communities to host species and habitat we conducted separate ANOVAs for each fungal community. The relationship between AMF and non-AMF richness and diversity per root sample was evaluated using linear regression. The above statistical analyses were conducted using the statistical program JMP (version 8.0.2; SAS Institute, Inc.). Overall sampling effort per host species was assessed using rarefaction analysis in ESTIMATES.



Differences in fungal community composition were analyzed using PC-ORD (version 4.1, [45]). The relative abundance of AMF and non-AMF OTUs based on 97% sequence similarity were used to calculate community distance estimates using the Sorensen (Bray-Curtis) index; these estimates were visualized using nonmetric multidimensional scaling [46]. The optimal number of dimensions ( $k$ ) was selected using a Monte Carlo test of significance at each level of dimensionality ( $k = 1$  to 6) by comparing 100 runs with empirical data to 100 randomized runs with a random seed starting value. The three-dimensional solution produced stress values significantly smaller than those in the randomized runs for both fungal communities. Differences in community composition based on this three-dimensional ordination were analyzed using Multiresponse Permutation Procedure (MRPP) [46]. Since MRPP cannot account for interactions between factors, we analyzed differences due to host identity and habitat separately. For this analysis we report the  $P$ -value and the chance-corrected within-group agreement ( $A$ ), which represents within-group homogeneity compared to the random expectation.

## Results

### *Sequencing analysis and bioinformatics*

After controlling for sequencing depth per sample and sequence quality (presence of the DNA tag and primer, sequence length, and ambiguous bases), we retained a total of 3921 sequences from 36 root samples collected from three host species and two habitats (average length = 250 bp, median length = 245 bp). The total number of sequences acquired per root sample (average = 109, standard deviation = 49) did not differ significantly among host species ( $F = 0.71$ ,  $df = 2$ ,  $P = 0.50$ , 4.2% variance), habitat ( $F = 0.55$ ,  $df = 1$ ,  $P = 0.46$ , 1.6% variance), or host species within habitat ( $F = 0.69$ ,  $df = 2$ ,  $P = 0.51$ , 4.1% variance) indicating no bias in average sequencing depth among treatments. However, there was substantial variation in sequencing depth among individual root samples (Supplementary Information, Table S2).

The aligned matrix of OTUs and reference taxa included 9148 nucleotide positions, of which 68.8% (6291 nucleotide positions) was variable. Based on the BLASTN search and neighbor-joining analysis 13.8% of the sequences represented AMF (44 OTUs at 97% sequence similarity). The remaining 86.2% of the sequences represented various non-AMF taxa (186 OTUs at 97% sequence similarity). While it was difficult to reliably assign non-AMF sequences to a specific taxonomic group, the phylogeny and BLASTN results generally agreed as to which phylum the remaining sequences belong (non-AMF OTUs were 58% Ascomycota, 26% Basidiomycota, 14% Zygomycota, and 2% Chytridiomycota). Rarefaction analysis indicates that we approached, but did not achieve OTU saturation for each host species (Fig. 1a).

To further characterize the distribution of OTUs within the phylum Glomeromycota we conducted a more thorough neighbor-joining analysis using the AMF taxa only. After trimming the ends of the matrix, the alignment was 2424 nucleotide positions in length and 56.6% (1369 nucleotide positions) variable. The neighbor-joining phylogram of AMF OTUs and reference sequences (Fig. 2, full tree with labeled tips is available in the Supplementary Information, Appendix B) recovered a topology generally congruent to the published hypothesis of phylogenetic relationships within Glomeromycota [42]. OTUs were not equally distributed taxonomically or phylogenetically within Glomeromycota (Fig. 2). Most AMF richness occurred in Glomeraceae, with *Glomus* group A containing 28 OTUs and *Glomus* group B containing 10, together representing over 80% of the total number of AMF OTUs and 92% of AMF sequences. OTUs represented the longest branches in the tree and tended to cluster in clades without reference sequences, which suggests that these OTUs represent divergent taxa largely uncharacterized by current fungal systematics. Additionally, our assembly parameters required a

relatively high number of nucleotide changes (>7 bp in a 245 bp sequence) to identify as sequence as a separate OTU; thus, it is unlikely that sequencing artifacts produced this level of divergence.

### *Richness and diversity indices*

The total number of AMF taxa based on 97% sequence similarity was similar in open meadow (37 OTUs) and willow understory (35 OTUs) habitats, and 28 of the OTUs were present in both habitats. In contrast, the total number of AMF taxa varied among *T. ceratophorum* (36 OTUs), *T. officinale* (17 OTUs), and *P. viscosum* (25 OTUs). Eight AMF OTUs were present in all three host species, while 18 were present in only one host species. Individual root samples were colonized by 0–12 AMF OTUs (Supplementary Information, Table S2). AMF richness per root sample was significantly higher in the open meadow than the willow understory (Table 1, ANOVA, habitat effect,  $P = 0.05$ ). On average, there were 6.3 OTUs per root sample from the open meadow and 5.6 OTUs per root sample from the willow understory. The difference between habitats was only significant when OTUs were designated based on 97% sequence similarity (Supplementary Information, Fig. S1a). AMF richness and diversity per root sample were significantly higher in *T. ceratophorum* compared to *T. officinale* and *P. viscosum* (ANOVA, host effect,  $P = 0.003$  and  $0.01$ , respectively; Table 1 and Figs. 3a,b). The observed host effect on AMF richness and diversity was significant regardless of what level of sequence similarity was used to designate OTUs (Supplementary Information, Figure S1d,e). AMF evenness per root sample did not differ among host species or between habitats (Table 1 and Fig. 3c). Host identity explained more than 30% of the variation in AMF richness and diversity (Table 1). In contrast, habitat explained only 11.5% of the variation in AMF richness and 6.9% of the variation in AMF diversity (Table 1). Rarefaction analysis further supports the greater effect of host species compared to habitat on AMF richness (Fig. 1b,c).

The total number of non-AMF taxa based on 97% sequence similarity was similar in open meadow (135 OTUs) and willow understory (134 OTUs) habitats, and 83 of the OTUs were present in both habitats. In contrast, the total number of non-AMF OTUs varied among *T. ceratophorum* (115 OTUs), *T. officinale* (93 OTUs), and *P. viscosum* (105 OTUs). Thirty-seven of these OTUs were present in all three host species, while 95 non-AMF OTUs were present in only one host species. Individual root samples were colonized by 7–31 non-AMF OTUs (Supplementary Information, Table S2), and average non-AMF richness per root sample was similar across treatments (Table 1, ANOVA,  $P > 0.1$ ). Average non-AMF diversity and evenness per root sample varied among host species, but only in the willow understory where non-AMF diversity and evenness were reduced in *T. officinale* compared to *T. ceratophorum* and *P. viscosum* (Table 1, ANOVA, habitat  $\times$  host interaction,  $P = 0.007$  and  $0.01$ ; Fig. 3e,f). The observed habitat by host interaction was not significant at 99% sequence similarity (Supplementary Information, Figure S2b,c). Neither host identity nor habitat explained more than 6% of the variation in non-AMF richness; however, the interaction between host identity and habitat explained over 20% of the variation in non-AMF diversity and evenness (Table 1). Interestingly, non-AMF richness and diversity per root sample were not significantly correlated with AMF richness and diversity (linear regression,  $P = 0.12$  and  $0.50$ , respectively).

### *Community composition*

The most abundant AMF OTU represented 26–46% of the total number of AMF sequences for a given host species, and the identity of this OTU differed among the three host species. OTU 20 (HQ256922, *Glomus* group A, unable to identify a close reference species in the neighbor-joining tree), OTU 13 (HQ256915, *Glomus* group A, *Glomus aureum*), and OTU 2 (HQ256904, *Glomus* group A, *Glomus*

*hoi*) were the most abundant OTUs associated with *T. ceratophorum*, *T. officinale*, and *P. viscosum*, respectively (Fig. 2). The AMF community was characterized using a three-dimensional ordination solution representing 72% of the variation in AMF composition per root sample (Fig. 4a). MRPP analyses indicate that the AMF community differed among host species ( $A = 0.12$ ,  $P = 0.002$ ), but not between habitats ( $A = 0.006$ ,  $P = 0.33$ ). Pair-wise species comparisons indicate that the community colonizing *T. officinale* was significantly different from the community colonizing *T. ceratophorum* ( $A = 0.16$ ,  $P = 0.0008$ ) and *P. viscosum* ( $A = 0.14$ ,  $P = 0.003$ ). AMF composition was not significantly different between *T. ceratophorum* and *P. viscosum* ( $A = -0.02$ ,  $P = 0.74$ ).

1 pyrosequencing service. Gavin Conant assisted with post-sequencing analysis. Candace Galen, Lori Eggert, Marc Brock, Maarja Opik, and three anonymous reviewers commented on the manuscript.

## References

1. Lu X, Koide RT (1994) The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytol* 128: 211-218
2. Bever JD (2003) Soil community feedback and the coexistence of competitors: Conceptual frameworks and empirical tests. *New Phytol* 157: 465-473
3. van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72
4. Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417: 67-70
5. Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 316: 1746-1748
6. Hawksworth DL (2001) The magnitude of fungal diversity: The 1.5 million species estimate revisited. *Mycol Res* 105: 1422-1432
7. Buee M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F (2009) 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol* 184: 449-456
8. Jumpponen A, Jones KL, Blair J (2010) Vertical distribution of fungal communities in tallgrass prairie soil. *Mycologia* 102: 1027-1041
9. van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11: 296-310
10. Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *J Ecol* 90: 371-384
11. Harrison KA, Bardgett RD (2010) Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. *J Ecol* 98: 384-395
12. Vandenkoornhuysen P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Mol Ecol* 12: 3085-3095



13. Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Mol Ecol* 11: 2669-2678
14. Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M (2009) Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytol* 184: 424-437
15. Kernaghan G, Harper KA (2001) Community structure of ectomycorrhizal fungi across an alpine/subalpine ecotone. *Ecography* 24: 181-188
16. Körner C (2003) *Alpine plant life: Functional plant ecology of high mountain ecosystems*. Springer-Verlag Berlin Heidelberg, New York
17. Galen C (1990) Limits to the distribution of alpine tundra plants: Herbivores and the alpine skyliprot *Polemonium viscosum*. *Oikos* 59: 355-358
18. Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*. Academic Press, London
19. Öpik M, Moora M, Liira J, Zobel M (2006) Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *J Ecol* 94: 778-790
20. Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol* 188: 223-241
21. Gardes M, Dahlberg A (1996) Mycorrhizal diversity in arctic and alpine tundra: An open question. *New Phytol* 133: 147-157
22. Olsson PA, Eriksen B, Dahlberg A (2004) Colonization by arbuscular mycorrhizal and fine endophytic fungi in herbaceous vegetation in the Canadian High Arctic. *Can J Bot* 82: 1547-1556
23. Kytoviita M-M (2005) Asymmetric symbiont adaptation to arctic conditions could explain why high arctic plants are non-mycorrhizal. *FEMS Microbiol Ecol* 53: 27-32
24. Read DJ, Haselwandter K (1981) Observations on the mycorrhizal status of some alpine plant communities. *New Phytol* 88: 341-352
25. Pietikainen A, Kytoviita MM, Husband R, Young JPW (2007) Diversity and persistence of arbuscular mycorrhizas in a low-arctic meadow habitat. *New Phytol* 176: 691-698
26. Bever JD (1994) Feedback between plants and their soil communities in an old field community. *Ecology* 75: 1965-1977
27. Bever JD (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant Soil* 244: 281-290
28. Schmidt SK, Lipson DA, Raab TK (2000) Effects of willows (*Salix brachycarpa*) on populations of salicylate-mineralizing microorganisms in alpine soils. *J Chem Ecol* 26: 2049-2057
29. Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt CW (2008) Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. *Arct Antarct Alp Res* 40: 576-583

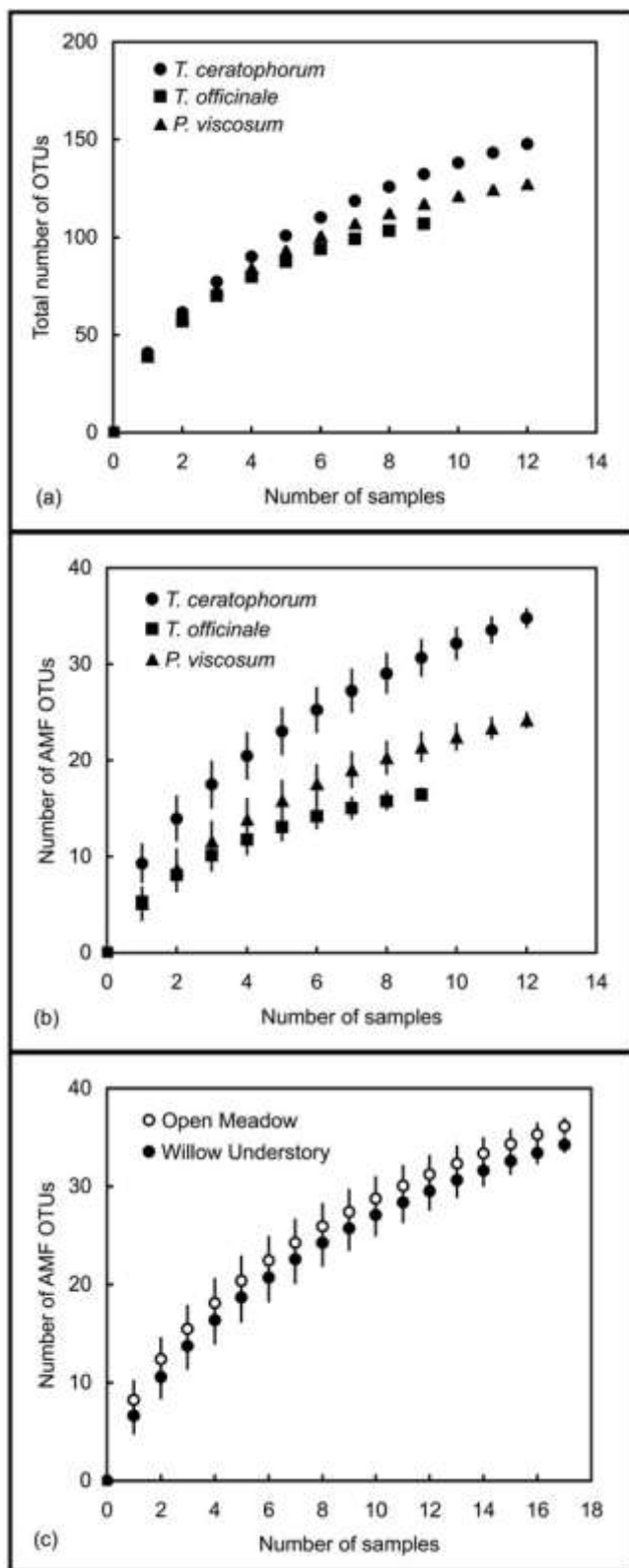
30. Becklin KM, Pallo ML, Galen C (2011) Willows indirectly reduce arbuscular mycorrhizal fungal colonisation in understory communities. *Journal of Ecology* doi: 10.1111/j.1365-2745.2011.01903.x
31. Dona AJ, Galen C (2007) Nurse effects of alpine willows (*Salix*) enhance over-winter survival at the upper range limit of fireweed, *Chamerion angustifolium*. *Arct Antarct Alp Res* 39: 57-64
32. Becklin KM (2010) Friends in high places: Ecology of mycorrhizal associations in alpine plant communities. Dissertation, University of Missouri <http://hdl.handle.net/10355/10239>
33. Becklin KM, Galen C (2009) Intra- and interspecific variation in mycorrhizal associations across a heterogeneous habitat gradient in alpine plant communities. *Arct Antarct Alp Res* 41: 183-190
34. Borneman J, Hartin RJ (2000) PCR primers that amplify fungal rRNA genes from environmental samples. *Appl Environ Microbiol* 66: 4356-4360
35. Redecker D (2006) Molecular ecology of arbuscular mycorrhizal fungi: A review of pcr-based techniques. In: Cooper, JE, Rao, JR (eds.) *Molecular approaches to soil, rhizosphere and plant microorganism analysis*. CABI, pp. 198-212
36. Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: Peeking into the black-box. *Mol Ecol* 10: 1855-1871
37. Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, Saar I, Bahram M, Bechem E, Chuyong G, Kõljalg U (2010) 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytol* 188: 291-301
38. Huang X, Madan A (1999) CAP3: A DNA sequence assembly program. *Genome Res* 9: 868-877
39. Quince C, Lanzen A, Curtis TP, Davenport RJ, Hall N, Head IM, Read LF, Sloan WT (2009) Accurate determination of microbial diversity from 454 pyrosequencing data. *Nat Methods* 6: 639
40. Reeder J, Knight R (2009) The 'rare biosphere': A reality check. *Nat Methods* 6: 636-637
41. Dickie IA (2010) Insidious effects of sequencing errors on perceived diversity in molecular surveys. *New Phytol* 188: 916-918
42. Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: Phylogeny and evolution. *Mycol Res* 105: 1413-1421
43. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32: 1792-1797
44. Colwell RK (2009) EstimateS: statistical estimation of species richness and shared species from samples. Version 8.2.0. User's Guide and application published at <http://purl.oclc.org/estimates>
45. McCune B, Meffort MJ (1999) PC-ORD: multivariate analysis of ecological data. Version 4.1. MjM Software, Gleneden Beach, Oregon, USA
46. McCune B, Grace JB (2002) *Analysis of ecological communities*. MJM Software Design, Gleneden Beach, OR
47. Helgason T, Merryweather JW, Young JPW, Fitter AH (2007) Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *J Ecol* 95: 623-630

48. Öpik M, Moora M, Liira J, Rosendahl S, Zobel M (2006) Comparison of communities of arbuscular mycorrhizal fungi in roots of two viola species. *Proc Estonian Acad Sci Biol Ecol* 55: 3-14
49. Jumpponen A, Jones KL (2009) Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytol* 184: 438-448
50. Jumpponen A, Jones KL, Mattox JD, Yaeger C (2010) Massively parallel 454-sequencing of fungal communities in *Quercus* spp. ectomycorrhizas indicates seasonal dynamics in urban and rural sites. *Mol Ecol* 19: 41-53
51. Arnold AE, Mejía LC, Kyllö D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. *Proc Natl Acad Sci* 100: 15649-15654
52. Sanders IR (2003) Preference, specificity and cheating in the arbuscular mycorrhizal symbiosis. *Trends Plant Sci* 8: 143-145
53. Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: Diversity and functional roles. *New Phytol* 182: 314-330
54. Parker IM, Gilbert GS (2004) The evolutionary ecology of novel plant-pathogen interactions. *Annu Rev Ecol Evol Syst* 35: 675-700
55. Munkvold L, Kjoller R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytol* 164: 357-364
56. Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytol* 177: 779-789
57. van der Putten WH, Kowalchuk GA, Brinkman EP, Doodeman GTA, Kaaij RMVD, Kamp AFD, Menting FBJ, Veenendaal EM (2007) Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. *Ecology* 88: 978-988
58. Setälä H, McLean MA (2004) Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. *Oecologia* 139: 98-107
59. Schwarzott D, Walker C, Schüßler A (2001) *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. *Mol Phylogenet Evol* 21: 190-197

**Table 1**

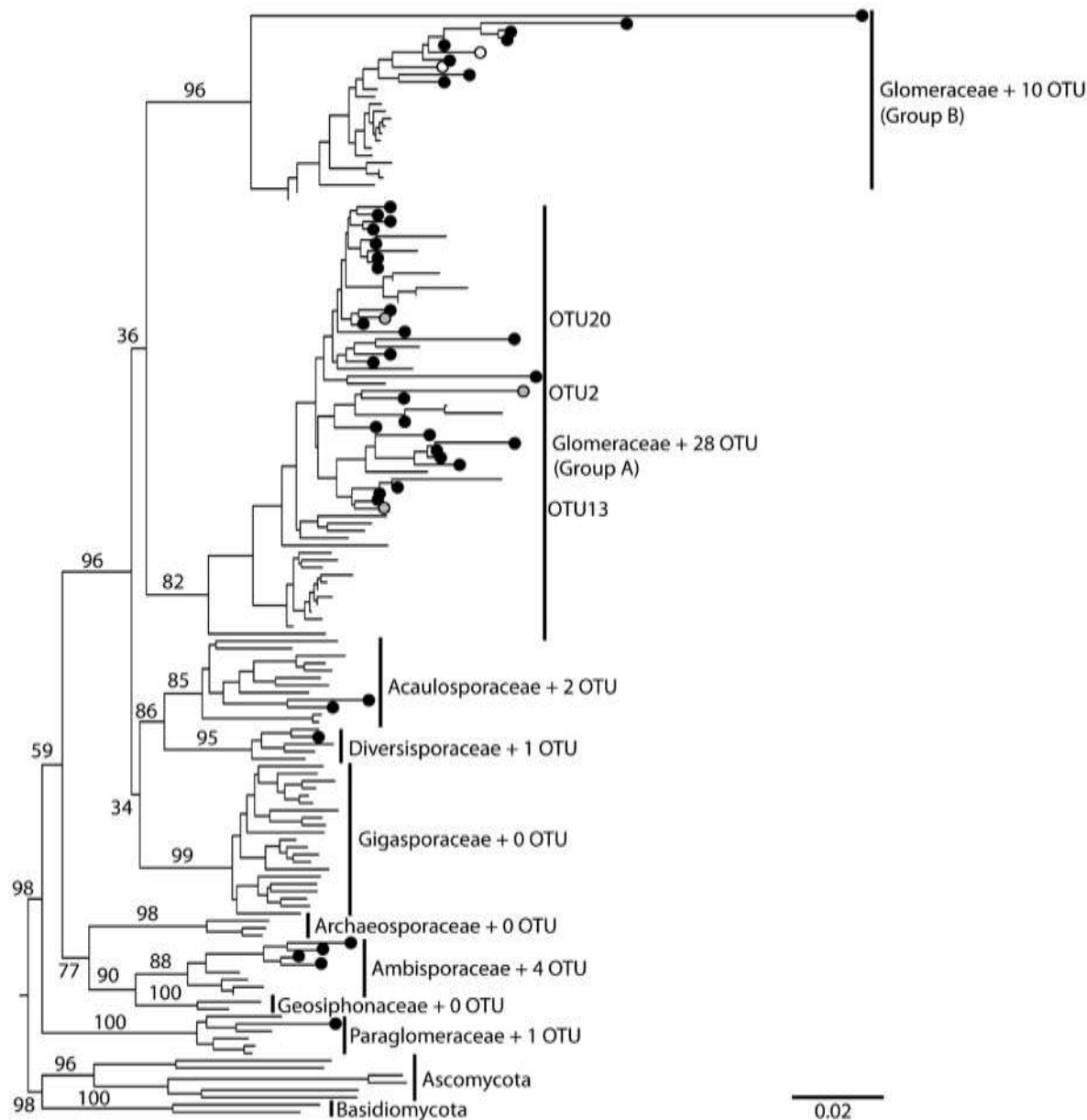
Analysis of variance on the effects of host identity and habitat on rhizosphere AMF and non-AMF communities. Richness and diversity estimates were calculated for OTUs designated based on 97% sequence similarity.

Response	Host Identity				Habitat				Host Identity x Habitat			
	<i>F</i>	<i>df</i>	<i>P</i>	% Variance	<i>F</i>	<i>df</i>	<i>P</i>	% Variance	<i>F</i>	<i>df</i>	<i>P</i>	% Variance
AMF												
OTU Richness (S)	7.88	2	0.003	39.3	4.59	1	0.05	11.5	1.21	2	0.32	6.0
Shannon's Index (H')	5.67	2	0.01	32.1	2.42	1	0.14	6.9	1.48	2	0.25	8.4
Evenness (H'/lnS)	0.31	2	0.74	2.7	1.52	1	0.23	6.7	0.83	2	0.45	7.4
Non-AMF												
OTU Richness (S)	0.09	2	0.91	0.5	2.13	1	0.15	6.0	1.74	2	0.19	9.9
Shannon's Index (H')	0.86	2	0.43	3.9	2.00	1	0.17	4.5	5.80	2	0.007	25.9
Evenness (H'/lnS)	1.35	2	0.27	6.1	0.19	1	0.67	0.4	5.08	2	0.01	23.1

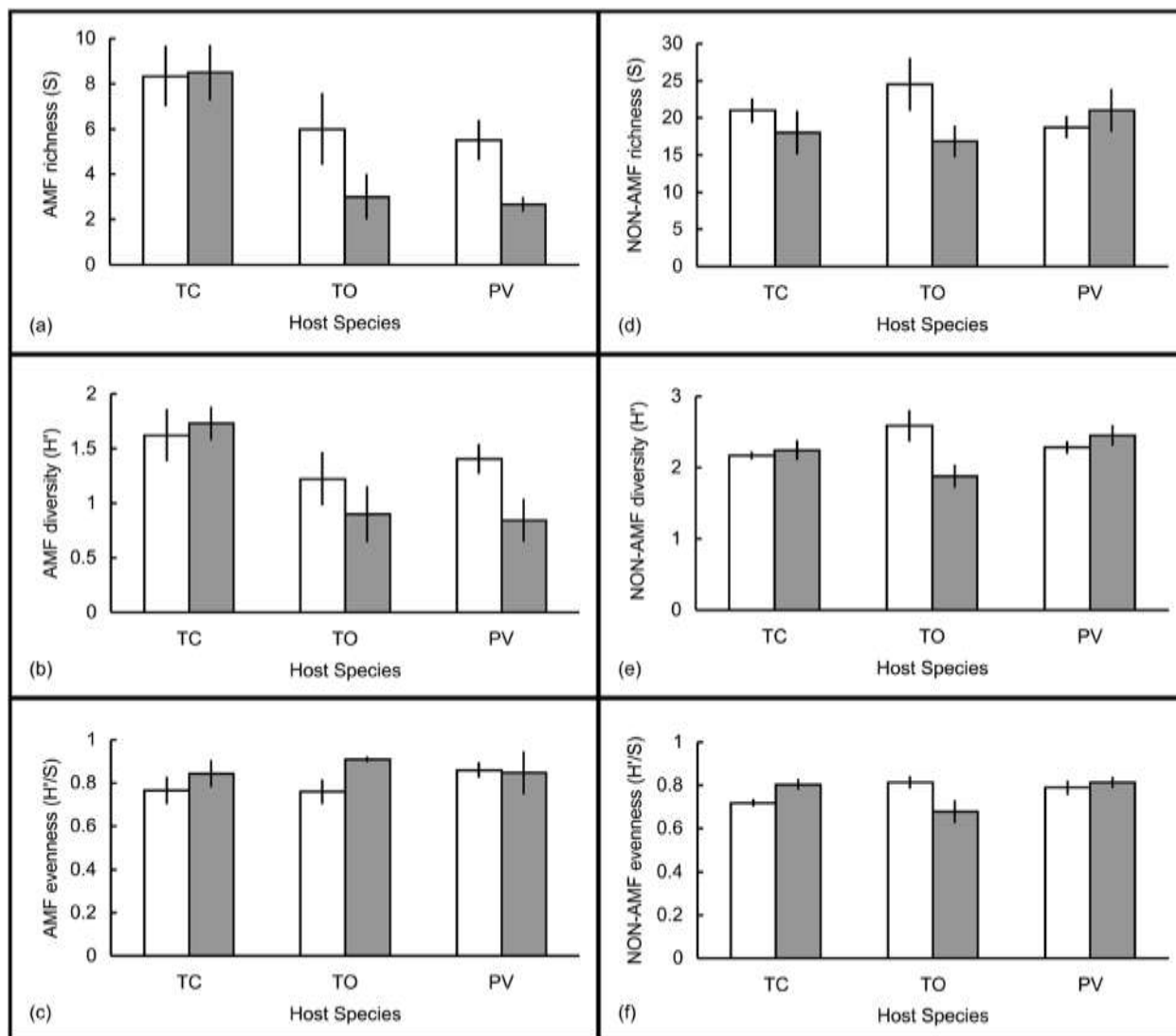


**Fig. 1** Species accumulation curves showing the number of fungal OTUs versus the number of root samples analyzed. (a) Total number of fungal OTUs detected in root samples from *T. ceratophorum* (circles), *T. officinale* (squares), and *P. viscosum* (triangles); (b) Number of AMF OTUs detected in root samples from each host species (symbols as described above); (c) Number of AMF OTUs detected in roots samples from open meadow (open circles) and willow understory (solid circles) habitats. OTUs were designated based on 97% sequence similarity.

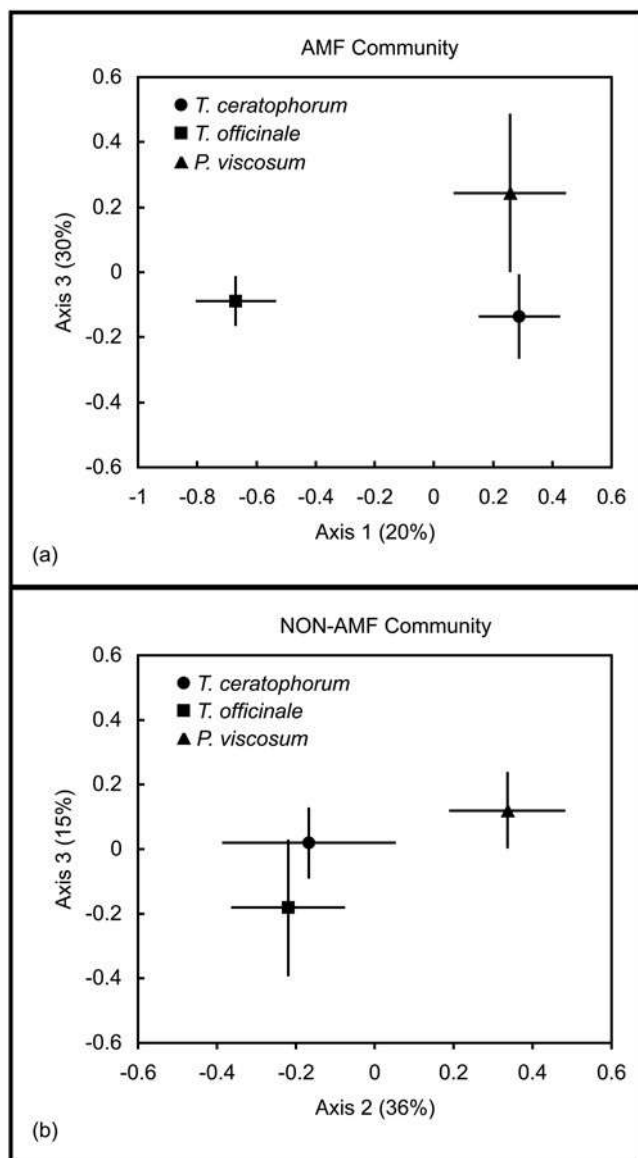




**Fig. 2** Neighbor-joining phylogram of AMF OTUs, top BLASTN reference sequences, additional AMF reference sequences from unrepresented Glomeromycota lineages, and outgroup taxa from Basidiomycota and Ascomycota inferred from small subunit ribosomal DNA sequences (alignment length = 2424 nucleotide positions). *Black circles* represent tips belonging to OTU sequences, *gray circles* indicate the most abundant AMF OTU in each host species (OTU 20 = *T. ceratophorum*, OTU 13 = *T. officinale*, and OTU 2 = *P. viscosum*), and open circles indicate two AMF OTUs removed from the study because of insufficient sampling depth. OTUs were designated based on 97% sequence similarity. Numbers by nodes along the tree backbone represent 1000 neighbor-joining bootstrap replicates. Clades are labeled according to the family assignments of reference sequences included in the lineage. The number of AMF OTUs found in each clade is noted after the '+' sign. Glomeraceae groups A and B follow the categories assigned by Schwarzott *et al.* [57].



**Fig. 3** AMF and non-AMF communities colonizing the roots of *T. ceratophorum* (TC), *T. officinale* (TO), and *P. viscosum* (PV) plants sampled from open meadow (open bars) and willow understory (solid bars) habitats on Pennsylvania Mountain. (a,d) Operational taxonomic unit (OTU) richness (S); (b,e) Shannon's diversity index (H'); (c,f) OTU evenness (H'/lnS). Means ( $\pm$  SE) were calculated using OTUs designated based on 97% sequence similarity. AMF richness and diversity were significantly higher in the open meadow than in the willow understory, and in *T. ceratophorum* compared to *T. officinale* and *P. viscosum*. non-AMF diversity and evenness were significantly reduced in *T. officinale* in the willow understory.



**Fig. 4** Nonparametric multidimensional scaling (NMS) ordination of (a) AMF and (b) non-AMF communities colonizing *T. ceratophorum* (circles), *T. officinale* (squares), and *P. viscosum* (triangles). Plotted are the mean scores ( $\pm$  SE) for each host species along the two axes that represent the greatest amount of variation among host species. *Taraxacum officinale* hosted a significantly different AMF community compared to *T. ceratophorum* and *P. viscosum*. *Polemonium viscosum* hosted a significantly different non-AMF community compared to *T. ceratophorum* and *T. officinale*. OTUs were designated based on 97% sequence similarity.

## Supplementary Materials

**Appendix A** Sequence alignment and neighbor-joining tree for the full dataset including AMF OTUs, non-AMF OTUs, and reference sequences. GenBank accession numbers are listed with abbreviated taxon names for reference sequences.

**Appendix B** Sequence alignment and neighbor-joining tree for the AMF dataset including AMF OTUs, reference sequences, and outgroup taxa. Reference sequences represent either the highest BLASTN matches from GenBank or consensus sequences from Schüßler *et al.* [42]. GenBank accession numbers are listed for the BLASTN matches. Accession numbers for all other reference sequences are listed in Schüßler *et al.* [42].

**Table S1** GenBank accession number, top BLASTN match, and phylogenetic assignment for each 454 operational taxonomic unit (OTU). OTUs were designated based on 97% sequence similarity.

**Table S2** Number of sequences, number of operational taxonomic units (OTUs), and relative abundance of each OTU per root sample collected from *T. ceratophorum*, *T. officinale*, and *P. viscosum* plants in open meadow and willow understory habitats. OTUs were designated based on 97% sequence similarity.

**Fig. S1** Mean ( $\pm$  SE) AMF richness, diversity, and evenness per root sample based on OTUs designated at 90, 95, 97, and 99% sequence similarity. (a,d) OTU richness by habitat (a) and host species (d); (b,e) Shannon's diversity index by habitat (b) and host species (e); (c,f) OTU evenness by habitat (c) and host species (f). In panels a–c open and solid circles represent samples from the open meadow and willow understory, respectively. In panels d–f symbols represent samples from *T. ceratophorum* (circles), *T. officinale* (squares), and *P. viscosum* (triangles). Asterisks indicate a significant habitat or host effect ( $*P \leq 0.05$ ,  $**P \leq 0.01$ ). The decrease in OTU richness and diversity at 99% similarity was due to an increased number of singletons, which were subsequently removed from the dataset.

**Fig. S2** Mean ( $\pm$  SE) non-AMF richness, diversity, and evenness per root sample based on OTUs designated at 90%, 95%, 97%, and 99% sequence similarity. (a) OTU richness by habitat and host species; (b) Shannon's diversity index by habitat and host species; (c) OTU evenness by habitat and host species. Symbols represent samples from *T. ceratophorum* (circles), *T. officinale* (squares), and *P. viscosum* (triangles). Open and solid symbols represent samples from the open meadow and willow understory, respectively. Asterisks indicate a significant habitat by host interaction ( $*P \leq 0.05$ ,  $**P \leq 0.01$ ).