Research Article

More closely related plants have more distinct mycorrhizal communities

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Abstract. Neighbouring plants are known to vary from having similar to dissimilar arbuscular mycorrhizal fungal (AMF) communities. One possibility is that closely related plants have more similar AMF communities than more distantly related plants, an indication of phylogenetic host specificity. Here, we investigated the structure of AMF communities among dominant grassland plants at three sites in the Northern Great Plains to test whether the pairwise phylogenetic distance among plant species was correlated with pairwise AMF community dissimilarity. For eight dominant and co-occurring grassland plant species, we reconstructed a phylogeny based on DNA data and characterized the AMF communities of their roots at each site. Community analyses revealed that AMF communities varied among sites and among plant species. Contrary to expectations for phylogenetic host specificity, we found that within a site more closely related plants had more distinct AMF communities despite their having similar phenologies. Associations with unique AMF communities may enhance the functional complementarity of related species and promote their coexistence.

Keywords: Functional complementarity; host identity; mixed-grass prairie; mycorrhizal community structure; niche partitioning; phylogenetic host specificity; phylogenetic signal; plant–soil (below-ground) interactions.

Introduction

Ninety percent of plants are estimated to associate with arbuscular mycorrhizal fungi (AMF) (Wang and Qiu 2006). Neighbouring pairs of plants can associate with similar or distinct AMF (e.g. Vandenkoornhuyse et al. 2003; Stukkenbrock and Rosendahl 2005; Montesinos-Navarro et al. 2012b; Osanai et al. 2013). A persistent challenge is to understand the source(s) of this variability and the interactions between plants and soil biota. Such knowledge may yield mechanistic insights into how AMF contribute to plant coexistence, plant diversity and plant productivity (e.g. Hart et al. 2003; Hausmann and Hawkes 2009; Davison et al. 2011; Montesinos-Navarro et al. 2012a; Morris et al. 2013).

Mutualist and antagonist host associations have been shown to exhibit phylogenetic signal (Kawakita et al. 2010; Poulin et al. 2011), which we define as the tendency for close relatives to resemble each other more closely in their characteristics than expected by chance (Wiens et al. 2010). For example, mycorrhizae (Jacquemyn et al. 2011),

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seed dispersers (Rezende et al. 2007a), plant pathogens (e.g. Gilbert and Webb 2007) and insect herbivores (e.g. Weiblen et al. 2006) are all known to associate with closely related host species. Phylogenetic conservatism in host use is also called ‘phylogenetic host specificity’ (e.g. Veresoglou and Rillig 2014). Understanding the causes of phylogenetic signal for even a single trait is difficult, as numerous factors can limit changes over evolutionary time (e.g. slow diversification rates, insufficient time, strong selection, morphological constraints and trade-offs; Wiens et al. 2010). Phylogenetic signal in mutualist and antagonist host interactions is even more complex, as multiple traits often mediate species interactions, selection on interactions can be indirect or highly variable in time, and each partner may reciprocally influence the other (e.g. coevolution between host and partner).

Plant associations with AMF and/or responses to AMF have been shown to exhibit phylogenetic signal, at both broad phylogenetic scales (Wang and Qiu 2006) and among local plant assemblages (Reinhart et al. 2012). A potential ecological explanation for the phylogenetic signal for AMF associations among co-occurring plants is phenological filtering (convergence of AMF communities among plants in response to their having similar phenologies). Phenological filtering may contribute to phylogenetic host specificity because (i) closely related plants often share phenological schedules (e.g. Davies et al. 2013) and (ii) AMF taxa are highly seasonal (e.g. Dumbrell et al. 2011). Phenological synchrony among plants and AMF would mean that plants with distinct phenologies are likely to associate with a different pool of AMF than plants with overlapping phenologies. This might then result in a positive correlation between the host phylogenetic distance and the distance in AMF associations. A perhaps less-likely explanation is that closely related plants may be interconnected by common hyphal networks that improve the establishment of relatives’ seedlings (Van Der Heijden and Horton 2009). Related plants may also associate with ‘more’ divergent AMF communities than expected by chance, based on one study (Veresoglou and Rillig 2014). Their study provided several interpretations for this including that closely related plants partition the AMF pool in ways that promote plant functional complementarity. For example, neighbouring grasses that share many traits may have increased their functional complementarity by associating with unique AMF. The well-known positive correlation of AMF diversity and plant productivity indicates a role of AMF in functional complementarity (Van Der Heijden et al. 1999b). The direction of the phylogenetic signal, then, may depend in part upon the hierarchical balance of abiotic and biotic filters.

Here our goal was to characterize the AMF taxa associated with eight grassland plant species within three sites in the Northern Great Plains of North America. We also tested whether related plant species have similar phenologies and AMF. In addition to testing for a phylogenetic signal in plant phenology, we tested whether pairwise phylogenetic distance among plants predicts the similarity in the AMF found in their roots in the field. Contrary to the expectation of phylogenetic host specificity, we found that phylogenetic distance and AMF distance were negatively correlated, indicating phylogenetic divergence in host use (e.g. close plant relatives hosted different AMF) and a possible role for below-ground plant–AMF associations in niche partitioning and coexistence of confamilial grasses.

**Methods**

**Study species, sites and system**

We used plant community composition data for the three sites from a previously published study on host-specific soil biota effects (also called soil feedback effects) (Reinhart 2012) to select the eight most frequent and abundant plants across all sites. Our focus on dominant species limited our sample size relative to all possible species occurring across sites but ensured pairs of species typically co-occurred in nature and did not occupy separate portions of an undescribed local gradient (Table 1). The sample included one perennial sedge (Carex filifolia), one exotic annual C3 grass (Bromus japonicus), three perennial C3 grasses (Hesperostipa comata, Koeleria macrantha, Pascopyrum smithii), one perennial C4 grass (Bouteloua gracilis), one exotic biennial forb (Tragopogon dubius) and one perennial subshrub (Artemisia frigida). In other words, the eight dominant plant species included five from Poaceae, one from Cyperaceae and two from Asteraceae. This distribution resulted in 35 % of our pairwise comparisons being intra-family comparisons of Poaceae taxa. A related study included relatively few studies (we determined 3 of 10 grassland studies) with two or more Poaceae taxa (Veresoglou and Rillig 2014). That said, our comparisons do not include very close or closest (i.e. sister) relatives. A review of the 342 plant species known to occur at the Fort Keogh Livestock & Range Research Laboratory (22 000 ha) indicates sister taxa exist at the site for half the species (i.e. Artemisia, Bromus, Bouteloua and Carex).

Most related studies that characterized AMF of more than one plant species (reviewed by Veresoglou and Rillig 2014) sampled only a single site. We sampled three replicate sites to avoid site-specific patterns. Sites were separated by 9–77 km in Custer County, Montana, USA, consisted of loamy soils and represented one of the
dominant grassland types in the region (H. comata, B. gracilis and C. filifolia) (Martin et al. 1998). The sites were located in the Northern Great Plains Steppe ecoregion of North America which is dominated by semiarid mixed-grass prairie that cover > 22 million hectares (Martin et al. 1998). Peak annual productivity for this system occurs between June and July reflecting its dominance by C3 graminoids and the grasslands are primarily nitrogen and not phosphorus limited (K.O.R., unpubl. data). Further details on the study sites (Reinhart 2012) and system (Martin et al. 1998) are reported elsewhere.

**Plant phylogeny construction**

We reconstructed a phylogeny for eight angiosperm species based on DNA data and the Bayesian analysis. We searched the GenBank database for sequences of five loci often used in published phylogenies: ITS1, matK, rbcL, trnL and 5.8s (e.g. Reinhart et al. 2012). Multiple loci are necessary to differentiate plant species because some loci represent conservative coding regions (e.g. rbcL) and others represent more rapidly evolving portions (e.g. matK) (e.g. Reinhart et al. 2012). When a sequence was not available for the exact same species for a marker, we used sequences available for species of the same genus, and also native to North America (acquired rbcL sequences for two species). All species had at least three loci represented for it or a congener in GenBank (http://www.ncbi.nlm.nih.gov). We included an ancestral angiosperm species (Magnolia grandiflora) as an outgroup to root the molecular phylogeny. We followed a previously described workflow to align sequences, edit sequences and determine the best-fit maximum likelihood models of nucleotide substitution (sensu Reinhart et al. 2012). Using the concatenated sequence alignments, we performed a partitioned Bayesian inference, estimating the posterior probability distribution of all possible phylogenies using a Markov chain Monte Carlo algorithm (i.e. Metropolis algorithm) implemented in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Two independent Markov chains were run, each with four heated
chains for 500 000 generations. The final average standard deviation of split frequencies was 0.0002, indicating good convergence of the two independent runs. We sampled runs every 50 generations and used a burnin of 500 trees to generate a majority rule consensus tree (i.e. phylogram). The resulting phylogram was transformed with non-parametric rate smoothing into an ultrametric tree using APE version 2.5 (Paradis et al. 2004) in R (R Developement Core Team 2011). The ultrametric tree, which has unit-less branch lengths, is shown in Fig. 2. We also ran divergence time estimation to get branch lengths in millions of years based on several calibration dates; we noted that measures of phylogenetic signal for phenology and AMF distance were qualitatively similar [see Supporting Information].

Root collection

We sampled eight mature plants per species and per site for a total of 192 samples (8 species × 8 plants × 3 sites). The plants were randomly sampled from each site in a 50 × 20-m plot. We did not measure the location of individual plants and are not able to address whether AMF community similarity exhibits spatial autocorrelation. We sampled at anthesis because AMF were most likely to be associated with roots when plants were actively photosynthesizing. Detailed description and justification of root sampling are provided [see Supporting Information].

AMF communities

We characterized the AMF community in each sampled plant using DNA-based methods involving the construction of a terminal restriction fragment length polymorphism (T-RFLP) library. A main aim of many microbial community ecology studies is to quantify microbial species richness and attach the best available names to their operational taxonomic units. Methods with a more limited capacity to differentiate and name microbial species (e.g. T-RFLP, phospholipid-derived fatty acid profiles) may still be used to quantify differences in microbial community composition. For example, a recent study determined that differences in fungal community composition among treatments were comparable whether based on data derived from T-RFLP or pyrosequencing despite the latter’s detection of many more ‘species’ (e.g. Dhami et al. 2013).

Sample processing, DNA extraction, amplification with nested polymerase chain reaction (PCR) and database T-RFLP characterization of AMF communities followed the methods of three previous studies (Renker et al. 2003; Aldrich-Wolfe 2007; Jordan et al. 2012). Detailed molecular protocols and analysis procedures are provided [see Supporting Information]. In brief, three root fragments (6 mm length) per sample were combined and used to extract DNA. The ITS region was amplified by nested PCR. PCR1 used the primer pair SSU-Glom1 and LSU-Glom1. LSU-Glom1 is specific to the Glomeromycota but also amplifies some basal Basidiomycetes (Renker et al. 2003). To further reduce the amplification of DNA from non-AMF, PCR1 amplicons were digested at 37 °C with AluI (Renker et al. 2003) [see Supporting Information]. The ITS region of most AMF lacks an AluI cut site, while the ITS regions of other fungi likely to have been amplified during PCR1 typically contain multiple AluI cut sites (Renker et al. 2003; Aldrich-Wolfe 2007; Jordan et al. 2012). The AluI digested products were then amplified for PCR2 with the fluorescently labelled universal fungal primers 6-FAM-ITS4 and HEX-ITS5.

Amplicons from PCR2 were then restricted digested with HinfI and MboI for T-RFLP. Restriction digested amplicons were submitted along with undigested amplicons to a DNA sequencing service centre. Lengths of terminal restriction fragments and undigested amplicons were examined with Peak Scanner Software, version 1 (Applied Biosystems, Foster City, California, USA).

The package TRAMPR implemented in the R environment was used to match PCR2 amplicons and terminal restriction fragments (TRFs) from root samples with a database of AMF knowns and to provide output for additional analyses (FitzJohn and Dickie 2007). A database of known AMF was built by aggregating two existing databases including one with isolates from around the world (Appendix C in Aldrich-Wolfe 2007) and another that expanded this original database by including isolates specific to the Northern Great Plains steppe ecoregion (Jordan et al. 2012). The two prior studies also characterized AMF communities with the same primers and T-RFLP. We supplemented the database further with additional isolates of local AMF as done by these prior studies. TRAMPR was used to accurately detect AMF by matching sets of TRFs and PCR2 amplicons from our samples with sets from our AMF knowns database. This was a conservative approach that helped reduce the probability that TRFs of non-AMF, persisting after AluI restriction enzyme digest, produced false-positive counts of AMF that were not actually present. Detailed protocols are provided [see Supporting Information]. TRAMPR was then used to generate a presence/absence matrix for each of the 192 samples. Hereafter, each known cluster profile is referred to as an operational taxonomic unit (OTU).

The distribution of AMF by site and species

Similarity of AMF communities within vs. among plant species and grassland sites was tested using the non-parametric permutation test ADONIS, with 1000 permutations, in the ‘vegan’ package in R (Oksanen et al. 2011).
Phylogenetic signal tests
We separately tested whether plant phenology and AMF associations varied by plant phylogeny and exhibited a phylogenetic signal. To assess the degree phylogeny explains the phenological similarity of species (i.e. trait conservatism), we quantified the phylogenetic signal using the descriptive K statistic using the package Picante (Kembel et al. 2010) in R. The K statistic compares the observed signal for a plant trait (i.e. mean Julian day of anthesis across sites) with the signal under a Brownian motion process and provides an estimate of phylogenetic signal or trait conservatism. K values <1 indicate that species resemble each other less than expected under Brownian motion evolution; K values >1 indicate that species resemble each other more than expected. Picante uses a permutation test to test the significance of the observed K value. To do this, the names of taxa are iteratively shuffled across the tips of the phylogeny 999 times. The significance of the K statistic is assessed with the quantile of the observed phylogenetically independent contrast variance vs. the null distribution, which provides a one-tailed P-value testing whether the phylogenetic signal is greater than expected. With only eight species, the significance test should be interpreted cautiously since tests with very few species are likely to fail to detect significant differences when they actually exist (Type II errors) (Rezende et al. 2007b) and justified use of a relaxed alpha level of 0.10.

To test for a phylogenetic signal in AMF host associations (i.e. phylogenetic host specificity), we determined whether variation in AMF distance (community dissimilarity) among pairs of host plant species was positively related to the phylogenetic (ultrametric) distance between this pair of plant hosts. Phylogenetic distance between each pair of plant species was determined with the program Patristic. Arbuscular mycorrhizal fungal distance among pairs of plant species per site was measured using Jaccard distance implemented with the package vegan (Oksonen et al. 2011) in R. Prior to Jaccard distance calculations, we summed the sample data (i.e. binomial presence/absence data) by species per site. This produced quantitative data of AMF OTU associations per plant species per site. We treated sites as a form of landscape-level replication and averaged dissimilarity measures for each pair of species (e.g. A. frigida and C. filifolia) which reduced the data to 28 species pairs (for a pool of 8 species there are 28 species pairs). This approach ensures that our pairwise description of pairwise AMF distance is not site specific. We used linear regression (LR) to determine the direction of the relationship between AMF distance and phylogenetic distance. A Mantel test was used to account for the non-independence in the data (not accounted for with LR), caused by a given plant species being present in multiple pairwise species combinations (e.g. Weiblen et al. 2006; Violle et al. 2011). The data were also analysed with quantile regression. Outliers were identified based on the maximum normal residual method (Snedecor and Cochran 1989) and P < 0.05. One outlier was detected, removed and the LR and Mantel analyses were repeated. We also tested the sensitivity of the LR results to any one particular species by removing data for each species and repeating LR analyses.

Results
The distribution of AMF by site and species
The AMF of mixed-grass prairie sites varied by plant species (R²_ADONIS = 0.15, P = 0.003) and grassland site (R²_ADONIS = 0.03, P = 0.026). We also detected an interaction between plant species and site (R²_ADONIS = 0.18, P < 0.001) (Fig. 1). Visual inspection of the NMDS plot (Fig. 1) confirmed the ADONIS results and illustrate the variation in AMF communities among plant species, among sites (coloured hulls), and the interaction between plant species and site. Sites had relatively unique AMF: Sites 1 and 2 shared 50 % of their OTUs, 1 and 3 shared 53 % of their OTUs, and 2 and 3 shared 58 % of their OTUs [see Supporting Information]. On the other hand, richness of OTUs per individual root sample did not vary by grassland site (F_2,191 = 1.96, P = 0.14) or plant species (ANOVA, F_7,191 = 1.51, P = 0.17), and no interaction between plant species and site was detected (F_14,191 = 0.60, P = 0.54).

Phylogenetic signal tests
Among the eight plant species, related species tended to have similar phenologies (i.e. anthesis) (phylogenetic signal test, K = 0.91, P = 0.052) suggesting trait conservatism.
The phylogenetic distance of pairs of plants, however, was negatively correlated with AMF distance ($b = -0.0008$, $R^2 = 0.15$, $F_{1,26} = 4.67$, $P = 0.040$) (Fig. 2) which was also significant according to the Mantel test (Pearson's product-moment correlation, $r = -0.39$, $P = 0.006$) and quantile regression ($P = 0.003$). In other words, related pairs of plants tended to have divergent AMF. Removing each plant species and repeating the regression analyses showed that the slope parameters were consistently negative, but only four of eight models were at least marginally significant ($P < 0.10$). We also detected and removed an outlier comparison (C. filifolia and T. dubius pair, Jaccard distance value = 0.25) which had relatively minor effects on the LR ($R^2 = 0.14$, $F_{1,25} = 4.17$, $P = 0.052$), Mantel test ($r = -0.38$, $P = 0.01$) and quantile regression ($P = 0.0001$) (data not shown). As noted in the subsection Phylogenetic signal tests, the Mantel test is the preferred analysis since it accounts for the non-independence in the data.

**Discussion**

**The distribution of AMF by site and species**

We characterized the AMF communities of eight dominant plant species co-occurring across three sites of semi-arid and temperate grassland of the Northern Great Plains of North America. In other systems, neighbouring grasses were found to have relatively distinct AMF communities (Vandenkoornhuyse et al. 2003; Osanai et al. 2013). We found that AMF associations among neighbouring plants and species were often distinct in some sites but not in others, reflecting a surprising degree of landscape variation in plant—AMF associations (plant species × site interaction, $P < 0.001$; Fig. 1). As has been shown in pot experiments (Smith and Read 2008), we found limited evidence for host specificity, especially among the more frequently detected AMF OTUs [see Supporting Information], and the few patterns of host specificity we found were site specific. Among the three comparison grasslands, we found that up to 50% of AMF OTUs may be site specific.

![Figure 1](image1.png)

*Figure 1.* Non-metric multidimensional scaling analysis of AMF community structure among dominant plant species at three grassland sites (Sites: 1 = green, 2 = orange and 3 = red). AMF OTU labels are in blue. Plant species abbreviations are as follows: Arfr, Artemisia frigida; Bogr, Bouteloua gracilis; Brja, Bromus japonicus; Cafi, Carex filifolia; Heco, Hesperostipa comata; Koma, Koeleria macrantha; Pasm, Pascopyrum smithii; Trdu, Tragopogon dubius. Coloured hulls enclose the plant species per site. The stress computed for this ordination was 0.09.

![Figure 2](image2.png)

*Figure 2.* Simple linear regression (solid line) and lower quantile regression (dashed line) for phylogenetic distance among pairs of plant species and mean community dissimilarity of AMF among pairs of host plant species from across three replicate mixed-grass prairie sites. A community dissimilarity value of one would indicate that the AMF communities for two plant species are entirely distinct with no shared associations and the opposite for values of zero. The lower quantile regression was based on the subset of data represented by open circles.
Phylogenetic signal tests

We predicted that related plant taxa would share traits (e.g. phenology and AMF associations), an indication of niche conservatism. We envisioned that AMF associations would be affected by a sequential hierarchy of abiotic and biotic filters. First, a temporal filter for co-occurrence of closely related plants and exposure to similar AMF (i.e. phenological filter) (Dumbrell et al. 2011; Davies et al. 2013). Second, local niche partitioning of AMF by close relatives. We confirmed that more closely related plants tended to have more similar phenologies as has been shown by others (e.g. Davies et al. 2013). Contrary to expectations for phylogenetic host specificity, we identified that phylogenetic distance among the dominant plants was negatively correlated with AMF distance (e.g. Fig. 2). Thus, closely related plants tended to have more divergent AMF communities than expected by chance. Another study on plant–AMF associations reported similar findings (Veresoglou and Rillig 2014).

Measures of phylogenetic signal, representative of the entire community, ideally require sampling a large number of species that are broadly distributed across the phylogeny for the community (e.g. Rezende et al. 2007b). While our study only contained eight species, these were the eight most dominant grassland species (mostly grasses) which helped ensure comparisons were of species that routinely co-occurred (Table 1). The significance of the LRs also depended on the species included in the analyses. However, the slopes of the relationship between phylogenetic distance and AMF distance (community dissimilarity) always remained negative, indicating that the direction of relationship between phylogenetic distance and AMF distance is robust to the influence of any one plant species. Further to the point, we failed to detect any evidence of phylogenetic host specificity (i.e. positive correlation between phylogenetic distance and AMF distance). The grass clade did disproportionately influence our results: the mean pairwise AMF distance among grasses was 0.86 (± 0.04 [95% CI]) vs. 0.75 (± 0.08) for grass–non-grass comparisons. Other studies confirm that AMF communities are often relatively distinct among grass species (Vandenhoekhuyse et al. 2003; Osanai et al. 2013). Unlike our results, a recent meta-analysis reported a marginally significant ($P < 0.1$) positive correlation between plant phylogenetic distance and AMF distance for the subset of data for grasslands ($P = 0.07$) (Veresoglou and Rillig 2014). Their main conclusion, however, was that phylogenetic distance was negatively related to AMF distance. We suspect their atypical finding for the subset of data for grasslands relates to the small number of studies (3 of 10) with more than a single grass species.

Arbuscular mycorrhizal fungal communities are thought to be affected by phenological filtering and temporal fluxes in host availability and photosynthate (e.g. Dumbrell et al. 2011). We confirmed that related plants tended to have similar timing of anthesis (e.g. $K = 0.91$, $P = 0.052$) but failed to detect evidence for a positive relationship between phylogenetic distance and AMF distance (e.g. Fig. 2) as done in another study (Veresoglou and Rillig 2014). Many other factors may affect mycorrhizal communities besides phenology and host phylogeny. Mycorrhizal communities may vary among plant species occupying different successional stages or niches (e.g. Davison et al. 2011) and vary over sites with varying properties (e.g. soil properties, climate differences, land-use history) (e.g. Hazard et al. 2013). Since we sampled plant species that were dominant and co-occurred, it is unlikely that our findings are mainly driven by plants occupying distinct points along local gradients. Other studies using greenhouse experiments have detected soil legacy (or priority) effects where the identity of the plant that initially conditioned or ‘trained’ the soil biota affected the mycorrhizal community of the next plant to establish (e.g. Hausmann and Hawkes 2010; Jordan et al. 2012). Natural grassland soils are, however, a diverse mixture of the roots of many species (Hiiesalu et al. 2012). Therefore, the findings from simple pot or field experiments may not be neatly extended to natural grasslands. Assuming that priority effects exist, we predict that many subordinate plant species will share mycorrhizal associations with the most dominant perennial plant (i.e. H. comata). With many grass tussocks per metre square, H. comata was often associated with relatively divergent AMF communities compared with other plant species including an annual grass (B. japonicus) and biennial forb (T. dubius) (Fig. 1).

The observed variation in AMF communities among the eight most dominant grassland plants (Figs 1 and 2) is likely of ecological importance and may help explain the coexistence of dominant plant species. Grassland ecosystems are often dominated by several grass species (Sims and Risser 2000) which tend to share traits due to shared ancestry (e.g. Davies et al. 2013). Plants with similar traits are likely to have similar niches and may engage in intense resource competition which may lead to competitive exclusion (Burns and Strauss 2011; Violle et al. 2011; but see Bennett et al. 2013). As AMF effectively extend plant roots and increase resource acquisition, grasses that associate with distinct AMF may experience enhanced functional complementarity relative to other grasses. Functional complementarity may be partly possible because individual AMF species are known to have host-specific effects (e.g. Van Der Heijden et al. 1998a;
Associations with divergent AMF may enable the more complete utilization of available resources and an overall increase in plant productivity (Van Der Heijden et al. 1998b; Montesinos-Navarro et al. 2012a). Increased niche partitioning among plants may partially explain how grasslands can be so productive despite their low levels of phylogenetic diversity (Bennett et al. 2013). Other studies have shown that AMF may influence plant coexistence (reviewed by Hart et al. 2003). However, effects of AMF on plant coexistence can range from positive (Hart et al. 2003; Van Der Heijden et al. 2003) to negative (Hartnett et al. 1993), and to neutral (Eissenstat and Newman 1990).

Conclusions

Across the three replicate grassland sites, we detected significant effects of both site and plant species on AMF communities. We found that related plant species (mostly grasses) tended to have more divergent AMF communities than distantly related plant species, suggesting that functional complementarity and local coexistence of otherwise ecologically similar grasses may be increased by association with distinct AMF taxa.

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Contributions by the Authors

The authors shared in constructing the phylogenies, analysing the data and writing.

Conflicts of Interest Statement

None declared.

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Supporting Information

The following Supporting Information is available in the online version of this article –

Appendix S1. Additional methods and results for a fossil calibrated phylogeny.

Appendix S2. Additional methods information on the root collection and T-RLFP.

Figure S1. Relative abundance of arbuscular mycorrhizal fungi OTUs.

Table S1. Information on the operational taxonomic unit (OTU) clusters in the ‘knowns’ library.

Literature Cited

Aldrich-Wolfe L. 2007. Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. Ecology 88:559–566.

Bennett JA, Lamb EG, Hall JC, Cardinal-McTeague WM, Cahill JF Jr. 2013. Increased competition does not lead to increased phylogenetic overdispersion in a native grassland. Ecology Letters 16: 1168–1176.

Burns JH, Strauss SY. 2011. More closely related species are more ecologically similar in an experimental test. Proceedings of the National Academy of Sciences of the USA 108:5302–5307.

Davies TJ, Wolkovich EM, Kraft NJB, Salamin N, Allen JM, Ault TR, Betancourt JL, Bolmgren K, Cieland EE, Cook BI, Crimmins TM, Mazer SJ, McCabe GJ, Pau S, Regetz J, Schwartz MD, Travers SE. 2013. Phylogenetic conservatism in plant phenology. Journal of Ecology 101:1520–1530.

Davison J, Ópik M, Daniell TJ, Moorea M, Zobel M. 2011. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiology Ecology 78:103–115.

Dhami MK, Weir BS, Taylor MW, Beggs JR. 2013. Diverse honeydew-consuming fungal communities associated with scale insects. PLoS ONE 8:e70316.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T. 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190:794–804.

Eissenstat DM, Newman EL. 1990. Seedling establishment near large plants: effects of vesicular–arbuscular mycorrhizas on the intensity of plant competition. Functional Ecology 4:95–99.

FitzJohn R, Dickie IA. 2007. TRAMPR: an R package for analysis and matching of terminal-restriction fragment length polymorphism (T RFLP) profiles. Molecular Ecology Notes 7:583–587.

Gilbert GS, Webb CO. 2007. Phylogenetic signal in plant pathogen–host range. Proceedings of the National Academy of Sciences of the USA 104:4979–4983.

Hart MM, Reader RJ, Klironomos JN. 2003. Plant coexistence mediated by arbuscular mycorrhizal fungi. Trends in Ecology and Evolution 18:418–423.
Hartnett DC, Hetrick BAD, Wilson GWT, Gibson DJ. 1993. Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. Journal of Ecology 81: 787–795.

Hausmann NT, Hawkes CV. 2009. Plant neighborhood control of arbuscular mycorrhizal community composition. New Phytologist 183:1188–1200.

Hausmann NT, Hawkes CV. 2010. Order of plant host establishment alters the composition of arbuscular mycorrhizal communities. Ecology 91:2333–2343.

Hazard C, Gosling P, Van der Gast CJ, Mitchell DT, Doohan FM, Bending GD. 2013. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. The ISME Journal 7: 498–508.

Hiiesalu I, Òpik M, Metsis M, Liihe L, Davision J, Vasar M, Moora M, Zobel M, Wilson SD, Pätär M. 2012. Plant species richness below-ground: higher richness and new patterns revealed by next-generation sequencing. Molecular Ecology 21:2004–2016.

Hulsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Jacquemyn H, Merckx V, Brys R, Tyteca D, Cammue BPA, Honnay O, Lievens B. 2011. Analysis of network architecture reveals phylogenetic constraints on mycorrhizal specificity in the genus Orchis (Orchidaceae). New Phytologist 192:518–528.

Jordan NR, Aldrich-Wolfe L, Huerd SC, Larson DL, Muehlbauer G. 2012. Soil-occupancy effects of invasive and native grassland plant species on composition and diversity of mycorrhizal associations. Invasive Plant Science and Management 5:494–505.

Kawakita A, Okamoto T, Goto R, Kato M. 2010. Mutualism favours higher host specificity than does antagonism in plant–herbivore interaction. Proceedings of the Royal Society B Biological Sciences 277:2765–2774.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464.

Klironomos JN. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Martin BS, Cooper S, Heidel B, Hildebrand T, Jones G, Lenz D, Lesica P. 1998. Natural community inventory within landscapes in the Northern Great Plains Steppe Ecoregion of the United States. Helena, MT: A report to the Natural Resource Conservation Service, Northern Plains Regional Office.

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M. 2012a. Plant facilitation occurs between species differing in their associated arbuscular mycorrhizal fungi. New Phytologist 196:835–844.

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M. 2012b. The network structure of plant-arbuscular mycorrhizal fungi. New Phytologist 194:536–547.

Morris EK, Buscot F, Herbst C, Meiners T, Obermaier E, Wäschke NW, Wubet T, Rillig MC. 2013. Land use and host neighbor identity effects on arbuscular mycorrhizal fungal community composition in focal plant rhizosphere. Biodiversity and Conservation 22:2193–2205.

Oksanen J, Blanchet FG, Kindt R, Legendre P, O’Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2011. Vegan: Community Ecology Package. R package version 1.17-9. http://CRAN.R-project.org/package=vegan.

Osanai Y, Bouguere DS, Hoyden HL, Hovenden MJ. 2013. Co-occurring grass species differ in their associated microbial community composition in a temperate native grassland. Plant and Soil 368:419–431.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Poulin R, Krasnov BR, Mouillot D. 2011. Host specificity in phylogenetic and geographic space. Trends in Parasitology 27:355–361.

R Development Core Team. 2011. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Reinhart KO. 2012. The organization of plant communities: negative plant–soil feedbacks and semiarid grasslands. Ecology 93: 2377–2385.

Reinhart KO, Wilson GWT, Rinella MJ. 2012. Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. Ecology Letters 15:689–695.

Renker C, Heinriches J, Kaldorf M, Buscot F. 2003. Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. Mycorrhiza 13:191–198.

Rezende EL, Jordano P, Bascompte J. 2007a. Effects of phenotypic complementarity and phylogeny on the nested structure of mutualistic networks. Oikos 116:1919–1929.

Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J. 2007b. Non-random coexistences in phylogenetically structured mutualistic networks. Nature 448:925–928.

Sims PL, Risser PG. 2000. Grasslands. In: Barbou MG, Billings WD, eds. North American vegetation. Cambridge: Cambridge University Press, 323–356.

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis, 3rd edn. New York: Academic Press.

Snedecor GW, Cochran WG, eds. 1989. Failures in the assumptions. In: Statistical methods. Ames, IA: Iowa State University Press, 273–296.

Stukkenbrock EH, Rosendahl S. 2005. Distribution of dominant arbuscular mycorrhizal fungi among five plant species in undisturbed vegetation of a coastal grassland. Mycorrhiza 15:497–503.

Vandenkroonenwyse P, Ridgway KP, Watson JI, Fitter AH, Young JP. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. Molecular Ecology 12:3085–3095.

Van Der Heijden MGA, Horton TR. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. Journal of Ecology 97:1139–1150.

Van Der Heijden MGA, Boller T, Wiemken A, Sanders IR. 1998a. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091.

Van Der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998b. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

Van Der Heijden MGA, Wiemken A, Sanders IR. 2003. Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plants. New Phytologist 157:569–578.

Veresoglou SD, Rillig MC. 2014. Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. Plant and Soil 377:395–406.

Violle C, Nemergut DR, Pu Z, Jiang L. 2011. Phylogenetic limiting similarity and competitive exclusion. Ecology Letters 14:782–787.
Wang B, Qiu Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**:299–363.

Weiblen GD, Webb CO, Novotny V, Basset Y, Miller SE. 2006. Phylogenetic dispersion of host use in a tropical insect herbivore community. *Ecology* **87**:S62–S75.

Wiens JJ, Ackerly DD, Allen AP, Anacker BL, Buckley LB, Cornell HV, Damschen EI, Davies TJ, Grytnes J-A, Harrison SP, Hawkins BA, Holt RD, McCain CM, Stephens PR. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters* **13**:1310–1324.