Ecological Applications, 28(3), 2018, pp. 736–748

Disturbance reduces the differentiation of mycorrhizal fungal communities in grasslands along a precipitation gradient

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Abstract. Given that mycorrhizal fungi play key roles in shaping plant communities, greater attention should be focused on factors that determine the composition of mycorrhizal fungal communities and their sensitivity to anthropogenic disturbance. We investigate changes in arbuscular mycorrhizal (AM) fungal community composition across a precipitation gradient in North American grasslands as well as changes occurring with varying degrees of site disturbance that have resulted in invasive plant establishment. We find strong differentiation of AM fungal communities in undisturbed remnant grasslands across the precipitation gradient, whereas communities in disturbed grasslands were more homogeneous. These changes in community differentiation with disturbance are consistent with more stringent environmental filtering of AM fungal communities in undisturbed sites that may also be promoted by more rigid functional constraints imposed on AM fungi by the native plant communities in these areas. The AM fungal communities in eastern grasslands were particularly sensitive to anthropogenic disturbance, with disturbed sites having low numbers of AM fungal operational taxonomic units (OTUs) commonly found in undisturbed sites, and also the proliferation of AM fungal OTUs in disturbed sites. This proliferation of AM fungi in eastern disturbed sites coincided with increased soil phosphorus availability and is consistent with evidence suggesting the fungi represented by these OTUs would provide reduced benefits to native plants. The differentiation of AM fungal communities along the precipitation gradient in undisturbed grasslands but not in disturbed sites is consistent with AM fungi aiding plant adaptation to climate, and suggests they may be especially important targets for conservation and restoration in order to help maintain or re-establish diverse grassland plant communities.

Key words: anthropogenic disturbance; arbuscular mycorrhizal fungi; grasslands; mutualisms; plant–fungal interactions; prairie ecosystems; soil microbial ecology.

Introduction

The conservation and restoration of ecosystems impacted by human activities remains a pressing need. To be successful, conservation and restoration practices should be informed by an understanding of the forces that structure and stabilize the ecosystems of interest. There is a growing appreciation of the role that soil microbial communities play in determining the structure and facilitating the function of plant communities (Mangan et al. 2010, Bever et al. 2015, Delgado-Baquerizo et al. 2016). Consistent with this, soil microbial amendments have been shown to improve the restoration of native plant diversity (Middleton and Bever 2012, Wubs et al. 2016), and soil microbes can mediate plant adaptation to the environment (Johnson et al. 2010, Rúa et al. 2016). Given their important role in terrestrial ecosystems, a greater focus needs be placed on the identification and conservation of critical native soil microbes (Fierer et al. 2013).

A major group of soil microbes that interact directly with plants are mycorrhizal fungi. These fungi form symbiotic associations with the roots of most terrestrial plant species, where they commonly provide the plants with phosphorus or other nutrients scavenged from the soil in exchange for photosynthetically fixed carbon. Plants in grassland ecosystems are predominantly colonized by arbuscular mycorrhizal (AM) fungi, which strongly increase the growth of some of the plant species with the highest conservation concern (Koziol and Bever 2015, 2016*a*), in addition to structuring plant communities (van der Heijden et al. 1998, Hartnett and Wilson 1999).

Because of the importance of AM fungi in promoting plant growth and structuring plant communities, considerable effort has gone into characterizing forces that affect their diversity, distribution, and abundance (Bever et al. 2001, Johnson 2010, Kivlin et al. 2011, Maherali and Klironomos 2012). AM fungal communities can be strongly affected by differences in precipitation (Egerton-Warburton et al. 2007, Hazard et al. 2013, Antoninka

Manuscript received 8 August 2017; revised 22 November 2017; accepted 11 December 2017. Corresponding Editor: Deborah Neher.
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et al. 2015), plant community composition (Bever et al. 1996, Eom et al. 2000, Hiiesalu et al. 2014), and soil characteristics, especially soil pH (Hazard et al. 2013, Jansa et al. 2014, Moora et al. 2014). AM fungal communities can also be impacted by anthropogenic disturbance, with generally less diverse communities persisting after mechanical soil disturbance (Oehl et al. 2003, Lumini et al. 2010, Moora et al. 2014), fertilization (Egerton-Warburton and Allen 2000, Liu et al. 2012), or the establishment of invasive plant species (Hawkes et al. 2006, Mummey and Rillig 2006, Pringle et al. 2009). The loss of AM fungal species that are particularly sensitive to disturbance may have functional consequences for plant communities. As evidence of this, the establishment and growth of plant species in grassland restorations is significantly improved when AM fungi from remnant grasslands are reintroduced into restorations (Middleton et al. 2015, Torrez et al. 2015, Koziol and Bever 2016*b*).

Here we investigate the environmental factors associated with AM fungal community structure in prairie ecosystems and their vulnerability to disturbance. Prior to European settlement, prairies extended across much of the central United States and parts of southern Canada, occurring in areas spanning a large gradient of average annual precipitation, from around 500 mm in the west to around 1,200 mm in the east. Due to the rich soils of tallgrass prairies in particular, most of their historic range has been converted to agriculture and generally <5% remains as remnants in protected areas (Samson et al. 2004). The conservation quality of isolated prairie remnants is usually monitored via the diversity and composition of their plant communities, which varies across the precipitation gradient spanned by prairie ecosystems (Daly et al. 2008). However, AM fungal communities can affect prairie plant community composition (Hartnett and Wilson 1999, Vogelsang et al. 2006) and the growth of individual plant species (Wilson and Hartnett 1998, Koziol and Bever 2016*a*). Because of this, the continued conservation of diverse plant communities requires a better understanding of AM fungal communities in prairie remnants, including how they may differ across the precipitation gradient and how they are affected by disturbance.

To help address this, we sampled plant roots from pairs of remnant and disturbed sites spanning western Oklahoma to eastern Illinois. We assessed AM fungal community composition by sequencing a portion of the nuclear large subunit ribosomal RNA (rRNA) gene from the sampled roots and then clustered similar sequences into operational taxonomic units (OTUs) that formed the basis for all community analyses. Using these data, we sought to address two main questions: (1) How are AM fungal communities in remnant prairies and nearby disturbed sites structured, and does community composition change across the precipitation gradient? (2) Are particular AM fungal OTUs consistently overrepresented in remnant sites compared to disturbed sites, and therefore sensitive to loss by disturbance?

Methods

Site selection

We sampled a total of 19 remnant and 15 disturbed sites across the midwestern United States (Fig. 1). We defined remnant sites to be locations that have not been tilled, but may be grazed or hayed and are still dominated by prairie plant species. Near each remnant site or group of remnant sites, we selected disturbed sites that were dominated by nonnative plant species, principally *Bothriochloa ischaemum* (L.) Keng (yellow bluestem), *Bromus inermis* Leyss. (smooth brome), or *Schedonorus arundinaceus* (Schreb.) Dumort. (tall fescue). We categorized these disturbed sites into three groups based on their land use histories. Seven of these disturbed sites had a known history of mechanical soil disruption caused either by plowing or construction. The two disturbed sites located in Oklahoma had no known history of mechanical soil disruption; however, in these sites, the establishment of the nonnative plant species may have been facilitated by overgrazing. The remaining six sites had unknown histories with respect to mechanical soil disruption. Each site was sampled once during mid-summer, either in 2013 or 2015. (See Appendix S1: Table S1 for sample details and information about soil composition for each site).

Sampling

Each sample comprised four soil cores (2 × 15 cm) collected within a 1-m^2 plot. After collection, samples were kept on ice until processing, generally within 12 h. At least 50 mg (wet mass) of fine roots were removed

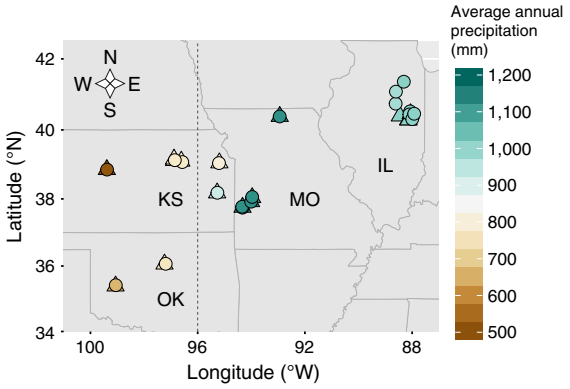

Fig. 1. Map of paired sampling locations from remnant sites (circles) and disturbed sites (triangles) across the precipitation gradient, with location markers colored by average annual precipitation from dark brown (least precipitation) to dark green (most precipitation). The west/east split of samples used for statistical tests coincides with average annual precipitation of 800 mm and is denoted by the dashed vertical line at 96° W. State abbreviations are OK, Oklahoma; KS, Kansas; MO, Missouri; and IL, Illinois.

from each sample, rinsed, and blotted dry; samples were then stored frozen or lyophilized (samples from Fort Riley, Kansas) until DNA extraction. Soil chemical analyses (pH, C:N, cation exchange capacity [CEC], Bray 1 phosphorus, Bray 2 phosphorus, bicarbonate phosphorus, magnesium, calcium, and potassium) were conducted for most soil samples (A&L Great Lakes Labs, Fort Wayne, Indiana, USA; see Appendix S1: Table S1).

DNA sequencing

Roots from each sample were finely chopped on disposable weighing paper using forceps and scissors that were ethanol treated and flamed between samples to minimize cross-contamination; 35 mg of chopped roots were used for DNA extraction (PowerSoil kit, Qiagen Carlsbad, California, USA). A roughly 850 bp portion of the nuclear large subunit ribosomal RNA (rRNA) gene was amplified from the DNA extractions using PCR with forward primer LROR (Bunyard et al. 1994) and barcoded reverse primer FLR2 (Trouvelot et al. 1999). PCR amplification proceeded as follows: 94°C for 5 min, then 35 cycles of (1) 94°C for 30 s, (2) 48°C for 30 s, and (3) 72°C for 45 s; ending with 72°C for 10 min. PCR products were purified (AMPure XP Beckman Coulter, Indianapolis, Indiana, USA) and the resulting concentration was quantified using PicoGreen (Thermo Fisher Scientific, Waltham, Massachusetts, USA). An equimolar amount of PCR product from each sample was pooled and sequenced using Illumina MiSeq (Center for Genomics and Bioinformatics, Indiana University) to produce paired 300-bp non-overlapping reads. We used this approach to generate paired sequences that covered portions of both the phylogenetically informative D1 and D2 variable regions of the large subunit rRNA gene (Hassouna et al. 1984).

Raw sequences were truncated to only retain bases with quality scores of at least 10 and were screened allowing a maximum of one expected error (Edgar and Flyvbjerg 2015) per read pair using a custom Python script. To keep roughly equal numbers of forward and reverse reads after standardizing their lengths, all forward reads were truncated to 235 bp and all reverse reads to 165 bp. Reads with shorter lengths were discarded and any previously paired reads where only one read remained after length trimming were removed. Remaining read pairs were then concatenated after taking the reverse complement of the reverse read, and identical sequences were de-replicated before identifying chimeric sequences using the –uchime_denovo function of VSEARCH (Rognes et al. 2016). Chimeric sequences were removed and the remaining concatenated read pairs were re-replicated before clustering.

Concatenated read pairs were clustered into OTUs with AbundantOTU (Ye 2010) using a 97% sequence similarity threshold; this method performs well for AM fungi although no clustering method can generate OTUs that consistently match morphologically defined AM fungal species (House et al. 2016). The consensus sequence representing each OTU was added to a reference alignment of AM fungal sequences (House et al. 2016) using MAFFT (Katoh and Standley 2013), and the gap in the aligned consensus sequences that represented the non-overlapping forward and reverse reads was manually deleted. Consensus sequences that aligned poorly with the reference sequences were removed before creating a rooted phylogeny containing both the consensus and reference sequences using RAxML (Stamatakis 2014) with the GTR-GAMMA model and using *Mortierella elongata* as an outgroup. Consensus sequences falling outside the AM fungal clade in the phylogeny were considered non-AM fungal and were discarded, leaving 199 AM fungal OTUs representing 499,507 sequences. Another rooted phylogeny was created using only the consensus sequences of these AM fungal OTUs with the same reference sequences as above, and was used to attribute each OTU to a taxonomic group using its position in the phylogeny (Appendix S1: Fig. S1). Most OTUs were identifiable to the genus level except 57 OTUs that could only be assigned to the family Glomeraceae (See Data S1 for OTU consensus sequences). After quality screening, the number of sequences per sample varied by more than an order of magnitude (minimum = 451; maximum = 26,254; median = 3,571) and we accounted for this in various ways during our analysis.

Effect of site history and environmental conditions on AM fungal communities

To summarize aboveground environmental conditions, we calculated the average annual aridity index for each site using the Penman-Monteith equation (Allen et al. 1998) that incorporates measures of precipitation, temperature, wind speed, and dew point that were measured at the National Weather Service locations closest to each site. In our analysis, average precipitation near each site for the five years preceding sampling (2011–2015) was highly correlated with the aridity index ($\rho_{(70)} = 0.92$) and the two metrics gave nearly identical results; therefore for simplicity we only use average precipitation. Some analysis methods we used required further simplification of the precipitation-based analyses by creating two groups of samples: one group representing western samples from sites with <800 mm annual precipitation ($n = 58$), and one group representing eastern samples from sites with >800 mm ($n = 50$) as denoted by the dashed vertical line in Fig. 1. When testing effects of precipitation on AM fungal community composition using PERMANOVA (Anderson 2001), the results were qualitatively comparable regardless of whether we used precipitation as a continuous or a categorical predictor with the two groups already defined. For ease of interpretation among analyses, we therefore used the two precipitation groups as a categorical predictor for

all analyses except when testing associations between soil nutrients, precipitation, and AM fungal communities. We used PERMANOVA to test whether site history (remnant/disturbed) and location along the precipitation gradient (West/East), added as marginal effects in the model, explained differences in AM fungal community composition across all sites, and visualized these differences at a community level using principal coordinates analysis (PCoA). For both PERMANOVA and PCoA, we used Morisita's dissimilarity index (Morisita 1959) calculated from the OTU table counts, because it is robust to differences in sample size (Morisita 1959, Wolda 1981). PERMANOVA tests were performed using the adonis and adonis2 functions and PCoA was performed using the capscale function, all from the vegan package in R (version 3.3.3). We also used PERMANOVA to determine the effects of soil factors on AM fungal communities, for the subset of samples described in the next paragraph.

Correlations among site history, precipitation, and soil variables were calculated using Spearman's rank correlation. To determine how soil variables may correlate with differences in AM fungal community composition between remnant and disturbed sites, we used partial constrained PCoA (Anderson and Willis 2003) controlling for geographic location. This analysis was performed using the capscale function in the vegan package of R using Morisita's dissimilarity index (Morisita 1959) calculated from the OTU table counts. We fit a surface for each soil variable to the constrained ordination plot of the first and second principal coordinates using the ordisurf function in the vegan package of R to determine the correlation between values of that soil variable and differences in AM fungal communities. All samples from Kansas, Missouri, and Illinois with soil nutrient results (72 of 141) were used, and the following soil variables were $\log_{10}$-transformed before analysis: CEC, Bray 1 phosphorus, Bray 2 phosphorus, bicarbonate phosphorus, magnesium, calcium, and potassium.

Random forest classifiers

Random forest classifiers are a commonly used method in machine learning to divide samples into groups based on their characteristics, and random forests have successfully been used in a range of ecological applications (Cutler et al. 2007). Here we used them to predict, using the presence or absence of each OTU in each sample, either the site history (remnant/disturbed) for all samples or the location along the precipitation gradient (west/east) separately for samples from remnant sites and samples from disturbed sites. A random forest classifier generates predictions using repeated subsampling of both samples and OTUs and therefore should be robust to uneven sequence numbers per sample. Because random forest classifiers use subsampling, they can also quantify the contribution of each OTU to the overall classification accuracy. We assessed the classification results using either 5- or 10-fold cross validation to give approximately 10 samples in the "test" set, and the classification accuracy was averaged across cross validation folds. All random forest classifiers were run using the RandomForest package of R and data partitions for cross validation were made with the createFolds function in the caret package of R.

Differential abundance of OTUs

We used the DESeq2 package in R (Love et al. 2014) to determine the differential abundance of each OTU in pairwise comparisons of different groups of sites while correcting for both variation in sequence number across samples and variance in sequence number for each OTU (McMurdie and Holmes 2014). The DESeq2 correction has limited power for OTU tables with any entries of zero; therefore we added a pseudocount of one to all OTU table entries. After the correction is calculated, DESeq2 can test for differences in OTU abundance between any two experimental factors. We used this pairwise contrast to determine (1) differences in OTU abundance between remnant and disturbed sites considered together and (2) differences in OTU abundance with location along the precipitation gradient (west/east) separately for only remnant sites and for only disturbed sites. Differential abundance values are given in Data S1.

Using these differential abundance results, we calculated the net relatedness index (NRI; Webb et al. 2002) to test for phylogenetic clustering among OTUs that were more abundant in either remnant or disturbed sites when considering all samples, or were more abundant in either western or eastern sites when considering only remnant samples or only disturbed samples separately. All NRI calculations were performed with the ses.mpd function in the picante package of R using the phylogeny of OTU sequences as placed in relation to reference sequences (Appendix S1: Fig. S1) after removing the leaves corresponding to the reference sequences (Appendix S1: Fig. S2), and including abundance weighting using the differential abundance values. Significance of NRI values was assessed using 1,000 permutations of the "richness" null model in the ses.mpd function, where OTU abundances were randomized within each comparison, for example all OTUs that were more abundant in remnant sites, but not randomized across comparisons.

We also used the OTU table after DESeq2 correction to conduct linear discriminant analysis (LDA), an alternative to a random forest classifier in grouping samples by site history or location on the precipitation gradient using the AM fungal community composition. LDA was run using both backward and forward selection of OTUs to include in the model using the stepclass function from the klaR package of R using the same cross validation partitions as for the random forest classifier and a stop rule of <1% accuracy improvement.

TABLE 1. Differences in arbuscular mycorrhizal (AM) fungal communities with site disturbance history (remnant/disturbed), side of the precipitation gradient (west/east), or history of mechanical soil disruption (disturbed sites only).

Predictor variables	*P* value
All samples	
Disturbance	**0.0002**
Side of gradient	**0.0002**
Disturbance × Side of gradient	**0.0004**
Sequence number	0.06
Remnant samples only	
Side of gradient	**0.0002**
Sequence number	**0.0016**
Disturbed samples only	
Side of gradient	0.3
History of mechanical soil disruption	0.1
Sequence number	0.5

Notes: Tests were performed using PERMANOVA for three data subsets: (1) all samples, (2) only remnant samples, and (3) only disturbed samples. All tests used Morisita's dissimilarity index calculated using the operational taxonomic unit (OTU) table counts and significance was assessed using 5,000 permutations. In all cases, the $\log_{10}$-transformed sequence number per sample was included as a covariate. Tests with $P \leq 0.05$ are shown in boldface type.

RESULTS

Strong AM fungal community differentiation in remnant sites

The interaction between site disturbance history (remnant/disturbed) and site location (west/east) explained general trends in AM fungal community differentiation (Table 1). This interaction was driven by AM fungal communities from remnant sites being significantly different compared to those from disturbed sites overall (Table 1, Fig. 2a), as well as communities from remnant sites being strongly differentiated across the precipitation gradient (Table 1, Fig. 2b). In contrast, AM fungal communities in disturbed sites were not significantly differentiated across the precipitation gradient, nor were communities significantly differentiated in disturbed sites that had different histories of mechanical soil disruption (Table 1, Fig. 2c).

The differential abundance of OTUs with either site disturbance history (for all samples) or side of the precipitation gradient (for remnant or disturbed samples) revealed differences both in terms of the number of OTUs with significant abundance skews as well as phylogenetic clustering (Fig. 3, Table 2). Although there was not a significant skew in the number of particularly abundant OTUs when comparing remnant and disturbed sites for all samples (Fig. 3a), there was significant phylogenetic clustering among the OTUs that were more abundant in remnant sites (Table 2). However when considering only samples from remnant sites, there was no difference in the number or the phylogenetic clustering of OTUs that were particularly abundant on either side of the precipitation gradient (Fig. 3b, Table 2). For samples only from disturbed sites, there were significantly more OTUs that had increased abundance in sites from the eastern side of the precipitation gradient compared to the western side (Fig. 3c, binomial test $P < 0.001$), and this was also true for OTUs from the genera *Rhizoglomus* (Fig. 3c; binomial test $P = 0.002$) and *Claroideoglomus* (Fig. 3c; binomial test $P = 0.016$) in particular. Although there were relatively few OTUs that were more abundant in western disturbed sites, they showed significant phylogenetic clustering (Table 2), due to the absence of OTUs representing genera in the families Diversisporaceae, Acaulosporaceae, Gigasporaceae, and Claroideoglomeraceae (Fig. 3c), while the OTUs with greater abundance in eastern disturbed sites were not phylogenetically clustered (Table 2). When we tabulated the joint abundance for

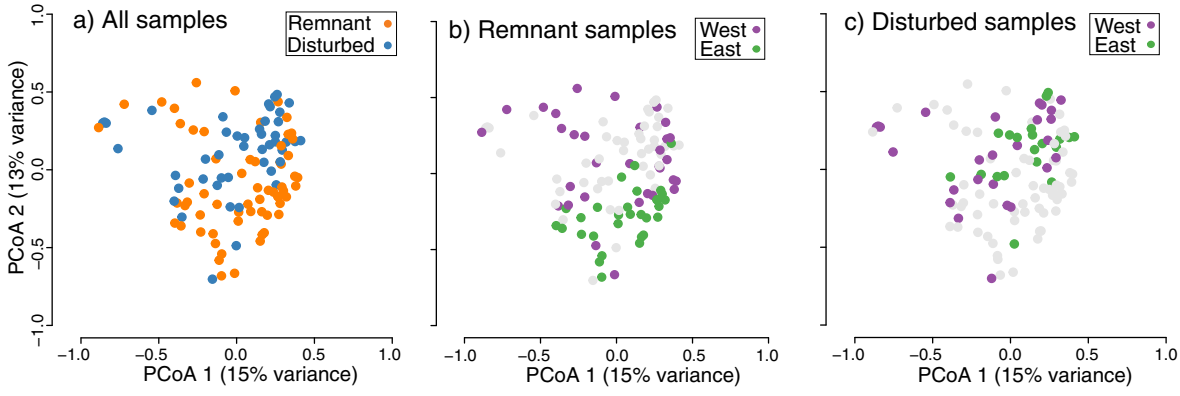

FIG. 2. Principal coordinates analysis (PCoA) of arbuscular mycorrhizal (AM) fungal community composition in all samples using Morisita's dissimilarity index calculated from the operational taxonomic unit (OTU) table counts, with highlighted comparisons between (a) site histories for all samples, and comparisons between the two sides of the precipitation gradient for (b) only remnant samples and (c) only disturbed samples. For panels b and c, samples from groups not being compared are denoted by gray dots. PCoA axis 1 explained 15% of the variance; PCoA axis 2 explained 13% of the variance.

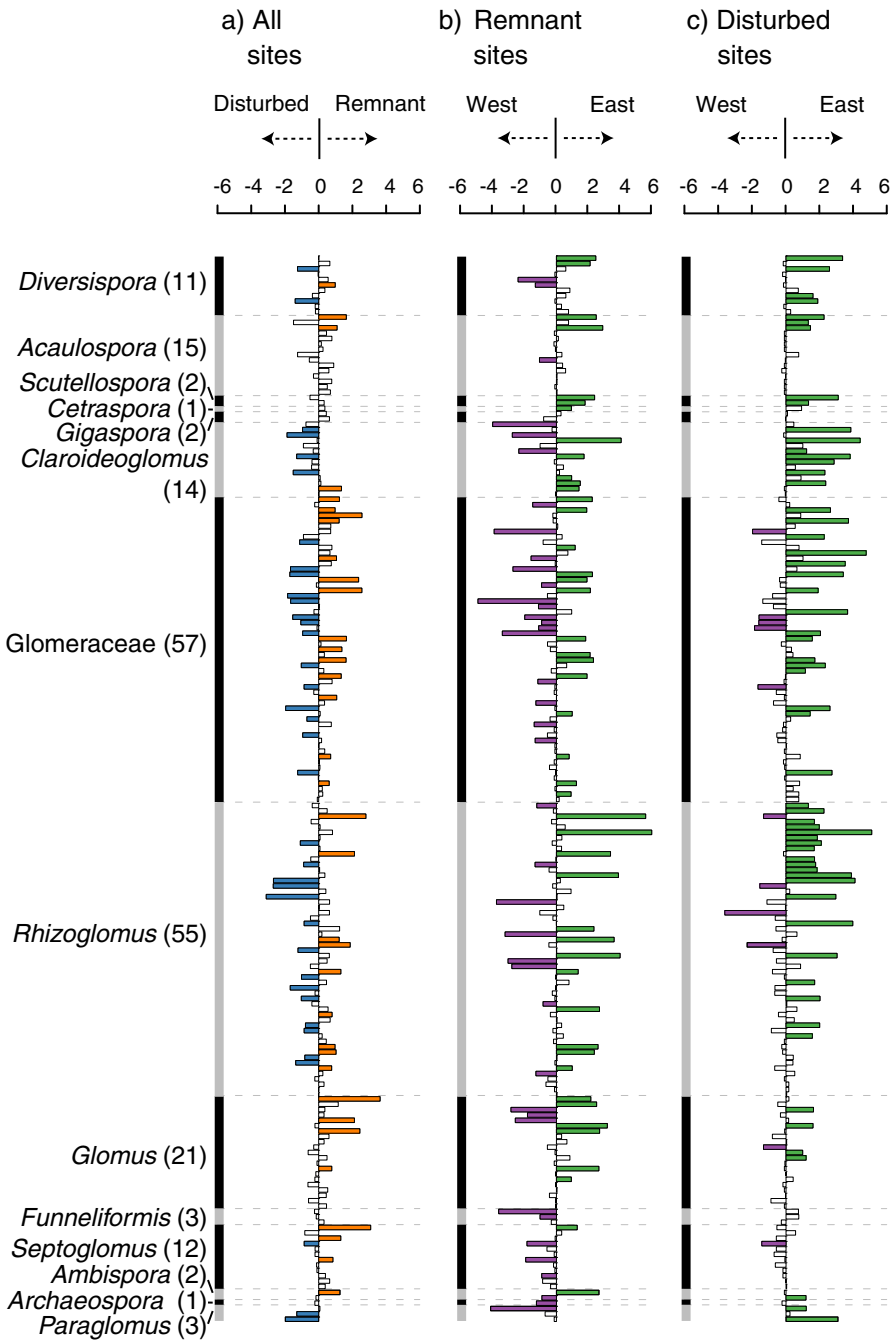

FIG. 3. Differential abundance (on $\log_2$ scale) for each of the 199 OTUs (bars) between (a) remnant (orange) or disturbed (blue) site histories for all sites and between the western (purple) or the eastern (green) side of the precipitation gradient for (b) only remnant sites and (c) only disturbed sites. Filled bars denote OTUs with a significant difference in abundance between the two groups being compared. The order of bars is the same for all panels, and is sorted by the phylogenetic relationships of each OTU's taxonomic attribution (genus level except the family Glomeraceae) as determined from Appendix S1: Fig. S1, with the number of OTUs represented in each taxon in parentheses. OTUs from different taxonomic groups are denoted by alternating black and gray vertical bands on the left side of each panel, and by dashed horizontal lines.

each of the 199 OTUs across the comparison with all samples (Fig. 3a) and the comparison with only remnant samples (Fig. 3b), a disproportionate number of remnant OTUs that were more abundant in eastern sites were also sensitive to anthropogenic disturbance, while OTUs that accumulated with disturbance were not differentially distributed along the precipitation gradient (Fig. 4; $\chi^2 = 51.6$, df = 4, $P < 0.0001$).

TABLE 2. Phylogenetic clustering of AM fungal communities with site disturbance history (remnant/disturbed) or side of the precipitation gradient (west/east) using the net relatedness index (NRI).

Sample group from differential abundance results used to calculate NRI	NRI	*P*
All samples		
Disturbed	−0.48	0.7
Remnant	2.38	**0.005**
Only remnant samples		
West	−0.20	0.6
East	−0.38	0.7
Only disturbed samples		
West	2.30	**0.008**
East	−0.90	0.8

Notes: NRI values are abundance weighted and were calculated using the differential abundance of OTUs shown in Fig. 3 and the phylogeny of all OTU sequences relative to reference AM fungal sequences (Appendix S1: Fig. S1), after removal of the tips representing the reference sequences (Appendix S1: Fig. S2). Positive NRI values indicate phylogenetic clustering of OTUs in the community. Significance of NRI values was assessed using 1,000 permutations, allowing OTU abundances to only be randomized within each sample group. Tests with $P \leq 0.05$ are shown in boldface type.

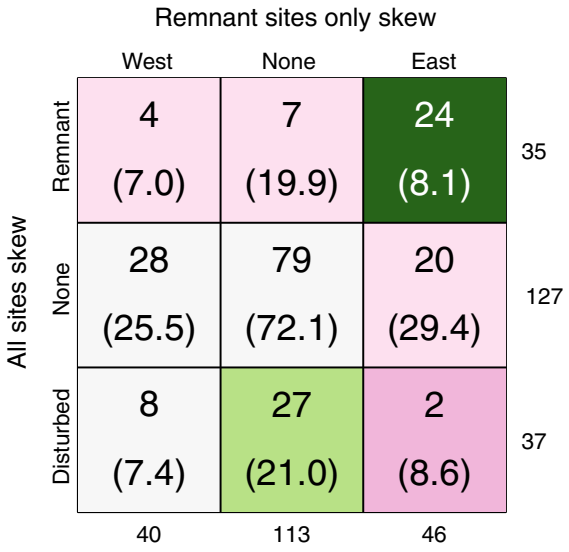

FIG. 4. OTUs that are significantly more abundant in remnant sites from the eastern side of the precipitation gradient appear more sensitive to loss by disturbance than those from the western side. OTUs were categorized into nine groups (boxes) based on their abundance skews (Fig. 3) between remnant and disturbed sites in all samples (rows; from Fig. 3a) as well as between western and eastern sides of the precipitation gradient for only samples from remnant sites (columns; from Fig. 3b); the top number in each group's box is the observed OTU count, with the expected count calculated from the marginal totals given in parentheses below. Boxes are colored by the ratio of observed to expected values, with ratios close to 1 being gray, ratios >1 being progressively greener, and ratios <1 being progressively pinker. Cells in the first row represent OTUs that are sensitive to loss by disturbance, and of these most of the OTUs are significantly more abundant in eastern remnant sites.

Despite samples from remnant and disturbed sites having contrasting amounts of AM fungal community differentiation across the precipitation gradient, this was generally not the case at the level of individual OTUs. For samples from both remnant and disturbed sites, either the random forest classifier or LDA were able to use OTU composition to correctly assign samples to the western or the eastern side of the precipitation gradient with at least 80% accuracy (Table 3). Overall, this was similar to the accuracy in correctly assigning all samples to their site disturbance history (remnant/disturbed; Table 3). Finally, combining the OTU-level random forest classification accuracies with the differential abundance analysis allowed us to better identify OTUs that were closely associated either with site history for all samples (Fig. 5a), or with the side of the precipitation gradient for only remnant samples (Fig. 5b) or only disturbed samples (Fig. 5c). Using these combined analysis results, we identified eight of the top 20 OTUs used by the random forest classifier as being significantly more abundant in remnant sites compared to disturbed sites (Fig. 5a). All of these OTUs represented the family Glomeraceae or genera within it, although other OTUs also representing the Glomeraceae were significantly more abundant in disturbed sites (Fig. 5a).

TABLE 3. Accuracies of different methods for predicting site features using AM fungal community composition.

Set of samples used	Predicting	Random forest	LDA model selection		No. cross-validation folds
			Backward	Forward	
All samples	remnant/disturbed	0.89	0.78 (192 OTUs)	0.85 (4 OTUs)	10
Remnant only	west/east	0.91	0.92 (195 OTUs)	0.97 (5 OTUs)	5
Disturbed only	west/east	0.89	0.82 (198 OTUs)	0.96 (3 OTUs)	5
Average accuracy		0.90	0.84	0.93	

Notes: Using the OTU composition of each sample in the same data subsets as in Table 1, a random forest classifier and linear discriminant analysis (LDA) were run to predict either site disturbance history (remnant/disturbed) or side of the precipitation gradient (west/east). The OTU table was coded by presence/absence for the random forest classifier, and was transformed to account for sequence variation between samples for the LDA. The classification accuracy was assessed using either 5- or 10-fold cross validation. The LDA was run using both forward and backward model selection with a stop rule of <1% accuracy improvement; numbers of OTUs in the final model are given in parentheses (of 199 total OTUs). The random forest used all 199 OTUs for each classification.

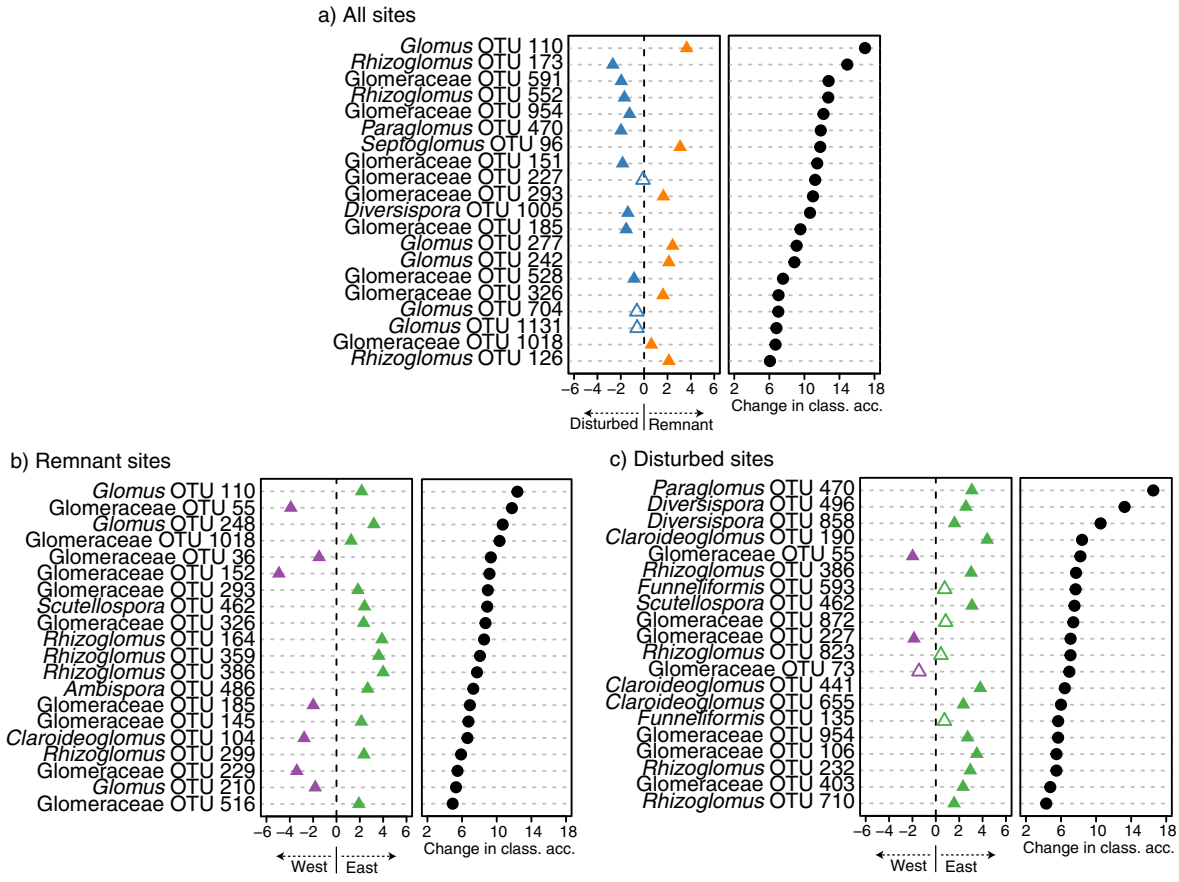

FIG. 5. The influence of specific OTUs (labeled with their taxonomic attribution from Appendix S1: Fig. S1) on the classification accuracy (class. acc.) of the random forest classifier using OTU presence/absence data (right side of each panel), and whether the same OTU was significantly more abundant in either of the two tested groups (left side of each panel) for comparisons between (a) site histories for all sites, and comparisons between the two sides of the precipitation gradient for (b) only remnant sites and (c) only disturbed sites. OTU abundance is on a $\log_2$ scale. Filled triangles represent significant differential abundance for that OTU in one of the two tested groups (colors match those in Fig. 3); open triangles represent trends in abundances that are not significant.

Contribution of environmental factors to AM fungal community differentiation

Precipitation, site history, and their interaction explained roughly 14% of the variation in AM fungal community composition after accounting for sequence number and site location (Table 4). Individual soil variables accounted for between 2.5% (magnesium) and 8% (Bray 2 phosphorus) of the variation in AM fungal community composition (Table 4). However, this was primarily in place of variation explained by precipitation or site history (Table 4) due to correlations between them: disturbed sites were most strongly correlated with increases in both Bray 2 phosphorus and soil pH as well as with decreases in soil organic matter (Appendix S1: Table S2), while increased precipitation was most strongly correlated with decreases in soil potassium, calcium, CEC, and pH (Appendix S1: Table S2).

Soil characteristics were correlated with site history to a large enough extent that when only the soil variables were used to generate a partial constrained PCoA ordination of AM fungal community composition accounting for geographic location, PCoA axis 1 still clearly separated communities from remnant and disturbed sites, regardless of the U.S. state in which the sites were located (Appendix S1: Fig. S3). In contrast, PCoA axis 2 mainly separated between-sample variation within each site history (Appendix S1: Fig. S3). Of the four soil variables that explained the most community variation in the constrained ordination, Bray 2 phosphorus primarily drove the community separation by site history along PCoA axis 1, similar to the results from PERMANOVA (Table 4), with consistently higher soil phosphorus in disturbed sites (Appendix S1: Fig. S3B). Soil organic matter, the soil variable that best explained the variation in AM fungal communities in the constrained ordination, was generally higher in remnant samples (Appendix S1: Fig. S3A, Table S2), while soil pH was consistently lower (Appendix S1: Fig. S3D, Table S2), and soil magnesium showed no clear pattern (Appendix S1: Fig. S3C), potentially because it was not significantly correlated with site history (Appendix S1: Table S2).

TABLE 4. Correlations between site features, soil variables, and AM fungal community composition.

Soil variable	R^2†	Resulting reduction in R^2 (%) when each soil variable was added individually to the basic model		
		Precipitation	Site history	Precipitation × Site history
pH	0.061	−40	−31	16
Bray1 phosphorus‡	0.045	−0.4	−20	**−19**
Bray2 phosphorus‡	0.080	−35	**−59**	−11
Bicarbonate phosphorus‡	0.033	−4	−0.4	−10
Cation exchange capacity (CEC)‡	0.038	−29	4	31
C:N	0.026	−2	−2	26
Organic matter	0.043	−2	−9	−0.2
Magnesium‡	0.025	2	4	−7
Calcium‡	0.041	−44	4	32
Potassium‡	0.076	**−64**	−20	−9

Notes: The (coefficients of determination R^2) without soil variables (basic model) are 0.055 for both precipitation and site history, and 0.031 for precipitation × site history. The R^2 values between AM fungal community composition and precipitation, site history, and soil variables were tested using PERMANOVA while accounting for sequence number ($\log_{10}$-transformed) and site location. All tests used Morisita's dissimilarity index calculated from the OTU table counts and significance was assessed using 5,000 permutations. Values in the first column are coefficients of determination between AM fungal community composition and each soil variable when it was added individually to the basic model. The last three columns give the percent change in the coefficients of determination for precipitation, site history, and their interaction caused by inclusion of each soil variable individually in the basic model. The soil variable resulting in the largest decrease in R^2 for each of the variables in the basic model is shown in boldface type.

†R^2 for each soil variable when added individually to the basic model.

‡Variables were $\log_{10}$-transformed in the model.

DISCUSSION

Differentiation of AM fungal communities in remnant sites

The AM fungal communities from remnant sites were significantly differentiated across the precipitation gradient (Table 1, Fig. 2b). Because precipitation was strongly correlated with soil characteristics, especially potassium, calcium, CEC, pH, and Bray 2 phosphorus (Appendix S1: Table S2), it is possible that these soil characteristics also helped to drive the community differentiation. Although the OTUs comprising these communities were taxonomically and phylogenetically diverse, representing at least 12 genera (Fig. 3b), they nonetheless showed significant phylogenetic clustering across the precipitation gradient (Table 2), and AM fungal communities in grassland ecosystems can also be phylogenetically clustered at more local scales (Horn et al. 2014). This phylogenetic clustering is consistent with filtering of AM fungal communities among all remnant sites compared to all disturbed sites, suggesting that site disturbance is associated with the loss of phylogenetic clustering, independent of precipitation. While other studies have also found variation in AM fungal community differentiation with precipitation in grasslands (Egerton-Warburton et al. 2007) or in sites with diverse environmental conditions and land use histories (Hazard et al. 2013, Antoninka et al. 2015), this study identifies that the differentiation of the remnant AM fungal communities along the precipitation gradient was weakened by disturbance. The OTUs that were abundant in the eastern remnant sites were particularly sensitive to disturbance (Fig. 4), suggesting that the AM fungi of the highly fragmented and rare eastern prairies are of particular conservation concern.

This AM fungal community differentiation and the phylogenetic clustering of OTUs in remnant prairies is consistent with the role of native AM fungi in promoting local adaptation of prairie plant communities. Experimental support for this has been provided by observations that AM fungal isolates from drier sites conferred greater drought tolerance to their hosts than AM fungal isolates from wetter sites (Stahl and Smith 1984). In addition, prairie grasses grown with their sympatric AM fungal community consistently had increased biomass and reproductive output relative to their growth with AM fungal communities from other prairie sites (Johnson et al. 2010), and these outcomes may be partially driven by the resource allocation strategies used by both plants and fungi (Revillini et al. 2016).

Functionally, AM fungal communities in grassland ecosystems help maintain diverse plant communities and improve soil stability. Inoculation experiments have demonstrated the importance of AM fungal isolates from remnant prairies in improving the establishment, growth, and reproduction of late successional stage prairie plants and in increasing prairie plant diversity (Middleton et al. 2015, Koziol and Bever 2016*b*). Conversely, the experimental reduction of AM fungal abundance in remnant prairies using fungicide has strong effects on both plant species richness and diversity (Hartnett and Wilson 1999). The proportion of water-stable aggregates, a measure of a soil's ability to resist erosion, has been shown to be significantly higher in remnants compared to sites with a history of soil disturbance (Jastrow 1987, Duchicela et al. 2012). There is also a strong correlation between the abundance of AM

fungal hyphae in the soil and the amount of water stable aggregates (Wilson et al. 2009), suggesting AM fungal communities are critical in promoting soil stability either directly through hyphal meshes or indirectly through the glycoprotein glomalin (Rillig and Mummey 2006).

Reduced AM fungal community differentiation in disturbed sites

In contrast to remnant sites, AM fungal communities in disturbed sites were not significantly differentiated across the precipitation gradient (Table 1, Fig. 2c), despite the various histories of mechanical soil disruption that are represented by the disturbed sites we sampled. Because our main focus here was not to comprehensively evaluate the effects of different disturbance types on AM fungal communities, we took a conservative approach when labeling the history of mechanical soil disruption at each of the disturbed sites by assigning any site not having a confident history of past disturbance to an 'unknown' disturbance category. Although classifications of disturbance histories are unavoidably at least somewhat subjective, these classifications can have substantial effects on the outcome of statistical tests. For instance, in this study, when we repeated the PERMANOVA analysis for disturbed samples (Table 1) but did not include the history of mechanical soil disruption as a predictor, the AM fungal communities in disturbed sites were then significantly differentiated across the precipitation gradient ($P = 0.04$). However, the AM fungal communities in remnant sites were always more strongly differentiated across the precipitation gradient compared to those in disturbed sites regardless of the model used. The early successional or invasive plant communities that dominated all of the disturbed sites sampled here are less reliant upon AM fungi than the late successional stage plants found in remnants (Wilson and Hartnett 1998, Pringle et al. 2009, Koziol and Bever 2015, 2016*a*). These plant community characteristics in disturbed sites may allow a range of AM fungal taxa to persist compared to remnant sites where reliance of the plants on AM fungi may place functional constraints on the AM fungal communities. This expectation is supported by the phylogenetically even representation of OTUs from disturbed sites compared to the significant phylogenetic clustering of OTUs from remnant sites (Table 2). While many factors can contribute to the local adaptation of plant communities, this lack of both AM fungal community differentiation and phylogenetic clustering across the precipitation gradient following anthropogenic disturbance may reduce the ability of these AM fungal communities to aid the local adaptation of plants.

Despite the lack of AM fungal community differentiation in disturbed sites, individual OTUs still had substantially different abundances across the precipitation gradient (Fig. 3c), facilitating the ability to classify communities on both sides of the gradient with high accuracy (Table 3). Although we found no evidence of phylogenetic clustering of AM fungal communities from eastern disturbed sites, there was strong phylogenetic clustering in communities from western disturbed sites (Table 2). This clustering may be partly due to differences in the dominant plant species between western and eastern sites, with mainly smooth brome and yellow bluestem in western disturbed sites and mainly tall fescue in eastern disturbed sites, due to differences in precipitation or related soil characteristics, or due to other factors we did not measure here.

Soil nutrients also predict AM fungal community differentiation

Among measured soil nutrients, differences in soil phosphorus were most strongly correlated with changes in AM fungal community composition (Table 4). This was not unexpected given the importance of phosphorus exchange in mycorrhizal associations. The fact that not all AM fungi provide the same growth benefit to plants over a range of phosphorus conditions (Vogelsang et al. 2006) may help drive shifts in AM fungal community composition between sites with different phosphorus levels. Concentrations of Bray 2 phosphorus in the soil most strongly separated samples from the two site histories, with disturbed sites consistently having more phosphorus (Appendix S1: Fig. S3B, Table S2), which is consistent with more intensive land use, including agricultural fertilization. Increased phosphorus levels in the soil can reduce plant preferential allocation of carbohydrates to the most mutualistic AM fungal strains (Ji and Bever 2016), which can allow the proliferation of less mutualistic, faster-growing strains of AM fungi (Bever 2015). In this study, we cannot isolate the direct effect of increased soil phosphorus from that of site disturbance because the two were highly correlated (Appendix S1: Table S2). However, the significantly greater number of OTUs that were more abundant in eastern disturbed sites where the disturbance was primarily due to soil disruption (Appendix S1: Table S1; Fig. 3c) is consistent with the establishment of less mutualistic AM fungal strains following soil disturbance and concomitant agricultural phosphorus fertilization allowing their persistence. In contrast, several other studies failed to find significant correlations between AM fungal community composition and soil phosphorus concentrations (Oehl et al. 2010, Jansa et al. 2014, Moora et al. 2014). This discrepancy could be due to differences in host plant communities, the form of available soil phosphorus, or how AM fungal communities were assayed (e.g., spores or molecular methods).

Changes in soil potassium and pH were also correlated with changes in AM fungal communities (Table 4). Soil potassium was negatively correlated with precipitation (Table 4; Appendix S1: Table S2), consistent with findings from other ecosystems (Austin and Vitousek 1998, Bachar et al. 2010). Soil pH was also negatively correlated with precipitation and was consistently lower in remnant sites (Appendix S1: Table S2). In contrast, soil organic matter was consistently higher in remnant sites (Appendix S1: Table S2) and was also most predictive of AM fungal

community composition in the constrained ordination (Appendix S1: Fig. S3A). These results are similar to those of a previous study in Switzerland (Jansa et al. 2014), suggesting the relationship between soil organic matter and AM fungal communities is not unique to the especially rich soils of tallgrass prairies. However, correlations between measured soil variables in this study (Appendix S1: Table S2) limit the utility of their individual interpretation. The negative correlation between soil pH and both soil organic matter and soil magnesium (Appendix S1: Table S2) in particular likely had the most influence on the constrained ordination results (Appendix S1: Fig. S3).

Identifying possible indicator OTUs of site history or site location

The combination of a random forest classifier based on OTU presence or absence together with differential abundance analysis (Fig. 5) provides a robust alternative to traditionally used but more rigidly calculated methods to identify indicator species (Dufrêne and Legendre 1997). Due to its repeated randomizations of both samples and OTUs, a random forest classifier is relatively unaffected by differences in sampling intensity (sequencing depth) between samples, and also differences in the number of samples collected per site. The model-corrected OTU table also takes these differences into account and linear combinations of relatively few OTUs (LDA forward model selection) generally gave equal or greater classification accuracy than the random forests (Table 3). However there were strong correlations between OTUs, as expected because single AM fungal cells may contain multiple OTUs (House et al. 2016). This prevents us from confidently isolating the effects of single OTUs using LDA as is possible using a random forest classifier because of its randomizations. We therefore believe that OTUs that both highly promote random forest classification accuracy as well as have significantly skewed abundances toward remnant sites (Fig. 5a) are promising indicators of remnant grasslands.

Three of the top five of these potential indicator OTUs were assigned to the genus *Glomus* specifically with the remainder also being from the family Glomeraceae. Even within only remnant samples, 17 out of the 20 top OTUs in predicting whether a sample was collected from the western or the eastern side of the precipitation gradient were from the Glomeraceae (Fig. 5b). The organization of these OTUs into species is unclear however, because the Glomeraceae have an especially large range of rRNA gene sequence variation that can result in multiple OTUs occurring within the same species (House et al. 2016). The identification of these potential indicator OTUs may enable more informed conservation planning based on characteristics of the soil microbial community. However, the uncertainty in linking OTUs to species means that further work is necessary before individual AM fungal species can be identified as targets for culturing efforts to grow AM fungal inoculum that can efficiently aid the establishment of grassland restorations (Middleton et al. 2015, Koziol and Bever 2016*b*). Finally, these indicator OTUs are just that: indicators of remnant sites. While reintroduction of AM fungi from remnant sites has been shown to benefit the growth of late successional stage prairie plants (Middleton et al. 2015, Koziol and Bever 2016*b*), it remains to be determined whether the AM fungi that contain these indicator OTUs are functionally better than other AM fungi found in remnant sites.

Conclusions

AM fungal communities in remnant grasslands were strongly differentiated across a precipitation gradient, while those in disturbed sites across the same gradient were not differentiated despite these sites having a range of disturbance histories. Late successional stage prairie plants are generally reliant upon AM fungi, and the differences in AM fungal communities that occur in remnant prairies across the precipitation gradient are consistent with the role of AM fungi in aiding local adaptation in plant communities. We specifically found that AM fungi characteristic of eastern prairie remnants are especially vulnerable to anthropogenic disturbance. Those taxa are of particular conservation concern given the small size of existing prairie remnants and evidence that native prairie fungi can provide strong growth benefits to late successional prairie plants.

Acknowledgments

K. Zaiger was instrumental in the planning and the sample collection/processing for this project. We thank J. Bauer, N. Craun, E. Duell, C. Gurholt, K. Hickman, J. Hopkins, L. Koziol, and P. Schultz for assistance with sample collection. We also thank M. Hahn, J. Lennon, R. Phillips, T. Cheeke, A. Tipton, and two anonymous reviewers for helpful comments and suggestions that greatly improved this manuscript. This work was supported by Strategic Environmental Research and Development Program (SERDP) grant RC-2330 to J. D. Bever and National Science Foundation grant DEB-1556664 to J. D. Bever.

Literature Cited

Allen, R. G., L. S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration. Guidelines for computing crop water requirements. FAO irrigation and drainage paper 56. FAO, Rome, Italy.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

Anderson, M. J., and T. J. Willis. 2003. Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. Ecology 84:511–525.

Antoninka, A. J., M. E. Ritchie, and N. C. Johnson. 2015. The hidden Serengeti—Mycorrhizal fungi respond to environmental gradients. Pedobiologia (Jena) 58:165–176.

Austin, A. T., and P. M. Vitousek. 1998. Nutrient dynamics on a precipitation gradient in Hawai'i. Oecologia 113:519–529.

Bachar, A., A. Al-Ashhab, M. I. M. Soares, M. Y. Sklarz, R. Angel, E. D. Ungar, and O. Gillor. 2010. Soil microbial abundance and diversity along a low precipitation gradient. Microbial Ecology 60:453–461.

Bever, J. D. 2015. Preferential allocation, physio-evolutionary feedbacks, and the stability and environmental patterns of mutualism between plants and their root symbionts. New Phytologist 205:1503–1514.

Bever, J. D., J. B. Morton, J. Antonovics, and P. A. Schultz. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. Journal of Ecology 84:71–82.

Bever, J. D., P. A. Schultz, A. Pringle, and J. B. Morton. 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. BioScience 51:923–932.

Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of plant species diversity by pathogens. Annual Review of Ecology, Evolution, and Systematics 46:305–325.

Bunyard, B. A., M. S. Nicholson, and D. J. Royse. 1994. A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. Mycologia 86:762–772.

Cutler, D. R., T. C. Edwards, K. H. Beard, A. Cutler, K. T. Hess, J. Gibson, and J. J. Lawler. 2007. Random forests for classification in ecology. Ecology 88:2783–2792.

Daly, C., M. Halbleib, J. I. Smith, W. P. Gibson, M. K. Doggett, G. H. Taylor, J. Curtis, and P. P. Pasteris. 2008. Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States. International Journal of Climatology 28:2031–2064.

Delgado-Baquerizo, M., F. T. Maestre, P. B. Reich, T. C. Jeffries, J. J. Gaitan, D. Encinar, M. Berdugo, C. D. Campbell, and B. K. Singh. 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications 7:10541.

Duchicela, J., K. M. Vogelsang, P. A. Schultz, W. Kaonongbua, E. L. Middleton, and J. D. Bever. 2012. Non-native plants and soil microbes: potential contributors to the consistent reduction in soil aggregate stability caused by the disturbance of North American grasslands. New Phytologist 196:212–222.

Dufrêne, M., and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs 67:345–366.

Edgar, R. C., and H. Flyvbjerg. 2015. Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31:btv401.

Egerton-Warburton, L. M., and E. B. Allen. 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecological Applications 10:484–496.

Egerton-Warburton, L. M., N. C. Johnson, and E. B. Allen. 2007. Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. Ecological Monographs 77:527–544.

Eom, A.-H., D. C. Hartnett, and G. W. T. Wilson. 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia 122:435–444.

Fierer, N., J. Ladau, J. C. Clemente, J. W. Leff, S. M. Owens, K. S. Pollard, R. Knight, J. A. Gilbert, and R. L. McCulley. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342:621–624.

Hartnett, D. C., and G. W. T. Wilson. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80:1187–1195.

Hassouna, N., B. Mithot, and J.-P. Bachellerie. 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. Nucleic Acids Research 12:3563–3583.

Hawkes, C. J., C. D'. Antonio. Belnap, and M. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281:369–380.

Hazard, C., P. Gosling, C. J. van der Gast, D. T. Mitchell, F. M. Doohan, and G. D. Bending. 2013. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. ISME Journal 7:498–508.

Hiiesalu, I., M. Pärtel, J. Davison, P. Gerhold, M. Metsis, M. Moora, M. Öpik, M. Vasar, M. Zobel, and S. D. Wilson. 2014. Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. New Phytologist 203:233–244.

Horn, S., T. Caruso, E. Verbruggen, M. C. Rillig, and S. Hempel. 2014. Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. ISME Journal 8:2231–2242.

House, G. L., S. Ekanayake, Y. Ruan, U. M. Schütte, W. Kaonongbua, G. Fox, Y. Ye, and J. D. Bever. 2016. Phylogenetically structured differences in rRNA gene sequence variation among species of arbuscular mycorrhizal fungi and their implications for sequence clustering. Applied and Environmental Microbiology 82:4921–4930.

Jansa, J., A. Erb, H.-R. Oberholzer, P. Šmilauer, and S. Egli. 2014. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Molecular Ecology 23:2118–2135.

Jastrow, J. D. 1987. Changes in soil aggregation associated with tallgrass prairie restoration. American Journal of Botany 74:1656–1664.

Ji, B., and J. D. Bever. 2016. Plant preferential allocation and fungal reward decline with soil phosphorus: implications for mycorrhizal mutualism. Ecosphere 7:e01256.

Johnson, N. C. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytologist 185:631–647.

Johnson, N. C., G. W. T. Wilson, M. A. Bowker, J. A. Wilson, and R. M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences USA 107:2093–2098.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30:772–780.

Kivlin, S. N., C. V. Hawkes, and K. K. Treseder. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 43:2294–2303.

Koziol, L., and J. D. Bever. 2015. Mycorrhizal response trades off with plant growth rate and increases with plant successional status. Ecology 96:1768–1774.

Koziol, L., and J. D. Bever. 2016*a*. AMF, phylogeny, and succession: specificity of response to mycorrhizal fungi increases for late-successional plants. Ecosphere 7:e01555.

Koziol, L., and J. D. Bever. 2016*b*. The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology. https://doi.org/10.1111/1365-2664.12843

Liu, Y., G. Shi, L. Mao, G. Cheng, S. Jiang, X. Ma, L. An, G. Du, N. C. Johnson, and H. Feng. 2012. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytologist 194:523–535.

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:550.

Lumini, E., A. Orgiazzi, R. Borriello, P. Bonfante, and V. Bianciotto. 2010. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environmental Microbiology 12:2165–2179.

Maherali, H., and J. N. Klironomos. 2012. Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. PLoS ONE 7:e36695.

Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752–755.

McMurdie, P. J., and S. Holmes. 2014. Waste not, want not: Why rarefying microbiome data is inadmissible. PLoS Computational Biology 10:e1003531.

Middleton, E. L., and J. D. Bever. 2012. Inoculation with a native soil community advances succession in a grassland restoration. Restoration Ecology 20:218–226.

Middleton, E. L., S. Richardson, L. Koziol, C. E. Palmer, Z. Yermakov, J. A. Henning, P. A. Schultz, and J. D. Bever. 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6:1–16.

Moora, M., J. Davison, M. Öpik, M. Metsis, Ü. Saks, T. Jairus, M. Vasar, and M. Zobel. 2014. Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiology Ecology 90:609–621.

Morisita, M. 1959. Measuring of interspecific association and similarity between communities. Memoirs of the Faculty of Science Kyushu University, Series E 3:65–80.

Mummey, D. L., and M. C. Rillig. 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288:81–90.

Oehl, F., E. Sieverding, K. Ineichen, P. Mäder, T. Boller, and A. Wiemken. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. Applied and Environmental Microbiology 69:2816–2824.

Oehl, F., E. Laczko, A. Bogenrieder, K. Stahr, R. Bösch, M. van der Heijden, and E. Sieverding. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biology and Biochemistry 42:724–738.

Pringle, A., J. D. Bever, M. Gardes, J. L. Parrent, M. C. Rillig, and J. N. Klironomos. 2009. Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40:699–715.

Revillini, D., C. A. Gehring, and N. C. Johnson. 2016. The role of locally adapted mycorrhizas and rhizobacteria in plant–soil feedback systems. Functional Ecology 30:1086–1098.

Rillig, M. C., and D. L. Mummey. 2006. Mycorrhizas and soil structure. New Phytologist 171:41–53.

Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.

Rúa, M. A., et al. 2016. Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BMC Evolutionary Biology 16:122.

Samson, F., F. Knopf, and W. R. Ostlie. 2004. Great Plains ecosystems: past, present, and future. Wildlife Society Bulletin 32:6–15.

Stahl, P. D., and W. K. Smith. 1984. Effects of different geographic isolates of *Glomus* on the water relations of *Agropyron smithii*. Mycologia 76:261–267.

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Torrez, V., T. Ceulemans, J. Mergeay, L. de Meester, and O. Honnay. 2015. Effects of adding an arbuscular mycorrhizal fungi inoculum and of distance to donor sites on plant species recolonization following topsoil removal. Applied Vegetation Science 19:7–19.

Trouvelot, S., D. van Tuinen, M. Hijri, and V. Gianinazzi-Pearson. 1999. Visualization of ribosomal DNA loci in spore interphasic nuclei of Glomalean fungi by fluorescence in situ hybridization. Mycorrhiza 8:203–206.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

Vogelsang, K. M., H. L. Reynolds, and J. D. Bever. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytologist 172:554–562.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475–505.

Wilson, G. W. T., and D. C. Hartnett. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85:1732–1738.

Wilson, G. W. T., C. W. Rice, M. C. Rillig, A. Springer, and D. C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters 12:452–461.

Wolda, H. 1981. Similarity indices, sample size and diversity. Oecologia 50:296–302.

Wubs, E. R. J., W. H. van der Putten, M. Bosch, and T. M. Bezemer. 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2:16107.

Ye, Y. 2010. Identification and quantification of abundant species from pyrosequences of 16S rRNA by consensus alignment. Pages *in* Proceedings IEEE International Conference on Bioinformatics and Biomedicine. 153–157. IEEE, Hong Kong.

Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1681/full

Data Availability

Data available from the following:

Raw sequences have been deposited in the NCBI Sequence Read Archive (SRA) as accession SRP106887 at https://www.ncbi.nlm.nih.gov/sra.

Analysis scripts and input data used to make all figures and tables are accessible from https://doi.org/10.5281/zenodo.1064315.