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Importance of dispersal and thermal environment for mycorrhizal communities: lessons from Yellowstone National Park

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Abstract. The relative importance of dispersal and niche restrictions remains a controversial topic in community ecology, especially for microorganisms that are often assumed to be ubiquitous. We investigated the impact of these factors for the community assembly of the root-symbiont arbuscular mycorrhizal fungi (AMF) by sampling roots from geothermal and nonthermal grasslands in Yellowstone National Park (YNP), followed by sequencing and RFLP of AMF ribosomal DNA. With the exception of an apparent generalist RFLP type closely related to *Glomus intraradices*, a distance-based redundancy analysis indicated that the AMF community composition correlated with soil pH or pH-driven changes in soil chemistry. This was unexpected, given the large differences in soil temperature and plant community composition between the geothermal and nonthermal grasslands. RFLP types were found in either the acidic geothermal grasslands or in the neutral to alkaline grasslands, one of which was geothermal. The direct effect of the soil chemical environment on the distribution of two AMF morphospecies isolated from acidic geothermal grasslands was supported in a controlled greenhouse experiment. *Paraglomus occultum* and *Scutellospora pellucida* were more beneficial to plants and formed significantly more spores when grown in acidic than in alkaline soil. Distance among grasslands, used as an estimate of dispersal limitations, was not a significant predictor of AMF community similarity within YNP, and most fungal taxa may be part of a metacommunity. The isolation of several viable AMF taxa from bison feces indicates that wide-ranging bison could be a vector for at least some RFLP types among grasslands within YNP. In support of classical niche theory and the Baas-Becking hypothesis, our results suggest that AMF are not limited by dispersal at the scale of YNP, but that the soil environment appears to be the primary factor affecting community composition and distribution.

Key words: arbuscular mycorrhizal fungi; community composition; dispersal; habitat; niche; pH; RFLP, restriction fragment length polymorphisms; soil characteristics; temperature; Yellowstone National Park.

Introduction

A long-standing goal of community ecology is to explain the spatiotemporal distribution of species. The classical approach is to use niche theory, which emphasizes species' differences in their responses to abiotic and biotic factors that dictate their fundamental and realized niches. An alternative model, neutral theory, has recently been proposed to explain species distributions. In contrast to niche theory, neutral theory assumes equivalence in species' traits related to vital rates and highlights the importance of dispersal and stochastic processes influencing species distributions (see Gaston and Chown 2005 for a comparative discussion). Although both niche and neutral theory have been utilized with varying success to explain distribution patterns for larger organisms (McGill et al. 2006), less is known about their applicability for microbial communities (but see Martiny et al. 2006, Lekberg et al. 2007, Dumbrell et al. 2010). The Baas-Becking hypothesis (Baas-Becking 1934) suggested long ago that microorganisms are ubiquitous and that their distributions are largely determined by environmental factors rather than dispersal, a notion consistent with classical niche theory. On the other hand, dispersal limitations have been identified for many microorganisms (Martiny et al. 2006), suggesting that aspects of both theories may be applicable.

The objective of this study was to characterize arbuscular mycorrhizal fungal (AMF) communities across habitat types with known spatial arrangements to quantify the relative importance of the environment and dispersal for structuring fungal communities. AMF are components of most ecosystems and form a root symbiosis with >80% of terrestrial plant species, where they exchange phosphorus for carbon assimilated by the

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plant (Smith and Read, 2008). The long-held assumption that AMF are functionally equivalent has been refuted by research showing that fungal species richness influences plant diversity and productivity (van der Heijden et al. 1998), as well as ecosystem properties such as soil aggregation and carbon and nutrient cycling (as discussed in Rillig 2004). A better understanding of factors that structure fungal communities is therefore not only of interest to microbial community ecologists, but also could increase our predictive ability regarding symbiotic function, because functional traits may be phylogenetically conserved (Powell et al. 2009).

The environment has been shown to influence the distribution of certain fungal taxa, and shifts in fungal assemblages can be driven by differences among plant communities (Öpik et al. 2006), soil types (Lekberg et al. 2007), production systems (Oehl et al. 2004), and seasons (Klironomos et al. 2001). The role of fungal dispersal in community structure has been considerably less researched (but see Lekberg et al. 2007, Dumbrell et al. 2010), but given the large difference in size among fungal individuals (Rosendahl and Stukenbrock 2004) and family-level disparities in infective propagules (Klironomos and Hart 2002), the degree of dispersal limitation most likely differs among taxa. Indeed, some fungal taxa show worldwide distributions, whereas others are endemic (Appoloni et al. 2008, Rosendahl et al. 2009).

Yellowstone National Park (YNP) offers a unique opportunity to compare the importance of dispersal and environment for two main reasons. First, AMF are largely restricted to geothermal and nonthermal grasslands, because surrounding coniferous forests harbor few hosts, resulting in a fragmented landscape of AMF-suitable habitat. Second, these grasslands differ greatly in plant communities, soil temperature, and chemical properties, providing considerable variation in biotic and abiotic factors that might select for particular fungal taxa. Distance among grasslands was used to estimate dispersal limitations (see Chase 2005, Cottenie 2005), which we deemed appropriate given that AMF disperse by wind (Warner et al. 1987) and animals (Mangan and Adler 2000). If dispersal dictates community composition, we hypothesize that distance among grasslands will be negatively associated with the similarity of the AMF communities. On the other hand, if the environment (grassland type) is more important than dispersal, we predict little or no correlation between distance and community similarity, but strong correlations among fungal community composition and grassland type. Indeed, a previous study of AMF communities restricted to acidic grasslands in YNP (Appoloni et al. 2008) suggested strong environmental effects on fungal communities, but soil chemical properties were not analyzed in that study and therefore could not be separated from soil temperature. We expand on that study by contrasting AMF communities in geothermal grasslands of disparate chemical properties with those found in interspersed nonthermal grasslands. This approach allowed us to compare the effects of grassland type (geothermal and nonthermal) and to look for associations between fungal taxa and soil chemical properties.

Given that the field survey was strictly correlational, we conducted a controlled greenhouse experiment to test whether soil chemical properties were indeed causing the distribution of selected taxa observed in the field. Finally, to elucidate factors involved in AMF dispersal within YNP, we assessed whether the widespread herbivores in the park (bison) can be a vector for AMF as they frequently occur in both grassland types.

Materials and Methods

Field sampling

Five geothermal (T1–T5) areas and three interspersed nonthermal (NT1–NT3) areas were identified along a north–south transect within YNP (Fig. 1; see Appendix A). The geothermal grasslands are dominated by the heat-tolerant hot-spring panic grass (*Dichanthelium lanuginosum* (Schmoll) Spellenb) that is active year-round; monkey flower (*Mimulus guttatus*) and two bentgrass species (*Agrostis scabra* and *Agrostis rossiae*) are also present, but in lower abundance. The nonthermal grasslands harbor a more diverse grass and forb community (see Appendix B for a species list) that typically has a growing season of less than 5 months. These two grassland types also differ greatly in soil temperatures. Soil temperatures in the geothermal grasslands increase with depth, often exceed 45°C at rooting depth during the summer months, and rarely go below 10°C in the winter (Stout et al. 1997, Tercek and Whitbeck 2004). Soil temperature in nonthermal grasslands, on the other hand, mirrors air temperatures at the surface, decreases with depth, rarely exceeds 30°C during the summer months, and drops down to freezing in the winter (Y. Lekberg, *personal observation*).

From within each grassland, roots were sampled by taking 20 soil cores (2 cm diameter $\times$ 7.5 cm deep) adjacent to randomly chosen plants during spring and early summer from an area no smaller than 50×30 m. Soil cores allowed us to remove part of the root system without killing the plant, which is crucial for collecting such a substantial number of samples within YNP. The geothermal grasslands were sampled earlier than the nonthermal grasslands (sampling dates are in Appendix A), but both grassland types were sampled at the peak of the growing season, which is earlier on thermal soils. Soil temperatures (5 cm depth) were recorded at each sampling point (Appendix A). We collected samples exclusively in *D. lanuginosum* patches in geothermal grasslands, whereas representative grasses and forbs were chosen within the nonthermal areas. Each core sample was placed in a plastic bag and transported to the lab and refrigerated within 10 h and processed within 3 d.

Roots were carefully picked from the soil and washed with distilled water. A subsample was stained with

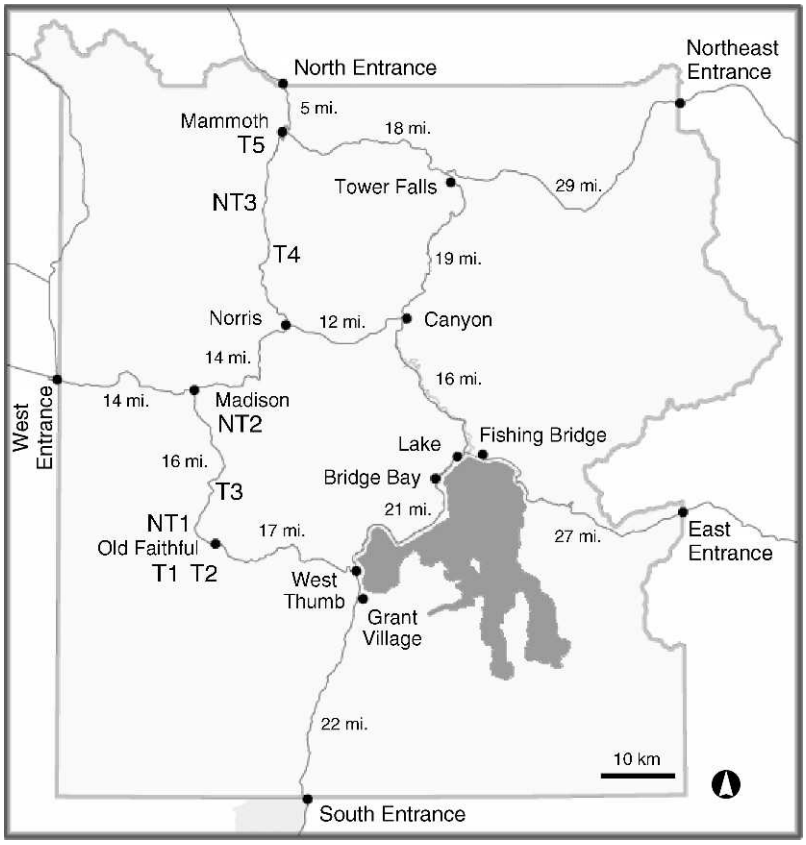

FIG. 1. Geothermal (T) and nonthermal (NT) sampling sites in Yellowstone National Park (YNP), USA, superimposed on a map produced by the U.S. National Park Service.

Trypan blue (Brundrett et al. 1996) for qualitative assessments of AMF colonization, and the remainder of the roots were dried with a desiccant (Drierite, W. A. Hammond Drierite Company, Xenia, Ohio, USA) and stored at −20°C awaiting molecular analyses. Soil from within each grassland was pooled, air dried, sieved through a 2-mm sieve, and sent to Agsource Harris Laboratories (Lincoln, Nebraska, USA) for soil chemistry analyses. Overall, the nonthermal grasslands had similar soil characteristics, with close to neutral pH, whereas four of the geothermal grasslands (T1–T4) were acidic with low base cation concentrations, and one (T5) was alkaline with high concentrations of Ca and S (Table 1).

Molecular identification of AMF

DNA was extracted from ~10 mg of dried frozen root tissue using the Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, California, USA) according to Koide et al. (2005), and amplified using a nested PCR (polymerase chain reaction) protocol described by Redecker et al. (2000). We used a hot-start Taq (ABgene House, Epsom, Surrey, UK) and the following thermocycling parameters for PCR 1: 15 min at 95°C, 31 cycles of 30 s at 95°C, 30 s of 51°C, and 2 min at 72°C, with a final extension phase of 5 min at 72°C. We used five separate primer pairs in PCR 2: ARCH1311AB/ITS4i, PARA1313/ITS4i, ACAU1661/ITS4i, GIGA1313/GIGA5.8R, and GLOM1310/ITS4i (Redecker et al. 2000, Redecker 2002), targeting members of the Archaeosporaceae, Paraglomeraceae, Acaulosporaceae, Gigasporaceae, and *Glomus* group A within the Glomeraceae, respectively. PCR 2 conditions were the same as those in PCR 1, except for an annealing temperature of 61°C. The primer pairs PARA1313/ITS4i and GIGA1313/GIGA5.8R were run under PCR 1 conditions, however, because preliminary trials indicated that the 61°C annealing temperature was too high for successful amplification of target DNA. Negative controls were included in both PCR reactions to exclude false positives. We did not use primers that amplify other *Glomus* groups because limited amplification and cloning of samples from the three nonthermal grasslands (Y. Lekberg, *unpublished data*) and previous analyses of samples from T1–3 (Appoloni et al. 2008) have indicated that these groups are absent or in very low abundance within YNP. Furthermore, because previous work on AMF from YNP showed that PCR products amplified with the five primer pairs that we use here rarely contained more than one AMF taxon (Appoloni et al.

TABLE 1. Selected soil chemical properties of the five geothermal (T) and three nonthermal (NT) sites in Yellowstone National Park, USA.

Site	OM (%)	pH (water)	Concentration (μg/g soil)							
			NO_3^-	P (Bray 1)	K	Ca	Mg	S	Zn	Fe
T1	3.2	4.8	4.0	11	93	256	35	14	1.0	167
T2	4.1	4.8	14	22	90	304	45	12	1.5	45
T3	7.5	4.7	17	24	234	352	28	44	2.1	121
T4	4.3	4.0	2.0	30	60	24	3.0	22	0.3	289
T5	4.3	7.9	15	6	183	5199	260	999	0.8	4.8
NT1	2.5	6.3	5.0	37	179	930	102	6	3.0	24
NT2	6.1	6.0	12	12	228	1322	152	9	6.0	81
NT3	5.3	6.5	5.0	27	219	1643	124	10	9.9	52

Note: Bray 1 is a soil extractant that removes primary sources of P from the solid state.

2008), we omitted the cloning step and directly digested PCR products with *Hin*fI and *Mbo*I (New England Biolabs, Beverly, Massachusetts, USA). Although this approach probably limited our ability to detect rare taxa within these grasslands, it allowed us to sample from replicated grassland types and to process significantly more samples than had we chosen to clone all positive PCR products. A minimum of two representatives of each RFLP (restriction fragment length polymorphism) type per grassland were cloned into a pGEM-T vector (Promega Corporation, Madison, Wisconsin, USA), except for T2, where RFLP types were compared with a previously generated clone library (Appoloni et al. 2008) and a clone library generated from the neighboring T1. Inserts were reamplified and digested with the restriction enzymes *Hin*fI and *Mbo*I to confirm the restriction type and then were sequenced in both directions. The glomeromycotan origin of the sequences was verified by BLAST (Altschul et al. 1997) and was aligned manually to previously obtained sequences from YNP (Appoloni et al. 2008) and elsewhere. In total, 42 sequences have been deposited in the European Molecular Biology Laboratory database under the accession numbers FR732065–FR732101 and FN869119–FN869123. Phylogenetic trees were obtained by distance analysis using the neighbor-joining algorithm in PAUP (Swofford 2001) with the Kimura two-parameter model and a gamma shape parameter = 0.5. Bootstrap analyses were performed using 10 000 replications, and results were verified by performing maximum likelihood analyses based on parameters estimated in Modeltest 3.5 (Posada and Crandall 1998). RFLP types consistently formed monophyletic groups in the phylogenetic trees, and their restriction lengths, BLAST matches, and accession numbers are in Appendix C. Presence/absence of RFLP types in each soil core was consolidated and sampling effort curves for each grassland were constructed in EstimateS (Colwell 2006) using the Mao Tau estimator.

Greenhouse study of direct soil effects

We conducted a greenhouse experiment to test whether the soil environment could explain the observed distribution of selected AMF within YNP. We used two morphospecies that had been isolated previously from acidic geothermal grasslands within YNP and were identified morphologically and genetically as *Paraglomus occultum* (corresponding to the RFLP type Para-2, isolated from T1) and *Scutellospora pellucida* (Giga-4, isolated from the Shoshone Geyser Basin, which is located ~10 km from T1), and one morphospecies that had been isolated previously from the alkaline geothermal grassland T1 and was morphologically and genetically identified as *Glomus intraradices* (Glom-A1). These cultures are maintained at Montana State University and have been given YNP catalog numbers 179 728, 179 731, and 179 732, respectively. *D. lanuginosum* seeds collected from greenhouse-grown plants originally from YNP were planted into an autoclaved 1:1 (v:v) mixture of T1 and T5 soil and grown for one month. The non-mycorrhizal status of seedlings was confirmed and any attached soil was washed off the roots prior to transplanting. Seedlings were inoculated with 2–10 mL soil inoculum (depending on the spore density) containing either 500 *P. occultum* spores, 50 *S. pellucida* spores, or 100 *G. intraradices* spores on 10 January 2008. Seedlings were grown in 100-mL Conetainer tubes (Stuewe and Sons, Corvallis, Oregon, USA) filled with autoclaved acidic T1 (pH 4.8) or alkaline T5 soil (pH. 7.9). Each treatment combination was replicated six times and two non-mycorrhizal control plants per soil were included to test for accidental cross contamination during the experiment, for a total of 40 tubes. Plants were grown in the greenhouse at 23°C/20°C (day/night) with 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of supplementary photosynthetically active radiation for 14 h/d. Because we had noticed a gradual increase in soil pH over time in acidic soils in previous greenhouse trials, we monitored the soil pH biweekly (once every two weeks), and seedlings in the acidic T1 soil were watered as needed with a weak citric acid solution (0.01 mol/L), whereas seedlings grown in the alkaline T5 soil were watered with distilled water. Plants were fertilized biweekly with ~10 mL of a half-strength Hoagland solution containing 6 μg P/L (Machlis and Torrey 1956).

Plants were harvested three months after transplanting. At this time, the acidic T1 soil inoculated with *G. intraradices* had a pH of 7.8, which was similar to the alkaline T5 soil (pH 7.9). This increase in pH was most likely the result of the small amount of alkaline T5 soil added with the AMF inoculum at the start of the experiment, combined with a low buffering capacity of the acidic T1 soil. Because soil pH was indicated as one possible important driver of the field AMF taxa distribution, the treatments inoculated with *G. intraradices* were excluded from further analyses. Soil pH also increased in T1 soil inoculated with *P. occultum* and *S. pellucida*, possibly due to the nitrate-based fertilizer solution used, but both soils remained acidic ($pH < 6.1$) throughout the experiment. Shoot tissue was dried at 60°C for 48 h and weighed. Spores were extracted from 5 g of air-dried soil through repeated washings and centrifugation in an aqueous sucrose solution (Brundrett et al. 1996), collected on a 45-μm sieve, and viewed under a dissecting microscope. Only healthy-looking spores that were likely to have been produced during the three-month experiment were counted. Spore production has been used for relative comparisons of performance and abundance among taxa across treatments (e.g., Bever et al. 1996).

Bison as a vector for AMF dispersal

To test whether bison feces contain viable AMF, we used a "bait plant approach" (Brundrett et al. 1996), which allows for identification of viable fungi that colonize host roots under controlled conditions. In the field, bison dung was collected at a location adjacent to T1 in March 2006 from one animal just after defecation on 1 m of snow to ensure that no contamination occurred in the field from surrounding soil and vegetation. Our purpose was to assess the possibility of dispersal through ingestion, but not the relative importance of bison as a vector. Feces were transported to the lab in a sealed plastic bag and stored in the refrigerator for 48 h until they were mixed with autoclaved soil (containing T1 soil : NT2 soil : Turface, 1:1:1 v:v:v) at a ratio of three parts feces to seven parts soil (v:v). Three 100-mL Cone-tainer tubes were filled with this mixture, and one-week-old sudan grass seedlings (*Sorghum sudanese* L.) were planted and grown in the feces–soil mixture in the same greenhouse conditions as previously described. To ensure that the autoclaved soil (excluding feces) did not contain viable AMF, and that no cross contamination occurred from other sources in the greenhouse, three additional seedlings were planted into autoclaved soil alone.

After three months, plants were harvested and roots were washed free of soil. A subsample of roots was stained with Trypan blue (Brundrett et al. 1996) for qualitative assessments of AMF colonization and the remainder of the root sample was used for molecular identification of AMF as previously described. Positive PCR products were cloned and screened using RFLP, and representative RFLP types were sequenced to ensure that the positive products were of AMF origin (EMBL accession numbers FR732068, FR732080, FR732101).

Statistical analyses

To determine the relationship between environmental variables and RFLP types, we first conducted a principal coordinates analysis (PCoA) based on Hellinger distance, where the axes represent hypothetical environmental gradients. We then used a distance-based redundancy analysis (db-RDA; with Hellinger distance), which constrains the taxa and samples to axes determined by supplied environmental variables (ter Braak and Prentice 1988). PCoA and db-RDA were selected because the average RFLP type response to the hypothetical gradient was linear (2.15 standard deviations) rather than unimodal. Consistency between the PCoA and db-RDA suggests that important environmental variables were quantified. Significant environmental variables ($P < 0.05$) in the db-RDA were identified using Monte Carlo permutations under the full model (using a forward stepwise selection procedure with 999 iterations). Ordination analyses and hypothesis testing were conducted using CANOCO 4.5 (ter Braak and Šmilauer 2002) based on Hellinger-transformed RFLP counts for each site and a focus on inter-taxa correlations. Biplots were created using CanoDraw 4.12 to display ordination results (ter Braak and Šmilauer 2002). The RFLP type scores were post-transformed so that correlations of the RFLP type and soil variables with the ordination axes could be inferred by perpendicular projection.

We used the methods of Peres-Neto et al. (2006) and the varpart function in the vegan R package (*available online*)[5] to determine how much of the variation in RFLP types was accounted for by (1) spatial variables alone, (2) soil variables alone, and (3) how much was shared by the two. Spatial predictors were the nine terms of a cubic trend-surface equation based on the *x*- and *y*-coordinates of each site (Borcard et al. 1992). Briefly, this variance-partitioning procedure entails extracting these three variance components and residual variation by first examining all environmental variables and identifying the minimal subset of those that are significant (pH and grassland type, using stepwise forward selection). Second, we examined all spatial predictors and identified those that were significant (xy^2, using stepwise forward selection). Third, we used RDA, partial RDA, and subtraction to calculate variation that was unique to the subset of significant environmental variables, variation that was unique to the subset of significant spatial variables, that shared by the two subsets, and residual variation. These analyses were conducted on Hellinger-transformed data because this

[5] ⟨http://cran.r-project.org/web/packages/vegan/vegan.pdf⟩

transformation performed best in RDA on RFLP data when compared to several other transformations (see Legendre and Gallagher 2001). Additionally, the Hellinger transformation controls for variation in abundance among the samples by dividing the abundance value for each RFLP type within a sample by the total abundance for the sample (i.e., giving each sample equal weight in the analyses). Hence, this transformation is insensitive to changes in abundance but does capture changes in community composition. The variance partitioning used adjusted R^2 values to account for the different number of predictors in the groupings of environment and spatial factors (Peres-Neto et al. 2006).

Finally, spore numbers, pH, and shoot dry mass from the greenhouse experiment were analyzed separately using the general linear model in Minitab Release 12.2 (Minitab 1998 State College, Pennsylvania, USA) with morphospecies and soil as crossed factors. Residuals were plotted and ln-transformations were performed to improve normality and equal variance when necessary.

Results

AMF community composition in geothermal and nonthermal grasslands

PCR products were obtained from all eight grasslands, but PCR success differed between sites (Appendix A). In particular, poor amplification was observed within T4, and this was supported by a surprisingly low AM colonization (estimated to be below 5%). All other grasslands showed intermediate to high AM colonization and a PCR success exceeding 65%. Sampling effort curves suggested that, within the restrictions of our extraction and amplification protocols, the sampling intensity was sufficient to characterize the majority of the abundant taxa in all sites but T4 (Appendix E). Additional sampling also may have revealed more rare taxa in T5, but is unlikely to have identified additional abundant taxa. Based on the low amplification success in T4, this site was excluded from further quantitative analyses.

All glomeromycotan lineages targeted by the primer pairs were detected, including *Glomus* group A within the Glomeraceae, Acaulosporaceae, Gigasporaceae, Archaeosporaceae, and Paraglomeraceae. We identified 12 RFLP types within the grasslands that corresponded to distinct phylotypes, nine of which clustered with known morphospecies (Appendices C and F–J). The most abundant RFLP type was Glom-A1 (closely related to *Glomus intraradices*), which was present in 54% of all samples and occurred in seven out of the eight grasslands (Appendix D). In contrast, all other RFLP types showed a more restricted distribution, but did not group within either the geothermal and nonthermal grasslands as expected. Instead, RFLP types clustered within either the acidic geothermal grasslands (Glom-A13, Giga-4, Arch-5, Para-2, Para-3) or the neutral to alkaline grasslands (Glom-A25, Giga-5, Acau-6, Acau-7, Arch-2, Para-1), of which one was geothermal (T5). For a more detailed description of the corresponding phyloypes, see Appoloni et al. (2008). The RDA supported this grouping (Fig. 2) and suggested that pH and/or pH-related soil chemistry may be important for the observed AMF distribution. Only pH ($P = 0.036$, first variable selected) and grassland type ($P = 0.002$, second variable selected) were significant based on the Monte Carlo permutation analyses. It is interesting to note that the few AMF detected at T4 were common in the other acidic grasslands (Appendix D). The variance partitioning revealed that the environmental factors and spatial arrangement of grasslands were relatively unconfounded, with only 13% of the variation shared by these two factors. Environmental factors alone accounted for 53% of the variation in the distribution of AMF ($P = 0.019$), whereas spatial factors alone, a proxy for dispersal limitation, accounted for only 4% of the variation ($P = 0.30$).

Greenhouse study of direct soil effects

Plants grown in autoclaved soil were non-mycorrhizal at harvest, suggesting that there was no cross contamination of AMF in this experiment. *Paraglomus occultum* and *S. pellucida* both produced significantly more spores in the acidic T1 soil than in the alkaline T5 soil ($F_{1,19} = 63.43$, $P < 0.001$; Table 2), suggesting that direct limitations posed by the soil environment may at least in part explain their absence in the neutral to alkaline soils in the field. The significantly higher shoot dry mass (DM) observed in mycorrhizal seedlings in T1 relative to T5 soil ($F_{1,19} = 5.60$, $P = 0.03$; Table 2) also show that the better performance of the AMF in the acidic soil has functional consequences for the plant. Without the alkaline *G. intraradices* in the analyses, it is possible that the T1 soil simply provided a better habitat in this greenhouse setting compared with the T5 soil, but we find this unlikely because there was no significant difference in shoot DM between non-mycorrhizal plants growing in the two soils (*t* test: $T = 1.06$, $P = 0.48$). Overall, *P. occultum* produced significantly more spores than *S. pellucida* ($F_{1,19} = 15.50$, $P = 0.001$), regardless of the soil, but seedlings inoculated with *S. pellucida* had close to a significantly higher shoot DM ($F_{1,19} = 4.02$, $P = 0.06$).

Bison as vectors for AMF

Roots of all three seedlings grown in bison feces were colonized by AMF, whereas roots of seedlings grown in sterile soil were non-mycorrhizal. Furthermore, PCR products were obtained from the feces-inoculated seedlings only. Thus, no contamination appears to have occurred during the bait period in the greenhouse, suggesting that bison-ingested AMF remains viable after passing through the digestive tract. RFLP types indicated (Fig. 3), and sequencing confirmed, that the feces contained Glom-A1, Acau-6, and Arch-2, which are closely related to *Glomus intraradices*, *Acaulospora morrowiae*, and *Archaeospora trappei*, respectively.

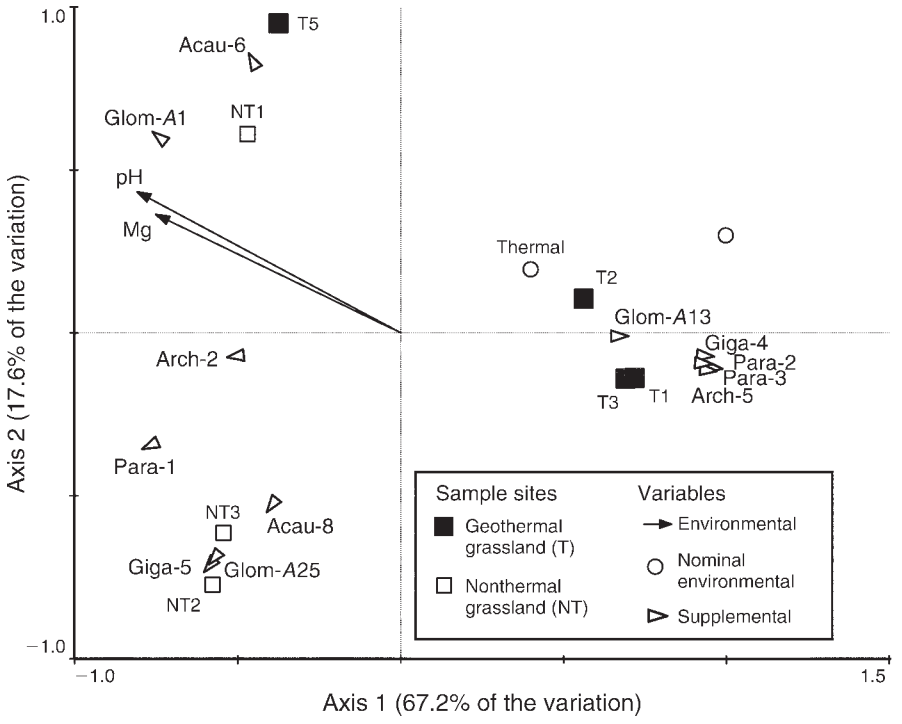

FIG. 2. Results of a distance-based redundancy analysis (based on principal coordinates and Hellinger distance) of AMF (arbuscular mycorrhizal fungi) community composition in four geothermal and three nonthermal grasslands within YNP. The grassland T4 was excluded due to the low amplification success. The six principal coordinates used in the analysis have been suppressed, and AMF phylotypes have been passively (post hoc) projected into the ordination space. The only significant environment predictors based on Monte Carlo permutation tests (999 iterations) were pH ($P = 0.036$) and site type (geothermal or nonthermal; $P = 0.002$). Only environmental variables with correlation coefficients outside the range of −0.7 to 0.7 were displayed; however, environmental predictors in the analysis were geothermal (nominal), and soil organic material (%), pH, cation exchange capacity, soluble salts, N, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, B, and Na. Distance of phylotypes and environmental predictors from the origin indicates their relative importance in the biplot. Perpendicularly projecting the environmental variables to the axes provides an estimate of the correlation coefficient of that variable with that axis. The angle between environmental variables is negatively proportional to the correlation of those variables. Distance among samples approximates their taxa dissimilarity.

DISCUSSION

Environmental variables were stronger predictors of AMF communities in the grasslands of YNP than were spatial factors. Thus, niche restrictions, rather than dispersal limitations, appear to structure these AMF communities. This result is consistent with the findings of a meta-analysis on 158 studies (Cottenie 2005), which revealed that the majority of the included communities were structured by environmental variables and very few by neutral processes alone. Strong environmental effects on AMF communities have been shown previously and lend support to our findings. For example, fungal communities shift significantly due to differences among soils properties (Lekberg et al. 2007), plant communities (Öpik et al. 2006), and seasons (Klironomos et al. 2001). It is interesting to note that fungal communities in the geothermal and nonthermal grasslands did not cluster separately in the RDA (Fig. 2), a somewhat surprising result given the drastic differences in both soil temperature and host plant communities experienced by AMF in these two grassland types. Instead, the alkaline

TABLE 2. Shoot dry mass (DM), soil pH, and spore numbers of seedlings grown in either autoclaved soil from the acidic site (T1) or the alkaline site (T5) inoculated with either *Paraglomus occultum* or *Scutellospora pellucida*.

Variable	T1, pH 4.8		T5, pH 7.9	
	P. occultum	*S. pellucida*	*P. occultum*	*S. pellucida*
Shoot DM (g)	154^{ab} (28.6)	260^{a} (30.2)	74.8^{b} (23.5)	138^{ab} (67.1)
Soil pH	5.76^{c} (0.10)	6.08^{b} (0.08)	7.77^{a} (0.04)	7.90^{a} (0.05)
Spores (no./g)	129.6^{a} (27.4)	66.0^{b} (12.4)	24.7^{c} $(3.6)^{c}$	5.3^{d} (2.3)

Notes: Values are means with SE in parentheses. Within rows, values with different superscripts differ from each other at $P \leq 0.05$ ($n = 6$).

geothermal grassland clustered with the nonthermal grasslands along the first axis that explained 67% of the variation in the AMF data. If this sole alkaline geothermal grassland is representative of other such grasslands, it may suggest that soil temperature and host plant community are weaker structuring forces for AMF communities than soil chemical properties. The RDA highlighted the potential importance of pH or pH-related influences on soil chemistry, and this is in accordance with previous studies that have identified pH as a strong predictor for AMF community structure (Fitzsimons et al. 2008, Dumbrell et al. 2010). Due to the correlative nature of these field surveys, causation cannot be implied, but pH manipulations under controlled conditions have shown differential ERH growth and phosphatase activity in two taxa (van Aarle et al. 2002), which provides a potential mechanism by which pH may cause community shifts.

Because the grasslands were not sampled at the same time but over a three-month period, AMF community differences could also be the result of seasonal shifts (e.g., Klironomos et al. 2001), but this is unlikely for two reasons. First, seasonal shifts are probably driven largely by changes in host vigor and/or temperature, both of which were minimized in this study by sampling at the peak of the growing season. Second, samples taken bimonthly (once every two months) over a one-year period at the T1 site revealed no seasonal shift in the AMF community composition (Y. Lekberg, *unpublished data*).

Further support for the direct effect of the soil chemical environment for structuring at least some RFLP types came from our greenhouse study. *Paraglomus occultum* and *S. pellucida* were found exclusively in the acidic geothermal grasslands (Appendix D) and they also produced significantly more spores and benefited plants to a greater extent when grown in the acidic T1 than in the alkaline T5 soil (Table 2). Based on this, we propose that the absence of these two taxa in the neutral to alkaline soils is at least partly due to restrictions in the fundamental niche, not dispersal limitations, because they were present in interspersed acidic geothermal sites. It is interesting to note that even though these isolates occur and may even prefer acidic soils, the low overall fungal abundance in T4 may indicate that pH 4 is a lower limit even for these AMF. After all, acidic soils have been shown to have a fungistatic effect on *Glomus mosseae* spores (Siqueira et al. 1984). The low AMF abundance at T4 could also be due to dispersal limitations, but this is unlikely because a subsequent sampling within an adjacent, nonthermal grassland showed highly colonized plants.

Contrary to the restricted distribution of *P. occultum* and *S. pellucida*, Glom-A1 was the most common RFLP type, occurring in all but one grassland. Glom-A1 is closely related to *Glomus intraradices*, which is found in almost all AMF habitats and appears to be a generalist (Koch et al. 2004, Öpik et al. 2006). However, whether

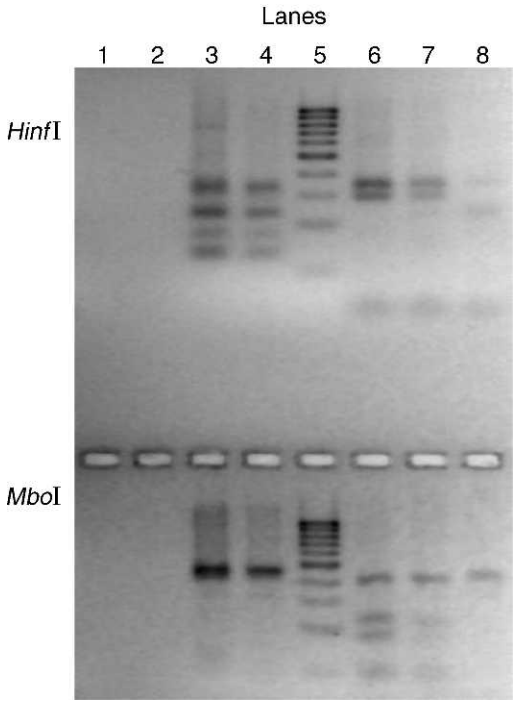

FIG. 3. RFLP (restriction fragment length polymorphism) patterns of cloned PCR products from roots grown in bison feces that were digested with the restriction enzymes *Hinf*I and *Mbo*I. Lanes 1 and 2 are control plants (grown only in autoclaved soil), row 3 and 4 are Glom-A1 (*G. intraradices*), row 5 is the ladder, row 6 and 7 are Acau-6 (*A. morrowiae*), and row 8 is Arch-2 (*A. trappei*). Family-specific primers were used during amplification.

or not the RFLP type found here consists of several locally adapted ecotypes or one generalist taxon will require more careful genetic analyses, as well as isolation and cross inoculations of fungi from different habitat types. It is interesting to note that Glom-A1 did not do equally well in all grasslands, and that the abundance was lowest in the acidic geothermal sites (Appendix D). This could be due to direct limitations posed by the soil environment, or competition with other AMF taxa for root space and host carbon (Pearson et al. 1993, Lekberg et al. 2007). Because of the failure with this isolate in our greenhouse experiment, we are unable to address this, but it is interesting to note that one *G. intraradices* isolate grow significantly less external mycelium at lower pH (van Aarle et al. 2002). Overall, it is important to remember that results from our greenhouse experiment only extend to the taxa researched and that the underlying reasons for the restricted distribution of the other taxa remain unknown.

We found little support for dispersal limitations in YNP, suggesting that most fungal taxa within the park are part of a metacommunity. This does not mean that dispersal should be disregarded, because dispersal limitations are probably scale dependent and might be

significant at greater distances (as discussed in Martiny et al. 2006). In fact, we tested for dispersal limitations at the level of the community, rather than RFLP type, and the restricted distribution of some RFLP types to a few grasslands (Appendix D) could indicate restricted dispersal. That the degree of dispersal limitation could differ among taxa should come as no surprise, given that AMF differ in their life history strategies (Hart and Reader 2002, Sykorova et al. 2007), as well as infective propagules (Klironomos and Hart 2002); taxa that form large mycelia and sporulate rarely (Rosendahl and Stukenbrock 2004) may show a more restricted distribution than sporulating taxa (Rosendahl et al. 2009). For example, it is interesting to note that we have been unsuccessful in isolating spores and subculturing Arch-5 (Y. Lekberg, *personal observation*), an RFLP type that may be endemic to YNP (Appoloni et al. 2008).

RFLP types in YNP might be broadly distributed partly because wide-ranging bison appear to be capable of vectoring AMF among grasslands separated by coniferous forests. We demonstrated that AMF can successfully pass through the gastrointestinal tract of bison and colonize host plants. Other researchers have also reported fungal dispersal by animals (e.g., Janos et al. 1995, Mangan and Adler 2000); indeed, the reestablishment of AMF after the eruption of Mt. Saint Helen may have been partly aided by elk dispersal (Allen 1987). Although our limited sampling demonstrated that AMF can disperse in this manner, further research is needed to estimate the relative importance of bison for AMF dispersal within YNP.

Many studies have addressed, and some have found, evidence of host preference within AMF communities (e.g., Husband et al. 2002). Niche differentiation among co-occurring host plants could be very important for AMF alpha diversity and might explain the coexistence of taxa, but whether it can increase our predictive ability regarding regional AMF distributions is uncertain. For example, a knowledge of soil chemical properties appeared to be more important than information on host plant community for predicting AMF communities in YNP, because the alkaline geothermal grassland T5 clustered with the nonthermal grasslands in spite of very different host plant communities. This is not surprising, given that a large proportion of the fungal biomass occurs, and directly interacts, with the soil. A continued effort to document AMF communities across environmental gradients is likely to yield repeatable community patterns across regions. Indeed, Glom-A13 that was found in three out of the four acidic geothermal grasslands is closely related to *Glomus diaphanum*, which has been identified previously in acidic mine sites in West Virginia (Morton and Walker 1984).

In conclusion, this study showed that niches, rather than neutral processes, structure AMF communities within YNP, and that the soil chemical environment is a strong structuring force for at least some taxa.

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APPENDIX A

GPS coordinates, harvest dates, soil temperatures, and PCR (polymerase chain reaction) success of the five geothermal and three nonthermal sites (*Ecological Archives* E092-108-A1).

APPENDIX B

Plant species observed within the three nonthermal grasslands (*Ecological Archives* E092-108-A2).

APPENDIX C

Ribotype name, their closest BLASTN match, fragment sizes with *Mbo*1 and *Hin*f1 digestion, and accession numbers (*Ecological Archives* E092-108-A3).

APPENDIX D

Presence of RFLP (restriction fragment length polymorphism) types in the five geothermal and three nonthermal grasslands sampled (*Ecological Archives* E092-108-A4).

APPENDIX E

RFLP species accumulation curves for the geothermal and nonthermal grasslands (*Ecological Archives* E092-108-A5).

APPENDIX F

Phylogenetic tree of *Glomus* group A based on ITS 1, 5.85 rDNA, and ITS 2 (*Ecological Archives* E092-108-A6).

APPENDIX G

Phylogenetic tree of *Scutellospora* based on 18S (*Ecological Archives* E092-108-A7).

APPENDIX H

Phylogenetic tree of *Acaulospora* based on ITS 1, 5.85 rDNA, and ITS 2 (*Ecological Archives* E092-108-A8).

APPENDIX I

Phylogenetic tree of *Archaeospora* based on ITS 1, 5.85 rDNA, and ITS 2 (*Ecological Archives* E092-108-A9).

APPENDIX J

Phylogenetic tree of *Paraglomus* based on ITS 1, 5.85 rDNA, and ITS 2 (*Ecological Archives* E092-108-A10).