

Diversity and Functionality of Arbuscular Mycorrhizal Fungi in Three Plant Communities in Semiarid Grasslands National Park, Canada

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Abstract Septate endophytes proliferating in the roots of grasslands' plants shed doubts on the importance of arbuscular mycorrhizal (AM) symbioses in dry soils. The functionality and diversity of the AM symbioses formed in four replicates of three adjacent plant communities (agricultural, native, and restored) in Grasslands National Park, Canada were assessed in periods of moisture sufficiency and deficiency typical of early and late summer in the region. The community structure of AM fungi, as determined by polymerase chain reaction-denaturing gradient gel electrophoresis, varied with sampling time and plant community. Soil properties other than soil moisture did not change significantly with sampling time. The DNA sequences dominating AM extraradical networks in dry soil apparently belonged to rare taxa unreported in GenBank. DNA sequences of *Glomus viscosum*, *Glomus mosseae*, and *Glomus hoi* were dominant under conditions of moisture sufficiency. In total, nine different AM fungal sequences were found suggesting a role for the AM symbioses in semiarid areas. Significant positive linear

relationships between plant P and N concentrations and active extraradical AM fungal biomass, estimated by the abundance of the phospholipid fatty acid marker 16:1 ω 5, existed under conditions of moisture sufficiency, but not under dry conditions. Active extraradical AM fungal biomass had significantly positive linear relationship with the abundance of two early season grasses, *Agropyron cristatum* (L.) Gaertn. and *Koeleria gracilis* Pers., but no relationship was found under dry conditions. The AM symbioses formed under conditions of moisture sufficiency typical of early summer at this location appear to be important for the nutrition of grassland plant communities, but no evidence of mutualism was found under the dry conditions of late summer.

Introduction

Arbuscular mycorrhizal (AM) fungi are found in the soil of most ecosystems where they form mutualistic associations with a large number of terrestrial plant species [64]. They are known as critical components of soil, and functional links between soil and plants [12, 27]. They can influence many important processes such as nutrient cycling [2, 31, 47], soil structure stabilization [61, 62], organic matter transformation and accumulation [49, 68], and the turnover of organic residues in soil [1]. AM fungi are important associates of plants and the composition of their community influences plant community structure [39], biodiversity [17, 22], plant drought resistance [19], primary production [23], and ecosystem dynamics [64].

Much research effort was spent to understand the interactions taking place between AM fungi and plants [28, 29, 60], but very few studies have attempted to clarify the functionality of AM fungi as influenced by variations in

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environmental conditions, especially in their natural environment [5, 34]. Previous research works have examined the distribution of AM fungi in sandy area [4], in agricultural soils [45], and in certain natural ecosystems [18], but few of them have looked into grasslands [56], especially in arid and semiarid areas [36]. Low AM fungal diversity was found in the dry plains of central Argentina [35] and a recent study in Kansas prairie ecosystems revealed that AM colonization of plant roots may be displaced by non-AM fungal root endophytes in the North American Great Plains, especially in the warm period of the growing season when water availability to plants is low [38]. We also observed abundant septate hyphae in the roots of plants growing in Southwest Saskatchewan prairie soils, which concur with reports from semiarid grasslands made by others [30]. Competition with other fungal endophytes for root occupation could limit the distribution of AM fungi in dry areas.

Grasslands National Park is located in semiarid Saskatchewan near the Canada–USA border, and has the mandate to preserve Canadian prairie grasslands. The Park offers different plant communities suitable for the study of AM fungi in dry environments. We used phospholipid fatty acid (PLFA) and DNA analyses to examine the AM symbiosis in three different plant communities of Grasslands National Park. In particular, we wanted to document AM fungal diversity and activity in different plant communities existing in a semiarid climate and to explore the relationship between AM fungal activity and plant nutrition in period of water sufficiency and in dry period.

Methods

Study Site

Four different locations in Grasslands National Park, Southwest Saskatchewan Canada, where three adjacent grassland plant communities (one agricultural, one native, and one restored at each of four locations totaling 12 research plots) met were examined. The latitude and longitude of the four sampling locations are: (1) N49°13'089, W107°36'563; (2) N49°07'329, W107°28'255; (3) N49°07'121, W107°28'168; and (4) N49°07'461, W107°29'328. Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) accounted for over 73% of total soil coverage in the agricultural plant communities examined. These crested wheatgrass stands were probably established by early settlers in the 1930s to stop wind erosion. Native plant communities were the unbroken native mixed grass prairie vegetation dominated by *Stipa comata* Trin. & Rup. and *Bouteloua gracilis* (BHK.) Lag. Restored plant communi-

ties were on land formerly in crested wheatgrass that had been re-seeded into native mixed grasses and forbs species at some point in the last 10 years by the Park officers and were in various stages of recovery. Total precipitation for May and June in 2007 was 67.8 mm, which was lower than normal for this location (Fig. 1); and average temperature was 13.9°C, which is closed to normal. The months of July and August 2006 had lower amount of precipitation (8.4 mm) than normal and similar temperature (20.8°C; Fig. 1). According to the United Nations Environment Program [63], the climate in this area is semiarid (aridity index was 0.28 for 2006 and 0.24 for 2007). Seasonal variation in climate creates a seasonal pattern in vegetation cover where cool-season plant species are followed by warm-season species.

Soil and Plant Sampling

Sampling took place on 29th and 30th of August 2006 and 4th and 5th June 2007. These sampling times were selected to correspond to the tail end of the cool and moist and the warm and dry periods characterizing the Southwest Saskatchewan summer. In each plant community of each location, the plants in five 0.25 m² randomly placed quadrates were identified. The percentage of soil coverage by each species was evaluated visually and used to describe plant community structure. Within these quadrates, the plants were harvested at the soil surface with a pruner and their shoots were dried in bulk, ground, digested with H₂SO₄/Se/Na₂SO₄, and analyzed for their N [44] and P [42] content on a segmented flow auto-analyzer (Technicon, AAI system, Tarrytown, NY, USA).

One soil core (0–20 cm depth) was taken from each quadrate using a 5 cm diameter hand-operated soil sampler. One soil core was used for soil bulk density, which was

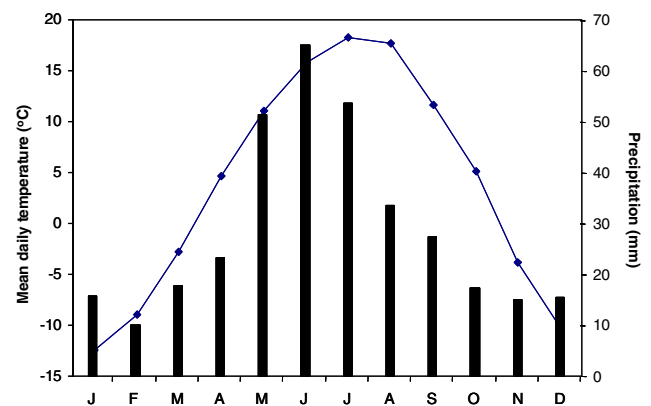


Figure 1 Average monthly temperature (*bar*) and precipitation (*line*) (1971–2000) in Val Marie, Saskatchewan, Canada, the study site (Environment Canada)

determined as the dry (105°C, until constant weight) mass of a soil core (5 cm diameter×20 cm depth) and expressed as g cm⁻³ [21]. The four other cores were pooled to yield one composite sample per plant community at each location. Half of each sample was stored at -20°C in a plastic bag before PLFA and DNA analyses of AM fungi. The other half of each sample was kept at 4°C, before determination of soil physicochemical properties. Soil moisture content was determined as the weight lost after drying 25 g of soil at 105°C, until constant weight, over the weight of the dry soil, and expressed on a percent basis [8]. Soil available N was determined by KCl extraction [40] and available P was extracted with sodium bicarbonate [46]. Soil pH was determined by the method of Peech [50]. Soil organic C was determined by the method of Baccanti and Colombo [2]. Soil electrolyte activity was tested by the method of Mckeague [41].

Phospholipid Fatty Acid Analysis

The abundance of active AM fungal hyphae biomass in soil samples was determined by quantification of PLFA 16:1 ω 5 [3]. Four grams of soil (dry weight equivalent) were extracted in 9.5 ml mixture of dichloromethane/methanol/citrate buffer (1:2:0.8 v/v/v) as described by Clapperton et al. [7]. Separation of the phospholipids fraction of soil lipid extracts on silica columns and the transmethylation and quantification of PLFA 16:1 ω 5 by gas chromatography also followed the method of Clapperton et al. [7].

DNA Extraction from Soil Samples and Nested PCR

AM fungal DNA was extracted from soil using UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). After 20 times dilution, DNA was subjected to a first polymerase chain reaction (PCR) amplification using universal primers GeoA2 and Geo11 targeting an approximately 1.8 kb fragment of the 18 S rRNA gene [55]. The first PCR product with a visible band on an agarose gel was used as template for a second PCR amplification using the reaction mixture described above except for primers in a nested protocol. The second stage primers used were AM1 [24] and NS31-GC, which is the primer NS31 described by Simon [57] plus a 5' GC clamp sequence producing an approximately 550 bp fragment. NS31 is a universal fungal primer and AM1 targets the AM fungi. Since AM1 is not specific enough to AM fungi [33] and produce sequences difficult to separate, primer Glo1 [9] was also used to amplify the DNA from the second PCR in a third PCR amplification. The PCR products coming from both the second and third amplifications were used to construct a clone library and denaturing gradient gel electrophoresis (DGGE) markers.

Denaturing Gradient Gel Electrophoresis Analysis

Twenty microlitres of nested PCR product were used for DGGE analysis as described by Ma [37]. Gels contained 6% (w/v) polyacrylamide (37:1 acrylamide/bis-acrylamide). The linear gradient used was from 35% to 55% denaturant, where 100% denaturing acrylamide was defined as containing 7 M urea and 40% (v/v) formamide. A 5 ml stacking gel containing no denaturants was added before polymerization was complete (~2 h). All DGGE analyses were run in Dcode Universal Mutation Detection system (Bio-Rad Laboratories, Hercules, CA, USA) at a constant temperature of 60°C. Electrophoresis was for 10 min at 75 V, after which the voltage was lowered to 60 V for an additional 13 h. Gels were stained in 1×Tris/acetic acid/EDTA buffer containing 4 μ l SYBR Safe DNA gel stain (Invitrogen) per 10 ml and visualized by ultraviolet illumination. Gel images were digitally captured by an OLYMPUS digital camera (SP-500 UZ) in Multimage Light Cabinet (Alpha Innotech Corporation, San Leandro, CA, USA) using a Sybr Safe filter.

Construction of Clone Library and DGGE Marker

To obtain enough DNA fragments in one clone library, products of PCR amplification with AM1/NS31 and Glo1/NS31 of selected samples (samples selected for their DGGE pattern to include all possible DNA fragments) were pooled to produce a clone library [51]. We transformed DNA fragments into *Escherichia coli* (strain TOP 10) using the TOPO TA Cloning Kit (Invitrogen, Cat#K4575-J10) following manufacturer's instructions. The transformed cells were plated onto solid Luria-Bertani (LB) medium containing ampicillin (50 μ g ml⁻¹) and incubated overnight at 37°C, then transferred into a 96-well plate filled with liquid LB medium and sent for sequencing at the Plant Biotechnology Institute of the National Research Council of Canada. Similarity to known 18 S rDNA sequences in GenBank was defined using the online program (BLAST).

We selected positive clones (clone with target DNA fragments) and ran a PCR using primer pair Glo1/NS31-GC under the PCR conditions mentioned above. Ten microliter of PCR product of each clone was submitted to DGGE to locate a distinct migration position for each clone on the gel. Then, 20 μ l of PCR product of each clone were pooled together and kept at -20°C. The DNA sequences in experimental samples were identified by comparison with these DGGE markers loaded (40 μ l) into a lane on each gel.

Statistical Analysis

The effects of location (block), sampling time, and plant community on soil conditions were tested using analysis of

Table 1 Soil conditions at the two sampling time

Soil conditions	pH value	Bulk-soil density (g/cm ³)	Soil moisture (%)	Soil electrolyte (mS)	Soil organic C (gkg ⁻¹)	Soil total N (mgkg ⁻¹)	Soil P (mgkg ⁻¹)
<i>P</i> value	ns	ns	<0.001	ns	ns	ns	ns
June sampling	6.787±0.143 ^a	1.247±0.029	13.56±0.609	0.295±0.026	1.205±0.028	2.659±0.330	7.218±1.419
August sampling	6.830±0.135	1.273±0.026	4.25±0.306	0.312±0.026	1.377±0.018	0.731±0.057	12.440±2.442

ns non-significant according to ANOVA; *N*=24

^a mean ± SE

variance in Network JMP (Version 3.2.6). Difference of AM fungal diversity between seasons and plant communities was analyzed by correspondence analysis (CA) using SYSTAT 12. Phylogenetic distance analysis was assessed by MEGA 4.0.2 using DNA sequences selected according to their sequence similarity to the reference data in Genbank, a *Mortierella verticillata* sequence was used as an out group to root the tree. The relationships between AM fungi active biomass (PLFA 16:1ω5) and plant tissue N and P concentrations were assessed by linear regression analysis and plotted using the R program (www.r-project.org/). The relationship between AM fungi active biomass and the abundance of plant species present in all systems was assessed for each sampling time using multivariate analysis of variance (MANOVA) in the R program (www.r-project.org/). Univariate F tests were used to detect the effects of plant species on AM fungi, and the Wilks's Lambda test was used to detect significant relationships ($P<0.05$) with the whole plant community.

Results

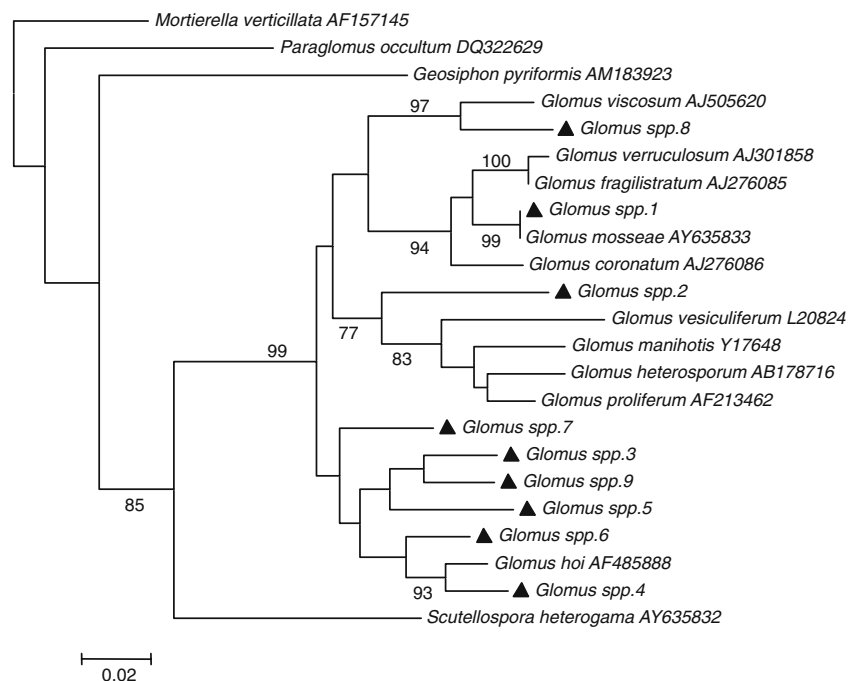
Temporal Effect on AM Fungi Habitat

Soil physico-chemical properties at the late August and early June sampling times were similar, except for soil moisture which was higher in June (13.7%) than August (4.1%; Table 1). This is consistent with the normal pattern of seasonal variation in the Park where a cool early summer characterized by soil moisture sufficiency is followed by a warm and dry period. At the late August sampling time, almost all plant species appeared to be dormant.

Temporal Effects on AM Fungal Diversity

A total of nine different AM fungal sequences were found in this study, all belonging to the genus *Glomus* (Fig. 2). All nine DNA sequences were found in early June and eight were found in late August. Thus, AM fungal richness

Figure 2 Phylogenetic distance analysis of identified AM fungi. The numbers in the branches are bootstrap values from 500 iterations. Non-significant values (<75%) were omitted. Names preceded by a triangle represent the sequences obtained in this research. Names followed by codes represent the sequences downloaded from Genbank



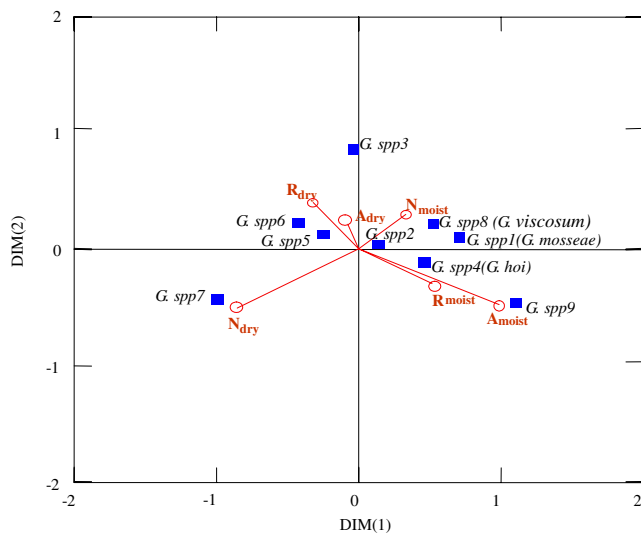


Figure 3 Relationship between the distribution of AM fungi and plant community at different sampling times, as revealed by correspondence ($P < 0.001$, $N = 24$). *N* native; *A* agricultural, and *R* restored ecosystems; *moist* June sampling, *dry* August sampling; circles represent different plant communities at different sampling times; squares represents different AM fungi. Dimension 1 (DIM(1)) explained 49.76% of the variation in AMF communities among three ecosystems, dimension 2 (DIM(2)) explained additional 21.03%

did not vary much with sampling time, but different AM fungal sequences were dominant in late August and early June samples (Fig. 3). Three AM fungi could be identified to the species level by blasting our sequences in GenBank (Table 2). These were frequently detected in early June samples, but seldom found in late August. Only one unreported species (*Glomus* 9) was found in the early June samples. Most of the AM sequences found in the late August sampling were yet unreported in public databases. These results suggest that in each plant community, the composition of AM fungal community had shifted from

one sampling time to the next. The CA reveals differences in the composition of the AM fungal communities of different plant communities within a sampling time (Fig. 3). For example, *Glomus* 9 was frequently detected under crested wheatgrass in June, whereas *G. viscosum* and *G. mosseae* were more frequent in the native prairie at that time.

Temporal Variation in the Relationship Between AM Fungal Active Biomass and Plant N and P Concentrations

Positive linear relationships were found between AM fungal active biomass and plant P ($P < 0.01$) and N ($P < 0.001$) concentrations (Fig. 4). Further examination and parsing of the data by season revealed that this positive linear relationships only existed at the early June but not at the late August sampling time, which indicates that the relationship between AM fungi active biomass and the N and P nutrition of plants may vary with environmental conditions. The active biomass of extraradical networks was lower ($P < 0.001$) in dry soil ($27.3 \pm 0.3 \mu\text{g g}^{-1}$ PLFA 16:1 ω 5 in early June and $15.1 \pm 0.2 \mu\text{g g}^{-1}$ in late August).

Temporal Effects on the Relationship Between AM Fungi Active Biomass and Associated Plant Community

We found different relationship between AM fungi active biomass and their associated plant community. Multivariate analysis of variance results showed that in early June, AM fungi active biomass was related with the abundance of crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), which was present at all sampling sites and dominant in agricultural communities, and junegrass (*Koeleria gracilis* Pers.), a dominant species in restored communities. But in late August, no significant relationship was found (Table 3).

Table 2 BLAST results for the AM fungal sequences found in Grasslands National Park

Identified sequences	Most related isolate from GenBank (% sequence similarity by BLAST ^a)	GenBank accession no.	Reference
<i>Glomus mosseae</i>	<i>Glomus mosseae</i> partial 18 S rRNA gene, isolate EEZ21 (100%)	AJ506089.1	[13]
<i>Glomus</i> 2	<i>Glomus</i> sp. MO-G14 partial 18 S rRNA gene, clone cMO108.6 (98%)	AJ496066.1	[48]
<i>Glomus</i> 3	Uncultured <i>Glomus</i> clone S3B 12 18 S small subunit ribosomal RNA gene, partial sequence (100%)	EU573742.1	[54]
<i>Glomus hoi</i>	<i>Glomus hoi</i> isolate sporocarp6 small subunit ribosomal RNA gene (97%)	AF485889.1	[25]
<i>Glomus</i> 5	<i>Glomus</i> sp. Glo4 18 S rRNA gene (99%)	AJ309440.1	[10]
<i>Glomus</i> 6	Uncultured <i>Glomus</i> clone JPC048 JP5 sequence type 18 S ribosomal (97%)	DQ085219.1	[70]
<i>Glomus</i> 7	Uncultured <i>Glomus</i> clone NS2D C7 2 18 S small subunit ribosomal (100%)	EU573758.1	[54]
<i>Glomus viscosum</i>	<i>Glomus viscosum</i> partial 18 S rRNA gene, isolate EFZ20 (98%)	AJ505813.1	[13]
<i>Glomus</i> 9	Uncultured <i>Glomus</i> clone Ca42 18 S ribosomal RNA gene, partial sequence (97%)	DQ357115.1	[52]

^a 97% sequence similarity is minimum requirement for identity to the species level [58]

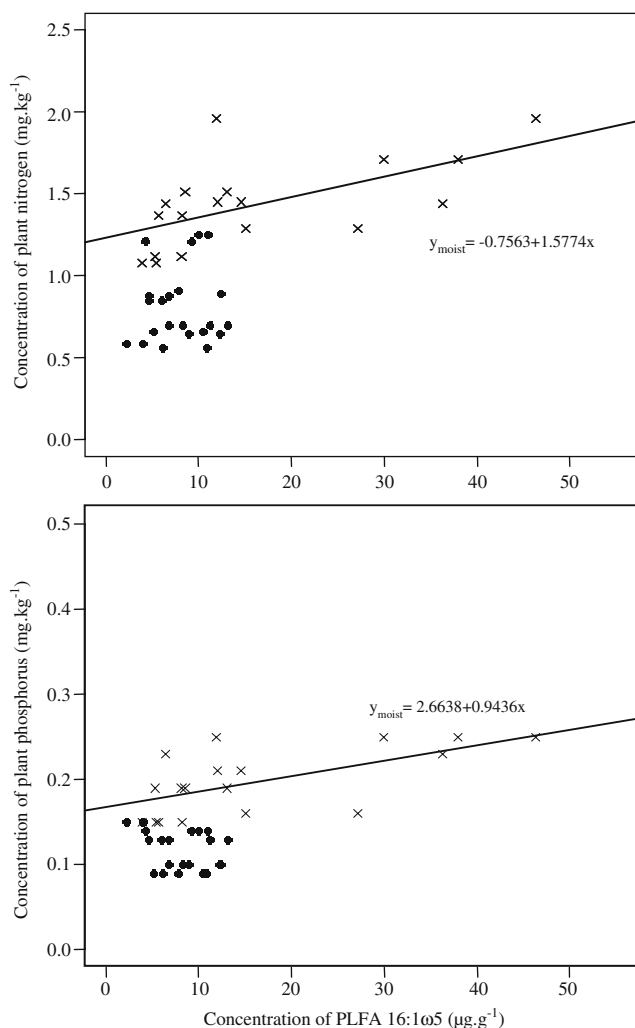


Figure 4 Relationships between AMF active biomass, as calculated by the abundance of PLFA 16:1 ω 5, and plant nitrogen ($P < 0.01$, $R^2 = 0.3212$) and phosphorus ($P < 0.01$, $R^2 = 0.3333$) in the moist period of summer 2007, as revealed by linear regression analysis ($P < 0.05$, $N = 24$). Dots represented plant nutrient concentration in July and August 2006 during the period of moisture deficiency and crosses-represented nutrient concentration in May and June 2007 during the period of soil moisture sufficiency

Discussion

Variation in AM Fungal Community

Seasonal variation in the relative abundance of AM fungi was found in all plant communities (Fig. 3), which agrees with earlier results [71]. Interestingly, phylogenetic distance analysis (Fig. 2) showed that *Glomus* 9 and 3 had very close genetic relationship even though they were dominant at different time. Adaptability to environmental change and biological functions of AM fungi were found to vary with isolates [67]. Our results concur with these findings and suggest that the soil AM fungi community can adapt to different environmental conditions and host plants.

The detection of similar number of AM fungal sequences in early June and late August, despite the contrasting conditions of temperature and soil moisture at these times, suggests that the arbuscular mycorrhizal symbiosis is, at all times, an important component of Grasslands National Park's soil-plant ecosystems. But the difference in composition of the AM fungal communities found in early June and late August conditions, suggests that the AM fungi making up a community are adapted to different environmental conditions. Better-adapted AM fungi appear to replace less-adapted AM fungi as environmental conditions changes. *Glomus viscosum*, *G. mosseae*, and *G. hoi* seems ill-adapted to the warm and dry environment of the native prairie in late August, as they were replaced by that of *Glomus* 5, 6, and 7 at that time. The absence of information on the AM fungal taxa most frequently found in late August, at the tail end of the warm and dry semiarid prairie summer, may indicate that much of the AM fungal diversity in dry environments remains to be discovered.

Adaptation to environmental conditions may occur within an AM fungal community or within a mycelium. Shift in the relative abundance of taxa making up an AM fungal community may occur with changes in environmental conditions, as shown by Oehl et al. [45] in a microcosm experiment. Adaptation could also involve changes in the abundance of some genes within rapidly evolving AM fungal mycelia. An AM hyphae contains dissimilar nuclei [26] and has a turnover time of 5-6 days [59]. Hyphal segments containing genetic information improving fitness under certain environmental conditions may preferentially proliferate at a certain time of the growing season. Variation in the frequency of detection of certain DNA sequences in June and August may reflect such selection of nuclei within AM mycelia.

Alternatively, different temporal pattern of sporulation in different AM fungi may explain the temporal variation in the frequency of detection of different AM fungal sequences observed in our study. Several AM fungi have seasonal pattern of sporulation. Whereas some AM fungi show a steady production of spores, sporulation in other taxa mainly occurs at different time in the growing season [35, 45].

Temporal changes in the frequency of detection of the different AM fungi was not restricted to the native prairie ecosystems, but also occurred in agricultural plant communities. Seasonal variations in the composition of the AM fungal community of Scottish grassland ecosystems were reported by Vandenkoornhuysen et al., [66]. Santos-González et al. [53], in contrast, found more non-AM fungal root endophytes in the fall, but no clear temporal variation in AM fungal community's composition in the roots of two grassland plant species during the growing season in Upland County, Sweden. Interestingly, these two plant species hosted different AM fungal communities. The succession of plants hosting

Table 3 MANOVA test of the relationship between the frequency of dominant plant species and AM fungi active biomass in cool season and warm season, respectively ($N = 24$)

Source	Average soil coverage (%)	Df	AM fungi activity in early season		AM fungi activity in late season		
			F ratio	P value	F ratio	P value	
Cool season plant	<i>Agropyron cristatum</i> (L.) Gaertn.	15.09	1	8.446	0.025	1.351	ns
	<i>Poa compressa</i> L.	2.5	1	2.671	ns	2.319	ns
	<i>Poa sanbergii</i> Vasey	2.5	1	0.01	ns	0.078	ns
	<i>Stipa comata</i> Trin. & Rup.	9.5	1	2.324	ns	0.217	ns
	<i>Taraxacum ceratophorum</i> (Ledeb.) D.C.	0.2	1	0.138	ns	0.985	ns
	<i>Koeleria gracilis</i> Pers.	4.8	1	6.807	0.034	1.535	ns
	<i>Carex filifolia</i> Nutt.	0.1	1	0.138	ns	3.697	ns
All species	34.69	7	4.705	ns	6.095	ns	
Warm season plant	<i>Bouteloua gracilis</i> (BHK.) Lag	8.5	1	0.352	ns	5.219	ns
	<i>Plantago patagonica</i> Jacq.	0.3	1	1.451	ns	3.326	ns
	<i>Artemisia frigida</i> Willd.	3.2	1	0.174	ns	0.001	ns
	<i>Malvastrum coccineum</i> (Pursh) Gray	0.9	1	0.86	ns	0.075	ns
	<i>Rabitiida columnifera</i> (Nutt.) Woot. & Standl.	1.1	1	4.522	ns	2.245	ns
	All species	14	5	1.515	ns	1.641	ns

ns non-significant according to ANOVA

different AM fungal communities could create a succession in their AM fungal associates. Soil moisture is another factor influencing AM fungal distribution [43, 65]. In the mixed grass prairie of Grasslands National Park, soil water availability varies and warm season plant species replace cool season plant species as summer progresses. Thus, variation in both plant species and soil moisture availability are possible drivers of shifts in AM fungal communities in the soil of this environment.

Environmental Effects on Relationship Between AM Fungal Activity and Plant Nutrition

The relationship between active AM fungal biomass and plant tissue N and P concentration found in early June suggests the involvement of AM fungi in nutrient cycling and in the nutrition of the plant communities. This finding is consistent with several previous studies [16, 20, 32, 39]. But more interestingly, in our study, no such relationship was detected at the late August sampling showing that changes in soil moisture modifies the relationship between AM fungal activity and their host plant. The concurrent changes in the composition of the AM fungal community can be one reason explaining the change in symbiotic functionality observed. In addition, July and August in 2006 was dryer than normal with only 8.4 mm of precipitation. Thus, the extremely dry environment in late summer 2006 could have inhibited the expression of a mutualistic relationship. The environment

might have been too dry for normal symbiotic function. The low extraradical AM fungal biomass at this time might also prevent the detection of any relationship in the dry period. Climate in Southwest Saskatchewan is characterized by a wide variability. A relationship between late season plant N and P concentration and extraradical AM fungi biomass might exist in years with better moisture.

Knowledge of the impact of drought on the arbuscular mycorrhizal symbiosis is still limited. Previous study on AM fungi sporulation in different seasons showed that heat can reduce AM fungal spore production in arid and semiarid areas [6]. Lugo et al. [35] found plant-specific and AM fungi-specific variations in the formation of intraradical AM fungal structures and spores in the moist and dry part of the year in an Argentinean dry plains with no seasonal variation in temperature. Soil water availability could be the factor explaining the change observed in the relationship between active extraradical AM fungal biomass and plant community. Although AM fungi are drought-tolerant fungi that can increase the drought tolerance of their host plant [14, 15], extreme drought can reduce both AM fungal growth and the activity of the associated plant community [6]. In our study, July and August had been much dryer than normal and almost all plants appeared to be dormant; but the possible occurrence of less mutualistic AM fungi in dry soil cannot be ruled out. The level of mutualistic ability of AM fungi dominating in dry soil remains to be tested.

Relationship Between AM Fungi Active Biomass and Plant Community

The AM fungi active biomass measured at the early June sampling time was significantly related to two cool-season grass species, crested wheatgrass, and junegrass. The lower abundance of some species may have prevented the detection of relationships, but it is possible that crested wheatgrass and junegrass are particularly supportive of soil AM fungal networks. Previous research found that AM fungi has close relationship with junegrass [11, 69] an observation that concurs with our results.

We did not find any significant relationship between the biomass of active AM fungal networks and plant community in late August. Even though some drought-tolerant AM fungi dominated at that time, we found no evidence of mutualistic relationship in agreement with previous research [6]. It remains unclear if the mutualism of AM symbioses breaks down under dry conditions or if the 2006 drought period in Grasslands National Park was just too severe for the expression of a mutualistic relationship.

In conclusion, the diversity of AM fungi in their natural habitats varies with changes in environmental conditions and seasonal plant succession. The biomass of their active extraradical networks is related to plant nutrient uptake and plant community structure, but these relationships can break down during dry periods.

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