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<td>Islam, M Nazrul; University of Saskatchewan, Soil Science Germida, James; University of Saskatchewan, Soil Science Walley, Fran; University of Saskatchewan, Soil Science</td>
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Responses of Arbuscular Mycorrhizal Fungal Communities to Soil Core Transplantation across Saskatchewan Prairie Climatic Regions

Authors
M Nazrul Islam (nazrul.islam@usask.ca)
James J Germida (jim.germida@usask.ca)
Fran L Walley (fran.walley@usask.ca)

Address
Department of Soil Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Canada

Corresponding author
M Nazrul Islam
Email: nazrul.islam@usask.ca
ABSTRACT

No information exists on the susceptibility of arbuscular mycorrhizal fungal (AMF) communities in Canadian prairie agriculture soils to climate change. An experiment was initiated in mid-May 2011 in which replicated soil cores were transplanted reciprocally from four cultivated prairie sites in Saskatchewan, Canada representing different regional climatic zones ranging from semi-arid to sub-humid regional climates, such that replicated (n=4) soil cores from each site were present at all sites. Field pea was grown in all cores and at harvest in early-September 2011, soil samples were collected to analyze the changes of AMF communities over the cropping season. A total of 82 operational taxonomic units belonging to eight AMF genera were identified using 18S rRNA gene pyrosequencing. When soils were transplanted to new environments, the relative abundance of AMF changed considerably. Typically, Shannon diversity declined when soil cores were transplanted to new environments. We present evidence that the altered climatic conditions following transplantation of soil cores, the relative abundance of AMF was significantly altered, and some taxa were enhanced, suppressed or disappeared in the home-away soils, compared to home-site soils. This study implies the future climate change effects on AMF may impact specific phylogenetic taxa differently, such that rare species or those with low abundance may increase or decrease with unknown consequences. Understanding the potential responses of AMF communities to soil-climate interactions is important when considering the impacts of climate change on soil microbial communities.

Key words: Climate change, soil core transplantation, Glomeromycota, arbuscular mycorrhizal fungi, Canadian prairies

INTRODUCTION
Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota are ubiquitous and abundant soil microorganisms. They establish symbioses with most species of land plants and occur in almost every terrestrial ecosystem (Smith and Read 1997). They play a crucial role in the functioning and sustainability of agroecosystems (Gianinazzi et al. 2010). They have been studied for decades because of their several important roles including nutrient and carbon cycling (Perry et al. 1990; Drigo et al. 2008), plant health (Song et al. 2011), pathogen protection and resistance (Azcón-Aguilar et al. 1996; Jung et al. 2012), plant fitness in polluted environments (Kaldorf et al. 1999), soil health (Rillig et al. 2004), water uptake (Augé et al. 2001), and plant productivity (Hamel and Strullu 2006).

Recent reports predict that climatic conditions in prairies will change. It has been suggested that there will be an increase in the frequency of droughts, particularly in the semi-arid regions of the prairie provinces, reducing crop yield in these locations (Mkhabela et al. 2011; Bonsal et al. 2012; Dumanski et al. 2015). Little is known regarding the impact of changing climate on the diversity of AMF in prairie soils. However, several studies examined the phylogenetic diversity of AMF communities in different climatic regions in Chernozemic soils of western Canadian cropping and natural ecosystems. For example, Yang et al. (2010) identified some specific AMF sequences, namely *Glomus viscosum*, *Funneliformis mosseae*, and *G. hoi* which were dominant under sufficient soil moisture conditions. The Black Chernozem soils harbored the most diversified AMF communities in crop soils compared to Brown and Dark Brown soils (Dai et al. 2012). Further, Bainard et al. (2014) demonstrated that the abundance of individual AMF genus, such as *Funneliformis* were correlated differently with soil phosphate levels, in particular, populations of *Rhizophagus* were significantly shifted to a dominance of *Funneliformis* taxa with increasing phosphate levels under wheat cultivation system. Similarly, a positive correlation was observed between the abundance of *Funneliformis* OTUs and soil pH under the semi-arid prairie agroecosystem. A better understanding of the impacts of climate change on AMF communities is vital for predicting the long-term impact on sustainable agriculture.
Transplanting undisturbed soils across multiple ecosystems presents an approach to examine the responses of microbial community dynamics and plant-microbe interactions to climate change conditions. For example, Bottomley et al. (2006) found that the reciprocally transplanted soil cores between two high elevation sites had different fungal and bacterial community composition compared to the site of origin and suggested this might be due to the temperature and soil moisture differences. Other studies showed a small change in atmospheric or soil temperature altered soil mineralization and decomposition processes (Parton et al. 2007; Adair et al. 2008; John et al. 2011) that eventually affected bacterial or AMF communities (Heinemeyer et al. 2004). Twenty years after soil transplantation between two sites approximately 1000 km apart, phylogenetic analysis revealed that bacteria, fungi, and nitrogen-cycling genes were clustered based on the site factors rather than soil characteristics (Sun et al. 2014). Moreover, a long-term soil transplant study over three agricultural research stations across warm, cold, and subtropical zones revealed a differential pattern of 16S rRNA bacterial genes and fluctuated OTU richness along with the cooling and warming conditions (Liang et al. 2015).

Soil transplantation studies are a useful way to evaluate the responses of microbial communities to changing climate conditions (Waldrop and Firestone 2006; Lazzaro et al. Vanhala et al. 2011; Zumsteg et al. 2013; Zhao et al. 2014). However, the responses of AMF communities to changing climatic conditions remains poorly understood, particularly in agricultural ecosystems of Canada. Thus, this study was initiated to examine potential responses of Canadian prairie AMF communities to changing climatic conditions. We established a soil transplant experiment at four different cultivated sites representing different regional climatic areas ranging from semi-arid to sub-humid, in Saskatchewan. Our research addresses two hypotheses: 1) soil core transplantation into different climates will alter the abundance and diversity of AMF communities; and 2) different AMF phylogenetic groups will show differential sensitivity to soil-climate interactions.

MATERIALS AND METHODS
Site description

Experimental sites were established at four locations in Saskatchewan, Canada, representing different climates and soils ranging from a semi-arid site at Swift Current (SW) to a sub-humid site at Melfort (MF) (~ 450 km distance between two sites) (Fig. 1A and B). The four sites were established within three different soil zones (i.e., ecoregions). Sites were located at Agriculture and Agri-Food Canada (AAFC) research sites in Saskatchewan at the following locations: 1) Swift Current Research and Development Centre, at Swift Current (SW), Brown soil zone (latitude: 50°18'00.000" N, longitude: 107°44'00.000" W, and elevation: 825.00 m); 2) Scott Research Farm at Scott (ST), Dark Brown soil zone (latitude: 52°21'35.064" N, longitude: 108°50'05.004" W, elevation: 659.60 m); 3) Melfort Research Farm at Melfort (MF), Black Soil zone (latitude: 52°49'00.000" N, longitude: 104°36'00.000" W and elevation: 480.10 m); and 4) Canada-Saskatchewan Irrigation Diversification Centre (CSIDC) at Outlook (OL), Dark Brown soil zone (latitude: 51°29'00.000" N, longitude: 107°03'00.000" W and elevation: 541.00 m).

Previous year (2010) cropping history on the sites were wheat, fallow, wheat, and pea at SW, OL, ST, and MF sites, respectively.

The 30 year (1980-2010) annual mean temperature and precipitation varied between 1.3 to 4.3 °C and 395.8 to 357.4 mm across the four study sites, respectively. The historical weather data for annual and crop season average temperature, precipitation, soil classification, and major soil physicochemical properties at each site are described in Tables 1 and 2. The regional data were obtained from Environment Canada (http://climate.weather.gc.ca) and the AAFC metrological databank.

Soil core establishment and experimental design

In May (15 to 22) 2011, 16 open-ended aluminum soil cores (20 cm diameter) were pushed into the soil to a depth of 37 centimeters using a truck-mounted hydraulic press at each of the sites. The undisturbed
soil cores were subsequently extracted manually and four cores from each site were transplanted (randomly) at the home (original) site. The remaining 12 cores from each site were transported to each of the three other sites where four cores from each site were transplanted in two rows 45 cm apart using a completely randomized design (Fig. 1C).

**Initial soil sampling**

Prior to seeding into the core, composite soil samples were collected from each site from the 0 to 15 cm depth (approx. 100 g core⁻¹) in May 2011 using a JMC Backsaver N-2 soil core (Clements Associates, Inc, IA, USA). Samples were stored in plastic bags and maintained at -20 °C. Soil pH and EC (1:2/soil: water suspensions) (Sparks et al. 1996), soil organic carbon (SOC) (dry combustion using LECO analyzer) (Wang and Anderson 1998), and soil organic matter (SOM) (Walkley and Black 1934) were performed in the Central Soil Science Laboratory at the College of Agriculture and Bioresources, University of Saskatchewan and other soil chemical analyses (mineral nutrients) were performed at the ALS Environmental Laboratory, Saskatoon, Canada and summarized in Table 2.

**Seeding in soil cores**

Field pea (*Pisum sativum* L., CDC Meadow) was seeded into the soil cores as the pea plant showed good arbuscular mycorrhizal root colonization (Jin et al. 2013a), inoculated with rhizobia at the recommended rate (equivalent to 3 mL kg⁻¹ seed N-Prove® containing *Rhizobium leguminosarum* bv. viceae 5.0 x 10⁸ viable cells g⁻¹ inoculant, Novozymes BioAg, Canada) was hand seeded into the soil cores at a depth of 4 cm and thinned to three pea seedlings per core on emergence. Weeds were controlled by hand three to four times during growing seasons. At seeding, 650 mL of water were applied to each core. Seeding and harvesting in 2011 were performed on 9th June and 10th September at the SW site, 8th June and 7th
September at the OL site, 6th June and 5th September at the ST site, and 7th June and 9th September at the MF site, respectively.

**AMF trap culture with field core soil samples**

At maturity, field pea were harvested by hand and soil samples (ca. 200 g) were retrieved from each core to 15 cm using an alcohol-sterilized soil probe. The seasonal decomposition of fine roots prevented collection of consistent root samples from the different soil cores for AMF community analyses. Consequently, we elected to use an AMF trap culture with field pea as the host plant grown in the soils collected at harvest for this purpose. For moist soils were passed through a 4-mm sieve and then mixed (1:1 w/w) with sterilized (autoclaved three times at 120 °C for 2 h) sand (Crystalline silica in the form of quartz, fine-grained particle size: 0.13 - 0.20 mm, Microcrystalline Silica CAS, Unimin Corp, USA) and 400 g of each soil/sand mix were placed in replicated (n = 4) 750 mL pots lined with plastic. Surface disinfested pea seeds (two plants per pot) were seeded in each pot. The trap cultures were maintained in a growth chamber with ambient day/night temperatures of 24 °C/18 °C with 16 h day lengths for 8 weeks.

At 8 weeks, roots were harvested, thoroughly rinsed in tap water then washed with deionized water to remove any residue soil particles and debris and blotted dry. The cleaned roots were immediately immersed in liquid N and preserved at -80 °C until molecular analysis.

**DNA extraction, PCR library preparation, and 454 pyrosequencing platform**

DNA was extracted from the AMF trap root using the Qiagen Plant DNeasy kit (QIAGEN, Mississauga, ON) according to the manufacturer’s recommended protocol. One hundred milligrams of freeze-dried pea root tissue were placed in a 2-mL screw-top micro-centrifuge tube with 5 mm ceramic beads and
pulverized to a powder using Precellys® 24 tissue homogenizer (Bertin Technologies, USA). Pure genomic root DNA was eluted in Tris EDTA (TE) buffer for further analysis.

A nested PCR protocol was used to amplify an ~800 bp partial fragment of AMF 18S rRNA gene for 454 pyrosequencing (Jin et al. 2013b; Bainard et al. 2014). The universal eukaryotic primers NS1 and NS4 (White et al. 1990) were used in the first round of PCR followed by AMF-specific primer pair AML1 and AML2 (Lee et al. 2008). The forward primer (AML1) and reverse primer (AML2) also included tags CS1 and CS2 (Fluidigm Corp., San Francisco, CA) that were anchors in a third PCR reaction adding Titanium multiplex identifiers (MIDs) and Lib-L adaptors sequences (Supplementary Table S1).

The first polymerase chain reaction (PCR) conditions were as follows: initial denaturing step at 95 °C for 15 min; 30 cycles at 95 °C for 30 sec; 50 °C for 30 sec; 72 °C for 1 min 30 sec; and a final extension step at 72 °C for 3 min, with a 5 µL reaction volume including 1 µL of 1/10 diluted DNA template, 1 mM dNTPs, 0.4 µM of each primer (NS1 and NS4) and FastStart High Fidelity (Roche, 04 738 292 001). Five microliters of the reaction mixture in the second round of PCR included FastStart High Fidelity, 1 µL of 1/10 diluted first PCR product, and 0.4 µM of each primer (AML1-CS1F and AML2-CS2R). The conditions for the second round of PCR were as follows: initial denaturing step at 95 °C for 15 min; 33 cycles at 95 °C for 30 sec; 60 °C for 30 sec; 72 °C for 1 min 30 sec; and the final extension step at 72 °C for 5 min.

The third PCR was performed to incorporate 10 nt-MIDs (Titanium Lib-L forward-MDs-CS1 and Titanium Lib-L reverse adaptor-CS2) and contained 0.5 µL of PCR, 1 µL of 2 µM barcodes, 0.5 µL of DMSO, 0.1 µL FastStart High Fidelity and 0.2 µL of 10 mM dNTP. The third PCR conditions were: initial denaturing step at 95 °C for 10 min; 15 cycles at 95 °C for 15 sec; 60 °C for 30 sec; 72 °C for 1 min; and a final extension step at 72 °C for 3 min.

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All final PCR products were run on 2% agarose gel and quantified using PicoGreen®. Samples were combined into pools of 64 samples based on their MIDs. Each pool was purified with three AMPure XP (Agencourt/Beckman Coulter) protocols (ratio 0.5) and quantified using Qubit 2.0 Fluorometer (Life Technologies, CA, USA). The samples were run on an Agilent 2100 Bioanalyzer using a high sensitivity DNA kit to confirm the size and quality of amplicons. Finally, unidirectional sequencing was performed in half region runs for each pool on a GS-FLX+ system (454 Life Sciences/ Roche Applied Science) at McGill University, Montréal and Génome Québec Innovation Centre (https://genomequebec.com), Quebec, Canada.

**Bioinformatics of AMF sequences**

The raw pyrosequencing reads were processed using MOTHUR pipeline version 1.31 (Schloss et al. 2009) to clean ambiguous nucleotides (average score of quality <30) (Huse et al. 2010). The excessively long and short homopolymers, low-quality, and chimeric sequences were removed from the dataset. The average 700 to 800 bp long 18S rRNA gene sequences were targeted for downstream analysis. The clean sequences were aligned against Silva eukaryotic reference sequences (http://arb-silva.de/). The reference sequence files were downloaded from public UCHIME domain (Edgar et al. 2011). Non-Glomeromycota sequences were also removed from the dataset. The representative sequences clustered into OTUs, based on 97% similarity, were performed using CD-HIT Suite (http://cd-hit.org).

**Phylogenetic analyses of AMF OTUs**

For taxonomic identification, the representative sequences of each AMF OTU were compared with GenBank reference sequences using BLAST search against NCBI nucleotide collection database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The OTUs and GenBank reference sequences (97% or above similarity) were aligned using ClustalW (Tamura et al. 2013). The neighbor-joining phylogenetic
reconstruction (Saitou and Nei, 1987) was used to build a phylogenetic tree using MEGA V.6. The tree was constructed using 82 OTUs and 45 GenBank reference sequences to group into representative AMF genera and species. The bootstrap replication method was set at a confidence level of 1000 with the Kimura 2 parameter model (Hall, 2004). The systematics of AMF taxa was used as the classification of Schüßler and Walker (2010) and identification of some Glomus spp. (Oehl et al. 2015).

**Shannon diversity index of AMF communities**

Species diversity in an ecosystem can be estimated using a variety of indices (Bonilla-Rosso et al. 2012). Our study focused on the effect of the environmental change on species abundance, diversity, and composition including low (rare) abundant species. The Shannon diversity index (SDI: $H$) accounts for both abundance and evenness/equitability ($EH$) of the OTUs belonging to specific phylogenetic taxa and sensitive to rare species present in the samples (Morris et al. 2014). The SDI formula refers to the proportion of species ($i$) relative to the total number of species ($p_i$) and then multiplied by the natural logarithm of this proportion ($lnp_i$) (Shannon 1948; Brodie et al. 2003). The resulting value is summed across species and multiplied by -1. The list file (number of observed OTUs) generated after clustering OTUs in MOTHUR bioinformatic pipeline was used as the total number of species ($p_i$) to formulate SDI.

**Data analysis**

For the estimation of percent relative abundance (PRA) of AMF sequences, the number of absolute sequences reads (ASR) present in each sample OTU was divided by the total number of ASR from the total OTUs in that sample and multiplied by 100 (Amend et al. 2010). Linear mixed models ANOVA using PROC MIXED in SAS (v.2.0.5) was applied to obtain $P$ values for the two fixed effects (soil and site) on the PRA of eight AMF genera (Funneliformis, Glomus, Rhizophagus, Septoglomus, Diversispora, Claroideoglomus, Archaeospora, and Paraglomus), three dominant OTUs (OTU65, OTU5, and OTU42),
and Shannon diversity index of AMF communities. Replication and randomization of soil cores within environments and their interactions considered as random effects. Soil, site, and soil x site interactions were the fixed effects and this model separated random errors (Type III) from the two fixed effects. Prior to statistical analysis, PRA data were subjected to a normality test to ascertain skewness and kurtosis and were transformed (i.e. square-root and arcsine) when required. The original (home-site) soil core was compared with the home-away soil cores (same soil transplanted into other three sites) and mean separation was performed using Tukey’s honestly significant difference (HSD) test at $P \leq 0.05$.

Permutational multivariate analysis of variance (PerMANOVA) using PC-ORD v.6.0 (McCune and Mefford, 1999) was applied to test the significance of soil and site (categorical variables/predictors) on the relative abundance and composition of AMF taxa. To visualize the difference in AMF communities among transplanted soil cores across the four sites, a distance matrix of AMF in various soil cores was ordinated using the principal coordinates analysis (PCoA). The Bray-Curtis distance algorithm and ‘slow and thorough’ autopilot mode were used for this analysis in PC-ORD v.6.0. A biplot PCoA analysis was performed with the whole data set (soil, climate/site, and AMF) as the soil-climate variables were included in the second matrix and relative abundance of eight AMF genera were arranged in the main matrix to identify the magnitude of the changes in AMF and environmental variables. The two most informative dimensions (axis 1 and 2) of the three-dimensional solution were used to construct the PCoA graph.

**Submission of nucleotide sequences**

The sequences representing 82 AMF OTUs analyzed in this study was deposited in the GenBank submission portal (https://submit.ncbi.nlm.nih.gov/subs/) under the accession numbers SUB6280397 (MN428685 to MN428766). The OTUs were also listed in the Supplementary Table S4.
RESULTS

Climate and soil variation across the study sites

According to historical (1980 to 2010) weather data, the ST Dark Brown and MF Black soil zones experienced relatively high amounts of precipitation and low temperatures (Table 1). In comparison, SW Brown and OL Dark Brown sites were drier and warmer. The average temperature and precipitation during the study period (May to September) were considerably increased across the four study sites compared to the last 30-year cropping season average temperature and precipitation (Table 1). The MF site had higher soil organic matter (SOM), and soil organic carbon (SOC) compared to SW, OL, and ST (Table 2). The soil pH was relatively lower in ST compared to SW, OL, and MF.

Pyrosequencing and identification of AMF communities

The DNA extractions from replicated 64 pea trap root samples were amplified successfully using nested PCR protocols. The universal fungal (NS1/NS4) and AM fungal (AML1/AML2) specific primer pairs were deemed to have good mycorrhizae specificity with an average of less than 19% of the sequences from non-Glomeromycota (data not shown). A total of 19 799 AMF sequence reads from 18S rRNA pyrosequencing platform grouped into 82 OTUs representing eight AMF genera were obtained (Fig. 2). For the identification of these OTUs, phylogenetic analyses with 45 GenBank AMF reference sequences were performed. Forty-seven OTUs clustered to species belonging to Glomeraceae family (27 OTUs to Glomus sp., 12 OTUs to Funneliformis sp., four OTUs to Septoglomus sp., and four OTUs to Rhizophagus sp.), 12 OTUs to Paraglomus sp., 11 OTUs to Claroideoglomus sp., 11 OTUs to Archaeospora sp., and one OTU to Diversispora sp. (Figs. 2 and 3).

AMF (genera) abundance and composition
The PROC MIXED analysis revealed a significant effect of soil and site on the relative abundance of the eight AMF taxa (Table 3). However, site environments had no significant impact on the AMF genera *Glomus* and *Paraglomus*. The most abundant sequence detected in the original and transplanted cores was *Funneliformis* sp., which represents 25% to 68%, followed by *Claroideoglomus* sp. (3% to 53%), *Glomus* sp. (2% to 25%), and *Paraglomus* sp. (1% to 29%) (Fig. 3). The *Diversispora* sp. (0 to 17%), *Rhizophagus* sp. (0 to 15%), *Archaeospora* sp. (0 to 9%), and *Septoglomus* sp. (0 to 9%) were either detected at relatively low abundance and/or were absent in some of the soils and sites (Fig. 3 and Supplementary Table S2).

Transplanting soil cores into the four different climate sites caused a substantial shift in the AMF communities (Fig. 3). For example, *Archaeospora* was detected in the MF soil at the original MF site but was not detected when the MF soil was transplanted to SW and OL sites. Similarly, both *Archaeospora* and *Septoglomus* were found in the ST soil but were not detected when ST soil was transplanted to SW. In contrast, some taxa that were not detected at the site of origin were detected when soil cores were transplanted to other sites. *Diversispora*, for example, was undetectable in SW soil at the original SW site but was detected in the SW soil transplanted at the OL site. Similarly, *Septoglomus* was absent in OL soil at the original OL site and was only detectable in the OL soil transplanted to the ST site.

An inconsistent shift in the PRA of the dominant AMF taxa (e.g., *Funneliformis, Glomus, Claroideoglomus*, and *Paraglomus*) was observed when home-site (original) soil cores were transplanted to new climatic ecoregions (Fig. 3). The genus *Funneliformis* in SW soil (32% PRA) at semi-arid SW site shifted when SW soil was transplanted to sub-humid MF and ST sites, accounting for 52% and 68% of the taxa, respectively. Similarly, a substantial increase of taxa belonging to the genus *Glomus* was detected when SW soil (6% PRA) was transplanted to the other three sites, increasing at OL (24%), ST (18%), and MF (14%). In contrast, a drastic reduction of the genus *Rhizophagus* was detected when MF
(15% PRA) soil was transplanted to ST (3%), OL (4%), and SW (11%) sites. Similarly, the genus *Claroideoglomus* in SW soil at the SW site accounted for 53% of the taxa; when SW soil core was transplanted to ST and MF sites, the proportion of *Claroideoglomus* was reduced to 32% for both sites. The *Paraglomus* community shifted greatly both in the original and new environments. For instance, *Paraglomus* was 8% in MF soil at the site of origin (MF) but was undetectable in MF soil transplanted to the ST site, and accounted for only 1% of the taxa at the OL site. However, the abundance of *Paraglomus* was enhanced from 8% to 13% in MF soil at the SW site.

PerMANOVA analysis revealed that the soil (*P* < 0.0001) and local climate (site) (*P* = 0.0030) had a significant impact on the composition of the AMF communities and the effect of soil were more pronounced than site and soil-site interactions on the composition of AMF taxa (Supplementary Table S3). These effects were also supported by the PCoA analysis (Fig. 4). The ordination plot revealed that there was a clear difference in AMF abundance among the soils (distance measured between the same soils at different sites and different soils at their home-sites) in response to soil-climate interactions. The soils (either same or different soil cores) had no distinct clusters and were scattered along with PCoA axis 1 and 2 which explained 52% and 32% variation. The greatest dissimilarity in AMF community abundance and composition along with the axis 1 was exhibited between SW soils followed by OL, ST, and MF soils. Relatively higher dispersion of AMF among the four different soil types (home-site cores) along with the axis 1 and 2 was plotted. The PCoA biplot ordination clearly showed that the soil (pH and EC) and climate (temperature and precipitation) variables had a strong influence on the changes in AMF taxa across the soils and sites (Fig. 4). The direction and length of the arrows (variables) revealed that changes in AMF were increased with the increasing geographical temperature and soil organic matter along with semi-arid (SW and OL) to sub-humid (ST and MF) regions. The scaling of AMF (genera) points within the PCoA plot showed that the relative abundance of certain taxa responded strongly to certain soil and site conditions. For example, *Claroideoglomus* was dominant in the higher temperature region (i.e. SW site), *Septoglomus*, *Paraglomus*, and *Rhizophagus* were associated with higher SOM sites.
(i.e. MF and ST), and *Funneliformis* was frequently distributed regardless of soil and site characteristics (Figs. 3 and 4).

**AMF (OTUs) abundance**

Three of the dominant OTUs, namely OTU65, OTU5, and OTU42 shared above 98% similarity with GenBank reference sequences for *Funneliformis* (accession no. MH629488.1), *Dominikia* (accession no. GU059535.1) and *Claroideoglomus* (accession no. KX879058.1), respectively and were frequently distributed across the soils and sites (Fig. 5). The relative abundance of these three OTUs was significantly changed in response to soil transplantation across the semi-arid to sub-humid regions (Table 3). The PRA of OTU65 (*Funneliformis* sp.) was significantly ($P \leq 0.05$) increased when original (home-site) soil core was transplanted to a new environment (Fig. 5A). In contrast, the PRA of OTU42 (*Claroideoglomus* sp.) was significantly ($P \leq 0.05$) reduced when the home-site soil was exposed to new climatic regions regardless of site characteristics (Fig. 5C). Interestingly, the shift in abundance of OTU5 (*Dominikia* sp.) was less predictable and depended on which soil type was moved to which climatic region, specifically, dry soils (i.e. SW and OL) moved to wetter sites (i.e., ST and MF) had a significant ($P \leq 0.05$) increase of the relative abundance of OTU5 (Fig. 5B). On the other hand, moving wetter soils (i.e. ST and MF) to the dry sites (i.e. SW and OL) had a drastic reduction of the abundance of OTU5 (Fig. 5B).

**AMF community diversity**

The impact of soil and regional climates on AMF community diversity was assessed using Shannon’s diversity index ($H$). According to PROC MIXED, the diversity of AMF communities across the semi-arid to sub-humid prairie regions were significant in response to soil type ($P < 0.0001$), site climate ($P = 0.0032$), and their interactions ($P < 0.0001$) (Table 3). The Shannon diversity indices showed that MF ($H$...
= 3.8) soil had the greatest diversity of AMF taxa while ST (\(H = 2.8\)) and OL (\(H = 2.6\)) soils harbored a moderate diversity, and a relatively low diversity was observed in SW (\(H = 2.5\)) soil (Fig. 6).

Transplanting soils to new environments had a significant impact on AMF diversity. The MF soil (\(H = 3.8\)) at the MF site (home-site) had higher diversity index than the other three soils (SW, OL, and ST), but the levels of diversity were significantly (\(P \leq 0.05\)) reduced when MF soil was transplanted to other sites (Fig. 6). For instance, the diversity in MF soil at SW site was reduced to less than half (from 3.8 to 1.7) compared to MF soil at the MF site. Similarly, diversity levels were nearly double (from 1.4 to 2.5) in SW soil at SW site compared to the SW soil at MF site (Fig. 6).

When the soils were transplanted to a new climate zone from the site of origin (home-site), the diversity index was significantly (\(P \leq 0.05\)) reduced (Fig. 6). This trend was consistent for all four home-site soils transplanted to the other three sites. However, OL soil at SW and MF sites, SW soil at ST and MF sites, and MF soil at OL and SW sites had no significant changes in diversity.

**DISCUSSION**

The 30 year average (1980 to 2010) temperature and precipitation varied between 14.3 to 15.4 °C and 47.9 to 53.0 mm across the study sites, respectively (Table 1). Both SW and OL represent semi-arid climates, with lower annual precipitation and higher temperatures than the sub-humid sites (ST and MF). In the 2011 study year, both SW and ST experienced higher than average precipitation and temperature, whereas MF experienced a relatively dry growing season. However, SW had 1.4 °C higher temperature and 17.7 mm lower precipitation than MF site during the cropping season. These four experimental sites provided a suitable range of soil and environmental conditions to examine the responses of the AMF communities to the differences within relatively short distances of prairie agroecological zones. Others have reported that small changes in atmospheric or soil temperature and moisture can influence microbial
processes such as soil organic matter decomposition (Adair et al. 2008; Parton et al. 2007). In the current study, soil organic matter increased along the ∼450 km apart reflecting the wetter and cooler environments at both MF and ST compared to other the two more southerly sites (i.e. SW and OL) (Les Fuller 2010; Anderson and Cerkowniak 2010) (Table 1). Soil fertility, which is largely influenced by the turnover of organic matter decomposition and mineralization, has been linked to levels of AMF root colonization (Smith and Read 1997). The results of this study showed that the AMF abundance and diversity was higher in the cooler and wetter sites (MF and ST) compared to the drier and warmer sites (SW and OL).

Our pyrosequencing data are consistent with the previous reports of AMF diversity of the prairie AMF communities. For instance, Dai et al. (2012) identified 33 dominant AMF OTUs using pyrosequencing, representing *Funneliformis, Rhizophagus, Claroideoglomus*, and *Diversispora* from 76 wheat fields across Dark Brown, Black, Dark Gray Chernozems, and Gray Luvisol in the Canadian prairies. Similarly, the 51 AMF OTUs in pea roots using MiSeq-Illumina were classified into four genera namely, *Rhizophagus, Glomus, Claroideoglomus* and *Funneliformis* from the four experimental sites representing Brown, Dark Brown, and Black Chernozems (Islam et al. 2014). However, the DGGE (Ma et al. 2005), and spore morphology (Talukdar and Germida 1993) analyses revealed a higher abundance of *Glomus, Acaulospora, Gigaspora*, and *Scutellospora* in the prairie soils, but in this study the latter three genera were not detected.

Consistent with our hypotheses, soil transplantation significantly altered the abundance, composition, and diversity of AMF community and some AMF groups (*Archaeospora, Diversispora, Paraglomus, Rhizophagus*, and *Septoglomus*) showed strong sensitivity to soil transplantation (Fig. 3), although, these AMF taxa were either low in abundance or undetectable in some soils among the transplanted soil cores. The differences in AMF taxa due to exposure to different environments suggest that the AMF were adapted to the home environment and transplanting the soil cores ultimately caused a significant shift of
AMF communities, presumably due to conditions that either stressed or promoted some taxa over others. Gai et al. (2012) also found that soil transplantation induced a significant shift in AMF communities under contrasting geographical elevation gradients and the AMF diversity (species richness) and root colonization decreased with increasing altitude. Other reports showed that soil transplantation from north to south boreal forest zones (Vanhala et al. 2011) and California oak to grassland ecosystems (Waldrop and Firestone 2006) resulted in a loss of microbial biomass, changes in the composition of fungal and bacterial communities, and altered functionality in response to warmer climatic conditions. However, inconsistent responses of soil fungal diversity and composition to environmental elevation frequently have been reported (Lugo et al. 2008; Margesin et al. 2009).

In this study, three AMF taxa, namely *Funneliformis* (12 OTUs), *Glomus* (27 OTUs), and *Claroideoglomus* (11 OTUs) were abundant at all study sites. Of these taxa, the PRA of *Funneliformis* and *Glomus* were increased by more than double and *Claroideoglomus* decreased by nearly double in the SW (warm and dry site) soil cores transplanted into MF and ST (wet and cool) sites (Fig. 3). This AMF shift indicates that the AMF communities residing in home climates either experienced stress or acclimated and adapted to the new environment. It is likely that edaphic factors, such as rapid fluctuation of soil moisture levels due to differences in temperature gradient, affected the AMF communities. Numerous reports revealed a significant alteration in soil microbial communities due to soil moisture changes (Schimel et al. 1999; Chen et al. 2007; Williams 2007). Moreover, the differences in the abundance of AMF genera in the transplanted soils strongly suggest that the observed alteration was driven by the regional climate gradients associated with the latitudinal locations.

Taxa with low abundance such as *Archaeospora, Diversispora, Paraglomus, Rhizophagus,* and *Septoglomus* were detected in home climates but were undetectable when transplanted to other climates (Fig. 3) resulting in a significant reduction in Shannon diversity indices in the soils transplanted to other sites (Fig. 6). The reduction in diversity indices in the soil cores transplanted into the new environment,
regardless of whether the sites were warmer or cooler, was observed for all four soils used in this study. This indicates that the shift in AMF likely reflects a “home-site” advantage rather than climatic preference based on moisture availability or warming condition, signifying the potential local adaptation impact on AMF communities under environmental stress gradient conditions (Thrall et al. 2007; Yang et al. 2018). However, little is known about the adaptation strategy of AMF communities under multiple environmental gradient interactions (Johnson et al. 2013).

The above changes in abundance and diversity of AMF taxa were not consistent across the four experimental sites. We observed an interesting shift in the AMF taxa with low abundance. Some taxa were present in the transplanted soils but were not detected in the soils at the original site suggesting that the home-site advantage may not be preferable to all AMF taxa (Fig. 3). Here, a distinct pattern emerges in which other inherent plant-AMF interaction factors such as AMF-soil compatibility for effective plant-AMF symbiosis may be involved along with the above mentioned local-site adaptation (Helgason and Fitter 2009; Herrera-Peraza et al. 2011; Yang et al. 2016). The AMF taxa “presence vs absence” is apparently due to spore germination and hyphal growth or spore dormancy under modified soil environmental condition or could reflect potential movement of AMF spores and hyphae (water or air borne inoculum) into the transplanted cores. It is known that some of the AMF taxa, such as Scutellospora, prefer to grow under a specific set of climates (Gai et al. 2012). Moreover, some AMF species, particularly lower abundance or rare species, exhibited a strong dependency on explicit environmental conditions (Van der Heijden et al. 1998; Klironomos, 2000, 2003; Macel et al. 2007).

In addition to the above explanation on the presence vs absence of low abundant AMF, specifically, Diversispora sp. belonging to a single OTU (OTU22) was detected only in the three soil cores out of 16 (Fig. 3). We anticipated that the detectability of this species might be partially associated with the higher number of PCR cycles (63 cycles in two round PCR), required to eliminate incorrect size fragments using universal fungal (NS1 and NS4) and AMF (AML1 and AML2) primer sets. Previous study harvested
AMF amplicons with 50 cycles with the similar primers under pyrosequencing platform (Bainard et al. 2014). Several studies recommend minimizing the number of PCR cycles in DNA library preparation as the possibility of formation of artifacts, chimeras, single-stranded DNA molecules and lowering the chance of amplification of the rare or low abundant sequences (Qiu et al. 2001; Bonnet et al. 2002; Schmidt et al. 2013). In contrary, the effect of cycle numbers had a minor importance in the microbial community analysis from environmental samples even at a high number of PCR cycles (Lueders and Friedrich, 2003; Acinas et al. 2005) and no effects on the Shannon diversity indices were observed with higher cycle numbers (Sipos et al. 2007).

This single crop season data demonstrates the usefulness of the soil transplantation technique as a tool to investigate the impact of soil-climate alterations on AMF communities in cropping ecosystem across Saskatchewan. we did find an indication from the PCoA analysis that the changes in AMF communities are susceptible to two major abiotic factors, soil organic matter and atmospheric temperature followed by soil pH and precipitation (Fig. 4). This also is apparent in three dominant OTUs which showed a preferential shift based on site and soil factors (Fig. 5). For example, *Funneliformis* (OTU65) responded positively when exposed to new environments, no matter if it was within a dry or wet climate (Fig. 5A). The observed abundance of *Funneliformis* was consistent with several other studies where *Funneliformis* specifically, *F. mosseae* was found abundantly distributed across the prairie regions and the world, regardless of climatic variations (Avio et al. 2009; Rosendahl et al 2009; Dai et al. 2013). An opposite trend was observed in *Claroideoglomus* (OTU42) that responded negatively to new environments (Fig. 5C). However, the genus *Dominikia* (OTU5) exhibited strong site selectivity. For example, dry soils (i.e., SW and OL) moved to wetter sites (i.e., ST and MF) significantly increased relative abundance and wet soils moved to dry sites significantly decreased relative abundance of OTU5 (Fig. 5B). This result confirmed a preferential response of different AMF clades to certain environmental conditions. A previous study on prairie cropping systems also showed that the specific group of AMF shifted along with the successive fluctuation of soil chemical properties during crop growing season (Bainard et al. 2014).
Responses to changes in soil-climate variables may vary between phylogenetic identities and growth habit of AMF (fast and slow growers), and thus certain AMF may have adopted contrasting sites to maximize their fitness in “warm or cold” and “dry or wet” environments.

In conclusion, drivers of changes in AMF communities in the soil transplants are not clearly understood. Additional research is required to provide a better picture of AMF community shifts and subsequent functionality (parasitism vs mutualism) under long-term climate change scenarios. In this study, we did not exclude the possibility that some of the observed shifts in AMF communities may be a transient response to the disturbance during excavation and reinstallation of soil core transplantation to the contrasting environmental sites. Furthermore, differences in root colonization detected using, AMF trap culture versus field root culture, were not compared. Nevertheless, the observed structural changes of AMF communities in this soil transplant study suggest soil microbiomes, especially prairie AMF resources will change under future climate.

ACKNOWLEDGMENTS

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Rosendahl, S., McGee, P., Morton, J.B. 2009. Lack of global population genetic differentiation in the arbuscular mycorrhizal fungus Glomus mosseae suggests a recent range expansion which may have coincided with the spread of agriculture. Mol. Ecol. 18:4316–4329.


Fig. 1 (A-D). A. Map of western Canadian prairie provinces showing Chernozemic soil zones, B. Map of Saskatchewan, Canada, showing the location of the four experimental sites (red dots) from semi-arid Swift Current (SW) to sub-humid Melfort (MF) climate regions, C. Experimental layout of the transplanted soil cores at Swift Current site, and D. Field pea growing inside the soil core during the study season. (Map source: http://malbarry.com/lowimpactfarmingjourney2013/wp-content/uploads/2013/07/soil-map.jpg).

Fig. 2. Phylogenetic analysis of 82 AMF OTUs. The OTUs are clustered into eight genera of the Glomeromycota namely, *Funneliformis* sp., *Septoglomus* sp., *Rhizophagus* sp., *Glomus* sp., *Diversispora* sp., *Claroideoglomus* sp., *Archaeospora* sp., and *Paraglomus* sp. Phylogenetic relationships were obtained by neighbor-joining analysis (Kimura 2 parameter model) of AMF 18S rRNA gene pyrosequencing. Forty-five GenBank reference sequences with accession number within parenthesis are indicated by the black and the OTUs by the red boxes. Tree branches contain the statistical distance frequency values which are greater than 30.

Fig. 3. The relative abundance of 82 AMF OTUs belonging to eight genera in the soil of field pea trap roots, trap plants were grown in the field core soil samples collected at harvest. Replicated (n = 4) intact soil cores were extracted at four sites representing different Chernozem soils, Swift Current (SW) Brown, Scott (ST) Dark Brown, Outlook (OL) Dark Brown, and Melfort (MF) Black. The cores demarcated by purple rectangles are the original soil cores at original sites (home-site), the undemarcated soils were transplanted at other three sites (home-away).

Fig. 4. A biplot ordination plot derived from a principal correspondence analysis (PCoA) showing the relationship between the relative abundance and composition of AMF communities (genera level) as observed under four soils transplanted at four sites. The percentages of variance explained by axes 1 and 2 are shown in parentheses. Each point represents the average of four replicate samples per treatment. A
legend of color symbols describing the soil types is presented in the figure. Dash purple circles are the original soil cores at original sites (home-site) and the red arrows evolved from second matrices (soil-climate variables) showing the direction and relationship with AMF in soil cores.

**Fig. 5.** (A-C) Relative abundances of the three dominant AMF OTUs representing *Funneliformis* sp. (OTU65) (A), *Dominikia* sp. (OTU5) (B), and *Claroideoglomus* sp. (OTU42) (C) distributed in the four soils and sites. OTUs were identified by the name and the percent identity with GenBank reference sequences. Purple bars are the original soil cores at original sites (home-site core). Error bars represent standard error (n = 4) of the mean. Different letters within the soils transplanted at four sites indicate significant changes ($P \leq 0.05$). SOM: Soil Organic Matter.

**Fig. 6.** Shannon diversity index ($H$) of AMF communities recovered from soil cores of Swift Current (SW) Brown, Outlook (OL) Dark Brown, Scott (ST) Dark Brown, and Melfort (MF) Black. Error bars represent standard error (n = 4) of the mean. Purple bars are the original soil cores at original sites (home-site). Different letters within the same soils transplanted at four sites indicate significant changes ($P \leq 0.05$).

**Table 1.** Climatic variables at four experimental sites.

**Table 2.** Soil variables at four experimental sites.

**Table 3.** $P$-values from the PROC MIXED analysis of variance (SAS) for the effects (fixed) of soil and site on the relative abundance of eight AMF genera, three dominant OTUs, and Shannon diversity index of AMF communities. Fixed effects of soil and site were extracted by separating random effects due to replicates within environments and their interactions with fixed effects.
Fig. 1 (A-D).
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5 (A-C).
Fig. 6.
Table 1. Climatic variables at four experimental sites.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Precipitation (mm)</td>
<td>Temperature (°C)</td>
<td>Precipitation (mm)</td>
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<tr>
<td>Swift Current (SW)</td>
<td>Semi-arid</td>
<td>357.4</td>
<td>4.3</td>
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<td>Outlook (OL)</td>
<td>Semi-arid</td>
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<td>3.8</td>
<td>47.9</td>
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<td>Scott (ST)</td>
<td>Sub-humid</td>
<td>366.2</td>
<td>1.8</td>
<td>50.4</td>
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<td>Melfort (MF)</td>
<td>Sub-humid</td>
<td>395.8</td>
<td>1.3</td>
<td>53.0</td>
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Note: Climate data at each site were extracted from Environment Canada and matched with the data from each study site AAFC and CSIDC weather station.
Table 2. Soil variables at four experimental sites.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Soil classification</th>
<th>Soil pH</th>
<th>Soil organic carbon (g kg(^{-1}))</th>
<th>Soil organic matter (g kg(^{-1}))</th>
<th>Total N (g kg(^{-1}))</th>
<th>Avail. P (mg kg(^{-1}))</th>
<th>Avail. K (mg kg(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>Swift Current (SW)</td>
<td>Brown Chernozem</td>
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<td>33.1</td>
<td>1.6</td>
<td>41.1</td>
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<td>Outlook (OL)</td>
<td>Dark Brown Chernozem</td>
<td>7.1</td>
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<td>1.7</td>
<td>66.88</td>
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<tr>
<td>Scott (ST)</td>
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<td>5.9</td>
<td>19.0</td>
<td>33.9</td>
<td>1.5</td>
<td>59.1</td>
<td>708</td>
</tr>
<tr>
<td>Melfort (MF)</td>
<td>Black Chernozem</td>
<td>7.9</td>
<td>55.0</td>
<td>94.3</td>
<td>5.0</td>
<td>34.4</td>
<td>371</td>
</tr>
</tbody>
</table>

Note: *Soils were classified according to the Canadian System of Soil Classification, Soil classification working group, 1998.*
Table 3. P-values from the PROC MIXED analysis of variance (SAS) for the effects (fixed) of soil and site on the relative abundance of eight AMF genera, three dominant OTUs, and Shannon diversity index of AMF communities. Fixed effects of soil and site were extracted by separating random effects due to replicates within environments and their interactions with fixed effects.

<table>
<thead>
<tr>
<th>Effects</th>
<th>Funneliformis</th>
<th>Glomus</th>
<th>Rhizophagus</th>
<th>Septoglomus</th>
<th>Diversispora</th>
<th>Claroideoglomus</th>
<th>Archaeospora</th>
<th>Paraglomus</th>
<th>OTU65</th>
<th>OTU5</th>
<th>OTU42</th>
<th>Shannon Diversity Index</th>
</tr>
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<tbody>
<tr>
<td>Site (Si)</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.2816</td>
<td>0.0161</td>
<td>0.0052</td>
<td>&lt;0.0001</td>
<td>0.0032</td>
<td></td>
</tr>
<tr>
<td>Soil (So)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0362</td>
<td>0.0021</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>
| Si x So          | <0.0001       | 0.0036 | <0.0001     | <0.0001     | <0.0001      | <0.0001         | <0.0001      | <0.0001    | 0.0606 | 0.0012 | <0.0001 |<0.0001

Note: “Effects: “Soil” refers to the different soil cores used in this study transplanted to the four sites and reflects the impact of differing soil characteristics. “Site” refers to the experimental sites to which the different soil cores were transplanted and reflects the soil cores exposed to different local climates.

Relative abundance termed as percent relative abundance of the sequences (18S rRNA) of the eight AMF genera distributed across the soils and sites.

Shannon diversity (combination of abundance and evenness) was determined through the MOTHUR bioinformatics pipeline using the observed AMF OTUs (list file).