Arbuscular Mycorrhizal Fungi Assemblages in Chernozems

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Arbuscular mycorrhizal fungi assemblages in Chernozem great groups revealed by massively parallel pyrosequencing

Mulan Dai, Chantal Hamel, Marc St. Arnaud, Yong He, Cynthia Grant, Newton Lupwayi, Henry Janzen, Sukhdev S. Malhi, Xiaohong Yang, and Zhiqin Zhou

Abstract: The arbuscular mycorrhizal (AM) fungal resources present in wheat fields of the Canadian Prairie were explored using 454 pyrosequencing. Of the 33 dominant AM fungal operational taxonomic units (OTUs) found in the 76 wheat fields surveyed at anthesis in 2009, 14 clustered as *Funneliformis – Rhizophagus*, 16 as *Claroideoglomus*, and 3 as Diversisporales. An OTU of *Funneliformis mosseae* and one OTU of Diversisporales each accounted for approximately 16% of all AM fungal OTUs. The former was ubiquitous, and the latter was mainly restricted to the Black and Dark Brown Chernozems. AM fungal OTU community composition was better explained by the Chernozem great groups ($P = 0.044$) than by measured soil properties. Fifty-two percent of the AM fungal OTUs were unrelated to measured soil properties. Black Chernozems hosted the largest AM fungal OTU diversity and almost twice the number of AM fungal sequences seen in Dark Brown Chernozems, the great group ranking second for AM fungal sequence abundance. Brown Chernozems hosted the lowest AM fungal abundance and an AM fungal diversity as low as that seen in Gray soils. We concluded that Black Chernozems are most conducive to AM fungal proliferation. AM fungi are generally distributed according to Chernozem great groups in the Canadian Prairie, although some taxa are evenly distributed in all soil groups.

Key words: arbuscular mycorrhizal fungi, biogeography, Glomeromycota, pyrosequencing, soil type.

Résumé : Les ressources en champignons mycorhizes arbusculaires (MA) de champs de blé des Prairies canadiennes ont été explorées par pyroséquençage 454. Parmi les 33 unités taxonomiques opérationnelles (UTO) dominantes de champignons MA trouvées dans 76 champs de blé examinés à l'anthèse, en 2009, 14 s'agrégeaient à *Funneliformis–Rhizofagus*, 16 à *Claroideoglomus* et 3 aux Diversisporales. Les UTO de deux MA, un *Funneliformis mosseae* et un Diversisporales, constituaient chacune approximativement 16 % de toutes les UTO de champignons MA. Le premier était ubiquiste alors que le deuxième était principalement limité aux chernozems noirs et brun foncé. La composition des UTO de la communauté de champignons MA s'expliquait davantage par les grands groupes de chernozems ($P = 0{,}044$) que par les propriétés mesurées du sol. Cinquante-deux pourcent desUTO de champignons MA n'étaient non reliées aux propriétés mesurées du sol. Les chernozems noirs abritaient la diversité la plus élevée d'UTO de champignons MA et presque deux fois le nombre de séquences de champignons MA observées dans les chernozems brun foncé, le grand groupe se classant deuxième quant à l'abondance de séquences de champignons MA. Les chernozems bruns abritaient l'abondance la plus faible de champignons MA et leur diversité était aussi faible que celle des sols gris.Nous avons conclu que les chernozems noirs sont les plus propices à la prolifération des champignons MA. Les champignons MA sont généralement distribués selon les grands groupes de chernozems dans les Prairies canadiennes, quoique certains taxons soient distribués aléatoirement dans tous les groupes de sols.

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Mots-clés : champignons mycorhiziens arbusculaires, biogéographie, Glomeromycota, pyroséquençage, type de sol.

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Introduction

The arbuscular mycorrhizal (AM) symbiosis can improve plant health (Song et al. 2011), nutrient uptake (Atul-Nayyar et al. 2009; Selosse and Roy 2009; Barrett et al. 2011), tolerance to water (Ruth et al. 2011) and to salt stress (Garg and Chandel 2011), and crop yield (Hamel and Strullu 2006; Wang et al. 2011). It is desirable to manage the AM symbiosis in agriculture.

Insufficient knowledge of the AM fungal resources naturally present in the landscape is a major impediment to the management of AM symbioses in crop production in the Canadian Prairie provinces where an important agricultural industry has developed over the last century. Knowledge of AM fungal diversity in Canadian Prairie soils is currently restricted to a few erratic reports (Boyetchko and Tewari 1993; Talukdar and Germida 1993; Ma et al. 2005; Yang et al. 2010).

The AM fungal resources present in Canadian soils could be inferred, at least to some extent, from the conditions of the soil environment. Previous research has reported relationships between AM fungal spore density, soil (Eom et al. 2001; Pande and Tarafdar 2004), and climate factors (Coelho et al. 1997; Cortes-Sarabia et al. 2009). Dumbrell et al. (2010) reported a relationship between soil pH and AM fungal community structure, and Verbruggen et al. (2010), relationships between various soil properties and AM fungal abundance. Oehl et al. (2010) found that soil type is a main driver of AM fungal diversity and community composition.

Soils were formed over long periods of time under the influence of climatic factors and living organisms, as conditioned by relief. Climate and living organisms act on geological materials and produce genetic horizons whose properties largely depend on the characteristics of the climate and organisms shaping the soil at different geographical locations. There is a strong relationship between soils and living organisms (Brady and Weil 2002) and it should be possible to infer properties of an AM fungal community from the properties of the soil. The knowledge on Canadian soils is organized in a usable way in the *Canadian System of Soil Classification* (Soil Classification Working Group 1998). The classes (taxa) are based upon properties of the soils and when the boundaries of these classes are shown on a map, interpretations can be made on the basis of properties implied in the class definitions. This organized database can be recalled systematically so that relationships may be seen among soil properties, environmental factors, soils suitability for various uses, and possibly, AM fungal resources for agriculture.

The aim of our study was to increase knowledge on the AM fungal resources in agricultural soils of the Canadian Prairie. We hypothesized that the distribution of different AM fungal species varies with soil class (taxa) and with certain soil properties. A study representing one of the largest investigations of the influence of the soil environment on soil microbes was set up to test this hypothesis. The composition of the AM fungal communities in 76 wheat fields distributed over 28 million ha in the Canadian Prairie was described using a tag-encoded massively parallel pyrosequencing protocol. The effect of the soil great group on AM fungal community structure in the Canadian Chernozems was tested, and possible relationships between AM fungi and soil properties were explored.

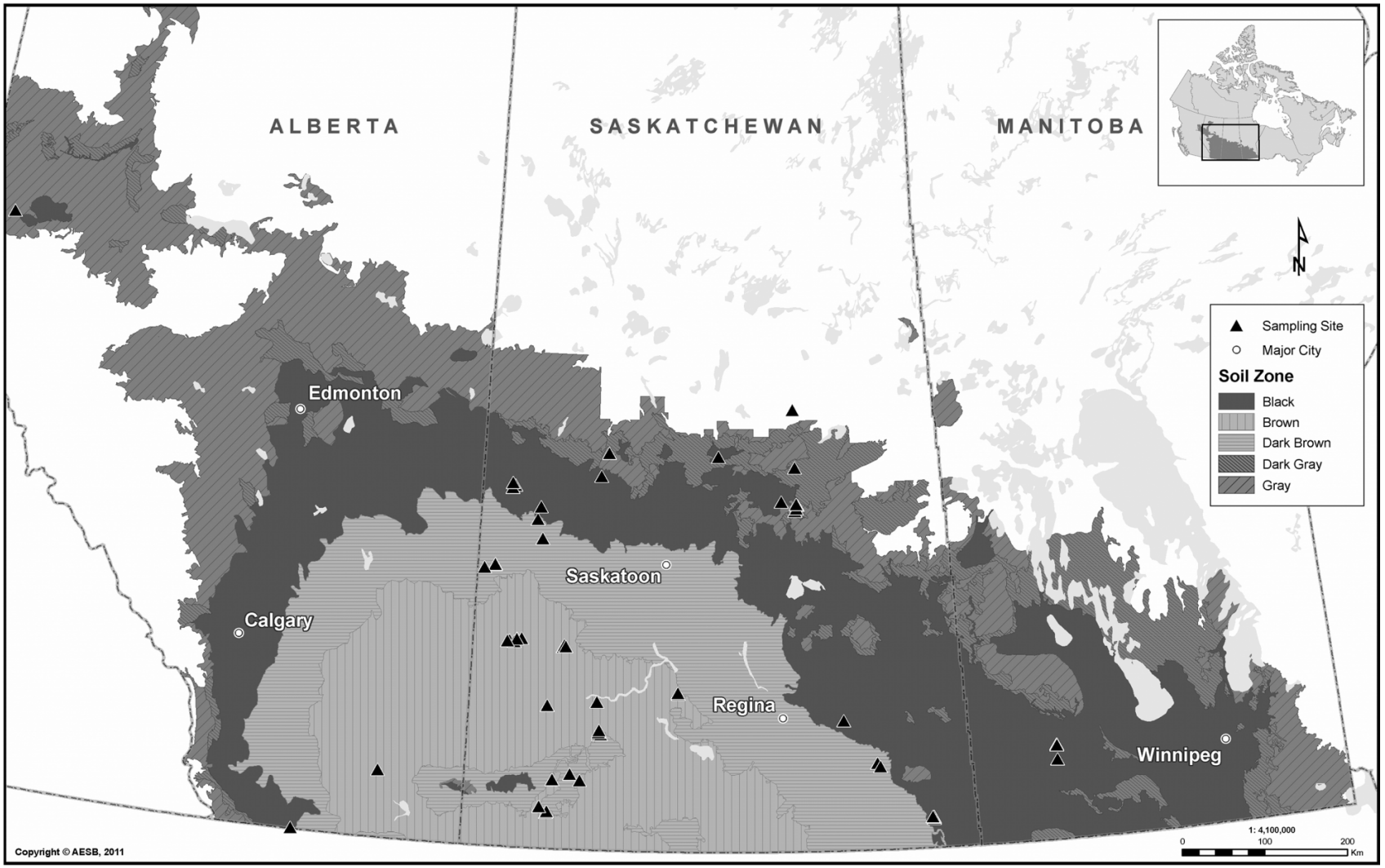
Materials and methods

Sampling site description

Samples were taken from 76 wheat fields located on Brown (26 fields), Dark Brown (18 fields), Black (23 fields), and Dark Gray (7 fields) Chernozems, and on Gray Luvisol (2 fields), over a vast area (28.3 million ha) in the Canadian Prairie. Details on the characteristics of these soils can be found in the *Canadian System of Soil Classification* (Soil Classification Working Group 1998). Soil zones are shown in Fig. 1, along with the location of sampling points. Essentially, Chernozemic soils cover most of the Canadian Prairie area and correspond to different ecological zones. The four great groups forming the Chernozem order are distributed along an increasing gradient of precipitation radiating outward from the United States border in southeast Alberta and southwest Saskatchewan, up to the boreal forest. The great groups of the Chernozem soil order are characterized by soil organic matter properties and accumulation. Brown Chernozems are the dryest Prairie soils; they are characterized by a brownish-colored A horizon and a poorly differentiated profile. The Brown soil zone is naturally populated by xerophytic and mesophytic grasses and forbs. Dark Brown Chernozems are characterized by a dark-colored A horizon that is usually darkest at the surface and becomes progressively lighter in color with depth. They are located in the Dark Brown soil zone immediately surrounding the Brown soil zone to the north (Fig. 1). The Dark Brown soil zone is populated by mesophytic grasses and forbs adapted to the prevailing cold semiarid climate. Black Chernozems have an A horizons darker in color and thicker than Brown and Dark Brown Chernozems, and form a zone next to the Dark Brown soil zone. The Black soil zone is subhumid and hosts a natural vegetation of mixed grasses, forbs, and trees. Dark Gray Chernozems and Gray Luvisols occur beyond the Black soil zone and together form the Gray soil zone. Dark Gray Chernozems have a Chernozemic A horizon with characteristics indicative of eluviation associated with soils developed under forest vegetation. They naturally occur with a mixture of trees, shrubs, forbs, and grasses in the forest–grassland transition zone and have a cold subhumid soil climate. In the Gray soil zone, Dark Gray Chernozems co-occur with Gray Luvisols, which are similar in many aspects, but Gray Luvisols show stronger evidence of eluviation.

Seventeen of these fields were under organic management, which includes regular tillage operations and green manure plough-down; the other 59 fields were under conventional

Fig. 1. The locations of the 76 wheat fields used in the survey of arbuscular mycorrhizal fungal diversity are indicated by triangles within the Brown, Dark Brown, Black, Dark Gray, and Gray soil zones in Alberta, Saskatchewan, and part of Manitoba, Canada. (Reproduced with permission of Agri-Environment Services Branch, Agriculture and Agri-Food Canada. This is a copy of an official work published by the Government of Canada. This reproduction has not been produced in affiliation with or with the endorsement of the Government of Canada.)



management, which includes fertilizer and herbicide applications, and may include tillage.

Soil sampling and determination of soil properties

The initial soil fertility was determined on soil samples collected soon after seeding, i.e., between 2 and 11 June 2009. Soil samples were taken from the tillage layer (top 7.5 cm) directly on the row. A composite sample of 700 mL was made at each field site by pooling 30 soil cores taken randomly with a soil probe from a 15 m^2 area seemingly representative of the field, based on topography. Field edges and access were avoided. Soil samples were kept on ice in a cooler during transportation and homogenized by sieving through a 2 mm sieve in the laboratory. Soil NO_3-N, NH_4-N, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, and S fluxes were measured using anion and cation exchange membranes. Two 18.7 cm^2 anion and two 18.7-cm^2 cation exchange membranes were placed in good contact with soil at field capacity and incubated for 24 h at room temperature. The nutrients on the membranes were extracted by 1 h elution in 0.5 mol/L HCl and quantified by inductively coupled plasma, except NO_3-N and NH_4-N, which were quantified by colorimetry on an autoanalyzer.

The profile of each soil was exposed by digging pits deep enough to reveal all the soil features required to identify each soil at the great group and soil association levels, according to the *Canadian System of Soil Classification* (Soil Classification Working Group 1998).

Fields were also sampled at the reproductive stage of wheat, between 10 and 17 July 2009. Most wheat crops were between booting and flowering (growth stage 10 to 10.5 on Feeke's scale) (Large 1954) at the time of sampling, but at nine sites, wheat plants were still jointing. A 5-cm-diameter metal cylinder was pushed into the top 7.5 cm soil layer at each sampling site using a custom-made foot sampler, capped on both ends, and carried to the laboratory. Soil cores were dried at 105 °C until constant mass. Soil bulk density was expressed as grams of dry soil per cubic centimetre. Composite soil samples were also taken from the tillage layer (top 7.5 cm) following the protocol used in June. A subsample from each soil was kept at 4 °C for a few days until determination of soil pH and electrical conductivity from 1:2 (soil–water) extractions using a pH and an electrical conductivity meter, respectively.

DNA extraction PCR conditions and pyrosequencing analysis

Another subsample of the sieved soil collected in July was stored in plastic bags at –20 °C until the construction of a DNA sequence library for each of the 76 sampling sites. Total DNA was extracted from 0.5 g of soil using an UltraClean Soil DNA Isolation kit (catalogue No. 12800-100, Mo Bio Laboratories, Inc.) according to the maximum yields protocol of the manufacturer, and stored at –20 °C.

The primer pair AMV4.5NF–AMDGR (Sato et al. 2005) were used to PCR amplify AM fungal 18S rDNA. These primers are known to amplify sequences in all four orders of the Glomeromycota, i.e., Archaeosporales, Diversisporales, Paraglomerales, and Glomales, and were successfully used for 454 pyrosequencing of AM fungal amplicons from environmental samples (Lumini et al. 2010).

A nested protocol was selected based on previous work (Van Tuinen et al. 1998; Jacquot et al. 2000) and on the recovery of a larger number of AM fungal OTUs with this approach than with a simple PCR, in preliminary tests. Each soil DNA sample was diluted (1:20) and amplified separately with the universal fungal primer pair NS1–NS4 for the first PCR (Lee et al. 2008; Alguacil et al. 2011) and AM fungi primer set AMV4.5NF–AMDGR for the second PCR (Sato et al. 2005; Lumini et al. 2010). Platinum PCR SuperMix (catalogue No. 11306-016, Invitrogen) was used in the PCR reactions. The final concentration of the reagent mix per 10 μL volume was 0.0165 U/μL *Taq* DNA polymerase, 1.24 mmol/L $MgCl_2$, 16.5 mmol/L Tris–HCl (pH 8.4), 41.25 mmol/L KCl, 165 μmol/L (each) dNTP, and 0.2 μmol/L (each) primer. Thermal cycling was conducted in an Veriti 96-well fast Thermal Cycler (Applied Biosystems) with the following conditions for the first PCR: 3 min of initial denaturation at 94 °C; 35 cycles of 45 s of denaturation at 94 °C, 45 s of annealing at 51 °C, and 1 min of elongation at 72 °C; followed by 7 min of final elongation at 72 °C. Conditions for the second PCR were 10 min of denaturation at 95 °C for the first step; 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 55 °C, and 1 min of elongation at 72 °C; followed by 9 min of final elongation at 74 °C. All amplicons from each sample were barcoded with one of 16 Roche's Multiplex Identifiers (Rothberg and Leamon 2008), thus allowing the sequencing of pools of 16 samples.

The PCR amplicons were checked on agarose gel and purified by cutting target DNA bands. We added 50 μL of sterile double-distilled water to each excised band, pounded the gel lump to pieces, incubated DNA samples at 4 °C for 24 h, and centrifuged the materials for 30 s at 10 000*g* to separate gel and water with DNA. This purified DNA was recovered and placed in a new centrifuge tube. The concentration of purified amplicons was measured using a Nano Drop-1000 spectrophotometer (Thermo scientific), and pyrosequencing was performed on the equimolar mixes of differently tagged samples in 8 × 1/8 of reaction at Génome Québec (Montréal, Quebec, Canada). Tags were extracted from the FLX instrument-generated composite fasta (FNA) file into individual sample-specific files based on the Multiplex Identifiers sequence, and only fungal primer pairs were left on read data.

Bioinformatic analysis

Stringent read quality filtering (average score ≥ 30) (Huse et al. 2010) and low clustering thresholds (97% similarity level) (Kunin et al. 2010) were applied in Mothur version 1.15.0 (Schloss et al. 2009) to clean ambiguous nucleotides and exclude possible erroneous sequences. A single nucleotide mismatch with the PCR primer and excessively long homopolymers were criteria for sequences rejections. We considered only the sequences reads between 230 and 250 bp in length (Lumini et al. 2010). The average length of trimmed sequences was 241 bp. AM fungal reads were identified by comparing the clean sequences with the Silva eukaryotic reference (appropriate for 18S rDNA sequences) using a *k*-nearest neighbor consensus and Bayesian approach and were then extracted. Among these, the AM fungal reads observed more than 30 times in the whole reads set were all considered to represent the dominant AM fungi in the land-

scape and were used in further analysis (Tedersoo et al. 2010). Threshold of 30 appearances was elected upon examination of a ranked abundance plot of AM fungal OTU sequences (not shown). AM fungal reads remaining were aligned against SILVA 18S rRNA alignment reference (http://www.arb-silva.de/) and OTU assignments were performed with the furthest neighbor clustering algorithm in Mothur. AM fungal OTUs corresponding to unique AM taxa were defined on the basis of a BLAST search of representative sequences from each OTU against the GenBank nonredundant nucleotide database (Zhang et al. 2000).

Phylogenetic analysis

Phylogenetic analysis was carried out on the AM fungal OTUs obtained (supplementary data[1]). The sequences used for this analysis where chosen among sequences published by Schüßler and Walker (2010). Reference sequences and representative consensus sequences from each OTU were aligned using the command ClusterW in MEGA 4.1 (Tamura et al. 2007), and neighbor-joining phylogeny reconstruction (Saitou and Nei 1987) was used to build a phylogenetic tree. Default parameters were used except that bootstrap replication was set at 500 (Barry 2004). A reference sequence of *Endogone pisiformis* was chosen as an outgroup to root the tree. The nomenclature used here was proposed by Schüßler and Walker (Schüßler and Walker 2010).

Statistical analysis

The Shannon diversity index (H') was calculated as a measure of diversity (McCune and Grace 2002). It was calculated by Mothur as

$$[1] \qquad H' = -\sum_{i=1}^{S_{obs}} \frac{n_i}{N} \ln \frac{n_i}{N}$$

where S_{obs} is the number of observed OTUs, n_i is the number of individual reads in OTU i, and N is the total number of reads obtained from the community. The effect of Chernozem great group on H' and on species richness, defined here as the number of OTUs, was tested by ANOVA using JMP version 3.2.6. Dark Gray Chernozems and Gray Luvisols were considered together as Gray soils in data analyses, as these soils are quite similar and rare in our data set. The significance of differences in AM fungal community structure between different Chernozem great groups was tested by the multiresponse permutation procedure (McCune and Grace 2002) in PC-ORD version 4.34 (McCune and Mefford 1999). Hierarchical clustering of Chernozem great groups based on AM fungal community structure used the group average method and Jaccard similarity index (McCune and Mefford 1999) in PC-ORD version 4.34. The AM fungal diversity in different Chernozem great groups was depicted in a heatmap with dendrograms, constructed with R version 2.10.1 (R Development Core Team 2011) based on the number of reads belonging to each OTU using the function heatmap.2 of the library gplots (Warnes 2011).

The relationship between the OTUs abundance data and soil properties was tested by redundancy analysis after Hellinger transformation of the OTUs abundance data (Borcard et al. 2011). The analysis was conducted using a covariance matrix with R version 2.13.1 (R Development Core Team 2011) and the library vegan (Oksanen et al. 2011). The function forward.sel of the library packfor 0.0-7 (Dray 2009) was also used to identify the variables in the soil property data that could be significantly related with the AM fungal OTU data. Pair-wise Spearman correlation coefficients were used to further examine the relationship between OTUs abundance and soil properties using JMP version 3.2.6.

Results

Molecular and bioinformatic techniques were applied to analyse the diversity of AM fungi present in the Canadian Prairie landscape, based on the 18S rDNA region. On average, 1189 sequences were obtained per sampling location after cleaning. Both singletons (5.5% of total AM fungal reads) and rare AM fungal OTUs (detected less than 30 times, i.e., 31.4% of total AM fungal reads) were ignored in this study. Of the 7086 AM fungi sequences obtained in total, 3382 were from Black Chernozems, 1518 from Dark Brown Chernozems, 1421 from Brown Chernozems, and 765 from Gray soils (Fig. 2).

The richness and abundance of AM fungi was highest in Black Chernozems (Table 1 and Fig. 2), but the AM fungal communities in the soils of this zone were characterized by the dominance of a few, over many subordinate OTUs (Fig. 3), resulting in similar H' in Dark Brown and Black Chernozems (Table 1). The abundance, richness, and diversity level of AM fungi were low in Brown Chernozem soils (Table 1).

Distribution of AM fungal OTUs in Prairie soils

Multiresponse permutation procedure showed that AM fungal community structure differed with Chernozem great groups ($P = 0.044$). The AM fungal communities of Black and Dark Brown Chernozem soils appeared to share some level of similarity, as shown by their close clustering, but those of the Brown Chernozem appeared to have a distinct structure (Fig. 3). The community structure of AM fungi in Gray soils differed most from the three other types of soil (Fig. 3). OTU12 was abundant in Gray soils but rare in other soils (Fig. 3), indicating that soil groups have distinct AM fungal community composition. The small number of Gray soil sampling sites may be partly responsible for the segregation of Gray soils in the cluster analysis, as AM fungal species that were absent in the sites sampled might be present in other Gray soils.

The multiresponse permutation procedure analyses indicated that the AM fungal community structure in Brown Chernozems was different ($P = 0.01$) from that found in Black Chernozems and tended to differ from that of Dark Brown Chernozems ($P = 0.06$). Brown Chernozems were characterized by the presence of OTU6, which was only found in these soils (Fig. 4). OTU4, OTU5, OTU11, OTU30, OTU32, and OTU33 were relatively rare and largely restricted to the Black Chernozem soils, and OTU7, OTU20, and OTU31 were preferentially associated with Dark Brown soils (Fig. 3). OTU26 and OTU19 stood out as the most abundant AM fungal OTU, particularly in Black Chernozem soils. They accounted for 16.41% and 16.38% of all AM fungal sequences, respectively.

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/w11-111.

Fig. 2. Rarefaction curves showing the number of arbuscular mycorrhizal (AM) fungal operational taxonomic units (OTUs) and AM fungal sequences, classified by soil type.

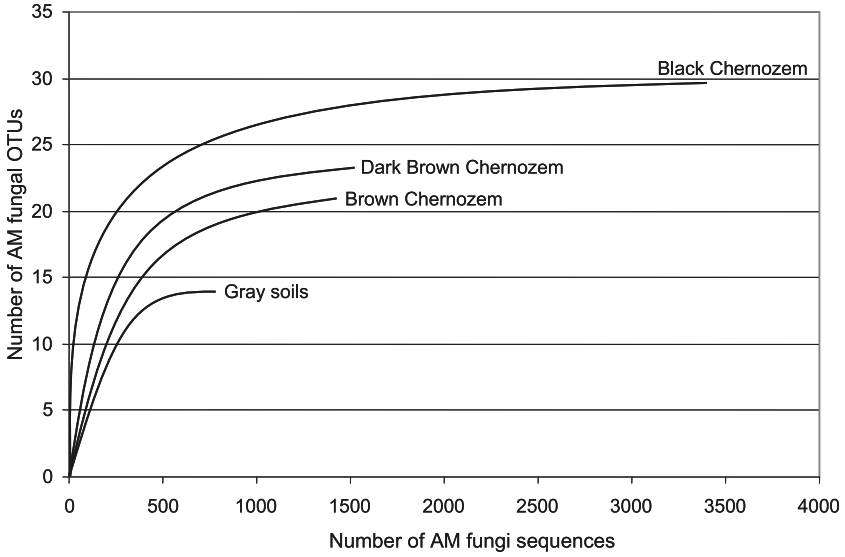


Table 1. Average abundance, species richness, and Shannon index of diversity (H') of arbuscular mycorrhizal (AM) fungal operational taxonomic units (OTUs) in an average wheat field of the Brown, Dark Brown, Black, and Gray soil zones, in the Canadian Prairie.

Soil type	*n*	Abundance (AM fungal sequence no.)	Richness	H'*
Brown Chernozem	26	54.7	6.2	1.58b
Dark Brown Chernozem	18	84.3	7.0	1.93ab
Black Chernozem	23	147.0	10.2	2.30a
Gray soils	9	85.0	7.1	1.59b
P value		0.073	0.194	0.011

*Values followed by the same letter are not significantly different, according to *t* tests ($\alpha = 0.05$).

OTU3 was also very frequent, in addition to having a wider distribution than OTU26 and OTU19. Different distribution patterns were found among AM fungal OTUs. Whereas some OTUs were widespread, others were more abundant in specific soil groups (Fig. 5). Most of AM fungal OTUs were observed in all Chernozem great groups, but their abundance greatly varied with great group (Figs. 3 and 5).

Some OTUs had the same pattern of distribution. This was the case for OTU14, OTU5, and OTU30, as shown in the heat map (Fig. 3). The phylogenetic analysis shows that the OTUs of these pairs belong to different taxonomic groups (Fig. 4).

Redundancy analysis revealed the absence of significant influence of the measured soil properties (Table 2) on the structure of AM fungal communities. Significant Spearman correlations were found between the abundance of individual OTUs and certain soil properties. The soil property most often correlated with the abundance of AM fungal OTUs was the thickness of the A horizon (six OTUs), which is consistent with the significance of Chernozem great group effects on AM fungal community structure.

Phylogenetic analysis of AM fungal groups

The neighbor-joining analysis grouped 14 AM fungal OTUs in the Glomeraceae, with reference sequences of the genera *Funneliformis* and *Rhizophagus* (Fig. 4). Three OTUs belonged to the order Diversisporales, and another 16 OTUs clustered in the family Claroideoglomeraceae (Fig. 4).

Four of the AM fungal OTUs encountered in the survey shared high levels of similarity with known sequences, according to BLAST in GenBank. OTU11 shared 100% similarity with *Funneliformis constrictum* (accession No. AJ534309); OTU24, 97% similarity with *Rhizophagus iranicus* (accession No. HM153424); OTU33, 99% similarity with *Claroideoglomus viscosum* (accession No. Y17652); and OTU26, 99% similarity with *Funneliformis mosseae* (*Glomus mosseae*, accession No. AY641819). OTU3, a highly dominant OTU, shared 95% similarity with *F. mosseae* (*G. mosseae* NG017178). The AM fungal OTU assignment procedure also yielded a good (>95%) match with many "uncultured" *Glomus*. This was the case for OTU7, OTU21, OTU20, OTU32, OTU22, OTU17, OTU28, OTU16, OTU8, and OTU12. These OTUs are all supported by the phylogenetic analysis as parts of the Claroideoglomeraceae (Fig. 4).

Discussion

Soil environmental effects on AM fungal diversity

Consistent with the hypothesis, the results demonstrate that

Fig. 3. Heat map depicting the diversity of arbuscular mycorrhizal (AM) fungi found in Prairie soil types. The color of each heat map rectangle indicates the abundance of the corresponding AM fungal operational taxonomic unit (OTU), as shown in the legend. Hierarchical clustering of soil types was performed using weighed centroid clustering method with Jaccard distance measure ($N = 76$). Significant Spearman's correlations between OTU abundance and soil properties are indicated at the right of the heat map with (*) $P < 0.05$ and (**) $P < 0.01$. Red stars indicate negative and black stars indicate positive correlations.

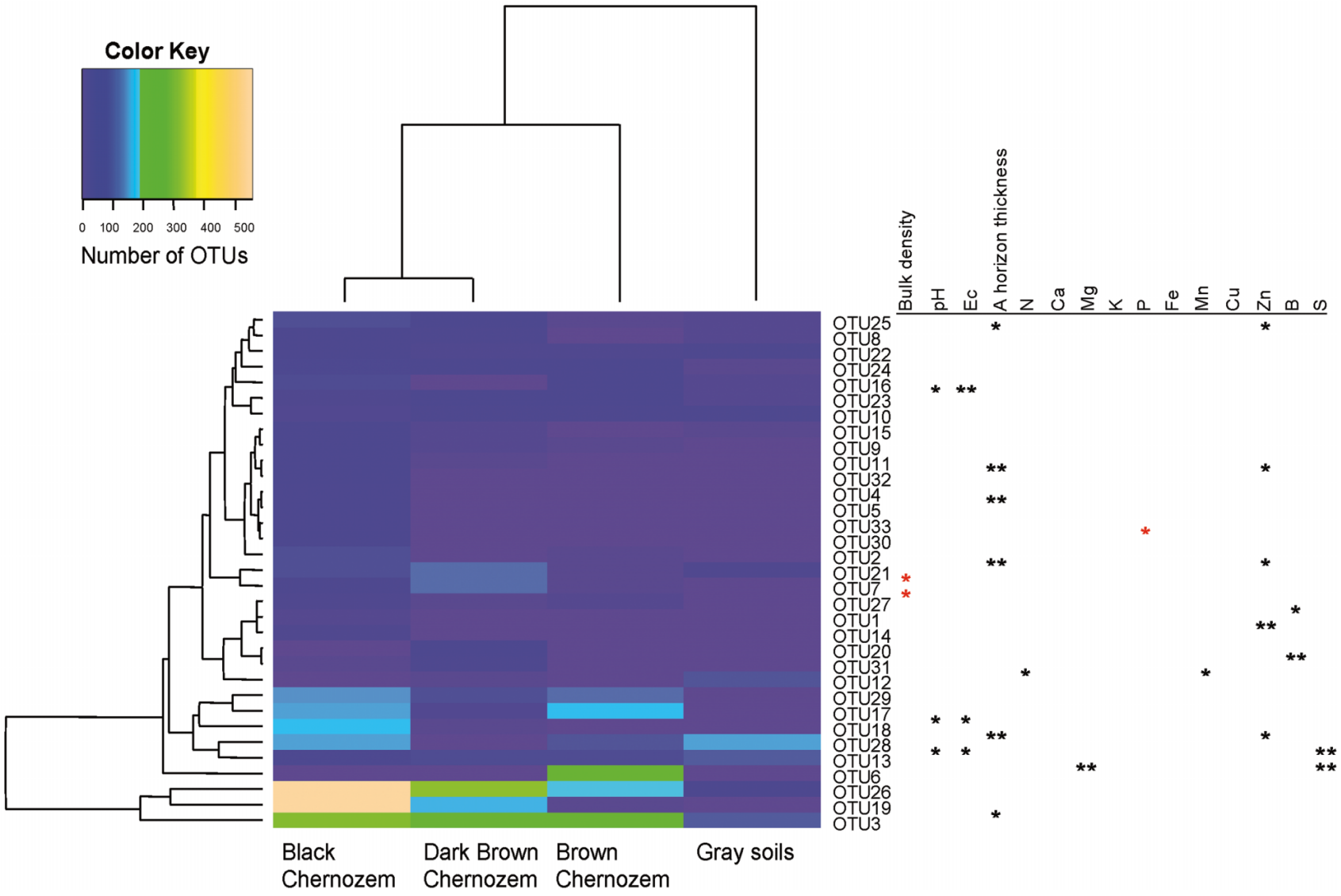


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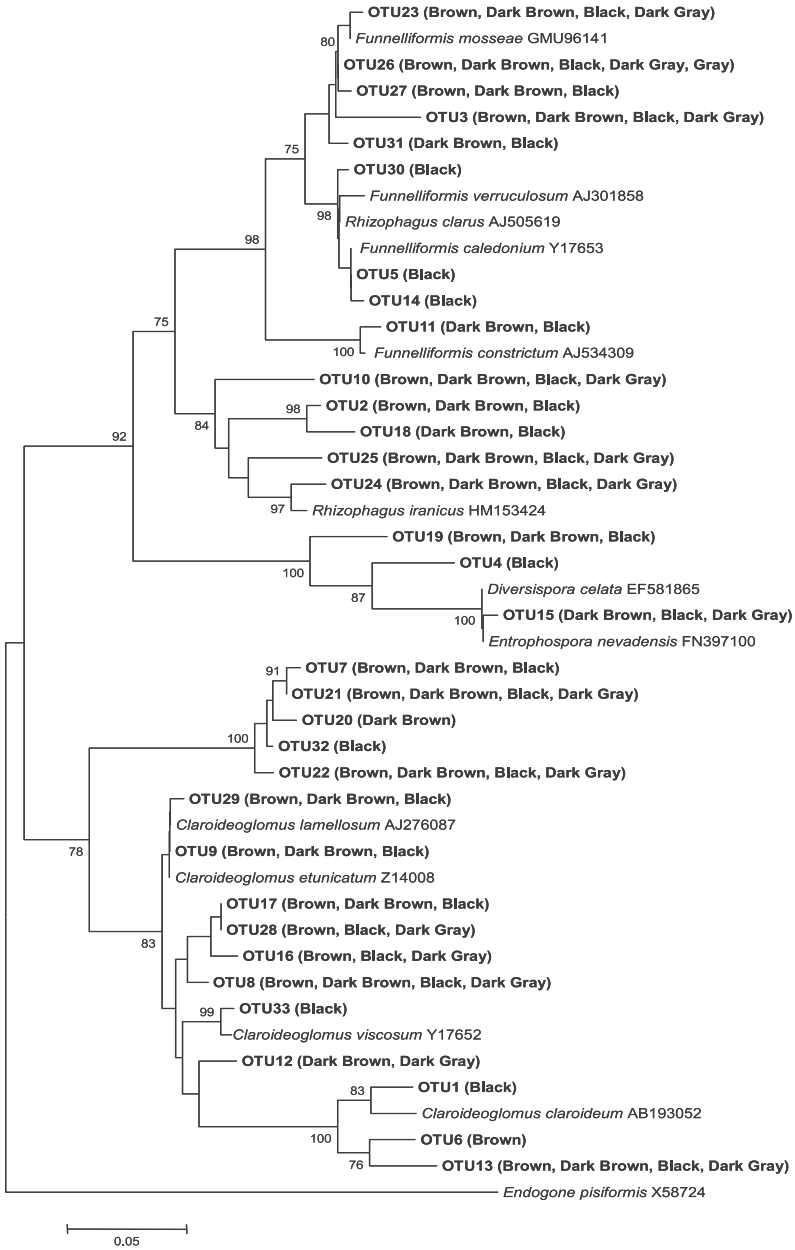
Fig. 4. Phylogenetic analysis of the 33 arbuscular mycorrhizal (AM) fungal operational taxonomic units (OTUs) found in 76 soil samples taken from different wheat fields of the Canadian Prairie. The OTUs are in bold font, and the soil types where they were found are indicated in parentheses. Reference sequences, in italic, are followed by their GenBank accession number. The scale represents substitutions per site. Values on branches are bootstrap values obtained with neighbor-joining; only values of ≥75 are shown.



AM fungal diversity and community structure in the Canadian Prairie landscape vary with the soil taxa. A recent study by Oehl et al. (2010) also supports that AM fungal community composition is related to soil taxonomic group, in this case, the soil order.

The species that can coexist in a community possess the ecological traits to effectively use and share limited resources with sympatric species in a given set of environmental conditions. The presence and abundance of different AM fungal taxa in different soil groups reflects the fact that these organisms exist within a range of environmental conditions where they are fit and can carry their function effectively (Bock 2003; Oehl et al. 2010). Reports of greater effectiveness of AM fungal isolates at promoting plant growth in soils with different properties (Herrera-Peraza et al. 2011) illustrate the existence of differential adaptation in AM fungi. Since AM fungi do respond to soil conditions, the soil environment was proposed as the major driver of selection in the Glomeromycota (Helgason and Fitter 2009).

The properties of the soil environment exert a selection pressure on soil communities, such as those of AM fungi, and it should be possible to infer mechanistically the composition of these communities based on soil properties. In a comprehensive study, Dumbrell et al. (2010) identified soil pH, C/N ratio, and phosphorus as the main factors determining the environmental niche of AM fungi and regulating the

Fig. 5. Distribution of the most abundant arbuscular mycorrhizal (AM) fungal operational taxonomic units (OTUs) across Chernozem great groups. Abundance is weighted to correct for the different number of sites per soil group. (N = 1109, 747, 728, 402, 357, and 355 for OTU26, OTU19, OTU3, OTU28, OTU13, and OTU29, respectively).

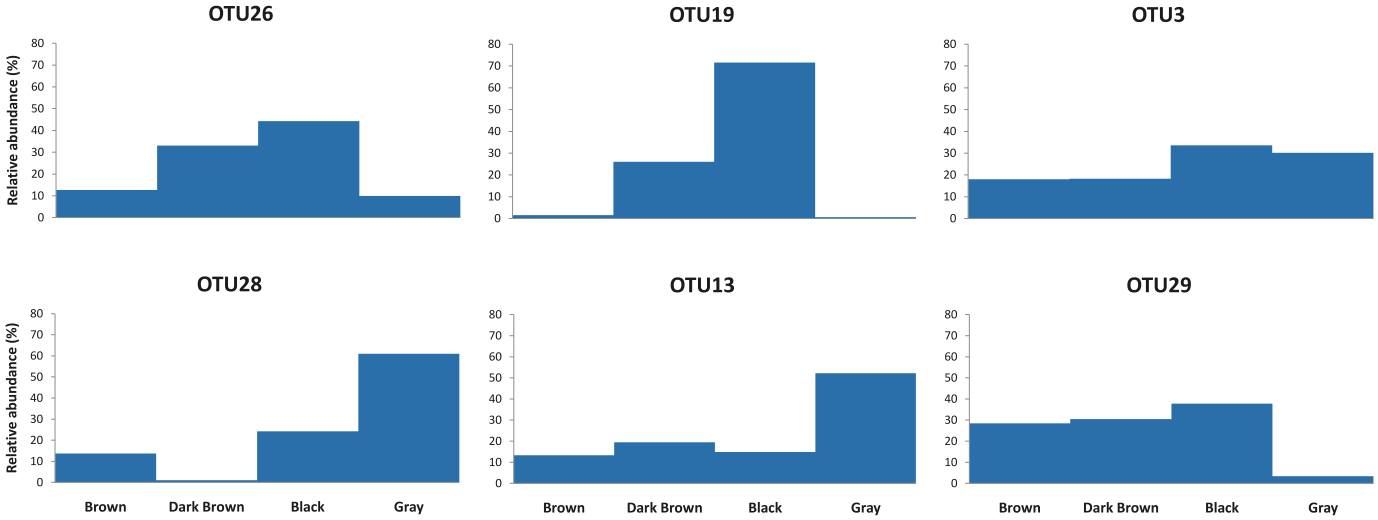


Table 2. Mean value and range (in parentheses) of soil properties measured in each ecozone of the study (n = 76).

	Ecozone			
Soil property	Brown	Dark Brown	Black	Gray soils
Bulk density (g/cm^2)	1.19 (0.54)	1.12 (0.74)	1.07 (0.66)	1.10 (0.93)
pH	6.59 (2.84)	6.42 (2.54)	6.75 (2.2)	6.40 (2.9)
Electric conductivity (dS/m)	0.55 (0.84)	0.66 (0.5)	0.65 (0.85)	0.58 (0.71)
A horizon thickness (cm)	11.2 (13.0)	12.5 (8.0)	15.4 (17.0)	17.3 (15.0)
Nutrient supply capacity				
Nitrogen	396 (839)	381 (1050)	518 (1298)	406 (704)
Calcium	1841 (2224)	1606(1833)	1675 (1351)	1679 (1634)
Magnesium	303 (446)	312 (283)	301 (273)	373 (546)
Potassium	108 (218)	129 (257)	147 (468)	79 (222)
Phosphorus	10.1 (33.6)	9.6 (57.4)	6.5 (21.6)	14.3 (54.4)
Iron	38.0 (59.8)	44.8 (80.2)	44.2 (82.0)	70.5 (63.4)
Manganese	32.9 (149.2)	38.7 (99.8)	30.1 (89.4)	24.7 (47.6)
Copper	1.00 (5.8)	0.62 (0.8)	0.55 (2.0)	0.40 (0.6)
Zinc	1.25 (15)	1.56 (2.6)	1.66 (6.2)	3.22 (7.6)
Boron	0.98 (1.6)	0.93 (1.6)	0.87 (1.6)	0.71 (0.4)
Sulfur	39.45 (217.4)	35.83 (78.8)	47.09 (211.0)	198.73 (746.4)

composition of these communities in a heterogenous 14-ha study site, in the UK. In the present study, the soil physicochemical properties (Table 2) were only a weak factor of the AM fungal community structure. By contrast and unsurprisingly, the Chernozem great groups could better explain the variation in AM fungal community structure encountered in cultivated Prairie soils. Soil taxa at the order level are based on the soil properties reflecting the effects of the dominant soil processes, and taxa at the great group level are based on properties reflecting the strengths of these dominant processes (Soil Classification Working Group 1998). Prairie soils are dominated by the effect of the water regime. In the Prairie, moisture availability is key to controlling important processes such as organic material deposition, biotic activity, and nutrient cycling. The concept of great groups in the Chernozem soil order captures the set of soil properties reflecting the dominant factor shaping soil ecosystems, most importantly the amount and properties of soil humic substances (Soil Classification Working Group 1998).

The concept of great group in the Chernozemic order also includes an important spatial component (Fig. 1), in addition to the dominant process shaping the soil ecosystems. Neutral processes of random genetic drift may be important contributors to ecological community structuration at large spatial scales, where distance may restrict the dispersal of organisms (Wagner 2008). A difference in the scale of the sampling area between the present study (28 million ha) and that of Dumbrell et al. (2010) (14 ha) may also explain why these authors reported a strong influence of soil physicochemical properties on AM fungal community structuration, contrasting with the weak influence found in the present study. Verbruggen et al. (2010) reported a significant linear correlation between soil physicochemical properties and the abundance of only 30% of the 15 AM fungal types they detected

over a large spatial scale of about 2 million ha. The present study examined AM fungal diversity at 76 sites; Dumbrell et al. (2010), 51 sites; and Verbruggen et al. (2010), 26 sites. The small size of these data sets prohibits the modeling of the complex interactions expected to influence the structure of AM fungal communities in soils. The soil great group concept best captures the drivers of AM fungal diversity.

Diversity of AM fungi

The results of this study shed some much needed light on the AM fungal resources for agriculture in the Canadian landscape. The dominance of members of the Glomeromycota, as previously determined based on spore morphology (Talukdar and Germida 1993) and rDNA sequence analysis (Ma et al. 2005), was confirmed here. But, a fine picture of AM fungal diversity in Canadian Prairie soil remains difficult to draw. Spores of *Rhizophagus fasciculatum*, *Claroideoglomus luteum* NT4, *Claroideoglomus etunicatum*, *F. mosseae*, *Glomus versiforme* (Talukdar and Germida 1993; Ma et al. 2005), *Glomus aggregatum*, *Glomus pansihalos*, and *Entrophospora infrequens* (Boyetchko and Tewari 1993) were found in cultivated Canadian Prairie soils. The dominance and ubiquity of *F. mosseae* in Prairie soils and the presence of *F. constrictum*, *R. iranicus*, and *C. viscosum* were found in this study. Sequences of *C. viscosum* were reported for samples collected under perennial plant cover in Brown Chernozems (Yang et al. 2010). Sequences of *Scutellospora calospora* were reported for Dark Gray Chernozems (Ma et al. 2005); spores of *Gigaspora decipiens* were found in Gray Luvisols and in Brown, Dark Brown, and Black Chernozems, whereas spores of *Acaulospora denticulata* were found in soils of all these great groups (Talukdar and Germida 1993). But the report of numerous unknown *Glomus* sequences by us and others (Ma et al. 2005; Yang et al. 2010) further underlines the important gaps in our knowledge of the Glomeromycota, which was reported earlier by Helgason and Fitter (2009), and calls for the construction of better reference collections for AM fungi.

Drivers of diversity in the Canadian Prairie soils

The diversity of AM fungal communities found in Prairie soils in our study varied with Chernozem great groups but was not related to the gradient of precipitation along which the soils of these groups have developed. Whereas diversity and abundance of AM fungi increased from the Brown Chernozems, to the Dark Brown, and to the Black Chernozems, along a gradient of increasing moisture, it decreased at the subhumid forest–grassland transition, i.e., from Black Chernozems to the Gray soil zone. Lower AM fungal diversity in Gray soils could be attributable to the forest influence on Gray soils. Considering the northernmost location of these soils, it is possible that AM fungal communities in Gray soils are in a period of transition from being part of a forest to being part of an annually cropped ecosystem (Eom et al. 2000). Neutral processes certainly contribute to the structuration of AM fungal communities in the Canadian Prairie. The Prairie provinces are young, and many Prairies soils, in particular Gray soils, may have only a short history of cultivation. The first settlers immigrated after 1904 to the rural municipalities of the Gray soil zone (Ka-iu 1999), and 60% of the 5 million ha of new land broken for crop production between 1957 and 1990 in Saskatchewan were on Gray and Black soils (Campbell et al. 1990).

Beside soil group, land use could be a factor shaping the structure of the AM fungal communities. Bare fallow has traditionally been more frequently practiced on Brown soils than elsewhere in the Canadian Prairie (Ka-iu 1999) and is still a soil water conservation strategy commonly used on Brown Chernozems. The absence of plants negatively impacts AM fungi (Douds et al. 2011), and the recurrence of bare fallow in Brown Chernozems may have contributed to the low abundance of AM fungi in these soils. Plants have a selective effect on AM fungi (Lang et al. 2011) and some crops are preferentially grown in certain soil zones (Gan et al. 2010). Higher proportions of wheat and summer fallow were historically used on Brown Chernozems. Although wheat was the dominant crop grown on Black and Dark Brown Chernozems, barley and canola were also occupying an important area of land (Campbell et al. 1990). Bare fallow frequency was traditionally practiced once every 2 years in the Brown soil zone, but once every 3–4 years in Black soils. Despite the important increase in crop diversification and decrease in bare fallow frequency observed in the Brown soil zone over the 15–20 years (Gan et al. 2010), historical land use may still influence the composition of AM fungal communities of Brown Chernozems. Land use legacies in soil microbial communities are long-term, and in the field, the soil microbiota is relatively insensitive to the immediate influence of plants (Kulmatiski and Beard 2011).

Low AM fungal diversity and abundance in Brown Chernozems of Canada were also found in an early study based on spore counts (Talukdar and Germida 1993). Lower AM fungal abundance in Brown than in Dark Brown and Black Chernozems may be a consequence of low plant productivity in these severely water-limited soils. Whether the lower abundance of AM fungi in Brown Chernozems corresponds to a smaller contribution of the AM symbiosis to crop production in these soils than in soils receiving more precipitation remains to be seen. But, in Brown soils, the abundance of AM fungi is associated with higher plant tissue nitrogen and phosphorus levels, suggesting a role for the AM symbiosis in dry soils (Yang et al. 2010).

Conclusions

Although some AM fungi are evenly distributed across Canadian Prairie soils, in general, different AM fungi have a different pattern of distribution. Variation in the structure of AM fungal communities at the large scale of this study is best explained by the Chernozem great group hosting a community. The concept of soil great groups reflects the strength of the dominant processes shaping the soil system, and seemingly, the distribution of AM fungal diversity. Land-use history and plant productivity are other possible drivers of AM fungal community composition in cultivated Prairie soils. Black Chernozems are most conducive to AM fungal proliferation and host a wide AM fungal diversity. Understanding the biogeography of the AM fungi could allow the management of the AM symbiosis in crop production.

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