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Farm system management affects community structure of arbuscular mycorrhizal fungi

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are considered to play a pivotal role in organic farming systems as they are known to enhance plant phosphorus (P) uptake from soils low in plant-available P. However, the structure and therefore function of AMF communities may be altered depending on environment and management conditions. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and quantitative real-time PCR (qPCR) were used to explore AMF community structure in soils from perennial forages (*Medicago sativa—Phleum pratense*) on three pairs of long-term (>20 yr) organic and conventional dairy farm soils in Ontario, Canada. There were no significant differences in DGGE band numbers (indicative of species richness) among organic and conventionally managed soils. Analysis of Similarity revealed significant differences in AMF community composition with both farming system management and farm pair location having significant effects ($R = 0.71$ and 0.91, respectively, $P < 0.0001$ for both). Real-time qPCR indicated greater abundance of *Funneliformis mosseae* under conventional management ($P = 0.036$), while organic management tended to support greater abundance of *Claroideoglomus claroideum* ($P = 0.067$). Such a compositional shift in AMF communities could have consequences for the growth and P-use efficiency of their host. The results of this study highlight that the structure of AMF community assemblages are co-determined by both local environmental conditions and farming system management, and importantly, demonstrate for the first time differences between organic and conventional management in dairy farm soils from mixed perennial forages.

1. Introduction

Recent studies exploring soil fertility on organic dairy farms in central Canada have reported low Olsen soil test phosphorus (STP) concentrations (<10 mg kg^{-1}), which is an index of plant-available phosphorus (P) (Main et al., 2013; Schneider, 2014). This is consistent with reports from the Canadian prairies and internationally where soil tests used to indicate plant-available P have been found to be low for organic farms (Gosling and Shepherd, 2005; Knight et al., 2010; Løes and Øgaard, 2001; Oberson and Frossard, 2005). These results may have implications for overall farm productivity, as soil P deficiencies can reduce crop yields and can negatively affect the ability of leguminous crops to biologically fix nitrogen from the air (Chalk et al., 2006; Parfitt et al., 2005). However, despite having low Olsen STP values, for which a high response to P fertilizer application would be expected, some organic farmers state that their crop yields are acceptable (Martin et al., 2007). This is consistent with a recent survey of forage fields on organic dairy farms in Ontario and Nova Scotia that did not find a significant relationship with Olsen STP and forage yield (Main et al., 2013). It has been suggested that increased soil biological activity, including the arbuscular mycorrhizal fungi (AMF) symbiosis, is involved in providing the crop with P not measured by conventional soil P tests (Main et al., 2013; Martin et al., 2007).

Colonization by AMF is known to enhance P uptake of plants (Jansa et al., 2011). For this reason, the mycorrhizal symbiosis is considered to play an important role in organic agriculture by enhancing host-plant P uptake and P-use efficiency (Gosling et al.,

Abbreviations: AMF, arbuscular mycorrhizal fungi; P, phosphorus; STP, soil test phosphorus.

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2006). Evidence indicates that conditions of organic agricultural systems including low levels of available soil inorganic P, a lack of synthetic fertilizer and pesticide use, and a tendency towards a more diverse crop rotation, may enhance AMF development (Gosling et al., 2006; Hamel and Strullu, 2006; Jansa et al., 2006). Finally, plant assemblage composition is also suggested to influence AMF communities (Johnson, 1993; Mira's-Avalos et al., 2011; Johnson et al., 2004; Mirás-Alvalos et al., 2001) and perennial legume-based forages may enhance the effect of AMF through the synergistic nature of the tripartite symbioses (Chalk et al., 2006).

Several studies have reported increased levels of colonization or spore abundance in fields from organically-managed agricultural systems compared with those under conventional management (Entz et al., 2004; Mäder et al., 2000; Oehl et al., 2004). However, colonization measurements do not provide specific information about the species present in the plant root or how beneficial they are to the plant. It has been shown that different AMF species vary in their functional abilities including P uptake efficiencies (Smith et al., 2000; Wagg et al., 2011). Helgason et al. (2002) found that during the growing season of *Hyacinthoides non-scripta* (common bluebell), plants were colonized by a succession of at least 8 different species of AMF. The authors proposed that AMF diversity is responsible for a vast array of benefits at many physiological levels and phenological stages.

Several polymerase chain reaction (PCR) based methods have been developed that provide useful analyses of AMF community structure, including restriction length polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE), and temperature gradient gel electrophoresis (TGGE) (Liang et al., 2008). Molecular approaches providing meaningful information at the AMF species level have been slow to develop due to complications relating to high DNA sequence diversity within individual isolates and to limited knowledge of the sequence diversity between species or taxa (Thonar et al., 2012). Thonar et al. (2012) appear to have made a breakthrough in being able to accurately detect and quantify the abundance of several AMF taxa using species-specific primers and fluorescently-labelled probes with qPCR technology. They have also developed a calibration system by calculating the number of target gene copies per unit of genomic DNA for each species in order to allow for a more useful interpretation of the numerical qPCR results.

The objective of this study was to compare the AMF community structure in soils from long-term, organically managed forage fields (with low Olsen STP concentrations (Olsen et al., 1954)), with that of soils from long-term conventionally managed forage fields (with relatively high Olsen STP concentrations). It was hypothesized that AMF community structure would differ under organic and conventional management, plausibly as a result of the historical differences in synthetic P fertilizer use. To test this hypothesis, we used PCR-DGGE and the recently established qPCR protocol and markers designed by Thonar et al. (2012) and Wagg et al. (2011). This study is unique in that it compares differences in AMF communities under organic and conventional management in dairy farming systems that are similar to each other in many aspects of management (e.g. similar crop rotation, similar tillage practices, similar herd size, both receive manure applications, etc.). This study is also the first to test on North American field soils the qPCR markers (primers and hydrolysis probes) developed by Thonar et al. (2012) and Wagg et al. (2011) in Switzerland.

2. Materials and methods

2.1. Site selection and description

Three organically-managed forage fields (*Medicago sativa* and *Phleum pratense*) were selected from an initial screening of ten organic dairy farms in south-western Ontario (data collected in 2008 by Main et al., 2013). The farms were located near Elora (43°43′20″ N, 80°27′44″ W), Stratford (43°25′22″ N, 80°50′37″ W) and Kincardine (44°13′15″ N, 81°31′30″ W), Ontario, Canada. The selected fields had relatively high forage yields despite having low Olsen STP concentrations (<10 mg P kg^{-1}) and were in either their second or third year of harvest for the 2009 growing season. The three farms had been managed organically for >20 yr and have

Table 1
Description of select management practices and soil properties (0–15 cm) of three paired organic (Org) and conventional (Con) dairy farm forage fields. Within columns, means followed by the same letter are not significantly different ($P < 0.05$).

Field	Crop rotation[a]	Fertilizer applied	Pesticide use	Soil texture[b]	Forage yield[c] (kg ha^{-1})	Legumes[d] (% composition)	OM[e] (%)	pH[f]	STP[g] (mg P kg^{-1})	Total P[h] (mg P kg^{-1})	Organic P[i] (mg P kg^{-1})
Org 1	O-B-H-H-H-H	Semi-composted manure	None	Silt loam	6089	66	7.2	6.9	6.9	1179	448
Org 2	RC-WW-O-SC-C-O-H-H-H-H	Semi-composted manure	None	Silt loam/ loam	9397	71	4.0	7.4	2.8	696	217
Org 3	O-O-B/P-H-H-H-H	Semi-composted manure	None	Loam	8617	58	5.2	7.2	5.5	810	222
Mean					8034a	65b	5.4a	7.2a	5.1a	895a	296a
Con 1	C-C-C-H-H-H-H	Mineral fertilizer and solid manure	Round-up as needed for corn	Silt loam	9422	17	4.7	7.4	17.1	1110	304
Con 2	C-S-C-O/B-H-H-H-H	Mineral fertilizer and semi-composted manure	Round-up for corn, fungicide for soybean	Silt loam	8547	44	4.8	7.3	6.8	827	272
Con 3	C-B-C-B-C-O/P-H-H-H-H	Mineral fertilizer, semi-composted and liquid manure	Round-up to kill hay	Silt loam	10449	31	4.5	7.5	11.2	699	145
Mean					9472a	30a	4.7a	7.4a	11.7b	879a	240a

[a] H—hay, RC—red clover, WW—winter wheat, O—oats, SC—sweet clover, C—corn, B—barley, P—peas, S—soybean.
[b] Particle size distribution was measured by the pipette method (Sheldrick and Wang, 1993).
[c] Forage yield determined during the 2009 growing season where 3 cuts of hay were taken (with the exception of Org 1, which took 2 cuts).
[d] All fields had similar planting ratios of legume: grass.
[e] Organic matter was determined according to the method of Tiessen and Moir (1993).
[f] pH was measured as a soil–water saturated paste (Miller et al., 1997).
[g] Soil test phosphorus as indicated by $NaHCO_3$ extractable P (Olsen P) (Olsen et al., 1954).
[h] Total P was determined according to the wet digestion method of Parkinson and Allen (1975).
[i] 0.5 M NaOH-EDTA extractable organic P (Bowman and Moir, 1993).

been consistent in their nutrient management practices, and crop rotations. Conventional dairy farms with forage fields located in close proximity (<2.3 km) to the organic sites were selected to enable an organic/conventional pairwise comparison. The conventional farms had a long-term (>20 yr) history of synthetic P fertilizer application, an established second or third year forage field that would be left in forage for the 2009 growing season, and similar level topography. All farms had similar tillage practices (when not in perennial crops, soils are ploughed in the fall and typically cultivated twice in the spring). A description of select management practices, soil characteristics and forage yield and composition of all fields are shown in Table 1. The soils were all Grey-Brown Luvisols according to the Canadian System of Soil Classification (AAFC, 1998) and Alfisols under the American System of Classification (Soil Survey Staff, 2014). Mean annual temperature and precipitation data are provided in Supplemental Information A. A more detailed description of site characteristics including crop yield and quality data, soil P forms characterization, AMF root colonization, and phosphatase activities can be found in Schneider (2014).

2.2. Soil sampling

Soil samples were collected from the forage fields in mid-June of 2009. A directed sampling approach was used to select four 20 × 20 m sample plots on each field. These four plots were selected to represent average areas of the field and were delimited based on topography, crop density and vigor, historical field management data, and any existing soil nutrient data. Within these 20 × 20 m plots, three 0.5 × 1 m subplots were randomly selected for soil sampling. From each sub-plot, nine soil cores (0–15 cm depth) were collected according to a grid pattern and composited to generate 12 samples per field. GPS coordinates were recorded for all sample plots.

Soil samples were kept in coolers with ice in the field and then stored at 4 °C until it was sieved (2 mm) the same day. The soil was then stored frozen (−20 °C) until further analysis. All equipment coming in contact with the soil (corers, sieves, etc.) was cleaned with a brush, washed with deionized water and wiped with ethanol between samples.

2.3. Soil DNA extraction

Frozen soil was lyophilised prior to DNA extraction. DNA was extracted from 5 g aliquots using the PowerMax™ Soil DNA Isolation Kit (cat no 12988-10 Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. The following modifications were made in an effort to encourage the extraction of DNA from AMF spores: (i) after addition of the lysis buffer solution (C1), the tubes containing the soil were incubated in a water bath for 65 °C for 12 min, and (ii) the bead solution tubes were shaken for 20 min as opposed to 10 min. The presence of DNA was confirmed by running a gel electrophoresis (1% agarose gel with 0.3 μg ml^{-1} ethidium bromide) and visualizing under UV light. The original stock of extracted DNA was kept frozen at −80 °C, with working stock subsamples kept at −20 °C.

2.4. AMF community structure

AMF community structure was compared among management systems using PCR-DGGE. The procedure consisted of a nested PCR according to the method of Gollotte et al. (2004), using the universal eukaryotic primer pair LR1-FLR2 followed by a nested PCR with AMF-specific primers FLR3 (5′-TTG AAA GGG AAA CGA TTG AAG T-3′) and FLR4 (5′-TAC GTC AAC ATC CTT AAC GAA-3′). A GC clamp was added to the primer FLR3 to prevent complete denaturation during DGGE. These primers amplify the 5′ end of the large sub-unit (LSU) ribosomal DNA and were selected as they result in less than 500 bp amplicons (300–380), which is a general requirement for DGGE analysis (Muyzer and Smalla, 1998), and have been successfully used by other researchers in PCR-DGGE experiments (Mira's-Avalos et al., 2011; Rodríguez-Echeverría et al., 2009). Also, they have good specificity for Glomeromycota with relatively good diversity coverage among AMF and allow for good discrimination at the species level (Mummey and Rillig, 2007). DNA from all twelve soil samples per field were extracted and used for the nested PCR, yielding 72 samples in total (12 per field × 6 fields). For DGGE analysis, nested PCR products from the same field quadrat were composited to facilitate the statistical analysis of the gel, yielding 4 samples per field and 24 samples in total.

2.4.1. PCR amplification of AMF DNA

PCR amplification was performed using a Mastercycler epgradient S thermocycler (Eppendorf, Hamburg, Germany). A total volume of 20 μL containing 2 μL 10x PCR buffer, 200 μM deoxynucleotide triphosphate, 500 nM of each primer, 1.5 mM $MgCl_2$, Go Taq Flexi polymerase (2 units added per reaction) (Promega, Madison, WI, USA) and 1 μL of template DNA was used for each reaction (adapted from Gollotte et al., 2004). The thermocycling conditions for the PCR were: an initial cycle of 3 min at 95 °C, 1 min at 58 °C and 1 min at 72 °C, followed by 35 cycles of 45 s denaturation at 94 °C, 45 sec at an annealing temperature of 61.5 °C (annealing temperature was optimized in a pre-test), and 1 min extension at 72 °C, with a final extension at 72 °C for 10 min (Van Tuinen et al., 1998). PCR products were confirmed using gel electrophoresis. After confirmation, the PCR products were diluted 1/100 and used as a template for the nested PCR, which was run under the same conditions as described above. Positive (DNA extracted from a *Rhizophagus irregularis* culture (commercial inoculant from Mikro-Tek Inc., Timmins) and negative (sterile nanopure water) controls were included in both PCR reactions in place of sample DNA in order to confirm successful amplification of the targeted DNA.

2.4.2. Characterization of AMF community structure using DGGE

DGGE was performed using a D-Code system (BioRad, Hercules, CA, USA). DGGE gels were 8% bis-polyacrylamide in 0.5× TAE buffer with a 25–50% concentration of the denaturant (7 M urea, 40% (v/v) formamide). Six μL of PCR product was mixed with 3 μL of loading dye and loaded into wells of a stacking gel that was denaturant free. Initial electrophoresis was performed at 20 V with the pump off until the temperature reached 60 °C, after which it was run at 75 V with the pump on for 17 h at 60 °C. After electrophoresis, gels were stained with SYBR Green I Dye (Invitrogen, S7563) diluted 5000× in TAE buffer (Nakatsu and Marsh, 2007) and visualized under UV light. Images were viewed and DGGE banding patterns were photographed as described above.

Select DGGE bands were excised under UV light, and placed in PCR tubes containing 100 μL sterile, nanopure water and kept at 4 °C overnight to allow DNA elution. An additional PCR of the eluted DNA using the FLR3 and FLR4 primers was performed using the same conditions as described above. The PCR products were cleaned using UltraClean Microbial DNA Isolation kit (MoBio, Carlsbad, CA) and submitted to the Genomic Facility at the University of Guelph (Guelph, ON, Canada) for sequencing using a 3730 DNA Analyzer (Applied Biosystems, CA, USA) to confirm the amplified product was AMF DNA (Supplemental information B).

2.4.3. Comparison of abundance of specific AMF taxa in soil using qPCR

Frozen DNA was thawed at 65 °C, mixed well, and 100 μL of each sample was dried down using a DNA 120 SpeedVac (ThermoSavant, Holbrook, NY). Samples were shipped by mail

to ETH Zurich, Echikon Research Station, Lindau, Switzerland, where the samples were reconstituted with sterile Milli-Q water and qPCR work was completed.

The qPCR method used was that of Thonar et al. (2012), who developed taxon-specific primers and hydrolysis probes for rapid and specific quantification of *Rhizophagus irregularis*, *Claroideoglomus claroideum*, *Funneliformis mosseae*, *Gigaspora margarita*, and *Scutellospora pellucida*. Further, we also used the system described by Wagg et al. (2011) to detect and quantify *Diversispora celata*.

The qPCR cycling conditions were: initial denaturation at 95 °C for 15 min, followed by 45 cycles with denaturation at 95 °C for 10 s, annealing at the temperature optimized for each primer/probe combination for 30 s (Thonar et al., 2012; Wagg et al., 2011), and elongation at 72 °C for 1 s. Each PCR sample contained a total volume of 9 μL, consisting of 4.55 μL water, 500 nM each of primer 1 and primer 2, 100 nM Taqman probe, 1.8 μL Roche Master Mix TaqMan 5*x* + 2.25 μL template DNA. The qPCR was carried out using a LightCycler 2.0 (Roche Diagnostics, Rotkreuz, Switzerland). DNA concentrations in samples were determined using UV spectrophotometry and PicoGreen fluorescence (Singer et al., 1997).

2.4.4. Statistical analysis

Digital images of the DGGE gel were imported into GeneTools (Syngene, Cambridge, UK) for analysis. In GeneTools, the images were visually analysed to ensure bands assigned by the software program were appropriate. The corrected DGGE profile was then imported into GeneDirectory (Syngene, Cambridge, UK). The data were standardized by expressing the area under each peak (corresponding to the intensity of each band) as a proportion of the total area, using 1% minimum peak volume as a threshold for inclusion of a given band in the analysis (Rees et al., 2004). Cluster analysis using the unweighted pair group with the mathematical average method (UPGMA) and Dice's coefficient of similarity were performed to generate a dendrogram to illustrate the AMF communities detected in the paired organic and conventional farms. A similarity matrix was created in GeneDirectory and subsequently analysed with Primer V6 (Primer-E Ltd., Plymouth, UK) using Analysis of Similarity (ANOSIM). A 2-way crossed ANOSIM was used to analyse the data using management (organic or conventional) and location (the three-paired locations) as factors. ANOSIM provides a *P* value indicating whether the treatments are significantly different and a global *R* statistic between 0 and 1, which indicates how different the AMF community structure is as a result of an effecting factor. An *R* value of 1 indicates that the null hypothesis that DGGE profiles between treatment groups are not any different from the profiles within a group should be rejected (Rees et al., 2004). A tolerance value of 1% was used for the similarity analysis.

Statistical analyses comparing the number of bands in the DGGE profiles and the abundance of the specific AMF taxa determined by qPCR were performed using the statistical software program SAS ver. 9.2 for Windows (SAS Institute, Cary, NC, USA). Treatment effects were estimated using the Proc Mixed procedure using management type as the single treatment effect and location as a random effect. If necessary, log transformation of the data was performed to ensure validity of the statistical analysis (i.e. that the residuals were normally and homogenously distributed). In the case of zeros in the qPCR data set (values below the detection limit), half of the minimum value in the sample set was added to all values before performing data transformation. A type I error rate of $P = 0.05$ was used for determining significant differences and a *P* value between 0.05 and 0.10 was regarded as marginally significant.

3. Results

3.1. Species richness

Visual observation of the DGGE gel (Fig. 1) revealed the presence of multiple bands in all samples (in theory, a band is presumed to represent a distinct taxon (Fromin et al., 2002)). The number of DGGE bands above the applied threshold (equivalent to a minimum peak volume of 1%) in all the samples ranged from 10 to 17. Mean number of bands (SD) for each of the fields were as follows: Org 1 = 11.3 (1.3), Org 2 = 13.8 (2.2), Org 3 = 13.3 (1.3), Con 1 = 13.3 (2.6), Con 2 = 12.5 (1.7) and Con 3 = 12.3 (1.9). No significant

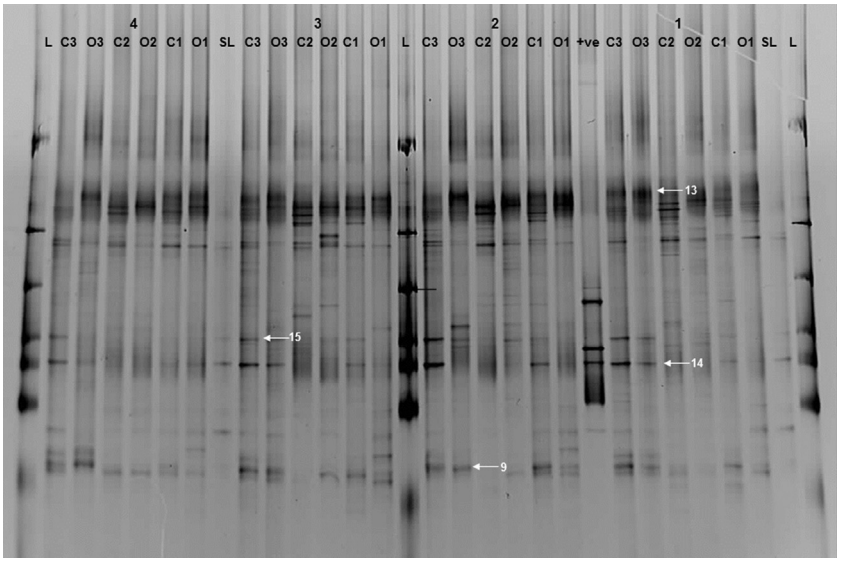

Fig. 1. DGGE image of DNA extracted from three organic (O1, O2, O3) and three conventional (C1, C2, C3) forage field soils and PCR amplified for AMF DNA. The four samples from each field are four unique composite samples (numbers 1–4). *L* = 100 bp DNA Ladder (Promega, Madison, WI, USA), SL = internal standard ladder and +ve = DNA extracted from *Rhizophagus irregularis* and amplified using the same primers. Numbered arrows indicate excised bands that were sequenced and identified as AMF-like sequencesin the GenBank. The numbered bands correspond with the numbered sequences in Supplemental material B and closely resembling (>99%) the following accession numbers: DGGE band 9 (HM216131); band 13 (HM216086); band 14 (HM216175); band 15 (HM216086).

difference in number of bands was found between organically (mean = 12.8) and conventionally (mean = 12.7) managed fields ($P = 0.93$).

3.1.1. AMF community analysis based on PCR-DGGE

The global R statistics generated from the ANOSIM were 0.71 and 0.91 ($P < 0.0001$) for the effects of farm management (organic or conventional) and location, respectively, indicating that both of these factors had a significant impact on the AMF community structure. The UPGMA dendrogram, generated using Gene Directory software, shows similarity among samples (Fig. 2) and illustrates that the AMF communities are most similar among samples from a similar geographical location. It also illustrates the divergence of the AMF communities among management types within a given location.

3.2. Real-time qPCR results

No significant difference in DNA concentrations were found among samples for the DNA extracted from soil (means ± SD for Org and Con were 12.3 ± 0.6 and 13.3 ± 1.7 ng μL^{-1}, respectively). All 72 DNA extracts tested were found to contain detectable amounts of *F. mosseae*. For *C. claroideum*, 70 of 72 samples had detectable amounts. *D. celata* was also frequently detected, with 61 out of 72 samples containing gene copy numbers above the detection limit; most of the samples with non-detectable amounts were from field Con 3. *R. irregularis* had moderate coverage, with 42 out of 72 samples containing detectable amounts of the targeted DNA. No detectable amounts of *R. irregularis* were found in DNA extracted from soil Org 2. *S. pellucida* was mostly found in fields Org 1 and Con 1, whereas this species was not detected in many samples in locations 2 and 3. None of the samples analyzed in this study contained detectable concentrations of *G. margarita* DNA.

Of the qPCR taxa tested, conventional systems were found to contain significantly more *F. mosseae* DNA as a proportion of total DNA (Table 2). On the contrary, amounts of *C. claroideum* DNA were marginally higher in DNA extracts from organically managed soils (Table 2). No significant differences between management types were found for *D. celata, R. irregularis,* and *S. pellucida* DNA abundances.

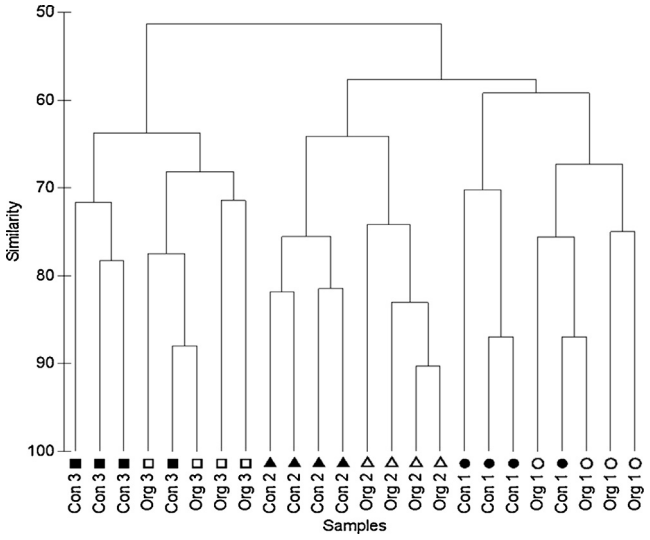

Fig. 2. UPGMA dendrogram showing %similarity (Dice's coefficient) among DGGE gel banding patterns obtained from nested PCR products of soil DNA extracts from three paired organic and conventional forage fields. The four samples from each field are four unique composite samples.

4. Discussion

4.1. Effect of farm system management on AMF community structure

4.1.1. AMF species richness (number of DGGE bands)

No differences in AMF species richness were detected between the different farming systems in this study, although the number of DGGE bands (species) counted (mean = 12.8 for organic and 12.7 for conventional) are within the range of those reported by other AMF diversity studies of agricultural soils (Douds and Millner, 1999; Hijri et al., 2006; Jansa et al., 2002). Lack of a strong effect of management systems on AMF species richness is in agreement with some previous studies (Franke-Snyder et al., 2001; Galva'n et al., 2009), but somewhat counter to others that have reported greater AMF diversity under organic as compared to conventional management (Dai et al., 2014; van der Gast et al., 2010; Verbruggen et al., 2010). Suggested reasons for greater diversity in organic systems were potential negative effects of mineral fertilizers and pesticides in the conventional systems, more diverse crop rotations and more frequent use of legumes and/or perennial forage in organic systems (Dai et al., 2014; van der Gast et al., 2010; Verbruggen et al., 2010). However, not only the management type (organic or conventional) is known to determine AMF species richness, but also management intensity and site history (Hijri et al., 2006; Oehl et al., 2003). The fact that the conventional farms in this study are not overly intensive, in that they do not rely solely on mineral fertilizers (they also apply manure), the soils have moderate STP concentrations, and they employ fairly diverse crop rotations including four years of perennial forage, may help to explain why we have observed no differences in AMF diversity between management systems.

4.1.2. AMF community structure

The composition of indigenous AMF communities was significantly affected by farm management type in this study, with the abundance of *Funneliformis* being higher in conventional fields and *Claroideoglomus* marginally lower, as compared to organically managed fields. This is in agreement with the findings of Verbruggen et al. (2010) and van der Gast et al. (2010) who conducted organic and conventional farm comparisons using similar community fingerprinting techniques (PCR-TRFLP) in the Netherlands and England, respectively. Dai et al. (2014), using high-throughput pyrosequencing to compare organic and conventional wheat farms in the Canadian prairies, also found consistent differences in the AMF community profiles between management types.

The organic fields in our study had not received synthetic fertilizers for over 20 yr, but routinely applied partially composted dairy manure, which could have supported the development of distinct AMF communities. The conventional fields have long term (>20 yr) histories of synthetic nitrogen and P fertilizer application, resulting in significantly higher STP values than in organic fields, which may alter the AMF assemblages in soil and plant roots (Jansa et al., 2006). AMF genotypes differ in their tolerance to high levels of available inorganic P (Ferna'ndez et al., 2011; Sylvia and Schenck, 1983) and even low levels of P additions can cause AMF community shifts (Del Mar Alguacil et al., 2010). The STP values recorded for our conventional soils were generally much lower than anticipated. Still, at a mean STP of 5.1 mg P kg^{-1} for the organically managed soils (Table 1), the regional recommendation for P fertilizer application is 120 kg P ha^{-1}, while the mean STP of 11.7 mg P kg^{-1} determined for the conventional fields would receive a recommendation of 30 kg P ha^{-1} (OMAFRA, 2009). We suspect that the latter STP concentrations were lower than expected because we collected our samples from alfalfa-containing perennial forage fields and alfalfa is known to be a crop that decreases available

Table 2
Real-time PCR results showing mean ($n = 3$) taxon abundance (ng fungal DNA mg^{-1} total DNA) ± se for AMF species: *Funneliformis mosseae*, *Claroideoglomus claroideum*, *Scutellospora pellucida*, *Diversispora celata*, and *Rhizophagus irregularis* obtained from three pairs of dairy farm soils (0–15 cm) under organic and conventional management. *P* values resulting from the mixed model (restricted maximum likelihood (REML) method) used to test for significant differences between management systems are shown.

AMF isolate	Mean taxon abundance (ng fungal DNA/mg total DNA)		*P* value
	Organic	Conventional	
F. mosseae[c]	4.9 ± 0.6	6.1 ± 0.6[a]	0.036
C. claroideum[c]	7.1 ± 0.1	6.5 ± 0.1[b]	0.067
S. pellucida[c]	2.8 ± 0.8	2.7 ± 0.8	0.689
D. celata[c]	5.1 ± 0.6	3.7 ± 0.6	0.137
R. irregularis	1628 ± 680	2530 ± 680	0.445

[a] Indicates significantly different ($P < 0.05$).
[b] Indicates differences are marginally significant ($0.05 > P < 0.10$).
[c] Statistical analysis was performed using log-transformed data.

inorganic P more than other crops (Bell et al., 2012; Campbell et al., 1993; Entz et al., 2001).

The shift from *Funneliformis*-dominated AMF communities in conventionally managed fields to communities with a more prominent *Claroideoglomus* component in organic fields is a very interesting finding of our study that deserves more detailed analysis. There is some evidence that *F. mosseae* is more P-tolerant and can thrive in soils containing high amounts of available P (Troeh and Loynachan, 2009). In a survey of 154 soils in Switzerland, Jansa et al. (2014) found *F. mosseae* to be positively correlated with mineral fertilizer use and the authors suggested this species might be used as a bioindicator of mineral fertilizer use or overuse. *F. mosseae* is also known to be a fairly aggressive species that can quickly colonise roots in early successional stages (Sýkorová et al., 2007; Troeh and Loynachan, 2009 Troeh and Loynachan, 2009). This would make it a likely candidate to increase in abundance if other species were reduced due to synthetic fertilizer or pesticide applications used in conventionally managed systems.

In agreement with our results, in a recent study comparing AMF profiles in soils from 72 organic and 78 conventional wheat fields, Dai et al. (2014) found sequences belonging to the *Claroideoglomus* genus to be more abundant in soil from organic production systems in comparison to those from conventional systems. We also observed greater abundances of *D. celata* DNA under organic management (Table 2), though differences were not statistically significant ($P = 0.137$). Interestingly, Jansa et al. (2014) noted that a decrease in abundance of both *C. claroideum* and *D. celata* was associated with the use of mineral fertilizers. Moreover, in a long-term (>40 yr) P fertilization trial of pasture soils in New Zealand, Wakelin et al. (2012) noted that *G. claroideum* was a dominant species in the control plot that did not receive synthetic P additions, and it was surmised that this species may have high fitness under low P conditions.

Synthetic pesticides can also affect the community assemblages of AMF in an agricultural system (Jansa et al., 2006). In the conventionally managed systems of this study, fungicide use was limited to seed treatment, but the herbicide glyphosate (round-up) was routinely used. Sheng et al. (2012) investigated the effects of glyphosate on AMF diversity in wheat cropping systems in the Canadian prairies and found glyphosate to impact AMF community structure in the second year of the field trial. Their study provided evidence that glyphosate use increased the abundance of *F. mosseae* and reduced other *Glomus* spp, which provides an alternative explanation to the effect of mineral P application for the observed high abundance of *Funneliformis* in conventional fields. Further research is warranted in order to better understand the effects of glyphosate use on AMF communities and on individual AMF species.

It is established that AMF community structure is linked with above-ground plant diversity (van der Heijden et al., 1998). In general, a greater diversity of crops is associated with greater diversity in AMF communities and organic farming systems tend to favor more diverse crop rotations (Gosling et al., 2006). In this study, the organic farms did not necessarily have more diverse crop rotations than their conventional counterparts; however, corn was grown more frequently in conventional systems and this may have impacted the AMF community composition. Finally, despite similar seeding ratios of alfalfa to grass at the time of planting, the organic fields supported greater legume proportions in the forage mixture (Table 1 and Schneider, 2014), which might also play a role in shaping the AMF communities. The reasons for this different plant composition between the farm management types are not fully known, but it seems likely that avoidance of synthetic fertilizers in organic fields plays an important role (Schneider, 2014).

4.2. Effect of geographical location on AMF communities

The results of this study indicate a surprisingly strong influence of geographical location on AMF species assemblages, with each location being different from the others (Fig. 2), in spite of similar soil and climatic properties (Table 1 and supplementary Table A1). Some previous studies have also indicated the presence of a strong geographic effect on AMF community profiles. For example, van der Gast et al. (2010) reported a significant influence of both management and location on AMF diversity among 9 field pairs in England, with the location having a comparatively stronger effect. Differences in AMF diversity observed among locations were suspected to partially result from differences in soil type, which is known to affect AMF diversity (Hazard et al., 2013; Jansa et al., 2014; Oehl et al., 2010). In the present study, sites were selected in an effort to minimize environmental heterogeneity, however, subtle differences in soil characteristics were likely present and it is possible that these differences affected AMF community structure.

4.3. Real-time qPCR results in Ontario field soils

Five out of the six tested AMF species were detected in the Ontario field soils used in this study with the qPCR method of Thonar et al. (2012). This result is significant as the primers and probes used for the quantitative detection of these taxa were designed using DNA sequence data of AMF isolates from grassland and arable soils in Switzerland. This methodology has mainly been used to detect these specific taxa in pot trials (for example, see Wagg et al., 2011), and more recently in agricultural soils in New Zealand (Wakelin et al., 2012) and Switzerland (Jansa et al., 2014). This study thus provides experimental evidence that calcareous soils in Ontario, Canada contain these same AMF taxa, which is consistent with the notion that many AMF species have a global distribution (Douds and Millner, 1999; Öpik et al., 2006).

Relative to the other species, large concentrations of *R. irregularis* DNA were detected. However, as it is difficult to correlate amounts of fungal DNA with more traditional biological units, such as hyphal length density or fractional root length colonization (Gamper et al., 2008), the comparability between the different AMF species is restricted.

So far, the availability of qPCR molecular markers limits the breadth of analyses possible to a few taxa for which the primers and probes have been developed and tested. Thus, there is a need to continue to derive pertinent sequence information for more AMF genotypes (Gamper et al., 2009) and to develop specific quantitative markers for them so as to address their abundance in the different soils.

4.4. Potential functional implications of observed differences in AMF communities

Although there is significant evidence that AMF communities differ under organic and conventional management, the functional differences of these communities are still largely unclear. There is some evidence that AMF in soils under organic management may provide greater benefits to their host crop in terms of yield and P-use efficiency (Johnson, 1993; Scullion et al., 1998), which our research indirectly supports. This is indicated because the abundance of *C. claroideum* DNA was greater in DNA extracted from soils under organic management in comparison with conventionally managed soils (results were marginally significant, $P = 0.067$) (Table 2). In the study by Dai et al. (2014), the efficiency of P uptake, defined as wheat P uptake / P flux (as determined using anion exchange resins), was found to be greater in organic compared to conventional fields. This increased efficiency was directly associated with the number of *Claroideoglomus* reads for organic wheat production, indicating a functional distinction for this group of AMF. Moreover, *C. claroideum* has been linked with greater host-plant P-use efficiency, in terms of plant yield per unit of P taken up, over other AMF species when tested in pot trials with *Medicago trunculata* (Lendenmann et al., 2011; Lendenmann et al., 2011).

There was also a trend for greater abundances of *D. celata* in organically managed systems in our research, though the overall effect of management on *D. celata* abundance was not significant ($P = 0.137$). Dai et al. (2014), comparing organic and conventional wheat fields in western Canada, found that the number of *Diversispora* reads was also associated with greater P uptake efficiency in organic wheat fields, suggesting potentially important involvement of this group in host plant P uptake.

Finally, in an experimental grassland model, Wagg et al. (2011) found *C. claroideum* and *D. celata* to have the greatest yield benefit to *Trifolium pratense* when the planting ratio of legume to grass (*Lolium multiflorum*) was high. This suggests that *C. claroideum* and *D. celata* may preferentially benefit the legume in a legume-grass mixture. This is interesting considering greater legume (alfalfa) yields were noted for the organic fields in this study (Table 1) while grass yields were higher in the conventional systems.

5. Conclusions

With easily-soluble mineral P fertilizer use prohibited under organic management, the role of soil biology in promoting efficient nutrient cycling is of paramount importance. This is especially critical under conditions of low STP, where AMF are known to greatly assist the plants with P uptake. Here we demonstrate that soil AMF communities are different between organic and conventionally managed dairy farms in southwestern Ontario. The detection and quantification of five AMF taxa in the Ontario forage fields in this study provides valuable insight into the AMF communities present and how they are influenced by farm management practices. More *F. mosseae* DNA was found under conventional management, further supporting that conventional practices, including synthetic fertilizer and pesticide use, promote greater abundance of this taxon. On the contrary, greater abundance of *C. claroideum* in organically managed soils is indicated here. These results predict differential contribution to plant growth and nutrition under the different farming systems. Based on available data, it can be speculated that organically-managed forage fields may support AMF communities that better promote host crop yield, P-use efficiency and legume development under low Olsen STP conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2015.07.015.

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