Impact of a 5-year winter cover crop rotational system on the molecular diversity of arbuscular mycorrhizal fungi colonizing roots of subsequent soybean

ARTICLE *in* BIOLOGY AND FERTILITY OF SOILS · MARCH 2014

Impact Factor: 3.4 · DOI: 10.1007/s00374-014-0912-0

11 AUTHORS, INCLUDING:

Rhae A. Drijber
University of Nebraska at Lincoln
81 PUBLICATIONS **1,420** CITATIONS

Biol Fertil Soils (2014) 50:913–926
DOI 10.1007/s00374-014-0912-0

ORIGINAL PAPER

Impact of a 5-year winter cover crop rotational system on the molecular diversity of arbuscular mycorrhizal fungi colonizing roots of subsequent soybean

Masao Higo · Katsunori Isobe · Rhae A. Drijber · Takuya Kondo · Moe Yamaguchi · Saki Takeyama · Yasuhito Suzuki · Daisuke Niijima · Yukiya Matsuda · Ryuichi Ishii · Yoichi Torigoe

Received: 19 October 2013 / Revised: 11 February 2014 / Accepted: 14 February 2014 / Published online: 4 March 2014

Abstract The impact of winter cover crops, specifically wheat (*Triticum aestivum* L.), red clover (*Trifolium pratense* L.), and rapeseed (*Brassica napus* L.) or winter fallow, on community composition of arbuscular mycorrhizal fungi (AMF) in subsequent soybean roots was investigated in a 5-year field trial on andosolic soils in Japan. Soybean roots were sampled at full-flowering and analyzed for AMF communities using a partial LSU rDNA region. Phylogenetic analysis detected 22 AMF phylotypes, including eight *Glomus*, three *Gigaspora*, two *Scutellospora*, three *Acaulospora*, two *Rhizophagus*, and one of *Funneliformis*, *Diversispora, Paraglomus*, and an unknown glomeromycete in the roots. The 5-year rotation of different winter cover crops or winter fallow did not impact the molecular diversity of AMF communities colonizing the roots of subsequent soybean. In all of the rotations, *Glomus* and *Gigaspora* phylotypes were common to soybean roots over the 5-year period. Redundancy analysis (RDA) demonstrated that AMF communities in the roots of subsequent soybean were not significantly different among winter cover crop rotations or fallow. However, AMF communities in soybean roots were clearly influenced by rotation year suggesting that climate or other environmental factors were more important than winter cover cropping system management.

M. Higo (✉) · K. Isobe · T. Kondo · M. Yamaguchi · S. Takeyama · Y. Suzuki · D. Niijima · Y. Matsuda · R. Ishii · Y. Torigoe
College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan
e-mail: higo.masao@nihon-u.ac.jp

R. A. Drijber
Department of Agronomy and Horticulture, University of Nebraska-Lincoln, 316 Keim Hall, Lincoln, NE 68583-0915, USA

Keywords Arbuscular mycorrhizal fungi · Community structure · Cropping system · Soybean · Winter cover crop

Introduction

Arbuscular mycorrhizal fungi (AMF) are widespread in most terrestrial ecosystems where they form mutualistic associations with the majority of plants to facilitate nutrient uptake from the soil via an extensive extraradical mycelium (Smith and Read 2008). This association includes most agricultural crops with benefits of increased plant productivity (Lekberg and Koide 2005), improved soil structure (Piotrowski et al. 2004), and increased pathogen resistance (Sikes et al. 2009). Moreover, AMF abundance or diversity is important to overall biodiversity, productivity, and stability of terrestrial ecosystems (Kennedy et al. 2007; Rosendahl 2008). Thanks to advances in molecular techniques in recent years, it is possible to apply PCR-based molecular methods to analyze the abundance or diversity of AMF colonizing the roots of an individual plant or in soil at any given time (Simon et al. 1992; Helgason et al. 1998; Öpik et al. 2009, 2013). Traditional identification based on spore morphology is often problematic, and the abundance of spores in the soil may not accurately reflect AMF community composition and dynamics (Clapp et al. 1995). Currently, the abundance or diversity of AMF in agricultural soils, especially under intensive management, or most natural ecosystems has been investigated by various molecular techniques such as traditional cloning and sequencing (Schreiner and Mihara 2009; Balestrini et al. 2010; Sasvári et al. 2011; Borriello et al. 2012) or new generation sequencing (Lumini et al. 2010; Lekberg et al. 2012; Öpik et al. 2013). Several researchers have showed low levels of mycorrhizal diversity related to the intensification of agriculture which

may actually diminish the contribution of AMF in intensive agriculture (Gosling et al. 2006; Bedini et al. 2007; Negrete-Yankelevich et al. 2013). However, intensive agricultural management may not always reduce the abundance or diversity of AMF. Mathimaran et al. (2007) has shown that phosphorus (P) application had no significant effect on AMF community composition in fields cropped to corn and crotalaria. Furthermore, other studies have shown agricultural practices, such as tillage (Lekberg et al. 2008; Wang et al. 2009; Borriello et al. 2012) or application of biocides (Gosling et al. 2006), may not negatively impact the abundance or communities of AMF (Hijri et al. 2006). In addition, AMF communities colonizing roots and rhizosphere soil are diverse among crop species (Higo et al. 2011a; Isobe et al. 2011; Gosling et al. 2013), and different crop rotations may induce different growth responses in crops (Higo et al. 2010, 2013; Karasawa and Takebe 2012; Isobe et al. in press).

Cover crops have been shown to provide many environmental and agronomic services within agroecosystems. These include reduced soil erosion, increased biological diversity (e.g., microbes, insects, and birds), increased nutrient cycling and biological N_2 fixation, increased soil organic matter content, improved weed control, and increased crop yield (Sainju and Singh 1997; Williams et al. 1998; Altieri 1999; Reddy et al. 2003). The use of winter cover crops by farmers in Japan has recently decreased leading to increased winter fallow (Ministry of Agriculture and Fisheries 2012). The addition of winter cover crops, such as wheat, barley, rapeseed, or leguminous crops, into agricultural production systems reduces seasonal fallow and thus provides many benefits to subsequent crops and soil fertility (Clark 2007). Moreover, frequent fallowing without organic matter inputs has been linked to losses in soil organic matter (Campbell et al. 1991; Power and Peterson 1998). Rotations with frequent fallow also increase the risk of erosion more so than rotations with grasses and/or legumes (Michalson 1999).

Soybean is an important crop for the production of vegetable oil for human consumption, and more recently of biodiesel. AMF via their ability to supply P, particularly under soil P limitation, are essential to the growth and development of soybean (Fredeen and Terry 1988). However, continuous cropping of soybean is not commonly practiced due to yield declines of up to 30 % from the soybean cyst nematode (Donald et al. 2006). Therefore, soybean is traditionally grown as a summer crop in rotation with winter cover crops in Japan. Studies evaluating the effect of winter cover crops on AMF in agricultural soils date back to at least two decades (Isobe and Tsuboki 1999; Kabir and Koide 2000; Deguchi et al. 2007; White and Weil 2010; Higo et al. 2013). In previous studies, introduction of mycorrhizal crops as preceding crops has also been shown to increase P uptake and yield in subsequent crops (Buyer et al. 2010; Oka et al. 2010; Lehman et al. 2012). The difference in AMF spore density in soil after mycorrhizal cropping has been also shown to be much higher than after non-mycorrhizal hosts (Karasawa et al. 2002, 2012) or left fallow (Higo et al. 2010, 2013).

Introduction of mycorrhizal crops during the winter season can be important for the maintenance and increase of indigenous AMF inoculum or diversity in soils and roots for subsequent crops. In Japan, wheat and rapeseed are usually grown as winter cover crops in rotation with soybeans or other summer crops (Isobe and Tsuboki 1999; Oka et al. 2010; Uchida et al. 2011; Yasumoto et al. 2012). Thus, continuous rotations of the same combination of crops long-term may cultivate dynamic AMF communities best adapted to the individual crops and their management. Actually, cropping of soybean in rotation with mycorrhizal crops has been shown to increase yield (Oka et al. 2010; Isobe et al. in press); however, little is known regarding the impact of various winter cover crops on AMF communities in roots of subsequent soybean. We hypothesize that growing non-mycorrhizal winter cover crops such as rapeseed, or winter fallow, in rotation with soybean leads to a loss of AMF diversity colonizing roots of subsequent soybean, compared to rotation with mycorrhizal crops. To test this hypothesis, we investigated the diversity of AMF communities in roots of subsequent soybean over 5 years of three consecutive winter cover crop–soybean rotational systems or winter fallow. The innovative aspects of this study highlighted the importance of estimates of AMF communities in subsequent soybean to evaluate the effects of winter cover crops in rotation using molecular techniques.

Materials and methods

Experimental design

A 5-year field experiment was conducted at Nihon University, in Kanagawa, Japan (35°22′ N and 139°27′ E) on a volcanic ash soil (allophanic andosol, sandy loam texture). Soil pH at this field site ranged from 5.5 to 6.0 and available P content (Bray P) ranged between 9.3 to 16.4 mg per kg dry soil in this study. Total carbon (C) and nitrogen (N) ranged from 5.3 to 6.1 % and 0.33 to 0.45 %, respectively. In the field plots used for the experiments, soybean (*Glycine max* (L.) Merr., cv: Enrei) had been cultivated to standardize soil biochemical conditions before the experiment was started. Winter cover crop treatments were replicated three times in 4.5×2 m plots. Conventional tillage was over the whole area in 2008, and no tillage was adopted over the whole area to maintain AMF hyphal network to increase AMF biomass and activity to promote P uptake and crop growth in 2009 to 2012 in this study. Tables 1 and 2 showed the summary of the 5-year winter cover crops–soybean rotations. Winter cover crops were sown manually in rows with spacing of 60 cm (in 2008) and 30 cm (in 2009 to 2012) in the cropped treatment

Table 1 Summary of winter cover cop–soybean rotational systems in this study

Rotation	2008		2009		2010		2011		2012	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Wheat	Winter wheat	Soybean	Wheat	Soybean	Wheat	Soybean	Wheat	Soybean	Wheat	Soybean
Red clover	Spring wheat	Soybean	Red clover	Soybean	Red clover	Soybean	Red clover	Soybean	Red clover	Soybean
Rapeseed	Rapeseed	Soybean	Rapeseed	Soybean	Rapeseed	Soybean	Rapeseed	Soybean	Rapeseed	Soybean
Fallow	Fallow	Soybean	Fallow	Soybean	Fallow	Soybean	Fallow	Soybean	Fallow	Soybean

on 18 November, 2007, 31 October, 2008, 22 October, 2009, 18 November, 2010, and 16 November, 2011. In 2008, the seed of winter and spring wheat (*Triticum aestivum* L. cv: Bandowase) was sown 100 kg ha^{-1} and the amount of N (ammonium sulfate) and K (potassium chloride) applied rates were 100 and 90 kg ha^{-1}, respectively. Also spring wheat was sown on 5 April, 2009. In rapeseed (*Brassica napus* L., cv: Michinokunatane), the seed sown was 10 kg ha^{-1} and the amount of N and K applied rates were 100 and 50 kg ha^{-1}. In 2009 to 2012, the seed of wheat sown was 200 kg ha^{-1} and the amount of N and K applied rates were 100 and 90 kg ha^{-1}. In rapeseed, the seed sown was 30 kg ha^{-1} and the amount of N and K applied rates were 100 and 50 kg ha^{-1}. In red clover (*Trifolium pratense* L., cv: Hokuseki), the seed sown was 30 kg ha^{-1} and the amount of N and K applied rates were 30 and 50 kg ha^{-1}. The tops of the winter cover crops were cut close to ground and removed on 10 June, 2008, 2 June, 2009, 5 June, 2010, 16 June, 2011, and 2 June, 2012. At our field site, some weedy plants such as *Chenopodium album*, *Cyperus microiria*, *Digitaria ciliaris*, *Echinochloa crus-galli*, *Equisetum arvense*, *Eleusine indica*, *Lamium amplexicaule*, *Plantago asiatica*, and *Oxalis corniculata* were observed. In the all treatments including fallow rotation, however, these weedy plants were manually removed once a week throughout the year.

Seeds of soybean were sown in a spacing 60×15 cm on 11 June, 2008, 9 June, 2009, 22 June, 2010, 12 July, 2011, and 30 June 2012. In 2009 to 2012, the amount of N (ammonium sulfate) and K (potassium chloride) applied rates were 30 and 50 kg ha^{-1}. No phosphate fertilizer was applied to any of the plots in this study.

Root sampling, staining, and DNA extraction

Root samples of soybean were taken at full bloom (R2 growth stage) on 28 July, 2008, 23 July, 2009, 23 July, 2010, 19 August, 2011, and 8 August, 2012. This corresponds to the stage, when the mycorrhizal colonization of soybean roots is usually at its highest (Zhang et al. 1995). In each rotation, roots were randomly collected from five plants (depth 15 cm, diameter 20 cm) per replicate (a total of 15 plants per treatment), resulting in 300 root samples for the 5 years.

Root samples were stained with 0.05 % (*w*/*v*) Trypan blue (Phillips and Hayman 1970), and the AMF colonization in the soybean roots was calculated as described by (Giovannetti and Mosse 1980).

Three independent DNA extractions (five pieces of 1- to 2-cm-long root fragments per root sample) were performed using cetyl trimethyl ammonium bromide (Higo et al. 2011b), resulting in 15 independent DNA samples per replicate. A total of 45 independent DNA per treatment were extracted, resulting in 180 DNA samples in each year (a total of 900 independent DNA extractions were performed for the 5 years) (Higo et al. 2011a). The DNA pellet was washed once with 80 % ethanol, dried, resuspended in 100 μl of tris-ethylenediaminetetraacetic acid (EDTA) (TE) buffer [10 mM Tris–HCl (pH 8.0) and 1 mM EDTA (pH 8.0)], and stored at −30 °C until use polymerase chain reaction (PCR).

Nested PCR

The 45 independent DNA samples extracted from roots in each treatment were used as PCR templates after 20-fold

Table 2 Timing of field operation and data collection for each year of the study

Operation	Year				
	2008	2009	2010	2011	2012
Cover crop sowing	18-Nov-07[a]	31-Oct-08	22-Oct-09	18-Nov-10	16-Nov-11
Cover crop cultivation	10-Jun-08	2-Jun-09	5-Jun-10	16-Jun-11	2-Jun-12
Soybean sowing	11-Jun-08	9-Jun-09	22-Jun-10	12-Jul-11	30-Jun-12
Soybean roots sampling	28-Jul-08	23-Jul-09	23-Jul-10	19-Aug-11	8-Aug-12

[a] Shows dd/mm/yy and spring wheat was sown only on 5 April, 2008

dilution. The amplification of the D2 region in the fungal 25S large subunit ribosomal DNA (LSU rDNA) was conducted using a nested PCR approach (Gollotte et al. 2004). The fungus-specific primers LR1 (5′-GCA TAT CAA TAA GCG GAG GA-3′) (van Tuinen et al. 1998) and FLR2 (5′-GTC GTT TAA AGC CAT TAC GTC-3′) (Trouvelot et al. 1999) were used in the first PCR to amplify the 5′ end of the LSU rDNA region. The 45 first PCR products were diluted 100-fold and used as templates for the second PCR using the nested primers FLR3 (5′-TTG AAA GGG AAA CGA TTG AAG T-3′) (Gollotte et al. 2004) and FLR4 (5′-TAC GTC AAC ATC CTT AAC GAA-3′), which amplifies about 400-bp fragment of LSU rDNA under the same PCR conditions. PCR was performed in a 10-μl reaction mixture containing 1 μl of template DNA, 1 μl of 10×PCR buffer, 0.2 mM of each deoxyribonucleotide triphosphate, 0.3 μM of each primer, and 0.25 U of TaKaRa Taq (Takara Shuzo), using a thermal cycler (Mastercycler Ep Gradient, Eppendorf, Hamburg, Germany). The PCR protocol was composed of an initial treatment at 94 °C for 1 min; 30 cycles of treatments at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min; and a final treatment at 72 °C for 10 min. Amplification products were separated by gel electrophoresis on 1 % agarose gel in tris-acetate-EDTA buffer (40 mM Tris, pH 8.0, 40 mM acetic acid, and 1 mM EDTA) and DNA was visualized by staining with ethidium bromide.

Cloning, sequencing, and construction of LSU rDNA libraries

The 45 independent second PCR products per replicate were combined prior to cloning; this approach has been shown to detect similar levels of AMF diversity as that involving cloning and sequencing of individual replicates (Renker et al. 2006). One microliter of the 45 independent second PCR products yielding positive amplicons from DNA extracts per rotation were pooled to one representative sample per rotation, which were subcloned into pT7 Blue using the Perfectly Blunt cloning kit (Novagen, Madison, WI, USA) following the manufacturer's instructions. One clone library per rotation in each year was constructed in this study, resulting 20 clone libraries for the 5 years. The plasmid DNA was extracted from transformed *Escherichia coli* cells suspended in 50 μl of sterile water in a 1.5-ml tube by boiling for 5 min. The plasmid-containing supernatants were amplified by PCR using M13 forward and reverse primers, and the products were electrophoresed on 1 % agarose gels to confirm the insertion of the second PCR fragments into the plasmids. The plasmids containing the second PCR fragments were sequenced in both directions using M13 forward primers by cycle sequencing using a DNA sequencer (ABI 3130xl, Applied Biosystems, Tokyo, Japan) and an ABI BigDye terminator v3.1 cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan).

Reconstruction of phylogenetic trees, rarefaction curves

The AMF species were inferred from sequence homologies with sequences registered in the DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp/). Multiple alignments were performed using CLUSTAL W algorithm. Sequences were analyzed with the basic local alignment search tool through the NCBI GenBank database. Phylogenetic analyses were performed using the Neighbor-joining (NJ) phylogenic trees (Tamure-Nei model) and maximum likelihood (TN93+G) algorithms implemented in the programs MEGA 5.0 (Tamura et al. 2011). *Mortierella verticillata* (Accession no. AF157199) was used as an outgroup, and bootstrap values were estimated from 1,000 replicates. The AMF community was characterized on the basis of the LSU rDNA allowing the identification of phylotype. Nucleotide sequences with greater than 97 % identity were assigned the same AMF phylotype group. Also, the AMF groups were classified according to Redecker et al. (2013). Representative sequence phylotypes, defined as groups of closely related sequences with a high level of bootstrap support in the phylogenetic analysis, were selected. If more than one sequence from our study was present in the same phylotype cluster, one was chosen as the representative sequence.

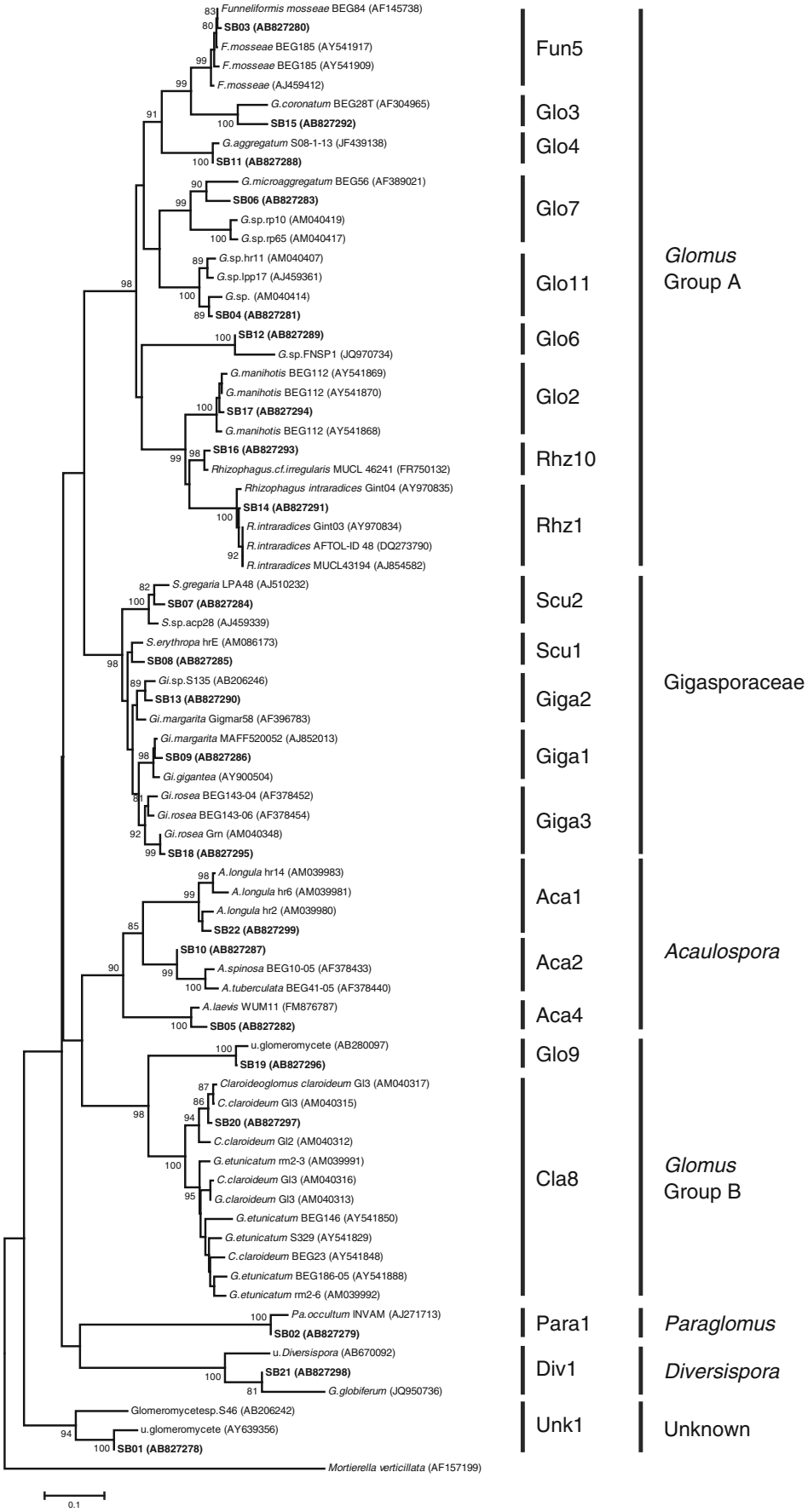
Rarefaction curves were constructed with the freely available software Analytic Rarefaction Program version 1.3 (http://www.uga.edu/strata/software/Software.hyml/) in order to determine whether the number of sequenced samples tested

Table 3 Climate conditions in each year from sowing to full-flowering stage

Year	Precipitation (mm)	Accumulated temperature (°C)	Mean temperature (°C)	Max. temperature (°C)	Min. temperature (°C)
2008	228.0	1,136.0	25.6	28.7	23.5
2009	194.0	1,051.6	24.5	27.1	22.5
2010	127.0	807.4	26.1	28.9	23.9
2011	144.5	1,763.8	26.7	33.2	19.7
2012	73.5	1,614.5	27.7	35.9	23.2

Table 4 Relative abundance (%) of detected phylotypes in the subsequent soybean roots at different rotations

Phylotypes	2008				2009				2010				2011				2012			
	Winter wheat	Spring wheat	Rapeseed	Fallow	Wheat	Red clover	Rapeseed	Fallow	Wheat	Red clover	Rapeseed	Fallow	Wheat	Red clover	Rapeseed	Fallow	Wheat	Red clover	Rapeseed	Fallow
Rhz1	3.2	16.1	3.7	0.8	11.6	5.8	5.7	2.8	21.3	28.6	21.5	9.7	49.6	26.9	12.2	4.2	25.2	60.1	23.8	25.3
Glo2		0.4		2.4			0.4	4.4		3.5		2.2	0.7	3.8	13.9	14.4			12.2	1.2
Glo3			0.8								1.5		0.7			1.3			9.1	1.8
Glo4	51.2	25.9	33.3	43.1	17.0	24.7	47.8	49.6	13.4	27.7	13.7	54.3	11.1	24.2	26.1	29.2	29.0	11.7	14.0	6.6
Fun5	5.5	8.0	8.5	0.4	3.5	2.5	6.9	3.6	0.2		1.8	1.1		4.3	1.7		3.3		1.8	3.0
Glo6		4.9	4.1	5.5	3.9	0.8	4.5	3.6	8.3	5.0		2.2	17.8	7.5	2.8	5.5	7.1	5.2	5.5	3.0
Glo7	24.0	12.9	1.2	6.7	10.0	39.9	2.0	5.2	15.6	7.5	3.9	2.5	5.9	7.5	11.1	11.0	6.2	6.0	9.1	7.8
Cla8	1.8	2.2	0.8		26.6	15.6	2.0	0.4	1.7	0.9		0.7	1.1	4.3	8.3	0.4	0.5			13.9
Glo9	0.9				0.4	1.2										3.8		0.8		7.2
Rhz10			0.4						10.4						9.4					
Glo11						0.4	0.8		0.9			1.1			5.6	7.6	2.4	3.6	11.0	
Scu1	0.5																			
Scu2	4.1	3.6	3.7	5.1	1.9	1.2		0.8	2.6	0.9	3.0	2.9	1.5	3.8		3.8	4.3	1.2	4.3	4.2
Giga1	10.1	18.3	30.1	29.6	15.8	4.9	19.2	18.3	13.2	17.6	46.3	16.5	7.4	12.9	8.9	16.1	13.8	8.9	6.7	15.1
Giga2	1.8	3.6	13.4	5.1	9.3	2.1	8.6	11.1	12.2	8.2	8.4	6.1		4.8		2.5	7.6	2.4	2.4	10.8
Giga3		0.4		1.2									1.5							
Aca1						0.4		0.4												
Aca2		0.5					2.0					0.4								
Aca4													1.5							
Div1						0.4														
Unk1									0.2								0.5			
Para1												0.4	1.1							
Phylotype richness	10	12	11	10	10	13	11	11	12	9	8	13	12	10	10	12	11	9	11	12
Diversity index (H')	1.52	2.00	1.73	1.57	1.99	1.70	1.67	1.61	2.15	1.87	1.63	1.67	1.62	2.01	2.10	2.09	2.03	1.52	2.29	2.29
AMF colonization (%)	15.5	12.9	12.7	15.1	16.3	4.7	7.6	17.6	6.7	6.4	2.9	3.1	9.1	7.3	4.0	5.0	10.9	11.2	9.1	5.8


Funneliformis mosseae BEG84 (AF145738)
SB03 (AB827280)
F.mosseae BEG185 (AY541917)
F.mosseae BEG185 (AY541909)
F.mosseae (AJ459412)
G.coronatum BEG28T (AF304965)
SB15 (AB827292)
G.aggregatum S08-1-13 (JF439138)
SB11 (AB827288)
G.microaggregatum BEG56 (AF389021)
SB06 (AB827283)
G.sp.rp10 (AM040419)
G.sp.rp65 (AM040417)
G.sp.hr11 (AM040407)
G.sp.lpp17 (AJ459361)
G.sp. (AM040414)
SB04 (AB827281)
SB12 (AB827289)
G.sp.FNSP1 (JQ970734)
G.manihotis BEG112 (AY541869)
G.manihotis BEG112 (AY541870)
SB17 (AB827294)
G.manihotis BEG112 (AY541868)
SB16 (AB827293)
Rhizophagus.cf.irregularis MUCL 46241 (FR750132)
Rhizophagus intraradices Gint04 (AY970835)
SB14 (AB827291)
R.intraradices Gint03 (AY970834)
R.intraradices AFTOL-ID 48 (DQ273790)
R.intraradices MUCL43194 (AJ854582)
S.gregaria LPA48 (AJ510232)
SB07 (AB827284)
S.sp.acp28 (AJ459339)
S.erythropa hrE (AM086173)
SB08 (AB827285)
Gi.sp.S135 (AB206246)
SB13 (AB827290)
Gi.margarita Gigmar58 (AF396783)
Gi.margarita MAFF520052 (AJ852013)
SB09 (AB827286)
Gi.gigantea (AY900504)
Gi.rosea BEG143-04 (AF378452)
Gi.rosea BEG143-06 (AF378454)
Gi.rosea Grn (AM040348)
SB18 (AB827295)
A.longula hr14 (AM039983)
A.longula hr6 (AM039981)
A.longula hr2 (AM039980)
SB22 (AB827299)
SB10 (AB827287)
A.spinosa BEG10-05 (AF378433)
A.tuberculata BEG41-05 (AF378440)
A.laevis WUM11 (FM876787)
SB05 (AB827282)
u.glomeromycete (AB280097)
SB19 (AB827296)
Claroideoglomus claroideum Gl3 (AM040317)
C.claroideum Gl3 (AM040315)
SB20 (AB827297)
C.claroideum Gl2 (AM040312)
G.etunicatum rm2-3 (AM039991)
C.claroideum Gl3 (AM040316)
G.claroideum Gl3 (AM040313)
G.etunicatum BEG146 (AY541850)
G.etunicatum S329 (AY541829)
C.claroideum BEG23 (AY541848)
G.etunicatum BEG186-05 (AY541888)
G.etunicatum rm2-6 (AM039992)
Pa.occultum INVAM (AJ271713)
SB02 (AB827279)
u.Diversispora (AB670092)
SB21 (AB827298)
G.globiferum (JQ950736)
Glomeromycetesp.S46 (AB206242)
u.glomeromycete (AY639356)
SB01 (AB827278)
Mortierella verticillata (AF157199)
0.1
Fun5
Glo3
Glo4
Glo7
Glo11
Glo6
Glo2
Rhz10
Rhz1
Scu2
Scu1
Giga2
Giga1
Giga3
Aca1
Aca2
Aca4
Glo9
Cla8
Para1
Div1
Unk1
Glomus Group A
Gigasporaceae
Acaulospora
Glomus Group B
Paraglomus
Diversispora
Unknown

◀ **Fig. 1** Neighbor-joining tree of partial LSU rDNA sequences obtained from the roots of subsequent soybean based on TN93+G substitution model, rooted tree by *Mortierella verticillata* as an outgroup. Bootstrap values (only values>80 are shown) were estimated from 1,000 replicates. Representative sequences from roots are incorporated. Each individual sequenced sample from root samples is labeled with a prefix (*SB*), and GenBank accessions numbers (*bold*) indicated that AMF sequences obtained in the present study

sufficiently represents AMF phylotype. The sequences obtained in rotation of each year in this study have been deposited in the DDBJ database and assigned accession numbers from AB827278 to AB827299, AB889552 to AB889600, AB890384 to AB891932, and AB891933 to AB893119.

Molecular diversity of AMF communities in soybean roots

From the data we calculated AMF phylotype richness, expressed by the number of phylotypes in each root sample. The Shannon diversity index of each treatment was calculated as an additional measure of AMF diversity.

Statistical analysis

To analyze the relatedness of the winter cover crops in respect of AMF community structure (AMF communities), and sampling year on AMF communities, a preliminary detrended correspondence analysis (DCA) on the relative abundance per phylotypes by using the vegan package version 2.0–7 in R 3.0.1 (http://www.r-project.org/). During multivariate analysis procedure, the data were log-transformed to standardize. DCA indicated (the length of gradient, >4) that at least some species had a unimodal distribution (ter Braak and Smilauer 2002). Redundancy analysis (length of gradient<4) as multivariate analysis was performed. The species data matrix was composed of the abundance of AMF phylotypes, and winter cover crop management or cultivation year. During the RDA procedure, the Monte Carlo 999 permutation test for significance at the $P<0.05$ was used.

Climate data were calculated from Japan Meteorological Agency (http://www.jma.go.jp/jma/indexe.html).

Results

Climate factors such as precipitation, accumulated temperature, mean temperature, and maximum and minimum mean temperature from sowing to full-flowering stage differed among years (Table 3). Cumulative precipitation ranged from 73.5 to 228.0 mm, and mean temperature ranged from 24.5 to 27.7 °C. Maximum mean temperature ranged from 27.1 to 35.9 °C, and minimum mean temperature ranged from 19.7 to 23.9 °C. In addition, the accumulated temperature ranged from 807.4 to 1763.8 °C.

Partial LSU rDNA sequences from all root samples were successfully amplified by nested PCR. The length of PCR products by nested PCR varied with the AMF genera, about 310 and 330 bp for *Scutellospora* and *Gigaspora*, and about 330 bp *Acaulospora*, and about 380 bp for *Glomus*. A total of 5,599 clones were sequenced in the present study. Among 5,599 sequenced clones, 89.3 % (4,999 clones) LSU rDNA fragments were successfully cloned and closely related to Glomeromycota. All sequences had high similarity (97–99 %) to AMF and belonged to members of phylum Glomeromycota. Phylogenetic analysis revealed 22 AMF phylotypes including eight *Glomus*, three *Gigaspora*, two *Scutellospora*, three *Acaulospora*, two *Rhizophagus*, and one of *Funneliformis*, *Diversispora*, *Paraglomus*, and an unknown glomeromycete in this study (Table 4, Fig. 1). Rarefaction analysis revealed that cloning and sequencing of the pooled DNA extracts was an effective method for capturing the majority of the AMF community in root samples (Fig. 2). The rarefaction curves by analyzed sequence numbers in each clone library almost reached a plateau.

Several phylotypes were common to soybean roots across all crop rotations, but differed among years (Table 4). In 2008, there were six common phylotypes (Rhz1, Glo4, Glo7, Scu2, Giga1, and Giga2). In 2009, this increased to eight phylotypes (Rhz1, Glo4, Fun5, Glo6, Glo7, Cla8, Giga1, and Giga2). Five phylotypes (Rhz1, Glo4, Scu2, Giga1, and Giga2) were common across crop rotations in 2010, while seven phylotypes were common across rotations in 2011 (Rhz1, Glo4, Glo6, Glo7, Cla8, and Giga1) and 2012 (Rhz1, Glo4, Glo6, Glo7, Scu2, Giga1, and Giga2). Glo1, Glo4, and Giga1 were found in all crop rotations over the 5-year period. In general, *Glomus* group A was dominant and detected at a much higher frequency, although the distribution of phylotypes differed among years and crop rotations (Table 4). Phylotypes other than *Glomus* were detected at a much lower frequency. Rotation with different winter cover crops did not appear to cause specific shifts in the composition of AMF communities in the roots of subsequent soybean.

The AMF colonization in the subsequent soybean roots were significantly influenced by rotation ($P<0.001$) and year ($P<0.001$) from two-way ANOVA (Table 4). The observed absolute numbers of AMF phylotypes (phylotype richness) were not significantly different among the rotations and years using the data averaged across all years (Table 4). No apparent differences in the mean number of AMF phylotypes were found in each rotation and year. Overall, the diversity index (H') also did not differ among the composition of AMF in crop rotations and years (Table 4, statistical analysis not shown). However, cumulative precipitation was negatively correlated with the diversity index (Table 5). Moreover, mean

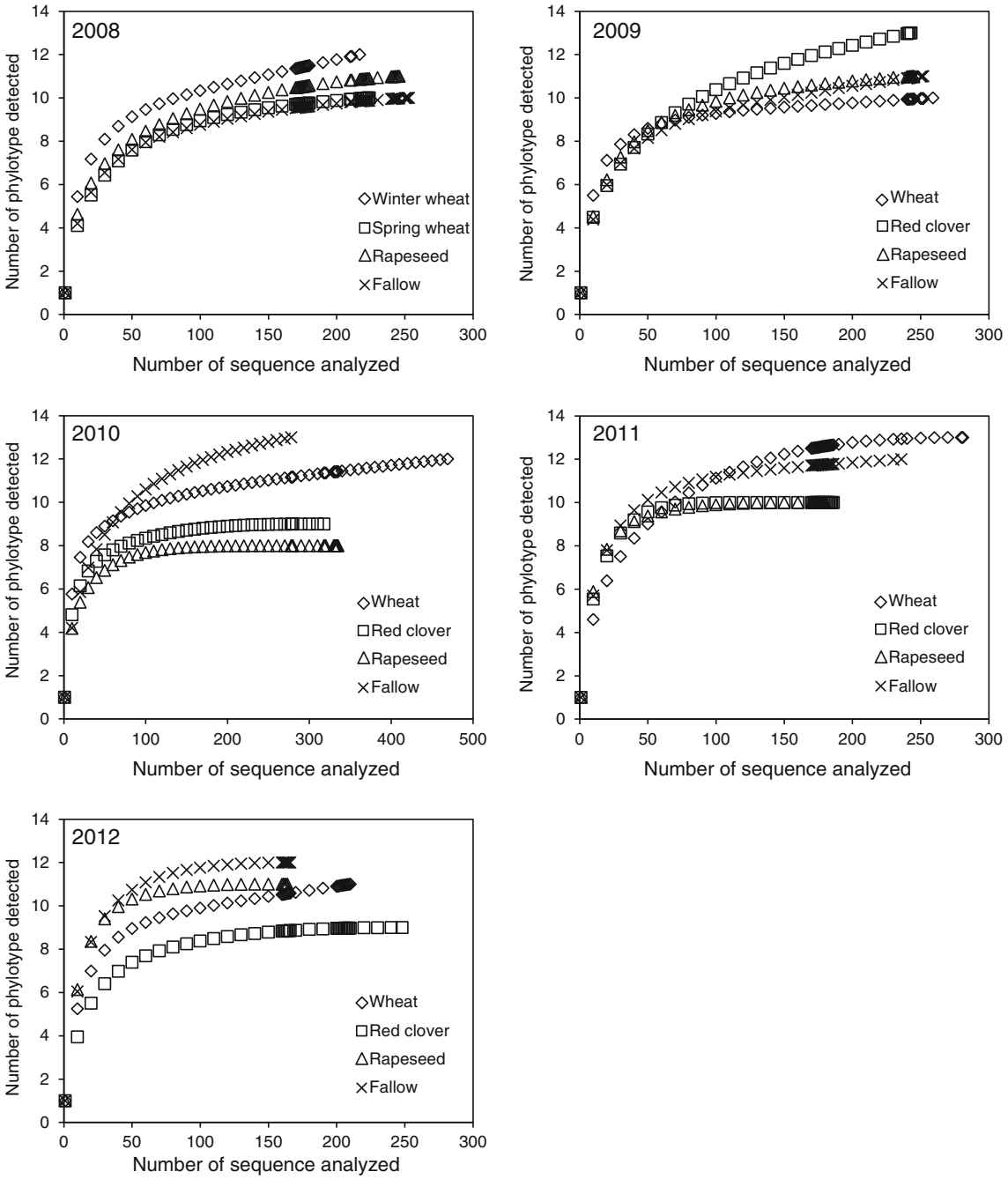


Fig. 2 Rarefaction curve of the phylotypes obtained from the soybean roots of each rotation treatment. Nucleotide sequences with greater than 97 % identity were referred as a same phylotype group

temperature and max mean temperature from sowing to full-flowering stage was positively correlated with the diversity index.

Redundancy analysis was used to identify relationships among AMF communities with winter cover crop management (Fig. 3a) and rotation year (Fig. 3b). RDA showed that composition of AMF communities were not significantly different by winter cover crop management (F=0.921, P=0.574). However, the composition of AMF communities were influenced significantly by rotation year (F=1.421, P=0.006).

Discussion

In general, the most frequently detected species or phylotypes in conventional agricultural systems belong to *Glomus* group A and B. *Glomus aggregatum* (Glo4), *Funneliformis mosseae* (Fun5), and *Claroideoglomus claroideum* or *Glomus etunicatum* (Cla8) are commonly found even in intensively managed arable lands (Oehl et al. 2003), in agreement with this study (Table 4, Fig. 1). It is not surprising that most of the AMF phylotypes detected belonged to *Glomus* given this is

Table 5 Correlations (*r*) between climate variables and the molecular diversity in this study

Climate variables	Phylotype richness	Diversity index (*H'*)
Cumulative precipitation	0.279	−0.904*
Accumulated temperature	0.465	0.752
Mean temperature	−0.321	0.901*
Max mean temperature	−0.030	0.948*
Minimum mean temperature	−0.797	−0.365

*$P<0.05$

the most prevalent genus in agricultural soils among AMF species described (Daniell et al. 2001; Jansa et al. 2003; Sasvári et al. 2011; Vestberg et al. 2011; Borriello et al. 2012). *Glomus* group A is particularly dominant in arable crops as their sporulation rates allow more rapid recovery in disturbed environments (Oehl et al. 2003). In addition, *Glomus* group A generally colonizes via fragments of mycelium or mycorrhizal root, readily forming hyphal anastomosis (Giovannetti et al. 1999, 2001), and thus can more rapidly re-establish a hyphal network after mechanical disruption. In contrast, Gigasporaceae (genus *Gigaspora* or *Scutellospora*) propagate via sporal dispersal or infection from an intact mycelium (Biermann and Linderman 1983; Daniell et al. 2001). Such differences support the dominance of *Glomus* phylotypes including *Glomus* group A over Gigasporaceae phylotypes in the roots of subsequent soybean regardless of

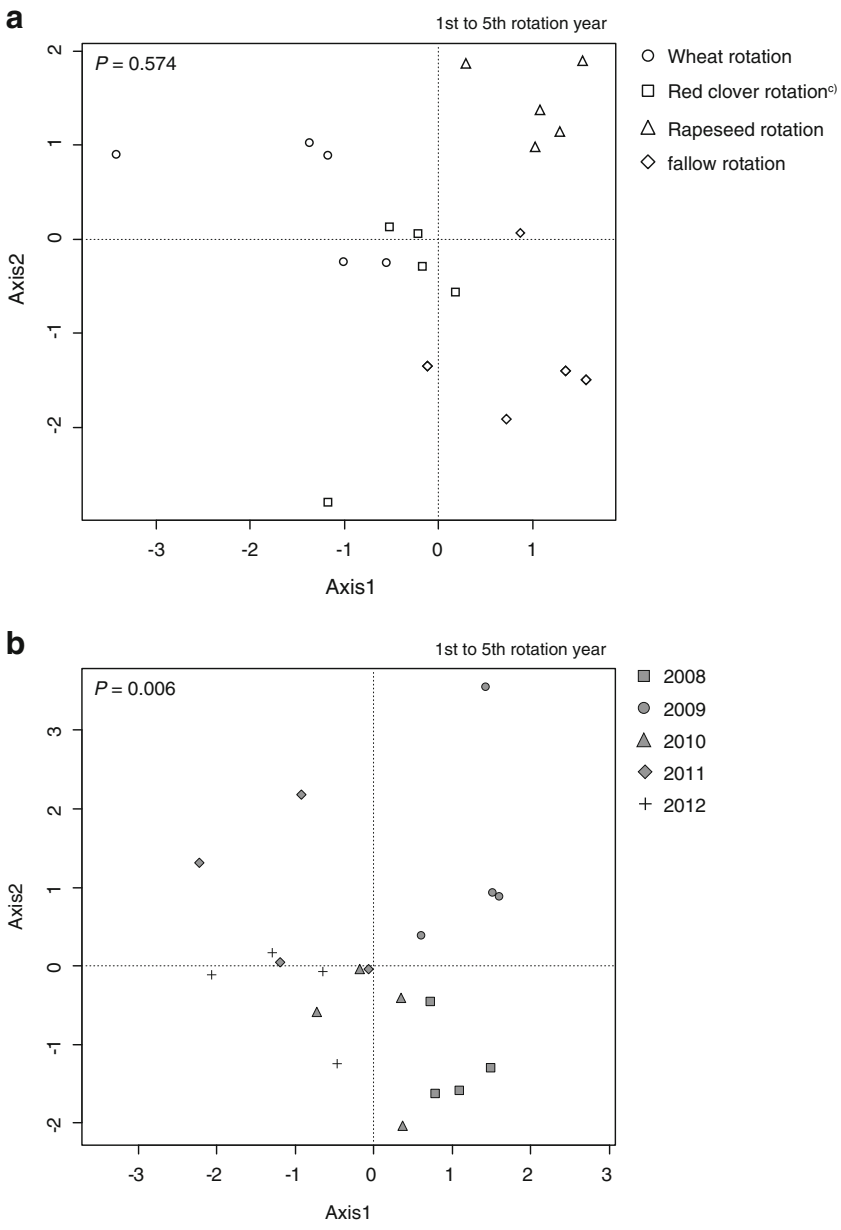


Fig. 3 Redundancy analysis biplot showing relationship between detected phylotypes and crop rotation (**a**), or rotation year (**b**) from 1 to 5 years. In crop rotation, the eigenvalues of the first and second axes were 1.777 and 0.902, respectively. **c** Spring wheat was included only first year in red clover rotation. In rotation year, the eigenvalues of the first and second axes were 2.771 and 1.436, respectively

winter cover crop management. Further investigation into the functional aspects of the different taxa within the genus *Glomus* or Giasporaceae would allow us to better assess the impact of AMF community composition on the productivity of subsequent crops in winter cover crop rotations.

The phylotype Giga1 of Gigasporaceae was found in the roots of subsequent soybean throughout the 5 years of consecutive winter cover crop rotations (Table 4). Johnson et al. (1991) reported that *Gigaspora* spp. dominate the soil after cultivation of soybean. Likewise, An et al. (1993) reported that *Gigaspora* spp. increased and became dominant under continuous cultivation of soybean. Phylotypes of Gigasporaceae have been detected in the roots of soybean grown under different agricultural managements (Alguacil et al. 2008; Chifflot et al. 2009; Bainard et al. 2011; Isobe et al. 2011), in agreement with this study. Thus, there appears to be some degree of host specificity or at least host preference between soybean and Gigasporaceae.

It has been shown that the composition of AMF communities are sensitive to agronomic practices such as crop rotation (Hijri et al. 2006; Oehl et al. 2009), winter cover crop management (Higo et al. 2013), geographic region (Isobe et al. 2007, 2008; Öpik et al. 2013) and AMF host plant (Higo et al. 2011a; Isobe et al. 2011; Torrecillas et al. 2012; Verbruggen et al. 2012; Gosling et al. 2013). Vandenkoornhuyse et al. (2002, 2003) and Gustafson and Casper (2006) have indicated a preference between host-plant and some AMF species. Alguacil et al. (2012) have shown that a significant difference in the composition of root AMF community when two agricultural crop species (*Jatropha curcas* and *Ricinus communis*) were established. In addition, Gosling et al. (2013) have also reported that the communities of AMF colonizing the roots of two crops (corn and soybean) after potato and wheat showed subtle differences. Higo et al. (2011a) have reported that the difference in crop type (wheat and red clover) and sowing date (winter and spring wheat) impacted the composition of AMF communities in its roots in andosols. Furthermore, Higo et al. (2013) have showed that the shift of compositions of AMF communities were observed based on winter cover crop management. Yet, in this study, differing winter cover crop rotations did not lead to changes in the composition of AMF communities colonizing the roots of subsequent soybean (Table 4, Fig. 3a). However, the composition of AMF communities in the roots of subsequent soybean at flowering were affected by crop year rather than winter cover crop management (Table 4, Fig. 3b). Vandenkoornhuyse et al. (2002) have shown year dynamic in the composition of AMF communities colonizing the roots of two plant species (*Agrostis capillaris* and *Trifolium repens*) over 2 years. Some dominant AMF groups were replaced by others and some disappeared over the 2-year experiment, in agreement with this research (Table 4). This pattern is consistent with the results of previous studies in different habitats (Liu et al. 2009; Sikes et al. 2012). Also, Bever et al. (2002) have indicated that different AMF communities differ in their response to changes in an abiotic environment, and the dominant AMF species is possible to shift as the environment changes over time (Husband et al. 2002). Guo et al. (2012) have shown that while most phylotypes of *Glomus* group A increased in abundance following liming, *Glomus* group B and Gigasporaceae/Acaulosporaceae phylotypes declined using a long-term liming trial. Our results showed that cumulative precipitation was negatively correlated with the diversity index (Table 5). Mean temperature and max mean temperature from sowing to full-flowering stage was positively correlated with the diversity index in the winter cover crop–soybean rotational system. Veresoglou et al. (2013) have indicated that a key factor that impacted odds of existence of Gigasporaceae was precipitation. On the contrary, odds of existence of Acaulosporacae increased in acidic environments and soils with high bulk density. Furthermore, Dumbrell et al. (2011) have found that significant differences in the composition of AMF communities and diversity during cooler and warmers periods of the year in temperate grassland and has indicated that change in the composition of AMF communities during the growing season can be induced by soil chemical properties. Therefore, we would expect that much more a longer-term trial may have clarified the relationships between time course and AMF community composition in winter cover crop–soybean rotation on function and role of AMF.

At our field site, some weedy plants such as *C. album*, *C. microiria*, *D. ciliaris*, *E. crus-galli*, *E. arvense*, *E. indica*, *L. amplexicaule*, *P. asiatica*, and *O. corniculata* were observed. We removed these weedy plants once a week throughout the year, however an unpredictable pathway or allelochemicals in these weedy plants might have impacted the composition of AMF communities colonizing the subsequent soybean roots throughout the cover crop rotations (Fig. 3b). Jordan et al. (2000) and Corneo et al. (2013) have suggested that the importance of interactions between AMF and weeds of agroecosystems. Actually, previous studies have showed substantial effects of weed species (e.g., *Centaurea maculosa*) on soil microbial group abundance and communities (Lutgen and Rillig 2004; Batten et al. 2006). Wortman et al. (2013) have reported that their results suggest that arable weed communities (*Abutilon theophrasti*, *Amaranthus retroflexus*, *C. album*, *Thlaspi arvense*, and *Setariaviridis*) altered soil microbial communities with and without cover crop. In addition, Batten et al. (2006) and Marler et al. (1999) have indicated that weedy species (e.g., *Aegilops triuncialis*, *C. maculosa*, and *Centaurea solstitialis*) could change soil microbial communities and increase the abundance of AMF. Conversely, Wortman et al. (2013), Lutgen and Rillig (2004),

and Mummey and Rillig (2006) have reported that *C. maculosa* reduces the abundance and diversity of AMF. Therefore, the weedy plants at this field could be one of the factors to change the composition of AMF communities colonizing subsequent soybean roots in each rotation in the different years. In this study, the weeds *C. album* are non-mycorrhizal plant species, while *Cyperus rotundus*, *D. ciliaris*, *E. arvense*, *E. indica*, and *P. Asiatica* are mycorrhizal plant species. Their occurrence in the rotation plots where winter cover crops were grown might have affected in an unpredictable way, depending on the relative occurrence of the different species in the different years to the results of this study. Moreover, their occurrence could have had an impact on *B. napus* or winter fallow rotation plots. In actual, the occurrence of mycorrhizal weeds could explain the maintenance of AMF species which were able to colonize the subsequent soybean, since Brassicaceae is a non-mycorrhizal genus. The same thing applies to winter fallow plots. In addition, since host identity strongly affects the composition of AMF communities (Johnson et al. 1992, 2004; Helgason et al. 2002; Scheublin et al. 2004; Torrecillas et al. 2012), the diversity of mycorrhizal weed species in the winter cover crop rotations may have described another factor affecting the occurrence and colonization ability of AMF in the subsequent soybean, but the reasons for these differences are not clear. As described above, the weedy plants which existed at the field site were routinely removed throughout the 5-year consecutive rotation. Further research would be needed to understand whether weeds at this site have negative or positive influences on AMF diversity in winter cover crop rotational systems.

In conclusion, the introduction of winter cover crops in rotation with soybean or winter fallow did not alter the diversity (phylotype richness and diversity index *H'*) and community composition of AMF in the roots of subsequent soybean at flowering over five consecutive cropping seasons. The high diversity of AMF communities found in soybean roots highlights the potential for P uptake and growth under winter cover crop rotations with soybean or other summer crops. Although choice of winter cover crop did not alter AMF community composition in soybean roots, maximizing agronomic benefits associated with cover crops will depend on appropriate species choices (Wortman et al. 2012). Traditionally, cover crop use and management have followed the conventional single species paradigm. Monoculture systems were developed to facilitate ease of mechanical cultural practices including planting, fertilization, weed control, and harvest. However, multi-species mixtures may increase productivity, stability, and resource-use efficiency of the AMF communities colonizing roots of soybean or other summer crops. Therefore, further investigation into more diversified cover crop rotations on crop productivity and associated ecosystem services will gain insight into the functional roles of AMF in long-term winter cover crop rotations with soybean or other summer crops.

References

Alguacil MM, Lumini E, Roldán A, Salinas-García JR, Bonfante P, Bianciotto V (2008) The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. Ecol Appl 18: 527–536

Alguacil MM, Torrecillas E, Hernández G, Roldán A (2012) Changes in the diversity of soil arbuscular mycorrhizal fungi after cultivation for biofuel production in a guantanamo (Cuba) tropical system. PLoS ONE 7:e34887. doi:10.1371/journal.pone.0034887

Altieri M (1999) The ecological role of biodiversity in agroecosystems. Agric Ecosyst Environ 74:19–31

An ZQ, Hendrix JW, Hershman DE, Ferriss RS, Henson GT (1993) The influence of crop rotation and soil fumigation on a mycorrhizal fungal community associated with soybean. Mycorrhiza 3:171–182

Bainard LD, Koch AM, Gordon AM, Newmaster SG, Thevathasan NV, Klironomos JN (2011) Influence of trees on the spatial structure of arbuscular mycorrhizal communities in a temperate tree-based intercropping system Agric. Ecosyst Environ 144:13–20

Balestrini R, Magurno F, Walker C, Lumini E, Bianciotto V (2010) Cohorts of arbuscular mycorrhizal fungi (AMF) in *Vitis vinifera*, a typical Mediterranean fruit crop. Environ Microbiol Rep 2:594–604

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. Biol Invasions 8:217–230

Bedini S, Avio L, Argese E, Giovanetti M (2007) Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. Agr Ecosys Environ 120:463–466

Bever JD, Pringle A, Schultz PA (2002) Dynamics within the plant–arbuscular mycorrhizal fungal mutualism: testing the nature of community feedback. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer, Berlin, pp 267–292

Biermann B, Linderman RG (1983) Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. New Phytol 95:97–105

Borriello R, Lumini E, Girlanda M, Bonfante P, Bianciotto V (2012) Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. Biol Fertil Soils 48:911–922

Buyer JS, Teasdale JR, Roberts DP, Zasada IA, Maul JE (2010) Factor affecting soil microbial community structure in tomato cropping systems. Soil Biol Biochem 42:831–841

Campbell CA, Lafond GP, Zentner RP, Biederbeck VO (1991) Influence of fertilizer and straw baling on soil organic matter in a thick black chernozem in western Canada. Soil Biol Biochem 23:443–446

Chifflot V, Rivest D, Olivier A, Cogliastro A, Khasa D (2009) Molecular analysis of arbuscular mycorrhizal community structure and spores distribution in tree-based intercropping and forest systems. Agric Ecosyst Environ 131:32–39

Clapp JP, Young JPW, Merryweather JW, Fitter AH (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytol 130:259–265

Clark A (2007) Managing cover crops profitably, 3rd edn. Sustainable Agriculture Network Handbook Series 9, Beltsville, MD

Corneo PE, Pellegrini A, Cappellin L, Gessler C, Pertot I (2013) Weeds influence soil bacterial and fungal communities. Plant Soil 373:107–123

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonizing arable crops. FEMS Microbiol Ecol 36:203–209

Deguchi S, Shimazaki U, Uozumi S, Tawaraya K, Kawamoto H, Tanaka O (2007) White clover living mulch increases the yield of silage corn via arbuscular mycorrhizal fungus colonization. Plant Soil 291: 291–299

Donald PA, Pierson PE, St. Martin SK, Sellers PR, Noel GR, Macguidwin AE, Faghihi J, Ferris VR, Grau CR, Jardine DJ, Melakeberhan H, Niblack TL, Stienstra WC, Tylka GL, Wheeler TA, Wysong DS (2006) Assessing Heterodera glycines-resistant and susceptible cultivar yield response. J Nematol 38:76–82

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol 190:794–804

Fredeen AL, Terry N (1988) Influence of vesicular–arbuscular mycorrhizal infection and soil phosphorus level on growth and carbon metabolism of soybean. Can J Bot 66:2311–2316

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Giovannetti M, Azzolini D, Citernesi AS (1999) Anastomosis formation and nuclear and protoplasmic exchange in arbuscular mycorrhizal fungi. Appl Environ Microbiol 65:5571–5575

Giovannetti M, Fortuna P, Citernesi AS, Morini S, Nuti MP (2001) The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phytol 151:717–724

Gollotte A, van Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi colonizing roots of the grass species *Agrostis capillaries* and *Lolium perenne* in a field experiment. Mycorrhiza 14:111–117

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Gosling P, Mead A, Proctor M, Hammond JP, Bending GD (2013) Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. New Phytol 198:546–556

Guo YJ, Ni Y, Raman H, Wilson BAL, Ash GJ, Wang AS, Li GD (2012) Arbuscular mycorrhizal fungal diversity in perennial pastures; responses to long-term lime application. Plant Soil 351:389–403

Gustafson DJ, Casper BB (2006) Differential host plant performance as a function of soil arbuscular mycorrhizalfungal communities: experimentally manipulating co-occurring *Glomus* species. Plant Ecol 183:257–263

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web? Nature 394:431

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. J Ecol 90:371–384

Higo M, Isobe K, Kang DJ, Ujiie K, Drijber RA, Ishii R (2010) Inoculation with arbuscular mycorrhizal fungi or crop rotation with mycorrhizal plants improves the growth of maize in limed acid sulfate soil. Plant Prod Sci 13:74–79

Higo M, Isobe K, Maekawa T, Ishii R (2011a) Community structure of arbuscular mycorrhizal fungi colonized in various winter crop roots. Soil Microorg 65:3–10

Higo M, Isobe K, Kang DJ, Maekawa T, Ishii R (2011b) Molecular diversity and spore density of indigenous arbuscular mycorrhizal fungi in acid sulfate soil in Thailand. Ann Microbiol 61:383–389

Higo M, Isobe K, Yamaguchi M, Drijber RA, Ishii R (2013) Diversity and vertical distribution of indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. Biol Ferti Soils 49:1085–1096

Hijri I, Sykorova Z, Oehl F, Ineichen K, Mäder P, Wiemken A, Redecker D (2006) Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. Mol Ecol 15:2277–2289

Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 11:2669–2678

Isobe K, Tsuboki Y (1999) Effects of winter crops on the density of arbuscular mycorrhizal fungi and the growth of succeeding kidney beans. Jpn J Crop Sci 68:118–125

Isobe K, Aizawa E, Iguchi Y, Ishii R (2007) Distribution of arbuscular mycorrhizal fungi in upland field soil of Japan 1. Relationship between spore density and the soil environment factor. Plant Prod Sci 10:122–128

Isobe K, Sugimura H, Maeshima T, Ishii R (2008) Distribution of arbuscular mycorrhizal fungi in upland field soil of Japan 2. Spore density of arbusclar mycorrhizal fungi and infection ratio soybean and maize fields. Plant Prod Sci 11:171–177

Isobe K, Maruyama K, Nagai S, Higo M, Maekawa T, Mizonobe G, Drijber RA, Ishii R (2011) Arbuscular mycorrhizal fungal community structure in soybean roots: comparison between Kanagawa and Hokkaido, Japan. Adv Microbiol 1:13–22

Isobe K, Higo M, Kondo T, Sato N, Takeyama S, Torigoe Y (in press, accepted in 2013) Effect of winter crop species on arbuscular mycorrhizal fungal colonization and subsequent soybean yields. Plant Prod Sci

Jansa J, Mozafar A, Kuhn G, Anken T, Ruh R, Sanders IR, Frossard E (2003) Soil tillage affects the community structure of mycorrhizal fungi in maize roots. Ecol Appl 13:1164–1176

Johnson NC, Pfleger FL, Crookston RK, Simmons SR, Copeland PJ (1991) Vesicular–arbuscular mycorrhizas respond to corn and soybean cropping history. New Phytol 117:657–663

Johnson NC, Tillman D, Wedin D (1992) Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034–2042

Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Booth RE, Grime JP, Young JPW, Read DJ (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytol 161:503–515

Jordan NR, Zhang J, Huerd S (2000) Arbuscular–mycorrhizal fungi: potential roles in weed management. Weed Res 40:397–410

Kabir Z, Koide RT (2000) The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. Agric Ecosyst Environ 78:167–174

Karasawa T, Takebe M (2012) Temporal or spatial arrangements of cover crops to promote arbuscular mycorrhizal colonization and P uptake of upland crops grown after nonmycorrhizal crops. Plant Soil 353:355–366

Karasawa T, Kasahara Y, Takebe M (2002) Differences in growth responses of maize to preceding cropping caused by fluctuation in the population of indigenous arbuscular mycorrhizal fungi. Soil Biol Biochem 34:851–857

Kennedy PG, Hortal S, Bergemann SE, Bruns TD (2007) Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. J Ecol 95:1338–1345

Lehman RM, Taheri WI, Osborne SL, Buyer JS, Douds DD (2012) Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. Appl Soil Ecol 61:300–304

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytol 168:189–204

Lekberg Y, Koide RT, Twomlow SJ (2008) Effect of agricultural management practices on arbuscular mycorrhizal fungal abundance in low-input cropping systems of southern Africa: a case study from Zimbabwe. Biol Ferti Soils 44:917–923

Lekberg Y, Schnoor T, Kjøller R, Gibbons SM, Hansen LH, Al-Soud WA, Sorensen SJ, Rosendahl S (2012) 454-Sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. J Ecol 100:151–160

Liu Y, He L, An LZ, Helgason T, Feng HY (2009) Arbuscular mycorrhizal dynamics in a chronosequence of *Caragana korshinskii* plantations. FEMS Microbiol Ecol 67:81–92

Lumini E, Orgiazzi A, Borriello R, Bonfante P, Bianciotto V (2010) Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environ Microbiol 12:2165–2179

Lutgen ER, Rillig MC (2004) Influence of spotted knapweed (Centaurea maculosa) management treatments on arbuscular mycorrhizae and soil aggregation. Weed Sci 52:172–177

Marler MJ, Zabinski CA, Callaway RM (1999) Mycorrhizae indirectly enhancecompetitive effects of an invasive forb on a native bunchgrass. Ecology 80:1180–1186

Mathimaran N, Ruh R, Jama B, Verchot L, Frossard E, Jansa J (2007) Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. Agric Ecosyst Environ 119:22–32

Michalson EL (1999) A history of conservation research in the Pacific Northwest. In: Michalson EL, Papendick RI, Carlson JE (eds) Conservation farming in the United States. The methods and accomplishments of the STEEP Program. CRC, Boca Raton, pp 1–10

Ministry of Agriculture, Forestry and Fisheries (2012) Statistical report on agriculture, forestry and fisheries. Available online at http://www.maff.go.jp/j/tokei/kouhyou/sakumotu/menseki/index.html

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosaalters* arbuscular mycorrhizal fungal communities in the field. Plant Soil 288:81–90

Negrete-Yankelevich S, Maldonado-Mendoza I, Lázaro-Castellanos O, Sangabriel-Conde W, Martínez-Álvarez JC (2013) Arbuscular mycorrhizal root colonization and soil P availability are positively related to agrodiversity in Mexican maize polycultures. Biol Fert Soils 49:201–212

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. Appl Environ Microbiol 69:2816–2824

Oehl F, Sieverding E, Ineichen K, Mäder P, Wiemken A, Boller T (2009) Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. Agric Ecosyst Environ 134:257–268

Oka N, Karasawa T, Okazaki K, Takebe M (2010) Maintenance of soybean yield with reduced phosphorus application by previous cropping with mycorrhizal plants. Soil Sci Plant Nutr 56:824–830

Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M (2009) Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytol 184:424–437

Öpik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I, Jairus T, Kalwij JM, Koorem K, Leal ME, Liira J, Metsis M, Neshataeva V, Paal J, Phosri C, Põlme S, Reier Ü, Saks Ü, Schimann H, Thiéry O, Vasar M, Moora M (2013) Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza 23:411–430

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–160

Piotrowski JS, Denich T, Klironomos JN, Graham JM, Rillig MC (2004) The effects of arbuscular mycorrhizae on soil aggregation depend on the interaction between plant and fungal species. New Phytol 164: 365–373

Power JF, Peterson GA (1998) Nitrogen transformations, utilization, and conservation as affected by fallow tillage method. Soil Till Res 49: 37–47

Reddy KN, Zablotowicz RM, Locke MA, Koger CH (2003) Cover crop, tillage, and herbicide effects on weeds, soil properties, microbial populations, and soybean yield. Weed Sci 51:987–994

Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C (2013) An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23: 515–531

Renker C, Weißhuhn K, Kellner H, Buscot F (2006) Rationalizing molecular analysis of field-collected roots for assessing diversity of arbuscular mycorrhizal fungi: to pool, or not to pool, this is the question. Mycorrhiza 16:525–531

Rosendahl S (2008) Communities, populations and individuals of arbuscular mycorrhizal fungi. New Phytol 178:253–266

Sainju UM, Singh BP (1997) Winter cover crops for sustainable agricultural systems: influence on soil properties, water quality, and crop yields. HortSci 32:21–28

Sasvári Z, László H, Katalin P (2011) The community structure of arbuscular mycorrhizal fungi in roots of maize grown in a 50-year monoculture. Biol Fertil Soils 47:167–176

Scheublin TR, Ridgway KP, Young JPW, van Der Heijden MGA (2004) Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appl Environ Microbiol 70:6240–6246

Schreiner RP, Mihara K (2009) The diversity of arbuscular mycorrhizal fungi amplified from grapevine roots (*Vitis vinifera* L.) in Oregon vineyards is seasonally stable and influenced by soil and vine age. Mycologia 101:599–611

Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. J Ecol 97:1274–1280

Sikes BA, Maherali H, Klironomos JN (2012) Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. Oikos 121:1791–1800

Simon L, Lalonde M, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular–arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58:291–295

Smith SE, Read DJ (2008) Arbuscular mycorrhizaes. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis, 3rd edn. Academic Press, London, pp 13–187

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

ter Braak C, Smilauer P (2002) CANOCO reference manual and Canodraw for Windows user's guide: Software for canonical community ordination, 45th edn. Microcomputer Power, Ithaca

Torrecillas E, Alguacil M, Roldán A (2012) Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. Appl Environ Microbiol 78:6180–6186

Trouvelot S, van Tuinen D, Hijri M, Gianinazzi-Pearson V (1999) Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hyhridization. Mycorrhiza 8:203–206

Uchida T, Kobayashi H, Yoshino N (2011) Effects of arbuscular mycorrhizal colonization on soybean nutrient uptake during ripening period with barley cover. Cropping Jpn J Crop Sci 80:277–283

van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol Ecol 7:879–887

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol Ecol 11:1555–1564

Vandenkoornhuyse P, Ridgeway K, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol Ecol 12:3085–3095

Verbruggen E, van der Heijden MGA, Weedon JT, Kowalchuk GA, Röling WFM (2012) Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. Mol Ecol 21:2341–2353

Veresoglou SD, Caruso T, Rillig MC (2013) Modelling the environmental and soil factors that shape the niches of two common arbuscular mycorrhizal fungal families. Plant Soil 368:507–518

Vestberg M, Kahiluoto H, Wallius E (2011) Arbuscular mycorrhizal fungal diversity and species dominance in a temperate soil with long-term conventional and low-input cropping systems. Mycorrhiza 21:351–361

Wang MY, Hu LB, Wang WH, Liu ST, Li M, Liu RJ (2009) Influence of long-term fixed fertilization on diversity of arbuscular mycorrhizal fungi. Pedosphere 19:663–672

White CM, Weil RR (2010) Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots. Plant Soil 328:507–521

Williams MM, Mortensen DA, Doran JW (1998) Assessment of weed and crop fitness in cover crop residues for integrated weed management. Weed Sci 46:595–603

Wortman SE, Francis CA, Bernards M, Drijber R, Lindquist JL (2012) Optimizing cover crop benefits with diverse mixtures and an alternative termination method. Agron J 104:1425–1435

Wortman SE, Drijber RA, Francis CA, Lindquist JL (2013) Arable weeds, cover crops, and tillage drive soil microbial community composition in organic cropping systems. Appl Soil Ecol 72:232–241

Yasumoto S, Suzuki K, Matsuzaki M, Hiradate S, Oose K, Hirokane H, Okada K (2012) Effects of plant residue, root exudate and juvenile plants of rapeseed (*Brassica napus* L.) on the germination, growth, yield, and quality of subsequent crops in successive and rotational cropping systems. Plant Prod Sci 14:339–348

Zhang F, Hamel C, Kianmehr H, Smith DL (1995) Root-zone temperature and soybean [*Glycine max*. (L.) Merr.] vesicular–arbuscular mycorrhizae: development and interactions with the nitrogen fixing symbiosis. Environ Exp Bot 35:287–298