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ORIGINAL PAPER

Molecular diversity of arbuscular mycorrhizal fungi in onion roots from organic and conventional farming systems in the Netherlands

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Abstract Diversity and colonization levels of naturally occurring arbuscular mycorrhizal fungi (AMF) in onion roots were studied to compare organic and conventional farming systems in the Netherlands. In 2004, 20 onion fields were sampled in a balanced survey between farming systems and between two regions, namely, Zeeland and Flevoland. In 2005, nine conventional and ten organic fields were additionally surveyed in Flevoland. AMF phylotypes were identified by rDNA sequencing. All plants were colonized, with 60% for arbuscular colonization and 84% for hyphal colonization as grand means. In Zeeland, onion roots from organic fields had higher fractional colonization levels than those from conventional fields. Onion yields in conventional farming were positively correlated with colonization level. Overall, 14 AMF phylotypes were identified. The number of phylotypes per field ranged from one to six. Two phylotypes associated with the *Glomus mosseae–coronatum* and the *G. caledonium–geosporum* species complexes were the most abundant, whereas other phylotypes were infrequently found. Organic and conventional farming systems had similar number of phylotypes per field and Shannon diversity indices. A few organic and conventional fields had larger number of phylotypes, including phylotypes associated with the genera *Glomus*-B, *Archaeospora*, and *Paraglomus*.

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This suggests that farming systems as such did not influence AMF diversity, but rather specific environmental conditions or agricultural practices.

Keywords *Allium cepa* · *Glomus* · Organic farming · Mycorrhizal colonization · Phylotype

Introduction

Onion (*Allium cepa* L.) has a sparse rooting system without root hairs which makes the crop dependent for water and nutrient acquisition on arbuscular mycorrhizal fungi (AMF) (De Melo 2003; Stribley 1990). This dependency is especially true in case of cultivation under nutrient-poor soil conditions as is frequently the case in low-input and organic agriculture. AMF enlarge the soil volume from which nutrients can be taken up, via an extensive mycelium network, enabling host plants to access more resources (Finlay 2004). As a consequence, AMF enhance uptake of nutrients, particularly phosphorus (Hayman and Mosse 1971), and may allow for a reduction of the amount of fertilizers applied (Linderman and Davis 2004). Furthermore, AMF can protect the plant against biotic (diseases) and abiotic (drought) stress, and improve soil aggregation (Gosling et al. 2006).

Research on *Allium* species and their interactions with AMF has a long history that dates back to 1884, when Mollberg described in roots of *Allium scorodoprasum* what we currently know as AMF (Koide and Mosse 2004). *Allium* species, and in particular onion, are excellent models for mycorrhizal research because they have a simple rooting system, slow growth, and high response to AMF. The knowledge on *Allium*–AMF interactions benefited greatly from the work of Mosse and co-workers, who presented detailed analyses of AMF functioning under field conditions (Hayman and Mosse 1971; Mosse and Hayman 1971; Mosse 1973; Owusu-Bennoah and Mosse 1979).

Onion, with a total annual acreage of 16,000–19,000 ha and an organically managed acreage of 600 ha, is an important crop in the Netherlands and a good model to monitor and compare the AMF status of agricultural soils. Numerous studies have shown that agricultural soils have low AMF species richness in comparison to natural ecosystems, such as woodlands and grasslands. The difference in AMF diversity is thought to be due to tilling-induced disruption of hyphal networks, rotation with non-mycorrhizal crop species, the occurrence of fallow periods, and the use of fertilizers and fungicides (Helgason et al. 1998; Daniell et al. 2001; Merryweather 2001; Jansa et al. 2002a). However, agricultural soils can differ in species richness and composition of AMF because their management systems differ significantly. This is the case for example in low- and high-input input farming systems (Ryan et al. 2000). In low-input and organic farming systems, synthetic fungicides and soluble phosphate fertilizers are limited or excluded. This may increase AMF inoculum potential and colonization levels compared to conventional farming systems, as has been observed for wheat (Douds et al. 1993; Ryan et al. 1994) and clover–ryegrass pastures (Eason et al. 1999; Ryan et al. 2000). Furthermore, recent research showed that AMF biodiversity was higher in low-input systems compared to high-input systems (Oehl et al. 2003, 2004), although the relationship is not always straightforward (Hijri et al. 2006).

The contribution of AMF to crop-production increase in high-input agriculture is low because phosphorus is amply available. In contrast, AMF might have a significant role in increasing crop production in low-input and organic agricultural systems. However, this concept is far from being practically applied due to the lack of understanding of the functioning of AMF species (Scullion et al. 1998).

The present research aimed to study AMF species richness and composition in onion fields in the Netherlands, by comparing organic and conventional cultivation systems. In this way, we investigate if the adoption of organic practices on formerly conventionally managed farmlands leads to higher AMF diversity. Unlike previous studies, this research was carried out in a large number of sites. The primer sets developed by Redecker (2000) and Redecker et al. (2003) were used to identify AMF phylotypes that colonize onion plant roots, and therefore, only the AMF assemblage of the target host species was analyzed rather than the complete diversity in the soil.

Materials and methods

Survey of onion fields and root colonization

Two traditional onion-growing regions in the Netherlands were sampled, namely, Zeeland in the southwest and Flevoland (the Flevopolder and the Noordoostpoder) in the center of the country. In Zeeland, onion cultivation takes place already for centuries, whereas in Flevoland, on the land recently reclaimed from the sea, onion cultivation only takes place since the second half of the twentieth century. In both regions, the soils are classified as clay to loess–clay soils. Clay content ranged from 23% to 40% for the soils investigated in Zeeland, and 7% to 55% in Flevoland (Table 1). Seed-onions in the Netherlands are cultivated in rotation with other field crops. The soils are ploughed every year, either before or after the winter, and seedbeds are prepared consisting of fine soil aggregates. Sowing date is at the end of March and early April, and harvest takes place in the second half of August (early cultivars) and

Table 1 Average soil chemical parameters and soil history of onion fields surveyed in 2004 and 2005, by cultivation system and region in the Netherlands

Cultivation system	Region	Number of fields	Years under organic cultivation[a]	Onion yield (ton/ha)	Soil properties				
					OM[b]	pH	Pw[c]	Ca[b]	*K*[d]
Organic		20	12.1	32	3.1	7.4	32	7.2	29
	Flevoland	15	13.8 (4–32)	33	3.1	7.4	32	6.5	30
	Zeeland	5	5.7 (1–12)	28	2.9	7.2	30	9.2	20
Conventional		19	–	70	3.0	7.3	44	5.1	26
	Flevoland	14	–	76	3.2	7.3	45	5.5	23
	Zeeland	5	–	59	2.5	7.5	42	2.8	33

[a] Average values. The range of values is indicated between brackets

[b] *OM* organic matter (%), *Ca* $CaCO_3$ (%)

[c] mg P_2O_5 l^{-1} of dry soil. The difference in P content between cultivation systems was almost significant (REML analysis, $p=0.071$)

[d] mg K kg^{-1} soil

September (late cultivars). In both years, samplings were done in the second half of June. Plants were in leaf development phase, with four to six expanded leaves.

Twenty onion fields were sampled in a balanced survey between cultivation systems and regions: five organic and five conventional fields in Zeeland, plus five organic and five conventional fields in Flevoland. Ten plants per field were randomly sampled. The surrounding soil was excavated in order to take out the rooting system as intact as possible. A new survey was done in June 2005, only in Flevoland, because the 2004 study suggested that management practices in this region could have an influence on AMF diversity. The 2005 study comprised ten organic and nine conventional onion fields. All of them were different from fields sampled in 2004, although in some cases, they were located within the same farm. Organic farm fields followed ecological or biodynamic practices, and fulfilled the basic standards for organic production (available from IFOAM 2007) as certified by SKAL (www.skal.com). Information on chemical characteristics of the soils (pH, organic matter, content of phosphorus, and other nutrients) and onion yields were obtained from the farmers for 28 of the 39 sites, and the number of years under organic management queried for all 20 organic fields (Table 1).

AMF colonization was estimated only for samples collected in 2004. Staining of fungal structures was done using trypan blue, and colonization was quantified following the magnified intersections method (McGonigle et al. 1990). Hyphal (HC), arbuscular (AC), and vesicular colonization (VC) were quantified separately. Data analysis was carried out via analysis of variance using Genstat 9.2 (Lawes Agricultural Trust, Rothamsted Exp. St., UK, 2006). The relationships between colonization parameters and environmental variables were studied using the Pearson correlation coefficient and linear regression analysis.

Molecular diversity analysis

AMF species colonizing onion roots were identified by sequencing the partial 18S-ITS1–5.8S-ITS2 rDNA region, as described by Redecker (2000), with minor modifications. DNA was isolated from 1-cm root pieces randomly taken from each onion sample. rDNA was amplified using a nested polymerase chain reaction (PCR) approach. The primers NS5/ITS4i were used in the first step (Redecker et al. 2003) with an annealing temperature of 51°C. For the second amplification, PCR products were diluted 1:100. This second step was performed using the primers ACAU1660, ARCH1311, GLOM1310, and LETC1670 in combination with ITS4i in separate reactions. Primer GIGA5.8R was used only in combination with NS5. Primer sequences and protocols for PCR amplifications are available from Redecker (2000) and Redecker et al. (2003).

PCR products having the expected size were cloned by the pGEM-T vector system (Promega, Madison, WI, USA). An aliquot of 8 µl of the successful clones were digested with restriction enzymes *Mbo*I, *Hinf*I, and *Alu*I in 15 µl at 37°C for 6 h. Restriction fragment patterns were run on a 3% gel made from RESponse agarose (Byozim group, Landgraaf, The Netherlands) and analyzed by the Phoretix 1D software (Nonlinear Dynamics, Durham, NC, USA). A representative clone for each distinct restriction profile was sequenced by the dideoxynucleotide chain termination method using BigDyeTM Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an automatic sequencer (ABI PRISM 3700 DNA Analyser, Applied Biosystems). After removing chimerical results, a set of 65 sequences were obtained from the 5.8S-ITS2 rDNA region and, when available, the partial 18S region (each region 400–500 bps in length). Sequences are deposited in the European Molecular Biology Laboratory

Table 2 Arbuscular mycorrhizal colonization parameters by cultivation systems and regions in the Netherlands (Survey 2004)

Cultivation system	Region	Arbuscular colonization (AC) (%)	Hyphal colonization (HC) (%)	Vesicular colonization (%)	Ratio AC/HC
Organic	Flevoland	62 a	89 a	9.2	0.69 a
	Zeeland	65 a	85 a	6.5	0.76 a
Conventional	Flevoland	67 a	91 a	7.7	0.73 a
	Zeeland	46 b	72 b	3.6	0.62 b

Means within each column followed by the same letter do not differ statistically (Fischer LSD test, $p>0.05$)

(EMBL and NCBI databases) under the accession numbers AM992799 to AM992864. These sequences were used for identification of AMF phylotypes after phylogeny analysis. In order to obtain that, sequences were submitted to the BLAST query tool (Altschul et al. 1997) and database *www.ncbi.nih.gov/blast/* for an initial similarity analysis. Sequence alignment was performed manually. Finally, a phylogenetic analysis was carried out by distance analysis using the neighbor-joining method in PAUP 4b10 (Swofford 2003) with the Kimura two-parameter model, and a gamma shape parameter of 0.5. Bootstrap analyses were done with 1,000 replications. Sequences selected from public databases belonging to known AMF species were included in the phylogenetic analysis.

Phylotypes were determined from cladograms as clearly distinct monophyletic taxa which were also present in the respective maximum likelihood trees. AMF genera or morphospecies associated to each phylotype were assigned on the basis of the position of already known sequences from databases (Table 3). Phylotypes were associated either to a group of related species (e.g., *Glomus caledonium–geosporum*) or a genus (e.g., *Paraglomus*), and therefore, the number of phylotypes is a conservative estimate for the number of morphospecies. Code names were assigned to phylotypes, as an acronym for the genus followed by a correlative number. *Glomus*-A and *Glomus*-B groups were distinguished according to Schwarzott et al. (2001).

AMF diversity was analyzed by integrating the data from 2004 and 2005, using the number of phylotypes found per onion field. In addition, Shannon–Weaver diversity indices (H') were calculated based on the relative abundance of phylotypes per field, as $H_i^{'} = -\sum[(n_i/N) \times Ln(n_i/N)]$, being n_i the number of observation for the i phylotype, whereas N is the total number of observations recorded (Mueller et al. 2004). Differences in AMF diversity indices between cultivation systems or between regions were tested by residual maximum likelihood analysis (REML) using Genstat 9.2. Besides, the associations between AMF diversity with chemical soil parameters and with the number of years under organic agriculture were studied by Pearson correlation coefficient and linear regression analysis.

Correspondence analysis (CA) was performed in CANOCO 4.53 (Ter Braak and Smilauer 2004). CA allows to study the contribution of each sampled site ($n=39$) to the total variation based on their AMF species composition, as well as the contribution from each phylotype ($n=14$) based on their abundance along sampled sites (Jongman et al. 1995).

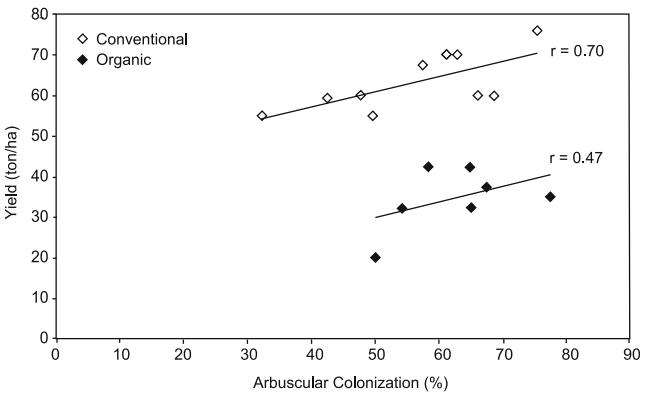


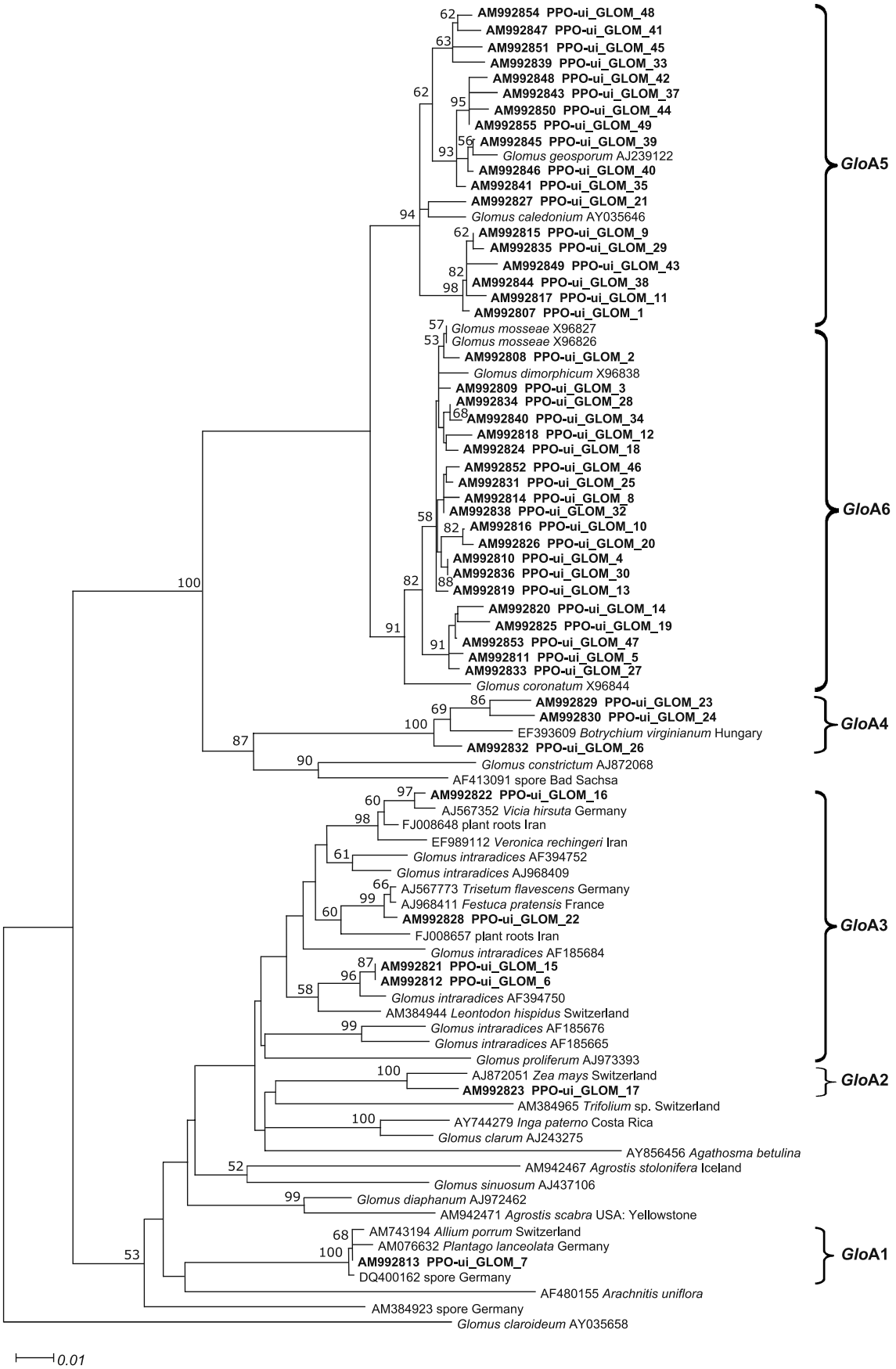
Fig. 1 The correlation between arbuscular colonization (%) and onion yield (tons/ha) for conventional ($n=10$) and organic ($n=7$) management systems (survey 2004)

Results

AMF colonization in onions

In 2004, all sampled onion plants were colonized. The average colonization in both sampled regions and cultivation systems was 60% for arbuscular colonization (AC) and

Fig. 2 Neighbor-joining phylogenetic analysis of arbuscular mycorrhizal fungal 5.8S-ITS2 rDNA sequences obtained from onion root samples in the Netherlands for *Glomus*-A (as defined by Schwarzott et al. 2001) phylotypes, rooted to *Paraglomus brasilianum* sequence AJO12112. The scale represents substitutions per site. Sequences obtained in the present study are shown in *boldface*. Sequences from databases isolated from roots were labeled with the accession number, host plant, and country of origin, whereas those isolated from spores, with fungal species and accession number. Bootstrap values from 1,000 replications larger than 50% are *indicated above branches*. *Brackets to the right* indicate phylotypes defined for the sequences obtained in this study


AM992854 PPO-ui_GLOM_48
AM992847 PPO-ui_GLOM_41
AM992851 PPO-ui_GLOM_45
AM992839 PPO-ui_GLOM_33
AM992848 PPO-ui_GLOM_42
AM992843 PPO-ui_GLOM_37
AM992850 PPO-ui_GLOM_44
AM992855 PPO-ui_GLOM_49
AM992845 PPO-ui_GLOM_39
Glomus geosporum AJ239122
AM992846 PPO-ui_GLOM_40
AM992841 PPO-ui_GLOM_35
AM992827 PPO-ui_GLOM_21
Glomus caledonium AY035646
AM992815 PPO-ui_GLOM_9
AM992835 PPO-ui_GLOM_29
AM992849 PPO-ui_GLOM_43
AM992844 PPO-ui_GLOM_38
AM992817 PPO-ui_GLOM_11
AM992807 PPO-ui_GLOM_1
GloA5
Glomus mosseae X96827
Glomus mosseae X96826
AM992808 PPO-ui_GLOM_2
Glomus dimorphicum X96838
AM992809 PPO-ui_GLOM_3
AM992834 PPO-ui_GLOM_28
AM992840 PPO-ui_GLOM_34
AM992818 PPO-ui_GLOM_12
AM992824 PPO-ui_GLOM_18
AM992852 PPO-ui_GLOM_46
AM992831 PPO-ui_GLOM_25
AM992814 PPO-ui_GLOM_8
AM992838 PPO-ui_GLOM_32
AM992816 PPO-ui_GLOM_10
AM992826 PPO-ui_GLOM_20
AM992810 PPO-ui_GLOM_4
AM992836 PPO-ui_GLOM_30
AM992819 PPO-ui_GLOM_13
AM992820 PPO-ui_GLOM_14
AM992825 PPO-ui_GLOM_19
AM992853 PPO-ui_GLOM_47
AM992811 PPO-ui_GLOM_5
AM992833 PPO-ui_GLOM_27
Glomus coronatum X96844
GloA6
AM992829 PPO-ui_GLOM_23
AM992830 PPO-ui_GLOM_24
EF393609 Botrychium virginianum Hungary
AM992832 PPO-ui_GLOM_26
GloA4
Glomus constrictum AJ872068
AF413091 spore Bad Sachsa
AM992822 PPO-ui_GLOM_16
AJ567352 Vicia hirsuta Germany
FJ008648 plant roots Iran
EF989112 Veronica rechingeri Iran
Glomus intraradices AF394752
Glomus intraradices AJ968409
AJ567773 Trisetum flavescens Germany
AJ968411 Festuca pratensis France
AM992828 PPO-ui_GLOM_22
FJ008657 plant roots Iran
Glomus intraradices AF185684
AM992821 PPO-ui_GLOM_15
AM992812 PPO-ui_GLOM_6
Glomus intraradices AF394750
AM384944 Leontodon hispidus Switzerland
Glomus intraradices AF185676
Glomus intraradices AF185665
Glomus proliferum AJ973393
GloA3
AJ872051 Zea mays Switzerland
AM992823 PPO-ui_GLOM_17
GloA2
AM384965 Trifolium sp. Switzerland
AY744279 Inga paterno Costa Rica
Glomus clarum AJ243275
AY856456 Agathosma betulina
AM942467 Agrostis stolonifera Iceland
Glomus sinuosum AJ437106
Glomus diaphanum AJ972462
AM942471 Agrostis scabra USA: Yellowstone
AM743194 Allium porrum Switzerland
AM076632 Plantago lanceolata Germany
AM992813 PPO-ui_GLOM_7
DQ400162 spore Germany
GloA1
AF480155 Arachnitis uniflora
AM384923 spore Germany
Glomus claroideum AY035658
0.01

84% for hyphal colonization (HC) as grand means (Table 2). The presence of vesicles was much lower, namely, 7% on average. While neither region nor cultivation system was a significant source of variation, the interaction between regions and cultivation systems was significant for AC (p=0.004) and HC (p=0.005). This was due to the fact that conventional cultivation in Zeeland had a mean AC of 46%, a mean HC of 72%, and both means differed significantly from the means of the other three combinations of region and cultivation system. Furthermore, onions grown in conventional fields in Zeeland had a significantly lower AC/HC ratio.

No correlation was found between average AC and HC and soil parameters, on the one hand, and the number of years under organic cultivation, on the other hand, except for the correlation between content of calcium in the soil and AC (r=0.55; p<0.05). In addition, a significant correlation was found between AC and onion yield (OY) under conventional management (r=0.70, p<0.05; Fig. 1), as well as between HC and OY (r=0.85, p<0.05). In case of conventional management (n=10), the linear regression was estimated as $\mathrm{OY}\left[\mathrm{ton}\times\mathrm{ha}^{-1}\right] = 0.38 \times \mathrm{AC}\% + 42.1$. With a smaller range of variation in AMF colonization level (AC, HC) and less data points available (n=7), in case of organic management, the regression was estimated as $\mathrm{OY}\left[\mathrm{ton}\times\mathrm{ha}^{-1}\right] = 0.38 \times \mathrm{AC}\% + 10.6$, though this regression was not significant.

AMF diversity in onion fields

Among the total plant samples, 56% in 2004 and 93% in 2005 yielded AMF amplification products and subsequent restriction profiles. The GIGA5.8R combined with NS5 did not yield any amplification product. After phylogenetic analysis, 65 rDNA-obtained sequences were clustered in 14 phylotypes, namely, six *Glomus*-A, five *Glomus*-B, two *Archaeospora*, and one *Paraglomus* phylotypes (Figs. 2 and 3). Phylogenetic analysis of the ITS2-5.8S sequences of *Glomus*-A species was in agreement with the phylogenetic tree obtained for the sequences from the 18S region (Figure S1). An exception was the distinction between phylotypes *Glo*A5 and *Glo*A6, which were clearly distinct regarding the ITS2-5.8S region, whereas the 18S region clusters did not hold high bootstrap values.

Glomus-A species were predominant in onion cultivation in the Netherlands, particularly phylotype *Glo*A6, which was present in 19 of the 20 onion fields in 2004 and all surveyed fields in 2005 (Table 3). Overall, this phylotype associated with the *G. mosseae–coronatum* complex was present in 70% of the individual sampled plants. Phylotype *Glo*A5 associated with the *G. caledonium–geosporum* complex was the second most abundant one and was found in 31 of the 39 fields.

The other phylotypes were present in a much lower frequency, three of them being exclusively found in conventional fields and seven only found in organic fields (Table 3). Some phylotypes were only found in 2004, such as *Glo*A1, *Glo*A2, and *Glo*A3, whereas others were found only in 2005, such as Arch1 (Table S1). Noticeably, onion fields of two organic farms harbored phylotypes *Par*1 (*Paraglomus*) and *Arch*2 (*Archaeospora*) in 2004, and the next year, sampling again on different fields of the same farms resulted in the finding of the same phylotypes.

The number of AMF phylotypes per field ranged from one to six. The highest diversity in phylotypes was found in two organic and two conventional fields. Organic and conventional management systems did not differ in the number of phylotypes per field (REML analysis, p=0.48), nor did the regions (p=0.99). In the same way, the Shannon–Weaver index calculated on the basis of the relative abundance of phylotypes per field (Table 4) did not significantly differ for the management systems studied and also not for the four management systems–region combinations (REML analysis).

The number of years a field was under organic management was neither correlated with the number of phylotypes per field, nor with the Shannon–Weaver index. Onion yields in organic or conventional farming were also not correlated significantly with AMF diversity indices (data not shown).

The contribution of phylotypes and sampled fields to the total variation in diversity was analyzed via correspondence analysis (CA; data matrix shown as Table S2). Figure 4 presents the biplot for the first and second CA axes, which explained 31.4% of the total variance (first axis: 16.8%, second axis 14.6%, eigenvalues 0.54 and 0.47, respectively; total inertia 3.24). Phylotypes *Glo*A6 (*G. mosseae–coronatum* complex) and *Glo*A5 (*G. caledonium–geosporum* complex) took a central position in the CA space, as these phylotypes did not contribute much to the variation between sampled sites. Other less frequent phylotypes, which took more extreme positions in the CA space, contributed more to the variation between fields. The larger proportion of sampled sites, 13 conventional and eight organic ones, took a central position in the chart, close to the most abundant phylotypes (*Glo*A5 and *Glo*A6), and irrespective of cultivation system and sampled region (Fig. 4). As a result, only a few other organic and conventional sites had larger AMF diversity, differing in community composition among them (Fig. 4).

Discussion

Molecular diversity

Only a few studies have addressed the question if and to what extent AMF species richness and composition differ

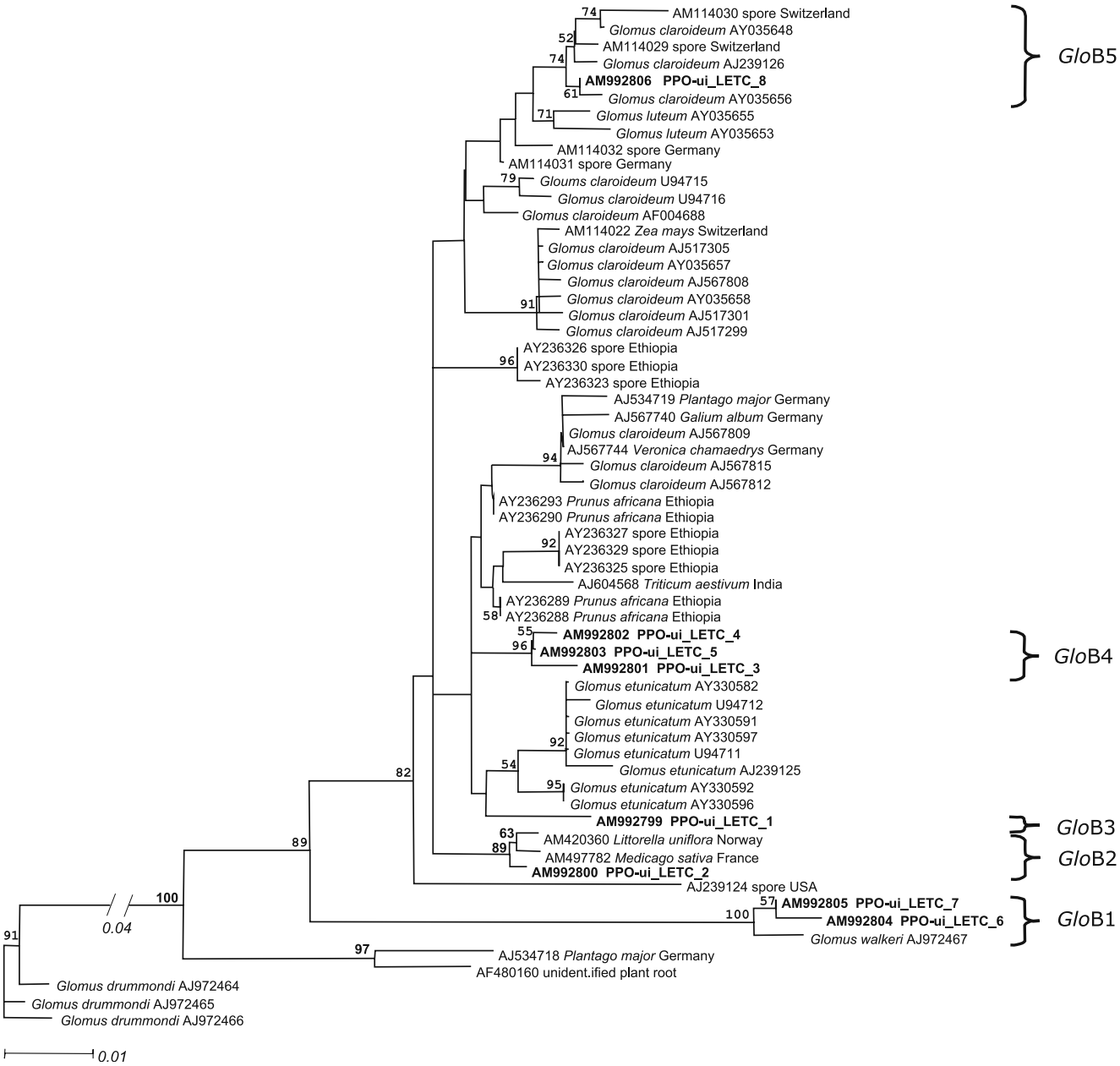


Fig. 3 Neighbour-joining phylogenetic analysis of arbuscular mycorrhizal fungal 5.8S-ITS2 rDNA sequences obtained from onion root samples in the Netherlands for *Glomus*-B (as defined by Schwarzott et al. 2001) phylotypes, rooted to the *G. drummondii* sequence AJ972466. The scale represents substitutions per site. Sequences obtained in the present study are shown in *boldface*. Sequences from databases isolated from roots were labeled with the accession number, host plant, and country of origin, whereas those isolated from spores, with fungal species and accession number. Bootstrap values from 1,000 replications larger than 50% are *indicated above branches*. *Brackets to the right* indicate phylotypes defined for the sequences obtained in this study

between organic and conventional farming systems (Oehl et al. 2003, 2004; Hijri et al. 2006). To contribute to this field of knowledge, the present research was carried out involving a larger number of sites compared to previous studies and different environmental conditions like a temperate sea climate.

AMF diversity was estimated via the number of different phylotypes per field, as no reliable molecular method has been developed to determine all different AMF morphospecies yet. This estimator can be seen as a lower limit for diversity estimation, as each phylotype may contain different AMF morphospecies. Phylotypes were defined as monophyletic groups in a conservative way. Since the taxonomic position of rDNA sequences from root samples is unknown, and rDNA sequence variation within a given morphospecies or even within a single spore exists (Jansa et

Table 3 Number of fields containing each AMF phylotype, by cultivation system and region in the Netherlands

Phylotypes [a]	Cultivation systems		Regions		Total nr of fields
	Organic	Conventional	Flevoland	Zeeland	
*Glo*A1 (*Glomus*-A)	1		1		1
*Glo*A2 (*Glomus*-A)	1			1	1
*Glo*A3 (*G. intraradices*)	3	1	2	2	4
*Glo*A4 (*Glomus*-A)	2		2		2
*Glo*A5 (*G. caledonium-geosporum)*	16	15	22	9	31
*Glo*A6 (*G. mosseae-coronatum)*	19	19	28	10	38
*Glo*B1 (*G. walkeri*)		2	2		2
*Glo*B2 (*Glomus*-B)		1		1	1
*Glo*B3 (*Glomus*-B)	1			1	1
*Glo*B4 (*Glomus*-B)	2		2		2
*Glo*B5 (*G. claroideum*)		1	1		1
*Par*1 (*Paraglomus* sp.)	5		5		5
*Arch*1 (*Archaeospora* sp.)	1	4	5		5
*Arch*2 (*Archaeospora* sp.)	2		2		2
Total number of surveyed fields	20	19	29	10	39

[a] Distinct monophyletic taxa after neighbor-joining analysis of rDNA sequences. Whenever known, species names associated with a phylotype are indicated. *Glomus*-A and B, as defined by Schwarzott et al. (2001)

al. 2002b), AMF diversity may be overestimated if smaller monophyletic groups are assumed as phylotypes. Furthermore, we used the Shannon–Weaver index calculated on the basis of the number of phylotypes per field.

A total of 14 AMF phylotypes were detected in onion roots, with one to six phylotypes per field. The total number of phylotypes and number of phylotypes per field were well in line with those obtained by Hijri et al. (2006) who studied AMF diversity in arable soils using the same primer set for AMF identification and a similar sampling effort. The most abundant phylotypes *Glo*A6 (associated with *G. mosseae–coronatum* species complex) and *Glo*A5 (*G. caledonium–geosporum* species complex) could be, respectively, phylotypes GLOM-A3 and GLOM-A4 in Hijri et al. (2006) and Appoloni et al. (2008). Phylotype *Glo*A3 was associated with *G. intraradices* and corresponds with GLOM-A1 in Hijri et al. (2006) and Sýkorová et al. (2007). Phylotype *Glo*A2 comprised the sequence AJ872051, which is representative of GLOM-A2 (Hijri et al. 2006).

Phylotype *Glo*A4 clustered with a sequence from naturally occurring *Botrychium virginianum* L. (Kovacs et al. 2007) and a *Glomus constrictum* sequence (Hijri et al. 2006). Phylotype *Arch*2 corresponds with phylotype ARCH-1 defined by Hijri et al. (2006) and ARCH-4 of Sýkorová et al. (2007), as these share the same *Archaeospora trappei* sequence and a sequence from a species of *Podocarpaceae*. Our *Paraglomus* sequences clustered in phylotype *Par*1 that corresponds to phylotype PARA-1 in Appoloni et al. (2008), as both clustered with the same sequence from *Zea mays*, except for our sequence PPO-ui_ARCH_7 which showed similarity to AJ564068 from *Dactylis glomerata* (Wirsel 2004).

Predominance of some *Glomus*-A phylotypes in AMF communities was in agreement with previous reports for agricultural lands. For example, analyzing SSU rDNA fragments, Helgason et al. (1998) found predominance of *G. mosseae* or closely related species, whereas Daniell et al. (2001) found predominantly *G. caledonium–G. geosporum*

Table 4 Means and their standard errors (±SE) of the number of phylotypes per field, and the Shannon–Weaver indices based on the relative abundance of phylotypes per field, for the cultivation systems and regions in 2004 and 2005

Diversity indices	Cultivation system	Survey 2004		Survey 2005
		Zeeland	Flevoland	Flevoland
Nr of phylotypes	Conventional	2.4±0.9	1.4±0.5	2.8±1.0
	Organic	2.6±0.5	2.6±2.1	2.7±0.7
Shannon index	Conventional	0.70±0.31	0.25±0.35	0.89±0.30
	Organic	0.66±0.22	0.66±0.69	0.88±0.20

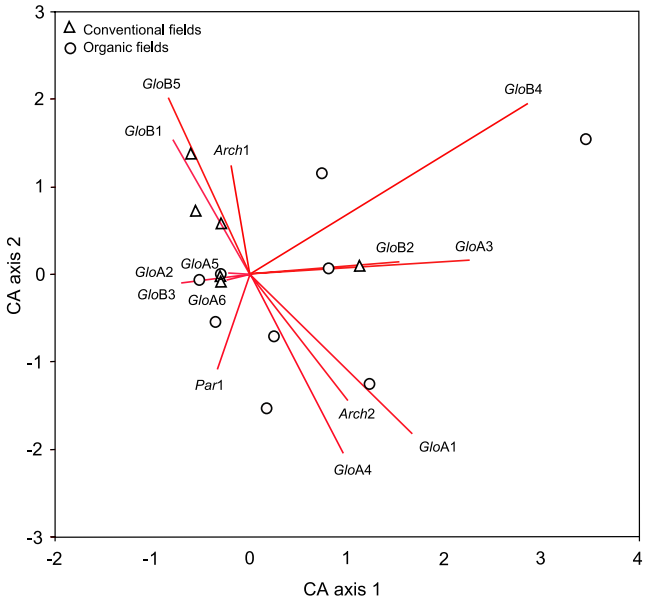


Fig. 4 Biplot for the first and second axis in the correspondence analysis for the number of AMF phylotypes in conventional onion fields (*open triangles*) and organic fields (*open circles*) in the Netherlands in 2004 and 2005. Some field positions overlap (same AMF species composition). AMF phylotypes are presented as vectors

sequences. The dominance of *Glomus*-A species was also reported in studies based on the morphology of spores, being *G. mosseae* as the most observed AMF species (Oehl et al. 2003; Cheng and Baumgartner 2004; Sjöberg et al. 2004; Wang et al. 2008). In other studies, however, *G. intraradices* was the dominant AMF species (Hijri et al. 2006; Mathimaran et al. 2005). The dominance of a few *Glomus*-A species is probably a consequence of the strong selection pressure imposed by agricultural practices leading to the predominance of fast root-colonizing species (Oehl et al. 2004) and species able to tolerate, among others, the repeated disruption of external hyphal networks, periods without mycorrhizal host plants, and the application of fertilizers and fungicides (Gosling et al. 2006).

In contrast, other AMF phylotypes were found at very low frequencies, which make it difficult to establish associations between farming systems with specific phylotypes or community composition. While some *Glomus*-B, *Paraglomus*, and *Archaeospora* phylotypes were occasionally found in some Dutch farm fields, neither *Acaulospora* nor *Scutellospora* rDNA sequences were detected at all. In previous studies, the presence of *Glomus*-B, *Archaeospora*, *Acaulospora*, *Scutellospora*, and *Paraglomus* species in arable lands was low in comparison to grasslands, or they were absent (Oehl et al. 2004; Hijri et al. 2006).

We found that AMF diversity in organically grown onions did not differ from conventional ones, with a considerable overlap between farming systems in the canonical analysis. This result is in contrast with Oehl et al. (2003) who reported that AMF diversity in organic fields (13–18 AMF species) took an intermediate position between conventional fields (eight to ten) and grasslands (20–25). However, Oehl and co-workers analyzed the morphology and number of AMF spores in soil samples and trap cultures, and therefore, they analyzed a broader part of the AMF species richness than the present research. Hijri et al. (2006) used an approach similar to our study, relying on the same primer set for AMF identification. Among four management systems established on the same loess soil (the DOK experiment), higher AMF biodiversity in organically managed plots than in conventional ones was observed, though not significantly. This trend is not in agreement with our results. Regional characteristics and specificities in AMF community composition may explain this difference. Furthermore, these authors compared a few fields at one location, whereas our study had a much broader setup, as we compared in total 20 organic and 19 conventionally managed fields belonging to commercial farms on clay and loess–clay soils.

Among the studied farmlands in the Netherlands, only a few had larger AMF diversity indices. This is in line with Hijri et al. (2006) who found, after studying two fields apart from the DOK experiment, that AMF diversity varied also within management systems depending upon the specific agricultural history. It was noteworthy, in the present study, that AMF diversity was larger in fields belonging to two farms sampled both years, in which onions were grown each year on different pieces of land within the farm. These observations suggest that farming system as such did not influence AMF diversity, but rather specific management practices or environmental conditions may contribute to the maintenance of more diverse AMF community in some farmlands. For instance, the continuous cultivation of mycorrhizal host crops in the rotations, or the use of green cover crops instead of fallow periods might favor the presence of more AMF species in the long term (Gosling et al. 2006; Hijri et al. 2006).

Onion yields were not correlated with AMF diversity indices. Nevertheless, the practical role of AMF diversity in agricultural systems should be further studied. First indications coming up from experimental setups in which mixed AMF species were applied as inocula point to the complexity arising from multiple fungi–host interactions. For instance, Van der Heijden et al. (2006) reported variable benefit in biomass increase from mixed inocula, whereas Jansa et al. (2008) reported only improved phosphorus acquisition in comparison to the same AMF species acting separately.

Colonization in onion roots

Onion roots in Dutch agricultural fields had high AMF colonization levels in comparison to levels reported for

naturally occurring AMF in other crops and environments (Ryan et al. 2000; Mäder et al. 2000). Although obtained at a single sampling date within a single growing season, our results revealed the abundance of AMF in agricultural soils in the Netherlands, including reclaimed lands.

As conventional farming in Zeeland had significantly lower AMF colonization levels compared to the other three management systems–region combinations (Table 2), the adoption of organic farming seemed to increase AMF colonization levels quickly. Indeed, the organic fields sampled in Zeeland were only 1 to 12 years taken out of conventional management. In contrast, mean colonization levels in Flevoland were high regardless of the cultivation system and not correlated with the number of years under organic management.

Furthermore, we found that colonization parameters were neither correlated with the concentration of readily available phosphorus in the soil, even when a trend toward a higher P content for conventional fields was observed (Table 1). Several authors reported larger AMF colonization for organically managed fields compared to conventionally managed fields. Among others, Ryan et al. (1994, 2000) found higher colonization levels in organically grown wheat and pastures than conventionally managed ones, whereas Mäder et al. (2000, 2002) reported higher colonization in organic winter wheat, vetch-rye, and grass-clover when comparing organic and conventional soil management systems. Similarly, Oehl et al. (2003) found higher colonization levels in plants grown on organic soils compared to conventional ones. In these studies, differences in colonization levels were explained mainly by the lower P concentration in organically managed soils, which was not clearly the case for the onion fields in the present research.

A striking finding of our survey was the significant correlation between colonization parameters of naturally occurring AMF and onion yields in conventionally managed onion farmlands, under moderate to high concentrations of readily available phosphorus (Table 1, Fig. 1). Organically managed fields followed the same trend as conventional ones, but the correlation coefficient was considerably lower and not significant for AC. The slope of both linear regression lines was 0.38 indicating that onions benefited similarly from AMF in both management systems. Alternatively, it could be speculated that AMF colonization and onion yields benefit simultaneously from yet unidentified environmental conditions. Anyhow, in order to practically exploit the fact that 10% increase in AMF colonization may represent an average yield increase of 3.8 tons·ha^{-1}, it would be necessary to better understand farm management practices or environmental factors leading to higher vitality of indigenous AMF.

Concluding remarks

This study did not demonstrate differences in biodiversity of AMF colonizing onion roots grown under organic and conventional cultivation, and this result raises at least two important questions. Firstly, is the lack of differences in AMF community composition between cultivation systems due to the fact that nutrient levels (especially phosphorus) are moderate to high in organic fields? If this is the case, it is uncertain that a further continuation under organic cultivation (with a concomitant decrease in soil nutrient levels) would increase mycorrhizal fungal diversity over a longer temporal scale.

Secondly, the positive correlation between mycorrhizal colonization and onion yield established for conventional fields suggests a mycorrhizal benefit even at high nutrient availability. It is therefore tempting to speculate that onion, with its depauperate root system, depends on mycorrhiza even at high nutrient levels, thereby preventing the selection for reduced functioning of the symbiosis under agricultural intensification postulated by Johnson (1993) and Kiers et al. (2002). Breeding onions with improved rooting system while retaining a diversity of mycorrhizal benefits would be an urgent further research step. Otherwise, onion cultivation under nutrient-poor conditions might result in yields that are too low to be economically attractive. In this context, the research of De Melo (2003) on the genetic basis of traits describing the *Allium* rooting system can be seen as a promising first step.

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