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# Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils

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## Summary

- The impact of various agricultural practices on soil biodiversity and, in particular, on arbuscular mycorrhizal fungi (AMF), is still poorly understood, although AMF can provide benefit to plants and ecosystems. Here, we tested whether organic farming enhances AMF diversity and whether AMF communities from organically managed fields are more similar to those of species-rich grasslands or conventionally managed fields.
- To address this issue, the AMF community composition was assessed in 26 arable fields (13 pairs of organically and conventionally managed fields) and five semi-natural grasslands, all on sandy soil. Terminal restriction fragment length polymorphism community fingerprinting was used to characterize AMF community composition.
- The average number of AMF taxa was highest in grasslands (8.8), intermediate in organically managed fields (6.4) and significantly lower in conventionally managed fields (3.9). Moreover, AMF richness increased significantly with the time since conversion to organic agriculture. AMF communities of organically managed fields were also more similar to those of natural grasslands when compared with those under conventional management, and were less uniform than their conventional counterparts, as expressed by higher β-diversity (between-site diversity).
- We suggest that organic management in agro-ecosystems contributes to the restoration and maintenance of these important below-ground mutualists.

## Introduction

Intensive agriculture has contributed substantially to the increase in food production over the past 50 yr (Foley *et al.*, 2005). This has been achieved by the application of fertilizers, pesticides, irrigation and high-yielding crop varieties. However, it is being increasingly recognized that some of these agricultural practices have a negative impact on the environment, resulting in reduced biodiversity, the eutrophication of surface waters and the accumulation of pesticides (Tilman *et al.*, 2002). Organic agriculture has been advocated as a potential strategy to mitigate against the loss of biodiversity in agricultural landscapes (McLaughlin & Mineau, 1995), as it is thought to mimic the natural ecosystem better via a richer crop rotation and a lack of synthetic pesticides and inorganic fertilizers.

Initial empirical evidence indicates that organic farming has a positive effect on the diversity of several organism groups, including carabids (Mäder *et al.*, 2002), birds and predatory insects (Bengtsson *et al.*, 2005), and earthworms and flora (Hole *et al.*, 2005). So far, however, only a few comparative studies have investigated whether organic

farming affects soil microbial biodiversity. Soil-borne biodiversity has been put forth as an important component of soil health, potentially enhancing plant productivity and ecosystem sustainability (Brussaard *et al.*, 2007; van der Heijden *et al.*, 2008), as has been observed for above-ground biodiversity (Tilman *et al.*, 1996; Hooper *et al.*, 2005). Here, we focus on the diversity of arbuscular mycorrhizal fungi (AMF), an abundant group of below-ground mutualists that is thought to play an important role in agricultural ecosystems by supplying limiting nutrients to plants (Jeffries *et al.*, 2003; Gosling *et al.*, 2006).

AMF form symbiotic relationships with the majority of land plants, including many crops (Smith & Read, 2008). All AMF are obligate plant symbionts, and generally provide nutrients, especially phosphorus, to plants in exchange for plant carbohydrates (Smith & Read, 2008). Moreover, AMF can provide protection against pathogens (Sikes *et al.*, 2009) and drought (Augé *et al.*, 2001). In addition, AMF have been shown to enhance the sustainability of ecosystems by improving the soil structure (Wilson *et al.*, 2009), and by reducing nutrient losses after rain-induced leaching events (van der Heijden, 2010). AMF species have been shown to be functionally distinct, in that some taxa provide disease resistance and others enhance nutrient supply to plants (Maherali & Klironomos, 2007). Different AMF species are also active at different times of the year (Merryweather & Fitter, 1998), and different plant species benefit from different AMF (Ravnskov & Jakobsen, 1995). These observations imply that diverse AMF communities may enhance plant productivity and ecosystem functioning as observed in several studies (van der Heijden *et al.*, 1998; Vogelsang *et al.*, 2006).

Among ecosystems in which AMF have been studied, agricultural sites have typically been the poorest in AMF diversity (Oehl *et al.*, 2003; Öpik *et al.*, 2006), although AMF abundance can be high (Treseder & Cross, 2006) and specific outcomes depend strongly on the study site (compare, for example, Helgason *et al.* (1998), Daniell *et al.* (2001) and Hijri *et al.*(2006)). In small-scale direct comparisons, organic management has been shown to enhance AMF richness (Oehl *et al.*, 2003; Hijri *et al.*, 2006) and colonization levels (Bending *et al.*, 2004), but it is not yet known whether organic management, in general, stimulates AMF diversity or abundance. Furthermore, it is not known whether organic management regimes lead to AMF communities that are more similar to natural assemblages, or whether such practices merely lead to AMF communities that are distinct from those found under conventional agriculture. Such data are critical for evaluating the impact of agricultural management on AMF communities in the light of sustainability goals.

Therefore, in order to interpret the extent and direction of AMF responses to different agricultural practices, it is essential to compare these responses against natural ecosystems. We hypothesized that a transition from conventional to organic farming would drive AMF communities to resemble those of (semi-) natural ecosystems, in terms of community structure and diversity, as a result of more frequent crop rotation and the discontinuation of mineral fertilizer and synthetic pesticide use.

We tested whether organic farming enhances AMF diversity and whether AMF communities from organically managed fields are more similar to those of species-rich natural grasslands or conventionally managed fields. Moreover, we aimed to investigate whether AMF species' richness increases with the duration of organic management. To test these hypotheses, we used genetic fingerprinting, targeting nuclear large subunit rRNA genes, to determine the composition and species' richness of AMF communities in roots across 13 pairs of adjacent organically and conventionally managed fields distributed throughout the Netherlands. Resulting AMF community patterns were compared with those determined for several undisturbed, semi-natural species-rich grasslands, which served as references of more 'natural' environments.

## Materials and Methods

### Study sites

Thirty-one field sites were examined within this study, representing 26 agricultural fields and five semi-natural grasslands. Half of the agricultural sites were managed organically and the other half conventionally. The sites were distributed throughout the Netherlands (Supporting information Fig. S1). All sites were on sandy soils, and cropped either with maize (*Zea mays* L.) or potato (*Solanum tuberosum* L.). The cropping season started in April 2007 and samples were collected between July 16th and July 29th, 2007. Near each organic field (< 2 km distance) a conventional field with the same crop was also selected. This pairing of close-by organic and conventional fields was performed to ensure that climate and soil conditions were approximately the same per pair of fields. All 16 maize fields were additionally sampled between September 17th and September 22nd, 2007. Five pairs of conventional and organic maize fields sampled in 2007 were sampled again in July and September 2008 (Table S1). Interviews with farmers with regard to their management practices revealed that all sites had been tilled in spring 2007, and all conventional farmers regularly applied herbicides and mineral fertilizers. In addition, all conventional potato farmers sprayed against potato blight.

(Semi-) natural grasslands were also on sandy soil and have been mown once a year or received irregular grazing by horses or cows (Notes S1 for plant species' lists). Grasslands have not been fertilized, except for one site (site 5 in Notes S1) which has been moderately fertilized yearly

with bovine slurry. This grassland was also cut twice a year. Geographical coordinates of all agricultural sites and grasslands are shown in Table S1.

Soil was sampled as follows: six cores (core length, 14.5 cm; diameter, 6.5 cm) were taken, evenly distributed within a hectare of a field, each adjacent (± 5 cm distance) to a standing plant, except for grasslands in which cores were taken from vegetation patches. Care was taken not to include weeds in order to compare only AMF colonizing the crop under study. The cores were pooled and stored at 4°C at the end of each sampling day. In parallel, soil for chemical analysis was taken with the same gauge adjacent to each root sample and subjected to chemical analysis by BLGG (Bedrijfslaboratorium voor Grond- en Gewasonderzoek; Oosterbeek, the Netherlands). The chemical measurements included: pH, cation exchange capacity, mineral nitrogen, organic matter content and plant available phosphate ($CaCl_2$ extraction). The sampling intensity of six cores was tested for the sufficiency of describing the AMF community of fields (Notes S2).

Roots were chosen as the subject of AMF community analysis as they probably represent the active AMF community (Hijri *et al.*, 2006), as opposed to resting spores present in soil. Within a few days after sampling (< 5 d), the crop roots from the pooled soil samples were washed and cut into small pieces (± 1 cm), mixed and surface dried with a paper towel. Thick roots (> 1 mm) were excluded from further analysis, as primarily fine roots are colonized by AMF (Guo *et al.*, 2008). Roots were freeze-dried overnight and stored at −20°C before further use. The percentage of root length colonized was determined using the modified line intersection method (McGonigle *et al.*, 1990) for all maize and grassland samples collected in 2007.

## DNA extraction and analysis

Dried root samples (± 50 mg; in duplicate) were ground in a microcentrifuge tube using a 4-mm glass bead in an FP120 (ThermoSavant, Holbrook, NY, USA) bead beater (4.0 m $s^{-1}$, twice for 10 s). DNA was extracted from the resulting powder using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA extractions and analyses were performed in duplicate. DNA extracts were diluted 10× in PCR-grade water before nested PCR (see below).

AMF community structure was determined by terminal restriction fragment length polymorphism (T-RFLP) of PCR-amplified large subunit rRNA gene fragments, as described by Mummey & Rillig (2007). This procedure involves nested PCR with the primer pair LR1–FLR2 (van Tuinen *et al.*, 1998; Trouvelot *et al.*, 1999) and a nested reaction with the AMF-specific primer pair FLR3–FLR4 (Gollotte *et al.*, 2004), which in our set-up were labelled with fluorescent dyes 6-FAM (FLR3) and VIC (FLR4). Thermal cycling was performed as in Mummey & Rillig (2007), except that the first PCR was run for 30 cycles, and the product of this PCR was diluted 500× before a second PCR. After the second PCR, 5 μl of product was cleaned up by incubation with 1 U of shrimp alkaline phosphatase (USB, Cleveland, OH, USA) for 1 h at 37°C, followed by 15 min at 65°C for shrimp alkaline phosphatase denaturation, and subsequent restriction digestion (6 h at 65°C with TaqI). Digests were purified by sodium acetate and ethanol precipitation and diluted in 30 μl of $H_2O$. Of this solution, 0.5 μl was added to 9.4 μl HiDi formamide (Applied Biosystems; ABI, Nieuwekerk a/d IJssel, the Netherlands) and 0.1 μl GeneScan 500LIZ sizer (ABI), denatured and run on an ABI 3100 automated sequencer (POP4 polymer and 36 cm capillaries). In cases in which the strongest peaks in the electropherograms were not in the range 2000–8000 relative fluorescence units (rfu), the sample concentration was adjusted accordingly and the sample was rerun.

## Terminal restriction fragment (T-RF) size calling and binning

Despite the cleanup of products, a relatively broad binning procedure was necessary because of irregular final adenosine incorporation by Taq polymerase and stutters resulting from residual polymerase activity acting on the 5′ overhang left after TaqI digestion (Hartmann *et al.*, 2007). The threshold for peak recognition was set at 50 rfu. The resulting profiles were subjected to the following procedure: (1) exclusion of peaks < 43 bp; (2) size binning of peaks within a size range of 1.2 bp, and assignment to the maximal peak size to account for A incorporation; (3) joining of bins < 2.3 bp apart and ascribing to the lowest size to account for 5′ overhang fillings by the residual Taq polymerase activity; (4) peak area calculation within the bins; peaks contributing < 1% to the total area were excluded to achieve the same sensitivity across runs with different total fluorescence; (5) calculation of a consensus T-RF profile from the duplicate sample analyses. Consensus profiles were obtained by applying the same binning procedure as under point (3). The resulting T-RF profiles were uploaded to the T-REX web application (Culman *et al.*, 2009) for final dataset-wide T-RF binning with a clustering threshold of 1.2 bp. On average, 60% of peaks, representing 85% of total profile peak area, were reproducibly found between the two duplicate DNA extractions. Peaks of relatively low intensity, in particular, were variable among duplicates, stressing the importance of duplicate extractions.

## Phylogenetic analysis

Seventy-one AMF sequences were obtained from a pool of PCR products (FLR3–FLR4 region) recovered from eight

fields, chosen for having rich and representative T-RFLP patterns. Randomly selected clones (pGEM®-T Vector System, Promega), containing a correctly sized PCR amplicon as insert, were sequenced to cover all different FLR3–FLR4 fragment lengths. The resulting sequences (GenBank accession numbers FN643081–FN643151) were subjected to phylogenetic analysis, together with 118 reference sequences from GenBank and four sequences from environmental spore samples of another study in the Netherlands (GenBank accession numbers GU322900–GU322903).

Sequences were aligned using the ClustalW algorithm and a neighbour-joining distance tree was constructed in MEGA version 4 (Tamura *et al.*, 2007) with 1000 bootstrap replicates. Terminal clades were named following the taxonomy of Schwarzott *et al.* (2001). All sequences were subjected to *in silico* restriction digestion with the program REMA (Szubert *et al.*, 2007) to predict the fragment sizes of each double-labelled sequence fragment. Using this procedure, predicted fragment lengths were calculated, taking the differences in migration caused by dye properties (VIC vs 6-FAM) into account. These *in silico* RFLP analyses were performed to putatively assign the most common T-RF pairs to specific sequences (AMF types). This procedure was only performed for T-RF pairs that were specific for a certain sequence. Related sequences having approx. 2 bp differences in T-RF sizes were considered to be the same, and this criterion was also used to match the observed T-RFs to sequences.

## Data analysis and statistics

AMF richness was assessed as the number of T-RF lengths per field divided by two, as each sequence theoretically gives rise to two peaks, assuming that both the 5′ and 3′ peaks are within the size range of detection. Similarly, one AMF type corresponds to two T-RFs: one 5′ end and one 3′ end fragment in the T-RFLP analysis. Crop rotation scores for each field were calculated as follows: the number of times a crop was grown in the previous 4 yr divided by four, yielding a number between zero (crop has been absent for the last 4 yr) and unity (repeated monoculture). The rotation score was calculated for all of the following crops: maize, potato, forage crops (either grass or a grass–clover mixture) and cereals (see Table 1 for rotation scores and soil chemical analyses, and management characteristics such as ploughing, herbicide and other pesticide usage).

The relationship between organic farming and AMF richness was analysed by linear regression using the number of years from conversion to organic management as a predictor of AMF richness. This regression was performed using data of organic farms only and data of both organic and conventional farms (where the number of years from conversion to organic management was zero for conventional farms). The first analysis with only organic farms was performed to ensure that there was no potential effect of management type on the outcome of the analysis.

In order to assess the diversity of AMF across fields (between-field diversity, or β-diversity) of treatments, we drew rank–abundance plots of the T-RFs (Whittaker, 1965). When a large proportion of total diversity is shared among sites, there is low β-diversity (Loreau, 2000), which is reflected in a steep decrease in rank–abundance. By contrast, when different sites contain different AMF, there is large between-site diversity, and subsequently there is no steep decrease in rank–abundance. Rank–abundance plots

**Table 1** Means (+SE) of several crop rotation, soil and management variables characteristic for organic and conventionally managed fields planted with maize or potato or for semi-natural grassland

| | | Maize | Maize | Potato | Potato | |
|---|---|---|---|---|---|---|
| | Crop management | Organic | Conventional | Organic | Conventional | Natural |
| Rotation variables | Grass in rotation | 0.32 (0.09) | 0.19 (0.12) | 0.45 (0.12) | 0 (0) | |
| | Maize in rotation | 0.42 (0.09) | 0.73 (0.13) | 0 (0) | 0.56 (0.21) | |
| | Potato in rotation | 0.03 (0.03) | 0.04 (0.04) | 0 (0) | 0.13 (0.07) | |
| | Wheat in rotation | 0.23 (0.13) | 0.04 (0.04) | 0.48 (0.07) | 0.06 (0.06) | |
| Soil variables | pH (KCl) | 5.1 (0.17) | 4.8 (0.16) | 4.9 (0.16) | 4.6 (0.21) | 5.0 (0.12) |
| | minN (kg N $ha^{-1}$) | 70 (21) | 48 (5) | 67 (11) | 46 (10) | 53 (9) |
| | P (mg P $kg^{-1}$) | 6.8 (1.2) | 8.1 (1.8) | 6.1 (3.3) | 6.3 (1.8) | 1.2 (0.5) |
| | OM (%) | 3.5 (0.3) | 3.1 (0.2) | 3.4 (0.4) | 2.5 (0.5) | 7.1 (1.4) |
| Management variables | Herbicides | No | Yes | No | Yes | |
| | Other pesticides | No | No | No | Yes | |
| | Ploughed | Yes | Yes | Yes | Yes | |

To describe different crop rotation schemes, the frequency with which a particular crop had been grown in the previous 4 yr is given. Management variables were uniform within groups (e.g. 'Yes' means that all fields of a specific crop management type were ploughed or received pesticides). 'Other pesticides' only included spraying against potato blight in this study. Soil variables did not differ significantly from each other between organic and conventional fields for either maize or potato. The rotation variable ''maize in rotation'' was significantly higher in conventional fields for both crops, and ''grass in rotation'' and ''wheat in rotation'' were significantly lower for conventional potato fields. OM, organic matter.

were drawn for each management type (organic farming, conventional farming and grassland). The rank number was determined as follows: the proportion of fields in which a given T-RF was found was determined for each management type, expressed as a percentage and $\log_{10}$ transformed. T-RFs present in the largest number of fields were assigned the first rank, and those that were present in only one field were assigned the lowest rank (highest rank number). To determine the difference in rank–abundance relationships between the three management types, a linear regression was applied to the log-transformed abundance data and the slopes were compared.

Principal component analysis (PCA) was performed to compare the AMF communities from the fields under the different agricultural management treatments using the T-RFs (AMF types) of each field as input data. PCA was executed for all samples together, as well as for the first sampling (July 2007) only, to prevent potential bias caused by repeated sampling of some fields. One sample was excluded from the analysis (first sampling of one organic maize field) because it only contained two T-RFs that were both unique in that dataset, and therefore strongly skewed the ordination plot, even though the pattern stayed the same. The scores of the first principal component axis were compared using Mann–Whitney *U*-tests, which take unequal variance into account. Two-sided, tie-corrected probabilities are reported, unless otherwise stated. In order to test whether a meaningful gradient for each management type was present, a Jonckheere–Terpstra test (Jonckheere, 1954) was performed with a hypothesized order of conventional–organic–natural. Correlation analysis (Spearman's rank) was performed between PCA axes 1 and 2 and the different environmental variables. All statistical analyses were carried out in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA), except for PCA which was performed in PAST (Hammer *et al.*, 2001).

## Results

### Effect of crop and agricultural management on AMF richness

The average root length colonized by AMF was similar between organic and conventional fields with relatively large variation within groups. Means ± SE were as follows: July 2007: organic, 33 ± 7.1%; conventional, 31 ± 6.0%; September 2007: organic, 39 ± 6.2%; conventional, 32 ± 7.0%. Colonization of semi-natural grasslands was higher than arable fields: 57 ± 5.7%. Overall, an estimated total of 42 AMF types (see the Material and Methods section for AMF richness estimation) were found in the whole dataset and, on average, 5.2 AMF types per field site.

Roots of crop plants from organically managed fields had a significantly higher AMF richness in September 2007 and for both dates in 2008 (Fig. 1) when compared with that of conventionally managed fields. In September 2007, the AMF richness was, on average, 1.6 times higher in organic vs conventional fields (average ± SE: 6.4 ± 0.6 vs 3.9 ± 0.9). In 2008, organic fields had an AMF richness that was more than twofold higher than that of conventional fields (July: 7.9 ± 1.0 vs 3.6 ± 1.3; September: 6.9 ± 0.9 vs 3.3 ± 1.1). For the July 2007 sample, organically and conventionally managed potato and maize fields were not significantly different from each other in terms of the number of AMF types (potato, 6.0 ± 1.1 vs 3.6 ± 0.6; maize, 5.4 ± 1.0 vs 4.7 ± 0.3). Semi-natural grasslands had the highest average AMF richness (8.8 ± 0.8).

A significant positive effect of organic agricultural management on AMF richness was also observed when the AMF richness was regressed against the time since conversion to organic management (Fig. 2). The duration of organic management explained 15–50% of the variance in AMF richness (Fig. 2). This relationship was also significant

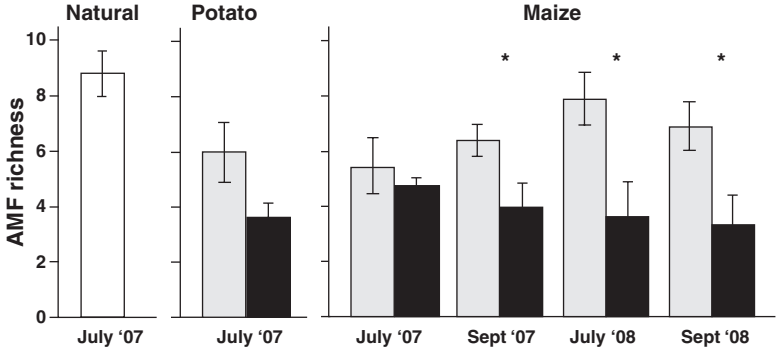


**Fig. 1** Mean arbuscular mycorrhizal fungal (AMF) richness ± SE in grasslands (natural) and in organic (grey bars) and conventional (black bars) potato and maize fields sampled at various sampling dates (July '07, July' 08, Sept. '07 and Sept. '08). The significance ($P < 0.05$) of pair-wise comparisons of organic vs conventional treatments is indicated by an asterisk. July 2007: maize ($n = 16$; $t = 0.703$; $P = 0.501$); potato ($n = 10$; $t = 1.986$; $P = 0.082$); September 2007: maize ($n = 16$; $t = 2.353$; $P = 0.034$); July 2008 ($n = 10$; $t = 2.722$; $P = 0.026$); September 2008 ($n = 10$; $t = 2.595$; $P = 0.032$). AMF richness is assessed as the number of forward and reverse terminal restriction fragments (T-RFs) divided by two.

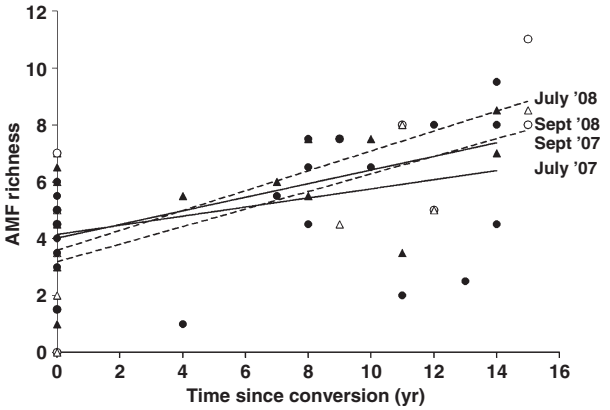


**Fig. 2** Relationship between time since conversion to organic management and arbuscular mycorrhizal fungal (AMF) richness. Linear regressions are shown for each sampling occasion separately, as repeated sampling was on the same sites and thus not independent. Lines represent: July 2007 (July'07, closed circles), $y = 4.15 + 0.16x$, adjusted $R^2 = 0.150$, df = 24, $F = 5.43$, $P = 0.029$; September 2007 (Sept'07, closed triangles), $y = 4.02 + 0.24x$, adjusted $R^2 = 0.255$, df = 14, $F = 6.13$, $P = 0.027$; July 2008 (July'08, open circles), $y = 3.58 + 0.35x$, adjusted $R^2 = 0.465$, df = 8, $F = 8.81$, $P = 0.018$; September 2008 (Sept'08, open triangles), $y = 3.18 + 0.31x$, adjusted $R^2 = 0.500$, df = 8; $F = 9.99$, $P = 0.013$. AMF richness is assessed as the number of forward and reverse terminal restriction fragments (T-RFs) divided by two.

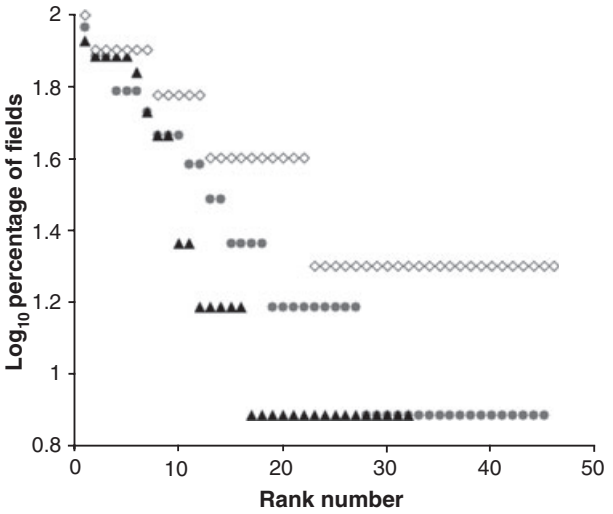


**Fig. 3** $Log_{10}$ rank–abundance plots of the frequencies in which particular arbuscular mycorrhizal fungal types (measured as terminal restriction fragments; T-RFs) were found across replicate fields for organic ($n = 13$; circles) and conventional ($n = 13$; triangles) agricultural management and semi-natural grasslands ($n = 5$; diamonds) in July 2007. The number of sites at which a given T-RF was present is divided by the total number of sites under the respective management type. T-RFs represent different AMF sequences.

when conventional fields (time since conversion = 0) were excluded from the regression analysis and all crop/sampling combinations were analysed together ($y = 2.93 + 0.33x$; adjusted $R^2 = 0.181$; df = 29; $F = 7.64$; $P = 0.01$).

The rank–abundance plot (Fig. 3) showed that natural sites had the shallowest slope, followed by organic and then conventional agricultural management. This indicates that conventional sites have many of the same AMF across sites, whereas organic and natural sites harbour more diverse AMF assemblages, and thus have larger between-site diversity. The 95% confidence intervals of the fitted slopes did not overlap, yielding the following values: conventionally managed fields, −0.046 to −0.033; organically managed fields, −0.029 to −0.024; natural fields, −0.019 to −0.014. Conventional, organic and natural sites had AMF richness values of 16, 22.5 and 23, respectively, in July 2007. Natural sites thus had the highest number of T-RFs even though only five sites were sampled, compared with 13 sites for organic and conventional fields. The 95% confidence intervals of the rank–abundance slopes for organically and conventionally managed fields also hardly overlapped, when separately analysed for each crop type and sampling time (Table S2).

From a total of 67 samples, only three failed to produce PCR products in duplicate DNA extractions. Two of these came from the same conventional maize site at different sampling times, September 2007 and July 2008, and the other sample, taken in September 2008, originated from a conventional potato field where maize had been grown in 2007. These samples produced PCR product in the first reaction, with the general fungal PCR primers (LR1–FLR2), and so the negative results were not caused by the inhibition of PCR.

## Effect of crop and agricultural management on AMF community structure

AMF community structure was compared across the different field types (crop/management/season combination) by PCA, and the means for each field type for the first and second PCA axes are shown for July 2007 (Fig. S2) and for all sampling times together (Fig. 4). In July 2007, the AMF assemblages of conventionally managed potato and maize fields were separate from those of semi-natural grasslands, with those from organically managed maize and potato fields falling in between (Fig. S2). The separation of the three management types along the first PCA axis suggests that agricultural management may explain a large degree of the differences among the AMF assemblages analysed. A Mann–Whitney *U*-test showed that only the AMF assemblages from conventional maize ($Z = -2.20$; $P = 0.028$) and potato ($Z = -2.20$; $P = 0.028$) fields differed significantly from natural sites, whereas the organic fields did not (maize: $Z = -1.38$; $P = 0.167$; potato: $Z = -0.52$; $P = 0.602$). Scores for the AMF assemblages from organic sites also differed significantly from those of the conventional sites ($Z = -2.12$; $P = 0.034$). The gradient of PC1

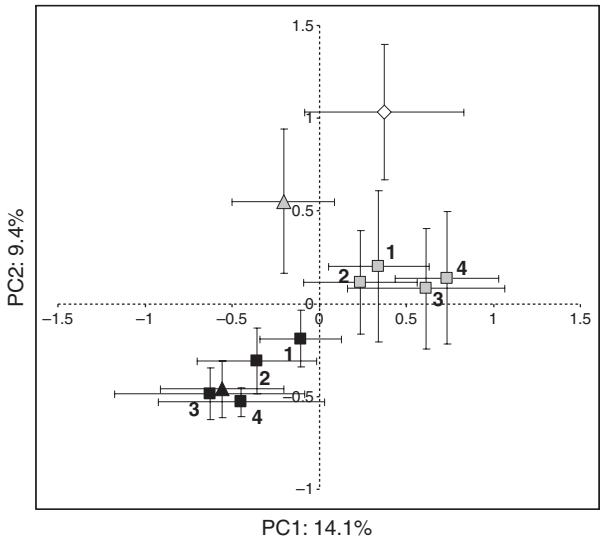


**Fig. 4** Means ± SE of crop/management combination scores for the first two principal component axes, with terminal restriction fragment (T-RF) presence/absence as dependent variable for all sampling times, as indicated next to the symbols for maize data (1, July 2007; 2, September 2007; 3, July 2008; 4, September 2008). Black symbols, conventional sites; grey symbols, organic sites; white symbols, semi-natural sites. Triangles, potato; squares, maize; diamonds, grasslands. T-RFs represent different arbuscular mycorrhizal fungal (AMF) sequences.

scores for AMF assemblages from conventional, to organic, to natural sites was highly significant according to the Jonckheere–Terpstra test (SD = 2.937; $P$ = 0.001; $n$ = 30).

For all sampling times together, no statistical tests were performed, because maize fields were overrepresented compared with potato and natural sites. Nevertheless, the same trend appeared in the data with small shifts between sampling times and field management schemes (Fig. 4). In particular, the AMF assemblages from the conventional maize fields separated well from the organic and natural fields at the second sampling (September 2007).

Several environmental variables (Table 1) were also examined as potential explanatory factors for differences in AMF community structure between management types. The variables with the highest differences between the various management types were the frequency of different crops in crop rotation, as well as mineral nitrogen and phosphorus availability. However, there were no significant differences between organic and conventional fields for any of the soil variables (Table 1). Correlations between these environmental variables and the PCA scores were performed to find trends in the data, independent of agricultural management technique. The first principal component axis of Fig. S2, which shows a trend from conventional to organic management to grasslands, showed a significant correlation with the frequency of maize in rotation (Spearman's $\rho = -0.45$; $P = 0.03$), the frequency of grass in rotation ($\rho = 0.41$; $P = 0.04$) and phosphorus availability ($\rho = -0.50$; $P = 0.01$). This suggests that previous crops and phosphate availability are predictors of the observed differentiation among the AMF assemblages (Figs 4, S2). For the second PCA axis of Fig. S2, significant correlations were found for organic matter content ($\rho = -0.42$; $P = 0.02$) and soil mineral nitrogen concentration ($\rho = -0.40$; $P = 0.045$).

## Identities of AMF and relationship with environmental parameters

The most frequently found AMF type in our dataset, *Glomus* Ab2 (5′, 182–184 bp; 3′, 44 bp; Table 2, Fig. S3), corresponded to a taxon affiliated with the species' group containing *Glomus intraradices*, which occurred in 79% of all root samples. The next most common AMF type, *Glomus* Aa5 (5′, 182–184 bp; 3′, 184 bp), corresponded to *Glomus mosseae*, with an overall occurrence of 70%, followed by the AMF type *Glomus* Aa1 (5′, 182–184 bp; 3′, 70 bp) for *Glomus coronatum*, which occurred in 67% of all samples. All other types occurred in < 50% of all samples. Several AMF types showed strong correlations with environmental variables, suggesting that these may correspond with habitat-specific AMF ecotypes. The strongest relationships were found with crop rotation variables (*Glomus* Aa1, *Glomus* A4 and *Glomus* Ab1) and pH (*Glomus* Ab2, *Glomus* Aa5, *Glomus* B1_1, *Glomus* B1_3 and *Glomus* B1_2). In addition, some AMF types correlated with soil phosphate availability (Clade B), mineral nitrogen concentration (*Glomus* B1_2) or soil organic matter content (*Glomus* Aa2; Table 2).

All partial nuclear large subunit rDNA sequences obtained in this study had closest BLAST hits with sequences of members of the Glomeromycota to which AMF belong. Sixty of 71 sequences obtained in this study fell within the order of Glomerales (Fig. S3). The other 11 sequences ('Clade B') did not show specific affiliations to any of the known orders, although they grouped most closely with members of the Diversisporales (i.e. Gigasporaceae, Acaulosporaceae and Diversisporaceae). The exact placement of these sequences remains to be clarified until large subunit sequences are available for at least all AMF families. *In silico* restriction digestions showed that T-RFLP patterns of the different sequences were distinguishable when using the restriction endonuclease TaqI. The occurrences of specific AMF types are shown in Table 2, together with correlations with environmental variables. These AMF types are composed of 32 out of 83 unique T-RFs found, which means that a considerable number of T-RFs are not represented. However, these AMF types covered between 78% and 96% of the total number of T-RFs in each group of fields (Table 2), indicating that the remaining fragments are relatively rare.

## Discussion

Our results show that (1) AMF diversity is higher under organic farming, and AMF richness increases significantly with time since conversion to organic farming, (2) AMF communities in conventionally managed farms are more distinct from those of undisturbed, semi-natural grassland than are those in organically managed fields, and (3) AMF between-site diversity (β-diversity) is higher among organically managed fields than among conventionally managed fields.

In many countries, schemes for sustainable land use have been implemented with an aim to protect biodiversity (Kleijn *et al.*, 2001; Billeter *et al.*, 2008). According to our results, organic farming appears to be a suitable agricultural management strategy with respect to beneficial effects on AMF biodiversity. Moreover, the fact that AMF communities of organically managed sites were more similar to those of semi-natural grasslands than to those of conventionally managed sites indicates that AMF assemblages have the potential to recover to their natural state after years of intensive agriculture. This is also indicated by the increase

**Table 2** Average abundance of specific arbuscular mycorrhizal fungal (AMF) types as a percentage of the total in each crop/management/time combination for 2007

| | Grasslands | 2007 July Maize Organic | 2007 July Maize Conventional | 2007 Sept Maize Organic | 2007 Sept Maize Conventional | 2007 July Potato Organic | 2007 July Potato Conventional | Environmental variable | | | | | | T-RFLP fragment | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AMF type** | | | | | | | | **Grass** | **Maize** | ***P*** | **pH** | **minN** | **OM** | 5'T-RF(bp) | 3'T-RF(bp) |
| G. Ab2 | | | | | | | | | | | **0.53 | | | 182, 184 | 44, 46 |
| G. Aa1 | | | | | | | | | *0.42 | | | | | 182, 184 | 70 |
| G. Aa5 | | | | | | | | | | | **0.50 | | | 182, 184 | 184 |
| G. B1_3 | | | | | | | | | | | *0.46 | | | 49, 51 | 212 |
| G. A4 | | | | | | | | *0.45 | *–0.49 | | | | | 45, 46 | 138 |
| G. B1_1 | | | | | | | | | | | **0.54 | | | 147 | 212 |
| G. Ab1 | | | | | | | | *0.47 | *–0.40 | | | | | 196 | 49,51 |
| Clade b | | | | | | | | | | *–0.40 | | | | 216, 215 | 84 |
| G. Aa2 | | | | | | | | | | | | | *–0.40 | 68 | 70 |
| G. B2_2 | | | | | | | | | | | | | | 333 | 329, 331 |
| G. Aa3 | | | | | | | | | | | | | | 182, 184 | 173, 174 |
| Gig. | | | | | | | | | | | | | | 86 | 84 |
| G. A1 | | | | | | | | | | | | | | 142 | 133 |
| G. B2_1 | | | | | | | | | | | | | | 365 | 362 |
| G. B1_2 | | | | | | | | | | | *0.42 | *–0.42 | | 150 | 212 |
| **Percentage covered** | 78 | 82 | 93 | 92 | 96 | 82 | 81 | | | | | | | | |
| *n* | 5 | 8 | 8 | 8 | 8 | 5 | 5 | | | | | | | | |

Shading from black to white (■, >80%; ■, 60–80%; ■, 40–60%; ■, 20–40%; ■, 0–20%; □, absence) indicates the relative abundance of AMF types across sites within a specific treatment. Each AMF type is characterized by a specific terminal restriction fragment (T-RF) pair. The last two columns of this table show the length (bp, base pairs) of the 5′ and 3′ end DNA fragment sizes affiliated to each AMF type (first column). When several T-RF pairs fell within the same higher order phylogenetic group, a further identity number was added after the group identifier (see Supporting Information Fig. S3). At the base, the percentage of all peaks covered by the depicted AMF types is given (percentage covered). For each AMF type, significant Spearman's ρ correlations with environmental variables and crop rotation indicators are shown for July 2007, excluding the semi-natural grassland sites. OM, organic matter. *, $P < 0.05$; **, $P < 0.01$.

in AMF richness with time since conversion to organic farming. Organic farming may thus sustain the mycorrhizal component of soil health and ecosystem functioning (Moonen & Bàrberi, 2008; Shennan, 2008).

The positive effect of organic management on AMF diversity could be explained by several factors. First, organic farm management utilizes a higher frequency of crop rotation with a grass–clover mixture as a forage crop. The inclusion of legumes in crop rotations has been shown to have a positive effect on several soil parameters (Drinkwater *et al.*, 1998), potentially including AMF diversity. Second, differences in AMF richness and community structure appear to be most pronounced late in the growing season, which indicates that organic farming may select for AMF with long life cycles. This finding agrees with those of Oehl *et al.* (2003), who found that AMF from organic agricultural systems sporulate later.

Earlier studies on nutrient-poor soils have shown that diverse AMF communities are beneficial for plants as they can enhance plant productivity (van der Heijden *et al.*, 1998) and provide protection against soil-borne diseases (Maherali & Klironomos, 2007). Hence, this may suggest that AMF-rich communities from organically managed fields contribute more to plant productivity and other ecosystem functions than do those of conventionally managed fields. In line with this, it has been observed that AMF from less intensively managed sites are more beneficial for plant biomass production than AMF from sites of higher management intensity (Johnson, 1993; Singh *et al.*, 2008b). However, it must be remembered that the beneficial effects of AMF decrease with increased nutrient fertility (Kiers & van der Heijden, 2006; Collins & Foster, 2009) and AMF may actually reduce plant growth at high fertility as a result of carbon loss (Smith *et al.*, 2009). Moreover, some crops (e.g. sugar beet and rapeseed) do not associate with AMF, and hence it is unlikely that such crops benefit from enhanced AMF diversity in organically managed fields. Thus, future work should test whether the more diverse AMF communities observed in organically managed fields have a positive effect on plant productivity. This may be strongly dependent on soil fertility and the crop/crop genotype under study.

It is important to note that soil variables, management and crop rotation varied considerably among the study sites, for both organic and conventionally managed sites. This is not surprising because all fields belonged to different farmers and were managed in different ways. In addition, some conventional farmers managed their fields closer to organic management (e.g. with respect to the amount of fertilizer input) and some organic farmers were very intensive in the way in which they treated their land with regard to crop rotation and manure inputs. Despite this large variation, it was interesting to see that the duration of organic management was positively related to AMF diversity. The root colonization levels of maize were also highest in the oldest organically managed sites (data not shown). Hence, this indicates that some of the characteristics of organic management (e.g. crop rotation, the inclusion of grass–clover in the rotation or the absence of pesticides) had a positive influence on AMF. Moreover, some of the soil variables (e.g. pH or phosphorus availability) influenced AMF composition and abundance, irrespective of organic or conventional management.

β-Diversity is a relatively understudied topic in AMF ecology. This diversity, the turnover of species' composition across sites, is considered to be a crucial aspect of biodiversity patterns (Ricklefs, 2004; Legendre *et al.*, 2005), and has been useful in describing species' richness in agroecosystems (Clough *et al.*, 2007). In this study, we found higher β-diversity of AMF communities across organically farmed and semi-natural sites, when compared with conventionally managed sites. The absence of mineral and chemical inputs and enhanced crop rotation and crop diversity in organic fields may explain this phenomenon. Conventional management may select for a small set of generalist AMF taxa (Helgason *et al.*, 2007), thereby leading to more uniform AMF assemblages. This notion is supported by the smaller range of PCA scores (smaller error bars) of the AMF assemblages in the conventionally managed fields, when compared with those in organically managed fields and semi-natural grasslands (Fig. S2). Moreover, the grasslands showed higher plant diversity and were not disturbed by ploughing, which probably explains the highest AMF diversity.

The most common AMF types in this study, as recorded by T-RFs, were affiliated with the family Glomeraceae, a finding that has been reported previously for arable fields (Daniell *et al.*, 2001; Hijri *et al.*, 2006; Toljander *et al.*, 2008) and several other temperate ecosystems (Öpik *et al.*, 2006). Three AMF types detected in this study were more common in either grasslands or organic farming systems than under conventional farming, and may thereby represent 'specialist' AMF (Helgason *et al.*, 2007; Sykorova *et al.*, 2007): *Glomus* Ab1, *Glomus* A4 and Gig., closely related to the Gigasporaceae. The first two are more prevalent at sites with a higher frequency of grass in the rotation. In fact, *Glomus* A4 occurred outside organic fields in only one conventional field that had been pasture for at least 4 yr before conversion to maize. Gig. was only observed in grasslands and organic sites. A higher sensitivity of many members of the family of Gigasporaceae to intensive farming with heavy phosphorus fertilization has been described previously (Jansa *et al.*, 2003; Singh *et al.*, 2008a). Indeed, the sites at which Gig. occurred had some of the lowest phosphorus availabilities among all our studied sites (data not shown).

All sequences obtained in this study were affiliated with the Glomeromycota (Schüßler *et al.*, 2001), which shows

that PCR with the primer pair FLR3–FLR4 is AMF specific, as described previously by Mummey & Rillig (2007). It has been reported, however, that this primer pair may not detect all AMF lineages equally well (Mummey & Rillig, 2007; Gamper *et al.*, 2009). Moreover, very rare AMF may have been excluded from the analysis because the smallest peaks in the T-RFLP analysis were excluded (see the Materials and Methods section), in line with other studies using this technique. Hence, the AMF richness observed in this study should be regarded as a conservative estimate. Moreover, size overlap of different T-RFs may lead to an underestimation of AMF richness by T-RFLP with only one restriction enzyme. Better resolution among sequence types could be obtained either by combining several restriction enzymes or by massive parallel pyrosequencing (Sogin *et al.*, 2006; Öpik *et al.*, 2009). In addition, with our study, we cannot conclude that specific AMF types are absent from fields, only that they did not colonize the roots sufficiently to reach detection level. Potentially, AMF types found to be absent could be present in deeper soil layers, as observed by Oehl *et al.* (2005), and recover when the environment is more suitable. Therefore, the recovery potential of AMF communities depends strongly on whether AMF are truly absent or only inactive, which should be the focus of future study.

### Conclusions

This study shows that organic management enhances the diversity of AMF assemblages, when compared with conventionally managed agricultural fields. AMF communities were richer and more diverse across organically managed fields and were more similar to those of (semi-) natural, undisturbed grasslands. Moreover, AMF richness increased significantly with the time since conversion to organic management. As AMF have been postulated as a keystone functional group, these results may have profound and positive ecosystem implications. Therefore, this research should function as an important stepping stone to disentangle potential AMF mediation in agricultural and environmental sustainability.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Map of the Netherlands showing the sampling sites of this study.

**Fig. S2** Principal components' plot of terminal restriction fragments (T-RFs) of first sampling.

**Fig. S3** Neighbour-joining distance tree of sequences used for terminal restriction fragment length polymorphism (T-RFLP) interpretation.

**Table S1** Coordinates of fields sampled in this study

**Table S2** Regression analysis of rank–abundance plots for separate management/crop combinations and sampling times

**Notes S1** Plant species' lists of the semi-natural grassland plots.

**Notes S2** Test for sufficiency of sampling intensity.

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