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Comparing arbuscular mycorrhizal communities of individual plants in a grassland biodiversity experiment

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Summary

• Plants differ greatly in the soil organisms colonizing their roots. However, how soil organism assemblages of individual plant roots can be influenced by plant community properties remains poorly understood.

• We determined the composition of arbuscular mycorrhizal fungi (AMF) in *Jacobaea vulgaris* plants, using terminal restriction fragment length polymorphism (T-RFLP). The plants were collected from an experimental field site with sown and unsown plant communities. Natural colonization was allowed for 10 yr in sown and unsown plots. Unsown plant communities were more diverse and spatially heterogeneous than sown ones.

• Arbuscular mycorrhizal fungi diversity did not differ between sown and unsown plant communities, but there was higher AMF assemblage dissimilarity between individual plants in the unsown plant communities. When we grew *J. vulgaris* in field soil that was homogenized after collection in order to rule out spatial variation, no differences in AMF dissimilarity between sown and unsown plots were found.

• Our study shows that experimental manipulation of plant communities in the field, and hence plant community assembly history, can influence the AMF communities of individual plants growing in those plant communities. This awareness is important when interpreting results from field surveys and experimental ecological studies in relation to plant–symbiont interactions.

Introduction

Understanding the factors that lead to the enormous diversity in communities of soil biota and the impacts on ecosystem functioning is one of the major challenges in soil ecology (Fitter *et al.*, 2005). A well studied group of soil organisms are the arbuscular mycorrhizal fungi (AMF; Glomeromycota), forming symbiotic associations with most terrestrial plant families. AMF have important functions in ecosystems, as they influence nutrient cycling, plant productivity and diversity (Van der Heijden *et al.*, 1998a; Klironomos *et al.*, 2000; Klironomos & Hart, 2002; Smith & Read, 2008). Less is known about the factors determining AMF community composition colonizing plant roots (Johnson *et al.*, 2004; Börstler *et al.*, 2006). Clearly, AMF propagules in the soil are important sources for the AMF community inside roots, but propagule composition does not necessarily reflect the community composition of AMF that colonize plant roots (Clapp *et al.*, 1995; Merryweather & Fitter, 1998; Rodríguez-Echeverría *et al.*, 2008). Also, host plants can be associated with very distinct mycorrhizal taxa (Vandenkoornhuyse *et al.*, 2003; Gollotte *et al.*, 2004; Scheublin *et al.*, 2004). Recently, Hausmann & Hawkes (2009) showed that the identity of surrounding plants can influence the composition of AMF in a focal plant. That study was performed in pots under glasshouse conditions. In the field, soil organism assemblages of individual plant roots could also be influenced by additional properties, such

as the assembly history and heterogeneity of the plant community. The new question addressed in the present study is how assembly history and heterogeneity of the plant community may affect the AMF community colonizing roots of individual plants.

The AMF spore community within the soil can be influenced by soil characteristics, such as disturbance history, soil type and chemistry (Helgason *et al.*, 1998; Egerton-Warburton *et al.*, 2007; Fitzsimons *et al.*, 2008). Other studies have shown that the composition of neighbouring plant species can affect AMF communities (Johnson *et al.*, 1992, 2004; Mummey *et al.*, 2005; Öpik *et al.*, 2006). Individual plant species can show preferences for specific AMF taxa (Scheublin *et al.*, 2004), so that changes in plant community composition may alter the relative proportion of AMF propagules in the soil, leading to shifts in AMF assemblages (Bever *et al.*, 1996; Burrows & Pfleger, 2002). Teasing apart the relative effects of these environmental influences on AMF community composition in plant roots would require experimental conditions where single factors are varied one by one. However, the number of such examples is limited, as in field studies where the plant community has been experimentally changed, other environmental parameters often co-vary or vice versa (Vandenkoornhuyse *et al.*, 2003; Börstler *et al.*, 2006; Egerton-Warburton *et al.*, 2007). As a consequence, the importance of individual parameters as determinants of AMF community composition in roots remains poorly understood.

We analysed the AMF community composition in individual *Jacobaea vulgaris* L. (synonym *Senecio jacobaea*; Pelser *et al.*, 2006) plants growing in plots with different assembly histories, using terminal restriction fragment length polymorphism (T-RFLP, Liu *et al.*, 1997; Mummey & Rillig, 2007) and cloning and sequencing. In early secondary successional stages on relatively dry sandy soils, *J. vulgaris* is a characteristic plant species (Bezemer *et al.*, 2006). We collected *J. vulgaris* plants from a long-term field experiment initiated in 1996 on ex-arable land. In this field, half the plots were sown with mid-succession grassland plant species, whereas the other plots were left to become colonized naturally (Van der Putten *et al.*, 2000). Natural colonization was allowed in all plots, but the sowing treatment has resulted in a plant community composition that diverged from unsown plant communities (Fukami *et al.*, 2005), and sown plant communities possessed higher temporal stability and lower diversity than unsown plant communities (Bezemer & van der Putten, 2007). One year after starting the experiment, *J. vulgaris* established spontaneously in all plots (Bezemer *et al.*, 2006). This enables us to analyse the influence of plant community composition and assembly history on AMF community composition using a single plant species that has been present for almost a decade in both the sown and unsown plant communities.

Here, we test the effects of plant community assembly history and spatial heterogeneity on the AMF assemblages colonizing the roots of individual *J. vulgaris* plants from sown and unsown plant communities 10 yr after their establishment. Our hypothesis was that individual pants growing in the two types of plant communities (sown and unsown) will have different AMF communities. In addition to our field study approach, we conducted a glasshouse bioassay in which we grew *J. vulgaris* plants in homogenized soils collected from the field plots. Homogenization of the field soil enabled us to reduce spatial variation that may have influenced the AMF community composition in the plants collected directly from the field. We hypothesized that in the glasshouse the community composition of AMF in individual plants grown in homogenized field soil would not differ between the two treatments. In this comparison, as glasshouse conditions can yield different AMF assemblages in plant roots, we considered the full AMF community composition as well as that part of the community that overlapped with the community observed in the field plants.

Materials and Methods

Study plant

Tansy or common ragwort, *Jacobaea vulgaris* ssp. *vulgaris* (synonym *Senecio jacobaea* L.; Pelser *et al.*, 2006), is a monocarpic perennial weed (Asteraceae) that spends its first year as a rosette. Flowering may take place in the second year, but is often delayed because of herbivory (Van der Meijden & Van der Waals-Kooi, 1979). *J. vulgaris* is an early successional plant species native to the Netherlands and Europe, but invasive in other continents. In the Netherlands, it is considered a problem weed in abandoned arable fields that are used for nature restoration (Bezemer *et al.*, 2006), because the plant contains pyrrolizidine alkaloids that are toxic for livestock (Cameron, 1935).

Field experiment

To study the influence of the surrounding plant community and biotic conditions on the AMF composition within individual *J. vulgaris* plants, we collected plants from experimental grassland field plots that differed in plant community composition. The experimental field is located near Ede, the Netherlands (52°04′N, 05°45′E), in a nature restoration area on arable land, which was abandoned in 1996. In 1996, the 0.5 ha field experiment was set up by ploughing and sowing 0 or 15 mid-successional grassland species in plots of 10 × 10 m. There are five replicate plots for both treatments, arranged in five blocks. After sowing, plots were left to be colonized by plant species from the seed bank and the surrounding area. Once a year, at the

end of the growing season, above-ground biomass was removed from all plots (see Van der Putten *et al.*, 2000 and Bezemer *et al.*, 2006 for further details). The plant community characteristics of the plots sown with 15 species (sown) and naturally colonized (unsown) treatments differed significantly and consistently over the years (Fukami *et al.*, 2005; Bezemer & van der Putten, 2007; Lepš *et al.*, 2007). *J. vulgaris* was not sown, but since 1997 this species has been present in varying densities in all plots (Bezemer *et al.*, 2006).

Plant community In July 2006, 10 yr after establishing the experiment, in all 10 plots we recorded the cover of all plant species, including *J. vulgaris*, in 12 permanent quadrats of 1 × 1 m. Plant productivity was estimated by clipping all above-ground biomass at 2 cm above the soil surface for an area of 0.25 × 0.25 m adjacent to each permanent quadrat. Above-ground biomass was dried at 70°C and weighed. Shoots of *J. vulgaris* were dried and weighed separately, and values of total and *J. vulgaris* above-ground biomass per m^2 were calculated. In all 10 plots, we also measured the plant height of 10 randomly chosen flowering *J. vulgaris* plants. Vegetation recordings in the 12 permanent quadrats were used to calculate mean species richness per m^2, Shannon diversity (H') and spatial heterogeneity for each plot. Spatial heterogeneity of the plant community was determined by calculating the dissimilarity (based on Bray–Curtis distance) among the 12 vegetation surveys per plot. Heterogeneity calculations were performed using Poptools version 3.06 (Hood, 2008) in Excel.

Soil chemistry In July 2006, from each plot we randomly collected 24 soil samples (2.5 cm diameter and 15 cm depth). The 24 samples from each plot were homogenized and sieved (< 0.5 cm). A subsample was dried for 3 d at 40°C. In this subsample, pH, plant available P and K were analysed in 1 : 10 (w/v) 0.01 M $CaCl_2$. Concentrations of available NH_4^+-N and NO_3^--N were determined colorimetrically in the $CaCl_2$-extract using a Traacs 800 autoanalyzer (TechniCon Systems Inc.).

Experimental *J. vulgaris* plants In July 2006, two flowering *J. vulgaris* plants were randomly selected in each plot. All plants were located at least 2 m apart. Plants were removed together with the soil underneath them (15 × 15 × 15 cm) and transported to the laboratory in individual plastic bags. Plant height was recorded, all adhering soil and roots of other plants were removed from the soil, and individual *J. vulgaris* roots were rinsed with tap water to remove adhering soil. Approximately 100 mg of fine root material was then collected, frozen immediately at −80°C and used for DNA extraction (Supporting Information, Methods S1). Shoots were oven-dried at 70°C and weighed.

Glasshouse bioassay

Soil collection In July 2006, field soil was gathered by randomly collecting 24 soil cores from each plot (2.5 cm diameter, 15 cm deep). Soil samples from each plot were homogenized to omit spatial variation present in the field and sieved (< 0.5 cm). To obtain sterilized soil, adjacent to the experimental field site soil from a depth of 5–20 cm was collected, sieved (< 0.5 cm), homogenized and gamma-sterilized (> 25 kGy gamma irradiation; Isotron, Ede, the Netherlands). The dry weight of each soil sample was determined gravimetrically (24 h at 105°C). *J. vulgaris* plants were grown in a 7 : 1 mixture (on a dry weight basis) of sterilized soil and field soil inoculum. Control plants were grown in a 7 : 1 mixture of sterilized soil and autoclaved field inoculum (autoclaved on three consecutive days, 20 min at 121°C) from sown and unsown plots of each block combined.

Growing conditions Plants were grown in pots of 0.9 l with 1.16 kg soil mixture (on a dry weight basis). There were three pots used for each field plot and those data were averaged. Therefore, we had two treatments (sown and unsown) × five replicate field plots × three pots per field plot + five pots with sterile soil serving as a control = 35 pots.

In order to carry out the experiment, seeds of *J. vulgaris* plants growing in the area adjacent to the experimental field site were surface-sterilized (30 s in 0.1% chloride solution), rinsed and germinated on glass beads. In each pot, three 1-wk-old seedlings were planted. Seedlings that died during the first week of the experiment were replaced. Pots were placed randomly in the glasshouse with 70% relative air humidity, temperatures of 21°C (day) and 16°C (night), and a 16 : 8 h day : night light cycle. Natural daylight was supplemented by metal halide lamps (225 μmol s^{-1} m^{-2} photosynthetically active radiation, one lamp per 1.5 m^2). Plants were watered every other day and initial soil moisture content (17% soil mass) was reset twice a week by watering to the original weight.

After 8 wk, all plants were harvested. Above-ground and below-ground plant material was separated for each pot, roots were rinsed with tap water and 0.5 g of fresh root material was stored in 50% EtOH at 4°C to determine mycorrhizal colonization. Also, *c.* 100 mg of fine root material was collected and frozen immediately at −80°C. For two of the three replicates this was used for DNA extraction (Methods S1). The remaining plant material was then oven-dried at 70°C and weighed.

AMF composition in *J. vulgaris* roots

AMF colonization To determine the AMF colonization of *J. vulgaris* bioassay plants, roots from the bioassay plants

were cleared for 1 h in 2.5% KOH at 90°C in a water bath, rinsed with water and left overnight in 1% HCl. Thereafter, roots were stained for 30 min at 60°C with 1% Parker Ink solution, destained and stored in lactic acid : glycerol : water (14 : 1 : 1) solution. Percentage mycorrhizal colonization was scored using gridline intersection with 100 intersections (McGonigle *et al.*, 1990).

Molecular characterization of AMF communities The AMF community composition in roots of *J. vulgaris* was determined by T-RFLP analyses of the FLR3/FLR4 fragments of the LSU rRNA gene (Liu *et al.*, 1997; Gollotte *et al.*, 2004; for AMF specificity see Mummey & Rillig, 2007; Krüger *et al.*, 2009). This method involves dual end-labelling of PCR amplicons and enzyme digestion of these fragments with the restriction enzymes *Alu*I, *Mbo*I and *Taq*I. Multiple enzymes were chosen to improve the discrimination of T-RFLP and these three enzymes have been used successfully in AMF T-RFLP analyses before (Mummey & Rillig, 2007). Digestion with restriction endonucleases yielded terminal restriction fragments (TRF) of different sizes, caused by sequence variation. The fragments are electrophoretically separated according to size and their presence/absence is scored. To identify the dominant community members, clone libraries were constructed and sequenced. Twelve clone libraries from the AMF-specific PCR amplicons were prepared for the root samples from the two plants from the sown and unsown plots of the experimental field blocks 2, 4 and 5, using the pGEM-T vector (Promega, Leiden, the Netherlands) and *Escherichia coli* JM109 High Efficiency Competent cells (Promega). Twelve to 30 clones per library (i.e. plant root system) were randomly selected for sequencing with the SP6 and T7 vector primers. Electropherograms of 143 successfully sequenced clones were checked in Chromas (version 1.45, Technelysium, Australia), before the sequences were compared against those in the public databases by BLASTN searches (http://www.ncbi.nlm.nih.gov; Altschul *et al.*, 1997). All nonredundant sequences were deposited in GenBank under the accession numbers FJ820857–FJ820960 (Fig S1). A more detailed description of the molecular analysis is given in Methods S1.

Statistical analyses

All data were analysed using univariate (GenStat version 11.1; VSN International Ltd, Hempstead, UK) or multivariate statistics (CANOCO version 4.55; Ter Braak & Šmilauer, 2002). Plot characteristics, biomass, bioassay data and number of TRFs were analysed using linear mixed models (residual maximum likelihood, REML) with treatment (sown or unsown) as fixed factor and block as random factor. For the bioassay, data from the three pots with soil from the same plot were averaged before univariate analyses. Compositions of AMF and plant community were analysed using multivariate analyses. Detrended correspondence analysis (DCA) was used to determine whether linear (principal components analysis (PCA), redundancy analysis (RDA)) or unimodal (correspondence analysis (CA), canonical correspondence analysis (CCA)) analyses were most appropriate for multivariate analyses (Lepš & Šmilauer, 2003). Significances in multivariate analyses were inferred by Monte Carlo permutation tests (999 permutations). Plant community composition (log (n + 1) transformed) in sown and unsown plots was compared using multivariate linear unconstrained (PCA) and linear constrained analyses (RDA).

Terminal restriction fragment incidence data of all enzyme–dye combinations together were analysed using unimodal multivariate analyses. Statistical analyses and results for the separate enzymes and dyes are given in Tables S1 and S2. Unimodal constrained analyses (CCA) were used to test for differences between mycorrhizal communities in sown and unsown plots, and to compare mycorrhizal communities originating from bioassay and field. For both glasshouse and field conditions, the two plants within each plot were analysed as split plots (not permuted) within each whole plot (field plot; permuted freely). There was one missing value, as the AMF community of one of the plants could not be successfully fingerprinted with the *Mbo*I enzyme. This plant was not included in the analyses in which all enzymes were combined.

To determine the dissimilarity in AMF communities between the two plants originating from the same plot (or growing in the same soil in the bioassay), we submitted the data, field and glasshouse samples separately to a unimodal unconstrained analysis (CA) and calculated the Euclidian distance between the two samples, based on the first three axes. The effect of sowing treatment on AMF community dissimilarity was then analysed using linear mixed models (REML). Finally, variance partitioning (Lepš & Šmilauer, 2003) was carried out using CANOCO to determine if AMF community composition of the field plants could be significantly explained by characteristics of the plant community, *J. vulgaris*, or soil chemistry.

Results

Field experiment

Plot characteristics Plant community composition in 2006 differed significantly (RDA; F = 4.951, P = 0.003, 38% explained variation) between sown and unsown plots (Fig. 1). Nevertheless, during the 10 yr following establishment, in plots that were not sown, annually 91 ± 2% (mean ± SE) of the plant cover was made up by species that were also found in the sown plots (individual plant species cover data not shown). Plant community heterogeneity,

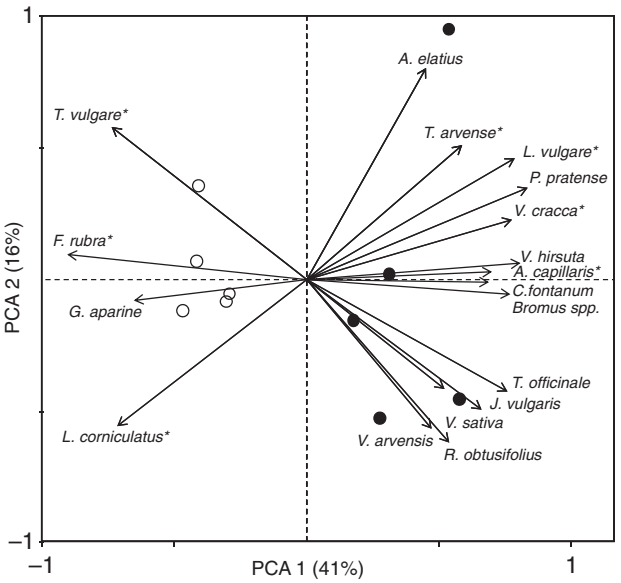

Fig. 1 Principal components analysis (PCA) of species scores of the 18 plant species with the highest scores present in the plant communities and PCA sample scores of sown (open) and unsown (closed) field plots. Amounts of explained variation by the first two PCA axes are given in parentheses. Species names are: *Agrostis capillaris*, *Arrhenatherum elatius*, *Cerastium fontanum*, *Festuca rubra*, *Galium aparine*, *Jacobaea vulgaris*, *Leucanthemum vulgare*, *Lotus corniculatus*, *Phleum pratense*, *Rumex obtusifolius*, *Taraxacum officinale*, *Tanacetum vulgare*, *Trifolium arvense*, *Veronica arvensis, Vicia cracca, Vicia hirsuta* and *Vicia sativa.* An asterisk (*) indicates species that were sown at the start of the field experiment in 1996.

Shannon diversity and species richness were all significantly higher in unsown than in sown plots, whereas above-ground productivity was significantly higher in sown plots (Table 1). In 2006, soil chemistry and the number of *J. vulgaris* individuals, above-ground *J. vulgaris* biomass per m^2 and abundance did not differ between sown and unsown plots (Table 1).

AMF communities of field plants Above-ground biomass of the sampled *J. vulgaris* plants did not differ significantly between the two types of plant communities (sown, 3.53 ± 0.76 g per plant; unsown, 3.18 ± 0.49 g per plant; $F_{1,4}$ = 0.15, P = 0.70). For all enzyme–dye combinations the number of TRFs did not differ significantly between plants growing in sown and unsown plots (Table S1). There was also no significant difference in the composition of TRFs between plants from sown and unsown plots (CCA, F = 0.67, P = 0.89; see Table S2 for individual enzyme–dye combinations). Of the TRFs found, 97% were found in both plants originating from the sown and unsown plots, and 3% were found only in plants from the unsown plots. However, the AMF communities of the two individual plants originating from the same plot were three times more dissimilar in unsown than in sown plots ($F_{1,4}$ = 22.94, P = 0.008; Fig. 2a) and there was a positive relationship between plant community heterogeneity and AMF dissimilarity ($F_{1,7}$ = 14.02, P = 0.007, r^2 = 0.67). Variance partitioning showed that AMF community composition could not be explained significantly by plant community characteristics (F = 1.07, P = 0.32), *J. vulgaris* field measurements (F = 1.24, P = 0.11) or soil chemistry (F = 1.12, P = 0.28). Analysis of partial LSU rDNA sequences from clone libraries containing 143 AMF clones of the field-collected plants demonstrated the specificity of the used PCR amplicons and revealed eight different clades (Schüβler *et al.*, 2001; Fig. S1). All plants revealed sequences from multiple clades (Table S3).

Table 1 Effect of sowing treatment on plant community, chemical and *Jacobaea vulgaris* characteristics in 2006

Measurement	Sown	Unsown	$F_{1,4}$	P
Plant community				
Spatial heterogeneity of plant community	0.37 ± 0.04	0.56 ± 0.02	14.80	0.02
Species richness (m^2)	10.8 ± 0.75	14.0 ± 0.77	10.35	0.03
Above-ground productivity (g m^{-2})	563 ± 80.6	298 ± 53.3	28.66	0.01
Diversity (Shannon *H*)	1.32 ± 0.04	1.87 ± 0.12	21.66	0.01
J. vulgaris				
Abundance (%)	0.65 ± 0.30	3.95 ± 1.28	6.72	0.06
Above-ground biomass (g m^{-2})	5.80 ± 3.96	21.4 ± 6.79	2.48	0.19
No. of plants per plot	43.4 ± 14.4	318 ± 109	5.36	0.08
Height (cm)	59.4 ± 3.69	62.8 ± 1.39	0.52	0.51
Soil chemistry				
P (mg kg^{-1})	4.77 ± 0.37	4.01 ± 0.33	3.90	0.12
K (mg kg^{-1})	42.3 ± 5.65	60.5 ± 5.16	8.31	0.05
NO_3 (mg kg^{-1})	1.57 ± 0.50	1.56 ± 0.12	0.00	0.99
NH_4 (mg kg^{-1})	0.17 ± 0.08	1.17 ± 0.78	1.66	0.27
pH $CaCl_2$	5.19 ± 0.07	5.26 ± 0.03	0.75	0.44

Means (±SE) are shown for sown and unsown plots (n = 5) and results of mixed model (residual maximum likelihood, REML) analyses, with treatment as a fixed factor and block as a random factor.

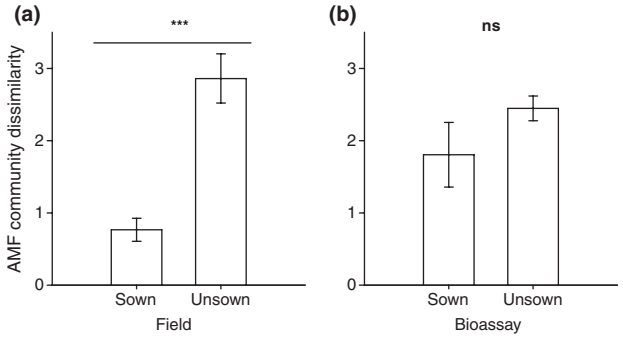

Fig. 2 Average dissimilarity (Euclidean distance) between the arbuscular mycorrhizal fungal (AMF) communities of two plants originating from the same field plot (a) or growing in soil obtained from the same field plot (b). Means (±SE; $n = 5$) are shown. ***, Significant difference between sown and unsown plots at $P < 0.01$ based on linear mixed models (residual maximum likelihood, REML), with treatment as fixed factor and block as random factor; ns, no significant difference.

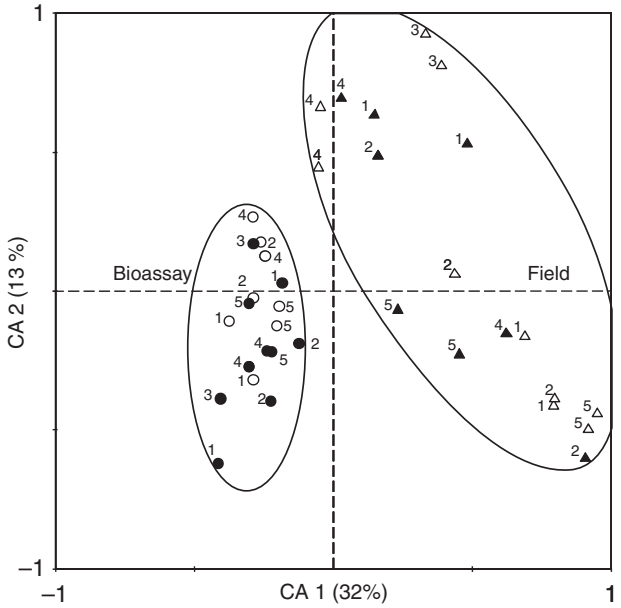

Fig. 3 Unconstrained unimodal canonical analysis (CA) of the arbuscular mycorrhizal fungal (AMF) community composition in *Jacobaea vulgaris* plants from the field (triangles) and from the glasshouse bioassay (circles). Open symbols, AMF communities from plants from the sown field plots; closed symbols, unsown plots. Symbols with the same number originate from the same field plot or were grown in soil from the same field plot. AMF communities in plants from the field differ significantly from those of bioassay plants (canonical correspondence analysis, CCA, $F = 7.17$, $P = 0.001$). Percentages of total explained variation by CA axes are given in parentheses.

Bioassay

In the glasshouse, plants grown in soil from sown plots had significantly more below-ground biomass than plants grown in soil from unsown plots (Table S4). The percentage AMF colonization did not differ between the two sowing treatments, while plants in sterilized soil remained almost completely devoid of AMF (Table S4). The average number of TRFs for the different enzyme–dye combinations also did not differ significantly between the two treatments (Table S1). Root samples of plants from sterilized soil did not yield any TRFs (data not shown). As was also observed in the plant roots collected directly from the field, the composition of TRFs in the bioassay plants was not significantly influenced by sowing treatment (CCA, $F = 1.16$, $P = 0.12$; Table S2). Of the TRFs found, 98% were found in both plants originating from the sown and unsown plots, and 2% were found only in plants from the unsown plots. However, in contrast to the field situation, the AMF assemblages of the two replicate plants per plot were not more dissimilar in soil from the unsown plots than in soil from the sown field plots ($F_{1,4} = 3.22$, $P = 0.16$; Fig. 2b). The AMF community composition of field and bioassay plants differed significantly (CCA, $F = 7.17$, $P = 0.001$; Fig. 3, Table S5). Moreover, there was considerably more variation in AMF composition between individual plants from the field than between plants from the bioassay, which can be seen by a more scattered distribution of the field samples than of the bioassay samples in the CA ordination (Fig. 3). Interestingly, 70% of all TRFs that were detected were present in plants from both experiments. When the similarity analyses were limited to those TRFs that were present in both field and bioassay plants, the pattern remained the same (field AMF dissimilarity, sown 0.86 ± 0.28, unsown 2.47 ± 0.50, $F_{1,4} = 10.11$, $P = 0.03$; bioassay AMF dissimilarity, sown 1.77 ± 0.52, unsown 1.70 ± 0.36, $F_{1,4} = 0.014$, $P = 0.93$).

Discussion

We studied the effects of plant community assembly history on AMF assemblages of individual *J. vulgaris* plants using experimental field plots. We examined AMF in plant roots that were directly collected from the field, as well as in roots of plants grown in homogenized field soil under glasshouse conditions. Despite the differences in plant community composition and assembly history of sown and unsown plant communities, we did not detect a difference in AMF community composition and richness in *J. vulgaris* plants of the sown and unsown plots, either in roots directly collected from the field or in roots of plants grown in the glasshouse. It is important to note that we used a qualitative method to determine AMF community composition. Hence, although we found no difference in the presence of TRFs, we cannot exclude the possibility that there were differences in the relative abundance of the TRFs between the treatments. Interestingly, in plant roots collected directly from the field, the average dissimilarity in AMF community was higher in the unsown than in the

sown plant communities. The unsown plots had the most spatially heterogeneous plant community. Moreover, the plant communities in these unsown plots had the lowest temporal stability, because over time they had the highest rates of extinction and colonization of species and the strongest fluctuation in productivity (Bezemer & van der Putten, 2007).

A controlled glasshouse bioassay with homogenized soil collected from the sown and unsown plots enabled us to study the composition of AMF communities in *J. vulgaris* roots under conditions without spatial variation. AMF diversity (number of TRFs) was higher in the bioassay plants than in plants collected from the field. This could be because bioassay plants were grown in homogenized soil, in which soil properties (e.g. mycorrhizal inocula) were homogeneously present throughout the soil. Moreover, the bioassay plants were still in the rosette stage, whereas the field plants were flowering. Studies on other plant species have shown that the development stage of the host plant can alter the AMF community (Šmilauer, 2001; Husband *et al.*, 2002). AMF communities in the roots of bioassay plants differed significantly from AMF communities in roots of field plants, as has been reported in other studies (Sýkorová *et al.*, 2007). This could be a result of the shorter growing period of the bioassay plants, or of the soil homogenization itself. Soil disturbance can favour colonization by fast-growing AMF (r-strategists) as discussed by Sýkorová *et al.* (2007). However, in our study, 70% of all TRFs that were detected were present in plants from both experiments. Since the field plants were growing in relatively old, and not recently disturbed plots, this suggests that the TRFs that make up this 70% do not resemble fast colonizing AMF taxa. Analyses based exclusively on the TRFs shared by the field and the glasshouse bioassay plants showed patterns that were similar to what we found for analyses with all TRFs present in the field or glasshouse. This indicates that the difference in dissimilarity found between field and glasshouse plants is not caused solely by the presence of different TRFs resulting from changes in environmental conditions. Our results therefore suggest that the sowing treatment caused differences in the spatial heterogeneity of the plant community and that this, in turn, has led to increased dissimilarity of AMF communities of individual plants growing in those communities. The positive relationship between spatial heterogeneity of the plant community and the dissimilarity among the AMF communities of two plant individuals supports this view.

Alternatively, it is possible that other external factors, for example differences in resource availability or AMF control by fungal grazers, or plant pathogen pressure may have enhanced spatial variation of AMF community composition between individual plants in the field. As pot experiments with a number of other plant species have pointed at direct plant neighbour effects on the AMF community composition (Hausmann & Hawkes, 2009), it is likely that our results also apply to plant species other than *J. vulgaris*.

The sowing treatment resulted in plant communities with largely the same plant species, but differing in plant community characteristics such as diversity, heterogeneity and stability (Bezemer & van der Putten, 2007). The sown plant communities were more homogeneous and stable than the unsown plant communities (Bezemer & van der Putten, 2007). The cause of these differences is unknown. However, what we have now shown is that individual plants from unsown communities, which are more heterogeneous and unstable, harbour much more dissimilar mycorrhizal assemblages than plants from sown communities. The observed relationship between instability and dissimilarity may be either causal or consequential, something that should be determined in further studies. Designing experiments that can separate cause from consequence will be challenging. The observed positive relationship between mycorrhizal community diversity and plant community diversity (Van der Heijden *et al.*, 1998b) suggests that increased spatial heterogeneity in plant–AMF interactions, as we observed, can ultimately influence diversity and functioning of ecosystems.

Symbiotic interactions with AMF can buffer plants against abiotic changes or disturbances (Smith & Read, 2008). Moreover, plant–mycorrhizal interactions have been proposed to increase the potential for redundancy of plant species and to weaken the relationship between plant diversity and ecosystem functioning (Johnson *et al.*, 1996; Loreau *et al.*, 2001). Our study does not provide direct evidence for such a feedback loop, but strongly suggests that in the further unravelling of the relationships between plant diversity and root symbionts, the effects of plant community characteristics, such as heterogeneity and stability, can play a profound role.

We detected up to 26 TRFs per enzyme–dye combination in T-RFLP analyses. TRFs that were present in less than three samples were excluded from further analyses and we were interested in heterogeneity rather than AMF identity; however, this number could be influenced slightly as a result of sequence heterogeneity within a single individual (Sanders *et al.*, 1995; Rosendahl & Stukenbrock, 2004). The AMF community of individual plants varied greatly between and within different plant communities. The diversity of TRFs and sequences found within one individual plant suggests that plant–AMF interaction studies carried out with only a limited set of AMF strains should be interpreted with caution, particularly since the effects of AMF on plant growth can differ greatly between AMF species and strains (Johnson *et al.*, 1997; Klironomos, 2003; Koch *et al.*, 2006). Earlier microcosm and macrocosm studies have pointed out the importance of AMF community composition for plant performance, plant community com-

position and ecosystem functioning (Van der Heijden *et al.*, 1998b). Most likely, this is not a one-way interaction. In nature, AMF community composition and functioning are also influenced by the plant community dynamics, so that plant and soil community composition are tightly intertwined, as was proposed in the 'driver/passenger' hypothesis (Hart *et al.*, 2001).

In conclusion, our results show that, in a long-term field experiment, AMF communities in plant roots were more dissimilar when collected from sown vs non-sown plant communities. As a major difference is that the non-sown plant communities were the most heterogeneous and the least stable over time, our results suggest that these factors may contribute to AMF dissimilarity among root systems of individual field plants. Reduced AMF dissimilarity among plants grown in homogenized field soil further supports this suggestion. The awareness that experimental manipulation of plant communities in the field, and hence plant community assembly history, can influence the AMF communities of individual plants growing in those plant communities is important when interpreting results from field surveys and experimental ecological studies in relation to plant–symbiont interactions**.**

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Supporting Information

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

Methods S1 Molecular characterization of arbuscular mycorrhizal fungal (AMF) communities.

Fig. S1 Phylogram derived of partial 28S rDNA sequences (LR1/FLR2 and FLR3-FLR4).

Table S1 Number of specific terminal restriction fragments (TRFs) and Euclidean distance between arbuscular mycorrhizal fungal (AMF) community compositions of the two plants originating from one plot per enzyme–dye combination

Table S2 Comparisons between arbuscular mycorrhizal fungal (AMF) communities in *Jacobaea vulgaris* plants from sown and unsown plots in the field, and from the glasshouse bioassay per enzyme–dye combination

Table S3 Overview of the clades from which sequences were present in the investigated plants

Table S4 Effect of soil treatment on biomass and arbuscular mycorrhizal fungal (AMF) colonization in the glasshouse bioassay

Table S5 Comparisons between arbuscular mycorrhizal fungal (AMF) communities in *Jacobaea vulgaris* plants from the field and from the bioassay

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