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ORIGINAL ARTICLE

The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale

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Arbuscular fungi have a major role in directing the functioning of terrestrial ecosystems yet little is known about their biogeographical distribution. The Baas-Becking hypothesis ('everything is everywhere, but, the environment selects') was tested by investigating the distribution of arbuscular mycorrhizal fungi (AMF) at the landscape scale and the influence of environmental factors and geographical distance in determining community composition. AMF communities in *Trifolium repens* and *Lolium perenne* roots were assessed in 40 geographically dispersed sites in Ireland representing different land uses and soil types. Field sampling and laboratory bioassays were used, with AMF communities characterised using 18S rRNA terminal-restriction fragment length polymorphism. Landscape-scale distribution of AMF was driven by the local environment. AMF community composition was influenced by abiotic variables (pH, rainfall and soil type), but not land use or geographical distance. *Trifolium repens* and *L. perenne* supported contrasting communities of AMF, and the communities colonising each plant species were consistent across pasture habitats and over distance. Furthermore, *L. perenne* AMF communities grouped by soil type within pasture habitats. This is the largest and most comprehensive study that has investigated the landscape-scale distribution of AMF. Our findings support the Baas-Becking hypothesis at the landscape scale and demonstrate the strong influence the local environment has on determining AMF community composition.
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Introduction

The biogeography of macroorganisms has been thoroughly investigated while that of microorganisms has not (Fitter, 2005). However, with improvements in the assessment of microbial diversity using molecular-based approaches, a new interest in understanding microbial biogeography has emerged (Martiny *et al.*, 2006; Prosser *et al.*, 2007; Ramette and Tiedje, 2007a; Nemergut *et al.*, 2011). Traditionally, microbial biogeographical patterns were assumed to follow the Baas-Becking hypothesis, which predicted that 'everything is everywhere, but, the environment selects' (Baas-Becking, 1934) or in other words, microorganisms are not dispersal limited and the resulting microbial community is shaped by the local environment. Recent research suggests that microbial biogeographical patterns are more complex than the Baas-Becking hypothesis predicts. The effect of geographical distance with or without an environmental effect has been shown, which suggests that past events (e.g., dispersal limitation, speciation and extinction) can affect current microbial distributions (Green *et al.*, 2004; Ramette and Tiedje, 2007b; Wang *et al.*, 2008; Oakley *et al.*, 2010; Schauer *et al.*, 2010; Martiny *et al.*, 2011; van der Gast *et al.*, 2011).

Soil microbes are genetically diverse, abundant and functionally important organisms (Roesch *et al.*, 2007; van der Heijden *et al.*, 2008), and thus understanding there biogeographical patterns and what drive them is key for maintaining ecosystems under a changing environment. Various biotic and abiotic factors and biological processes have been theorised to have an impact on microbial biogeographical patterns (reviewed by Martiny *et al.*, 2006 and Ramette and Tiedje, 2007a). Several observational

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studies have identified various edaphic variables that influence soil bacteria and fungal community compositions, and some of which have been related to driving distributions. Soil pH, e.g., has been identified to strongly influence the biogeographical patterns of soil bacteria (Fierer and Jackson, 2006; Lauber *et al.*, 2009). While, the gap in knowledge of microbial biogeography is narrowing, important groups of soil microbes have been neglected.

Arbuscular mycorrhizal fungi (AMF) are an important soil microbial group, which form a mutualistic symbiosis with plant roots. Through their impacts on plants, AMF affect multiple ecosystem functions and processes, including nutrient cycling, plant productivity and competition (Hartnett and Wilson, 1999; van der Heijden *et al.*, 2003; Scheublin *et al.*, 2007), and plant diversity (van der Heijden *et al.*, 1998a, b; Klironomos *et al.*, 2000; O'Connor *et al.*, 2002). Approximately 65% of the world's vascular plant species associate with AMF, the symbiosis occurring in nearly all terrestrial ecosystems (Wang and Qiu, 2006; Brundrett, 2009).

Currently, 226 AMF species have been described in the phylum Glomeromycota using spore morphology (Schüßler's Glomeromycota phylogeny, http://www.lrz-muenchen.de/~schuessler/amphylo/; 4 January 2011). However, this is likely an underestimation of AMF global richness (Opik *et al.*, 2010; Kivlin *et al.*, 2011). The current understanding of the geographic distributions of AMF species is limited. Several factors have been identified that may influence AMF distributions, including abiotic (e.g., soil physico-chemical properties) and biotic (e.g., host plant) factors, and intrinsic properties of species (e.g., dispersal ability) (reviewed by Chaudhary *et al.*, 2008). However, most data remain site specific, and there are relatively few studies at the landscape or larger geographical scales. The role of geographical distance and the local environment in shaping AMF distributions at various spatial scales and whether AMF distributions are more complex than the Baas-Becking hypothesis predicts remains to be determined.

Traditionally, surveys of AMF diversity have been based on the collection and morphological identification of spores; a method acknowledged as being limited in its detection capacity (Sanders, 2004; Chaudhary *et al.*, 2008; Rosendahl, 2008). With advances in molecular methods, enabling AMF actively colonising plant roots to be identified, AMF diversity studies have improved over the years, thus making AMF biogeography research more feasible. Recent evidence from a molecular-based study suggests that land management can impact the diversity and distribution of AMF over the landscape, with conventionally managed farm soils showing lower diversity and greater similarity of AMF communities than organically managed soil over the landscape scale (van der Gast *et al.*, 2011). Meta-analysis studies, availing of published DNA sequences and metadata, suggest that the local environment and geographical distance have a role in driving distribution patterns at the global scale (Opik *et al.*, 2006, 2010; Kivlin *et al.*, 2011). Factors such as climatic zone, habitat and plant community type, and soil temperature and moisture have been suggested to contribute to global patterns of AMF distribution (Opik *et al.*, 2006, 2010; Kivlin *et al.*, 2011).

Several studies have shown that co-occurring plant species can host different AMF communities (Helgason *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002, 2003; Sýkorová *et al.*, 2007a; Opik *et al.*, 2009). Such studies have investigated the AMF communities of co-occurring plants from only one or two sites. It is unknown whether AMF community differences between plant hosts only represents 'local host preferences' or is a phenomenon that also occurs at larger geographical scales (Sýkorová *et al.*, 2007a). Evidence from Opik *et al.* (2010), showing a significant relationship between AMF distribution patterns and occurrence in host angiosperm superorders, suggests that host preference effects may occur at the global scale. To make biogeographical inferences without the confounding effect of host preferences, AMF surveys across sites need to be carried out using the same plant species. As not all plant species will occur everywhere, a field soil bioassay bait-plant approach using a defined genotype could be a useful strategy in studies of AMF biogeography (van der Gast *et al.*, 2011).

Here, we investigate AMF distribution at the landscape scale and the role of the local environment and geographical distance in determining community composition. The community composition of AMF on field soil bioassay bait-plant roots was assessed for 40 geographically dispersed sites representing different land uses and soil types. Naturally occurring plants were also collected from a subset of sites to compare AMF communities of co-occurring plant species and between different experimental approaches (bait plants vs field plants). AMF community composition of plant roots was determined using terminal-restriction fragment length polymorphism (T-RFLP) of 18S rRNA, a method that has previously been shown to be useful for characterising AMF communities of plant roots (Vandenkoornhuyse *et al.*, 2003; Johnson *et al.*, 2004; Mummey *et al.*, 2005; Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010; van der Gast *et al.*, 2011).

Materials and methods

Field sites

Field sites were chosen from a pool of 1310 locations of the Irish National Soils Database (Fay *et al.*, 2007a). The 40 sites selected for this study represented the major Irish soil types and land uses and covered a geographically large area in the Republic of Ireland (Figure 1). Eight soil types were

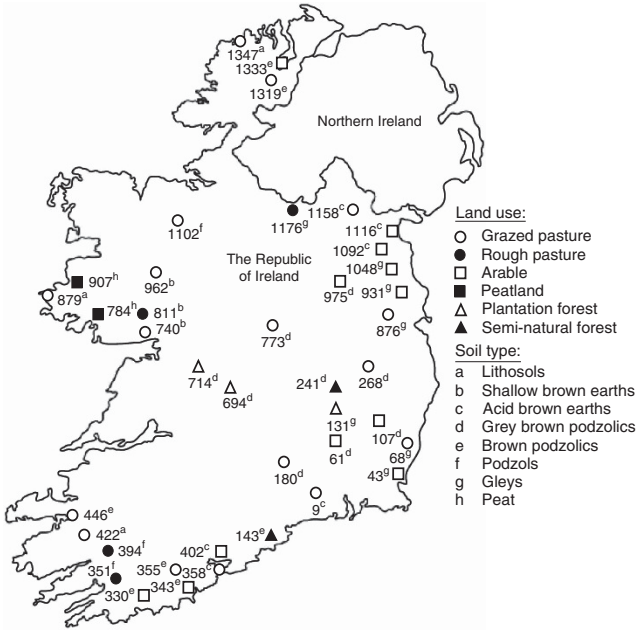

Figure 1 Site location, land use and soil type of the 40 Irish National Soils Database sites that were sampled for arbuscular mycorrhizal fungi in the Republic of Ireland.

considered; lithosols, shallow brown earths, acid brown earths, grey brown podzolics, brown podzolics, podzols, gleys and peat (for a detailed description of these soil types, see Fay *et al.*, 2007b). According to land use, the 40 sites included 21 pastures (17 grazed and 4 rough grazed pastures (sites with environmental characteristics and management between those of a pasture and peatland)), 12 arable fields (tilled), 2 peatlands (raised bogs) and 5 forests (3 plantations and 2 seminatural forests). The majority of sites consisted of pasture and arable sites, such that each soil type found in these two land-use categories was replicated by three sites (pasture and arable sites representing seven and four soil types, respectively). The distance between the sites ranged from 7 to 392 km. Further site descriptions, locations and soil chemical parameters (extractable phosphorus, % organic carbon, % nitrogen, organic matter content and pH; provided by Kelly and Carton, 2009) for each of the 40 sites are given in Supplementary Table S1.

Sampling strategy

A 30×30 metre plot was established at each of the 40 sites, with the plot centred on the sites' global positioning system point. Soil and plant samples were collected in August 2006. Within each plot, 20 soil samples were collected randomly, using a standard 20 cm depth $\times$ 5 cm diameter Edelman soil auger (Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands). The soil samples from each site were bulked, mixed and stored at 4 °C until use.

The field soil was used for a bioassay with *Trifolium repens* L. to bait for AMF. This plant species was chosen as the bait plant due to its abundance in Irish pastures, its ease in growing and because it is highly mycorrhizal. Sterile pots ($8 \times 8 \times 8$ cm) were filled with a mixture of field soil and autoclaved sand (1:1 mix), into which 10 surface-sterilised seeds of *T. repens* were sown. Seeds were surface sterilised in 2.5% sodium hypochlorite for 15 min and rinsed three times in sterile water. Three replicate pots were prepared per field site. Twenty-five negative control pots contained seedlings of *T. repens* grown in autoclaved field soil and sand (1:1 mix). Pots were randomised in a growth chamber where they were grown for 3 months under environmentally controlled conditions (8 h dark/16 h light (120 μmol photons $m^{-2} s^{-1}$) cycle, constant temperature of 20 °C) and watered twice a week. At harvest, all plant roots were carefully and thoroughly washed free of soil with water. From each of the three replicate pots per site, roots from five randomly selected plants were bulked ($n = 3$ bulk-root samples per site), and rinsed three times with deionised water, blotted dry, frozen with liquid nitrogen and stored at -80 °C.

Lolium perenne L. and *T. repens* L. plants were collected from a subset of the pasture sites (16 sites for *L. perenne*, 13 sites for *T. repens*, with 12 sites commonly sampled for both species). These plant species were chosen for collection as they were the most frequent and abundant plant species across the pasture sites. For each plant species, five random samples were collected in each plot using a shovel to excavate a block of turf 15×15 cm by 30 cm deep in which the plant species of interest dominated. These turf samples were stored at 4 °C and the roots were processed from these samples within 2 weeks from collection. Soil debris was thoroughly washed from plant roots with water. From each of the five turf samples per site, five randomly selected plants were bulked ($n = 5$ bulk-root samples per site), and the roots were further processed as described above.

Terminal-restriction fragment length polymorphism

Frozen root samples were homogenised (using a mortar and pestle) and total DNA was extracted from 100 mg of homogenate using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). A 550-bp region of the 18S rRNA was amplified using the universal eukaryotic primer NS31 (Simon *et al.*, 1992) and the AMF primer AM1 (Helgason *et al.*, 1998). NS31 and AM1 were 5′ end-labelled with the fluorescent dyes FAM and HEX, respectively. The primer set chosen amplifies most AMF species, except *Archaeospora*, *Paraglomus* and some *Glomus* group B species (Redecker, 2000). Also, some non-specific amplification can occur (Douhan *et al.*, 2005). To assess the amount of error due to non-specific amplification, representative samples were cloned and Sanger sequenced; amplification error was estimated based on these data and found to be minimal (see Supplementary Materials and methods).

PCR was conducted in a volume of 25 μl and contained 21 μl of Megamix (Microzone, Haywards Heath, UK), 25 pmol of each primer, 1 μl of bovine serum albumin and 2.5 μl of template DNA. Amplification was performed using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) and a programme consisting of: 3 min at 95 °C; 9 cycles of 1 min at 94 °C, 1 min at 58 °C, 2 min at 72 °C; 20 cycles of 30 s at 94 °C, 1 min at 58 °C, 3 min at 72 °C; a final extension for 7 min at 72 °C. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN).

For T-RFLP analysis, purified PCR products were digested with the restriction enzymes *Hin*fI (New England BioLabs, Ipswich, MA, USA) and *Hsp*92II (Promega, Madison, WI, USA) in two separate reactions. Although these enzymes have previously been shown to produce useful terminal-restriction fragments (T-RFs; Vandenkoornhuyse *et al.*, 2003; Johnson *et al.*, 2004), the adequacy of the produced T-RFs to represent the AMF community was verified in subsequent analyses (see Supplementary Materials and methods). Each 20 μl digestion reaction contained 15 μl of PCR product, 2 units of enzyme, 10 × manufacturer's buffer and was incubated for 4 h at 37 °C followed by 20 min at 80 °C to denature the enzymes. The digested products were purified by centrifugation through Millipore Multiscreen Filter Plates (Millipore, Bedford, MA, USA) filled with Sephadex G-50 Superfine (Sigma-Aldrich, St Louis, MO, USA). Mixtures containing 1 μl of each digest product, 0.15 μl of GeneScan-500 ROX internal-lane size standard (Applied Biosystems) and 9.85 μl of Hi-Di Formamide (Applied Biosystems) were denatured at 95 °C for 5 min and cooled on ice before being analysed on an automated capillary sequencer 3130X1 Genetic Analyser (Applied Biosystems). The resulting T-RF profiles were analysed using the programme GeneMarker v1.5 (SoftGenetics, State College, PA, USA), a threshold of 50 fluorescent units, a fragment range of 75–450 bp, and a bin width of 2 bp. A T-RF presence/absence matrix was constructed using the four restriction enzyme/primer combination, and for each site, single T-RF profiles from replicate bait *T. repens*, field *T. repens* and field *L. perenne* samples were combined, respectively.

Statistical analyses of data

The total number of T-RFs for each site was calculated and the significance of differences in the mean number of T-RFs across plant types, land uses and soil types was determined using one-way analysis of variance. Statistical tests were performed using SPSS v12.0.1 (SPSS Inc., Chicago, IL, USA). To assess sampling efficiency, estimates of the total number of T-RFs for each site, based on the first-order Jackknife estimate, were calculated in PC-ORD v5.0 (McCune and Mefford, 1999).

Multidimensional scaling and hierarchical cluster analysis were used to compare the AMF communities within the different plant types, land uses and soil types across sites. Bray-Curtis resemblance matrices were generated based on raw presence/absence T-RF data (Bray and Curtis, 1957; Clarke, 1993). The resemblance matrices were plotted in two dimensions by non-metric multidimensional scaling (NMDS) ordination (25 restarts, 0.01 minimum stress, Kruskall fit scheme 1, stress value $\leqslant 0.18$) (Kruskal and Wish, 1978). Stress (goodness of fit of the plot) was calculated as described by Kruskal (1964); a stress level of $\leqslant 0.1$ corresponds to an ideal ordination (Clarke, 1993). Dendrograms were constructed by hierarchical cluster analysis (group-average linking) using the Bray-Curtis resemblance matrices (Clarke, 1999). Clusters were superimposed on the NMDS plot to form ellipses at arbitrary resemblance levels of slices drawn through the dendrograms (20%, 40% and 60%). One-way ANOSIM (analysis of similarity) was performed on Bray-Curtis resemblance matrices (incorporating 999 permutations for R statistics) to determine the significance of differences between plant types, land uses and soil types. All procedures were computed using PRIMER v6.1.9 (Primer-E Ltd, Plymouth, UK).

Two complementary approaches, direct ordination and Mantel test (Tuomisto and Ruokolainen, 2006), were used to relate the variability in the distribution of AMF to environmental factors (land use, soil type, extractable phosphorus, nitrogen, organic carbon, organic matter, pH and rainfall) and geographical distance. For the direct ordination approach, principle coordinates of neighbour matrices (PCNM) were calculated from grid coordinates of the sites using the 'pcnm' function of the 'vegan' package with the R language (R Development Core Team, Vienna, Austria). PCNM were used as explanatory spatial variables for canonical correspondence analysis (Borcard and Legendre, 2002; Borcard *et al.*, 2004; Dray *et al.*, 2006). PCNM and environmental variables that significantly explained variation in AMF communities were determined with forward selection (999 Monte Carlo permutations; $\alpha < 0.05$) and used in canonical correspondence analysis (Peres-Neto *et al.*, 2006). Partial canonical correspondence analysis was performed when both PCNM and environmental variables were significant. Analyses were performed in CANOCO for Windows v4.5 (ter Braak and Smilauer, 2002).

For the Mantel approach (Mantel, 1967; Smouse *et al.*, 1986; Rossi, 1996; Martiny *et al.*, 2006), AMF similarity matrices for each plant type, using raw presence/absence T-RF data, were calculated using the Bray-Curtis index of similarity in PAST v1.22 (Hammer *et al.*, 2001). Similarity matrices for environmental factors were generated by calculating the absolute difference of values between sites for each quantitative factor and for the categorical data—sites with the same land use or soil type were coded with a zero and when different with a one. Lower tailed partial Mantel tests were conducted in

XLSTAT 2002 (Addinsoft, New York, NY, USA), with *P*-values based on 9999 permutations.

Results

Terminal-restriction fragments

A total of 446 T-RFs, ranging from 75 to 450 bp, were detected from the 265 bulk-root samples taken across the 3 plant types and the 40 sites surveyed. *Hin*fI-based analyses accounted for 108 FAM-labelled and 105 HEX-labelled T-RFs and *Hsp*92II for 124 FAM-labelled and 109 HEX-labelled T-RFs of the profiles. There was a greater proportion of rare T-RFs compared with frequent T-RFs; 75 T-RFs occurred in 1% of the bulk-root samples and only 38 T-RFs occurred in at least 20% of the samples. The mean number of T-RFs per bulk-root sample was 17 ± 13 for bait *T. repens*, 60 ± 25 for field *T. repens* and 29 ± 20 for field *L. perenne*. The mean number of T-RFs per site was 34 ± 19 ($n = 40$; all sites) for bait *T. repens*, 136 ± 18 ($n = 13$) for field *T. repens*, 67 ± 26 ($n = 16$) for *L. perenne* and 183 ± 30 ($n = 12$; pasture sites only) when including all plant types. Mean estimates of total T-RFs for sites based on first-order Jackknife estimates were 149 ± 22 for field *T. repens*, and 72 ± 38 for field *L. perenne*.

Effect of environmental factors and geographic distance on AMF communities

Based on the direct ordination approach, the AMF community composition on the bait *T. repens* was significantly influenced by only 1 of the 29 PCNM vectors, rainfall, peat land use, and the peat and lithosol soil types, and the field *L. perenne* by the gleys soil type, pH and rainfall. These variables were retained by the forward selection process and used in canonical correspondence analysis models, respectively. PCNM explained only 3.8% of the variation compared with 16% by environment of the bait *T. repens* AMF community (Table 1). Environment explained 31% of the variation in the field *L. perenne* AMF community (Table 1).

Table 1 Canonical correspondence analyses for determination of percent variation in arbuscular mycorrhizal fungal communities of the different plant types explained by environment and principle coordinates neighbour matrices (PCNM)

% Of variation	*Bait Trifolium repens*[a]	*Field Trifolium repens*[b]	*Field Lolium perenne*[c]
Environment	16.01	—	31.10
PCNM	3.84	—	—
Environment + PCNM	2.08	—	—
Undetermined	80.15	100	68.90

Abbreviation: AMF, arbuscular mycorrhizal fungi.
[a]Based on AMF terminal restriction fragment (T-RF) profiles of bait plants grown in soils from 36 sites.
[b]Based on AMF T-RF profiles of plants collected from 12 pasture sites.
[c]Based on AMF T-RF profiles of plants collected from 15 pasture sites.

Based on the partial Mantel approach, variation of the field *L. perenne* AMF beta diversity significantly correlated with soil pH ($r = -0.476$, $P < 0.0001$), and rainfall ($r = -0.332$, $P < 0.0001$) (Table 2). No significant correlation was observed between the variation in AMF beta diversity of the bait and field *T. repens* with environmental variables or geographic distance (Table 2).

AMF community of plant types: bait and co-occurring field plants

The majority of T-RFs were unique to one plant type (bait *T. repens*: 11%, field *T. repens*: 28% and field *L. perenne*: 24%) and only 10% of T-RFs were shared between all plant types, 6% between bait and field *T. repens*, 17% between field *T. repens* and *L. perenne*, and the remaining 4% between bait *T. repens* and field *L. perenne*. The mean number of T-RFs was significantly different between plant types sampled across common sites ($P = 0.001$; all pairwise comparisons being significantly different, $P < 0.007$) (Table 3). Higher numbers of T-RFs were obtained from field *T. repens* and *L. perenne*, as compared with the bait *T. repens* (numbers being greatest for field-collected *T. repens*) (Table 3). The AMF community of the different plant types clustered as distinct groups on the NMDS plot with each group having a within-group similarity of $>40\%$ (average similarity within bait *T. repens* was 51.97%, field *T. repens* with 70.72% and field *L. perenne* with 47.89%) and a between-group similarity of 20–29% (bait *T. repens*–field *T. repens* = 24; field *T. repens*–field *L. perenne* = 29), based on the cluster analysis (Figure 2a). The plant type groups were all significantly different from each other (global $R = 0.946$, $P = 0.001$; all pairwise comparisons, $P = 0.001$).

Differences in the frequency and abundance of phylogenetically defined sequence groups were observed when comparing between the plant type samples from site 1176 (Supplementary Figure S1). These differences in the distribution of AMF sequences between the three plant types correspond with the results of T-RFLP analysis, which demonstrated that the AMF communities of the plant types were significantly different.

AMF community of different land uses and soil types

The mean number of T-RFs across sites between land uses was not significantly different ($P = 0.144$) (Table 3). The AMF communities of the bait *T. repens* did not show distinct groupings based on land use, with 40% similarity ellipses superimposed on the NMDS plot encompassing a mixture of land uses (Figure 2b). The ANOSIM test, performed using the land use categories; arable, pasture (grazed and rough) and forest (plantation and seminatural), was not significant (global $R = 0.085$, $P = 0.125$).

Table 2 Partial Mantel test analyses for the association between arbuscular mycorrhizal fungi community structure and both environmental factors and geographical distance

Parameter	*Control for*	*Bait Trifolium repens*[a]		*Field Trifolium repens*[b]		*Field Lolium perenne*[c]	
		r	P	r	P	r	P
Soil type	Distance	0.040	0.839	0.178	0.924	−0.205	0.034
Land use	Distance	−0.037	0.181	—	—	—	—
Rainfall (mm year^{-1})	Distance	−0.067	0.045	−0.079	0.259	−0.332	**0.0001**
P (mg kg^{-1})	Distance	−0.104	0.005	−0.133	0.139	−0.129	0.097
C (%)	Distance	−0.092	0.011	0.019	0.567	−0.013	0.448
N (%)	Distance	−0.019	0.306	−0.019	0.436	−0.116	0.118
OM (%)	Distance	−0.095	0.010	0.037	0.611	−0.033	0.374
pH	Distance	−0.067	0.048	−0.118	0.180	−0.476	**0.0001**
Distance	Soil type	−0.037	0.180	0.019	0.562	0.047	0.652
Distance	Land use	−0.028	0.228	—	—	—	—
Distance	Rainfall	−0.007	0.425	−0.087	0.252	0.079	0.784
Distance	P	−0.021	0.290	−0.132	0.144	0.013	0.550
Distance	C	−0.019	0.327	0.007	0.517	0.011	0.541
Distance	N	−0.027	0.263	−0.059	0.328	−0.003	0.496
Distance	OM	−0.020	0.309	0.040	0.630	0.007	0.531
Distance	pH	−0.022	0.292	−0.121	0.172	0.011	0.539

Abbreviations: AMF, arbuscular mycorrhizal fungi; OM, organic matter.
P-values significant after Bonferroni correction for multiple comparisons (0.05/16 = 0.0031; 0.05/14 = 0.0036) are in bold.
[a]Based on AMF terminal restriction fragment (T-RF) profiles of bait plants grown in soils from 36 sites.
[b]Based on AMF T-RF profiles of plants collected from 12 pasture sites.
[c]Based on AMF T-RF profiles of plants collected from 15 pasture sites.

Table 3 Number of terminal-restriction fragments (T-RFs) between the plant types from commonly sampled pasture sites, and land uses based on the bait *Trifolium repens* from the 40 study sites

	No. of sites	*Total no. of T-RFs*	*Mean T-RFs across sites*	*s.d.*
Plant type				
Bait *T. repens*	12	121	42	17
Field *T. repens*	12	251	136	19
Field *L. perenne*	12	217	71	25
All plant types	12	403	183	30
Land use				
Pasture	21	165	39	18
Arable	12	105	25	19
Forest	5	71	34	13
Peatland	2	68	33	18
All land uses	40	179	35	19

The mean number of bait *T. repens* T-RFs among different soil types of pastures or arable sites was not significantly different (pasture: $P = 0.390$, arable: $P = 0.192$), nor were the field *L. perenne* mean number of T-RFs among different soil types of pastures ($P = 0.860$) (Table 4). The NMDS plot of the AMF community of *L. perenne* from pasture sites with different soil types shows groupings with 60% similarity ellipse deduced by cluster analysis (Figure 2c), and ANOSIM analysis indicated soil type-based discrimination of AMF community composition ($P = 0.008$; global $R = 0.315$). There were no significant groupings of soil types with the bait *T. repens* from pastures (global $R = 0.038$, $P = 0.355$) or arable sites (global $R = -0.029$, $P = 0.590$). Field *T. repens* were not included in the above analyses due to insufficient replication ($n < 3$) in the number of sites for some of the soil types.

Discussion

Our findings suggest that at the landscape scale the distribution of AMF was being driven by local environment rather than geographical distance. Therefore, our results do not reject the Baas-Becking hypothesis. There are very few studies on fungi that have investigated landscape-scale distribution patterns. Interestingly, Green *et al*. (2004) only detected small changes in ascomycete communities over increasing geographic distances (maximum distance 100 km) and suggested that abiotic variables at the local scale were driving community compositions. However, Lekberg *et al*. (2007) found that both soil texture and geographical distance (maximum distance 25 km) influenced the AMF community of *Zea mays* from 10 arable sites. This was recently supported by van der Gast *et al*. (2011), who showed that farming practice (organic vs conventional) and geographical distance (sites within 250 km) affected AMF community composition of bait plants from nine locations. A synthesis of microbial studies across different spatial scales suggests that the relationship between geographic distance and environmental effects on microbial communities may be related to the scale of the study, with a geographic distance effect at the large scale, an environmental effect at the small scale, and both effects or environmental only at the landscape scale (Martiny *et al*., 2006). Landscape-scale studies of AMF support this statement, but at the global scale

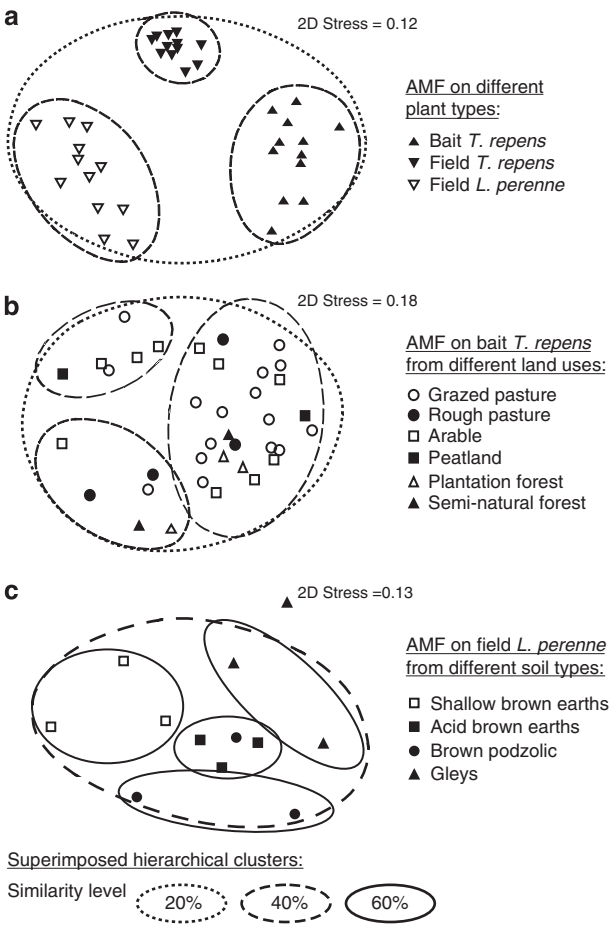

Figure 2 Non-metric multidimensional scaling plots of the arbuscular mycorrhizal fungal (AMF) community; (**a**) on different plant types sampled from common sites ($n=12$), (**b**) on bait *Trifolium repens* from sites with different land uses ($n=37$; 3 outliers sites are not shown), and (**c**) on field *Lolium perenne* from pasture sites with different soil types ($n=12$). Each point on the plot represents a site's AMF community. Plots were derived using Bray-Curtis resemblance matrices generated using terminal-restriction fragment data. Ellipses represent superimposed hierarchical clusters deduced using Bray-Curtis resemblance matrices.

do not, as both geographical distance and environmental effect were found to influence AMF distribution (Kivlin *et al.*, 2011).

Environmental variables explained variation in the AMF communities, and were correlated with the variation of the AMF beta diversity, unlike geographical distance. Soil pH and rainfall were significant drivers for the field *L. perenne* AMF, and rainfall for the bait *T. repens* AMF. Previous spore-based studies have shown soil pH and rainfall to influence AMF sporulation (Wang, 1993; Lovelock *et al.*, 2003), spore density and richness (Johnson *et al.*, 1991; Tchabi *et al.*, 2008), extraradical mycelium growth (van Aarle *et al.*, 2002) and spore community composition (Anderson *et al.*, 1984; Coughlan *et al.*, 2000; Fitzsimons *et al.*, 2008). In addition, AMF communities of *L. perenne* in pastures were influenced by soil type. These results are supported by the spore-based study of Oehl *et al.* (2010), which found several of their 61 detected AMF species to be associated with a specific soil type.

Several previous studies conducted in various ecosystems have shown that a wide variety of co-occurring plant species can harbour different AMF communities (Helgason *et al.*, 2002; Husband *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002, 2003; Gollotte *et al.*, 2004; Scheublin *et al.*, 2004; Santos-González *et al.*, 2007; Sýkorová *et al.*, 2007a). Only a few studies have found no significant differences between the AMF communities of co-occurring plant species (Opik *et al.*, 2003; Santos *et al.*, 2006). In our study, the distribution of AMF in the roots of co-occurring *T. repens* and *L. perenne* was non-random and these hosts possessed distinctly different AMF communities across the 12 pasture sites sampled. This was supported by the T-RFLP data (17% of T-RFs were common to both plant species across all sites), and to a lesser extent the sequencing data (50% of sequence groups being common to both plant species from pasture site 1176). AMF community differences between plant

Table 4 Number of terminal-restriction fragments (T-RFs) between the soil types

Soil type	*Bait T. repens from pasture sites*			*Bait T. repens from arable sites*			*Field L. perenne from pasture sites*		
	Total no. of T-RFs	*Mean T-RFs across sites*[a]	*s.d.*	*Total no. of T-RFs*	*Mean T-RFs across sites*[a]	*s.d.*	*Total no. of T-RFs*	*Mean T-RFs across sites*[a]	*s.d.*
Lithosols	74	28	16	—	—	—	—	—	—
Shallow brown earths	58	29	21	—	—	—	104	58	19
Acid brown earths	87	54	20	57	28	13	127	73	20
Grey brown podzolics	72	36	23	37	14	14	—	—	—
Brown podzolics	95	53	14	30	14	11	109	59	11
Podzols	72	44	9	—	—	—	—	—	—
Gleys	48	30	14	81	44	26	148	73	50

[a]Means based on three sites for each soil type.

species may not represent 'local host preferences', but is a phenomenon that occurs at larger geographical scales. How AMF plant-host preference is regulated in nature in terms of top-down (plant host) and bottom-up (fungal symbiont) control is not clearly understood. As two different types of plants were sampled, a grass (*L. perenne*) and a legume (*T. repens*), which have distinct differences in root morphology and phosphorus requirements (Schweiger, 1994), this may be a contributing factor to the host preference effect observed.

Somewhat surprisingly, there was little influence of land use on AMF T-RF richness and community composition of the bait *T. repens*. A wide range of land-use types were included in the study (e.g., arable, pastures and peatlands), which differed markedly in the associated vegetation and management practices. Previous studies have shown that plant community structure can influence AMF communities, and thus vegetation differences across sites were expected to have a community effect (Johnson *et al.*, 1992, 2004; Landis *et al.*, 2004; Fitzsimons *et al.*, 2008; Hausmann and Hawkes, 2009). In addition, different agricultural management systems have been shown to affect AMF community composition, with distinct communities found between organic and conventional farming (Oehl *et al.*, 2004; van der Gast *et al.*, 2011) and between agroecosystems varying from seminatural grasslands to intensively cropped arable fields (Oehl *et al.*, 2003, 2010). However, it appears that it is specific management practices, such as tillage (Jansa *et al.*, 2002) and grazing (Eom *et al.*, 2001), rather than broad management categories that drive differences between agroecosystems, and our results suggest that it is specific environmental variables of sites, such as soil pH and rainfall, that vary within land uses have a stronger effect than land use itself on AMF communities.

Alternatively, the lack of a land-use effect found on the AMF community of the bait *T. repens* could be a methodological artefact. The bait *T. repens* plants yielded a different AMF community composition than the field-collected *T. repens*. The bait plants highlighted different and lower numbers of T-RFs (only 6% of T-RFs were shared, and field *T. repens* had 2 × more T-RFs), and different sequence groups. Sequence data from previous studies, based on one or two field sites, have also shown differences in AMF communities between bait and field plants (Opik *et al.*, 2003; Sýkorová *et al.*, 2007b). Likely, the longer exposure time to a larger quantity of soil containing AMF propogules for field plants compared with bait plants contributes to the difference in the AMF communities observed. Also, differences between the community compositions could be attributed to the preferential occurrence of certain species in mature established root systems and later stages of succession, and the influential effects from plant neighbours in a natural system (Sýkorová *et al.*, 2007b; Hausmann and Hawkes, 2009).

The bait-plant method has the advantage of enabling comparisons of *in situ* AMF communities between multiple sites without confounding factors that affect the AMF colonisation of plant roots, such as various plant (e.g., plant species, genotype and development) and environmental (e.g., light, temperature and soil moisture) variables (Helgason and Fitter, 2009). However, using bait plants to infer AMF biogeographical patterns could be misleading. The AMF communities on the roots of the same plant species (*T. repens*), but from different approaches (bait vs field) shared few T-RFs and resulted in different relation to environmental variables. Further investigation is warranted to better understand this group of AMF, which colonises bait plants but not field plants. Due to the bias of the bait-plant approach and that AMF-plant host preferences exist at the landscape scale, to better capture AMF diversity a wide range of plant species should be collected from sites and the bait-plant approach used to complement that of field plants. Despite the difference in the AMF communities between the bait and field plants, both AMF compositions were not influenced by geographical distance.

Understanding AMF distributional patterns is important due to the role they play in ecosystem functioning, and because of the current environmental threats to AMF diversity (Turrini and Giovannetti, 2012). Here, we show that the landscape-scale distribution of the AMF communities on plant roots was driven by the local environment, with soil characteristics playing a vital role. Also, roots of *L. perenne* and *T. repens* were shown to support contrasting communities of AMF, and the nature of communities colonising each plant was consistent across pasture habitats and over distance. Our results also demonstrate the strong influence plant host and sampling method have on determining AMF community composition. Advances in sampling approaches and assessment of AMF diversity will further improve our understanding of AMF biogeography. To date, there are a limited number of studies on AMF spatial scaling. Additional studies would provide a more comprehensive view on whether geographical and/or environmental factors shape the distribution of AMF at various spatial scales.

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