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Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem

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Abstract

Arbuscular mycorrhizal (AM) fungi are biotrophic symbionts colonizing about two-thirds of land plant species and found in all ecosystems. They are of major importance in plant nutrient supply and their diversity is suggested to be an important determinant of plant community composition. The diversity of the AM fungal community composition in the roots of two plant species (*Agrostis capillaris* and *Trifolium repens*) that co-occurred in the same grassland ecosystem was characterized using molecular techniques. We analysed the small subunit (SSU) ribosomal RNA gene amplified from a total root DNA extract using AM fungal-specific primers. A total of 2001 cloned fragments from 47 root samples obtained on four dates were analysed by restriction fragment length polymorphism, and 121 of them were sequenced. The diversity found was high: a total of 24 different phylotypes (groups of phylogenetically related sequences) colonized the roots of the two host species. Phylogenetic analyses demonstrate that 19 of these phylotypes belonged to the Glomaceae, three to the Acaulosporaceae and two to the Gigasporaceae. Our study reveals clearly that the AM fungal community colonizing *T. repens* differed from that colonizing *A. capillaris*, providing evidence for AM fungal host preference. In addition, our results reveal dynamic changes in the AM fungal community through time.

Keywords: arbuscular mycorrhizal (AM) fungi, community composition, diversity, dynamics, host-plant preference, SSU rRNA gene

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Introduction

An enormous diversity of microorganisms is present in soils (e.g. Torsvik *et al*. 1990). Soil microorganisms can affect plant growth and reproduction powerfully, and can thus exert an important role in determining plant population structure. Arbuscular mycorrhizal (AM) fungi are probably the most important of those microorganisms. AM fungi play a key role in plant phosphorus supply, and in return the host plant provides carbon assimilates (Smith & Read 1997). In addition to an improvement of plant nutrition, AM fungi can protect the plants from pathogens (Newsham *et al*. 1995) and can influence plant growth traits (Streitwolf-Engel *et al*. 1997). Furthermore, a given AM fungal isolate can enhance, reduce or have no effect on plant growth depending on the plant species (van der Heijden *et al*. 1998a, 1998b). Therefore, it has been suggested that mycorrhizal fungal diversity is a determinant of plant diversity (van der Heijden *et al*. 1998b). However, we have insufficient knowledge about the diversity of AM fungi actually colonizing plant roots (as opposed to those present as spores in soil) to assess their diversity relative to that of other groups in the same ecosystems. Such data may additionally provide insight for conservation biology and environmental protection.

AM fungal taxonomy is based on spore morphology, and it is impossible to identify the fungal structures within plant roots below the order or the genus level from morphological data. Equally, the relative abundance of spores in the soil may not be a good estimator of AM fungal community composition and dynamics (Clapp *et al*. 1995; Morton *et al*. 1995; Merryweather & Fitter 1998). A molecular approach is needed to characterize the AM fungal species *in planta*,

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to focus on the actively growing and functional fungi (Helgason *et al*. 1999; Clapp *et al*. 2002). Specific primers for the amplification of the AM fungal SSU rRNA gene have been designed (Helgason *et al*. 1998), allowing a measurement of the AM fungal species diversity in roots (Helgason *et al*. 1998, 1999; Daniell *et al*. 2001). Using this strategy, Helgason *et al*. (1999) have shown differences in the AM fungal community associated with *Hyacinthoides nonscripta* on two sampling dates and in two environments. Despite the broad host range of AM fungi (Smith & Read 1997), the AM fungal communities in plants from arable soil were very different from those in nearby woodland soils (Helgason *et al*. 1998). Because arable and woodland soils support different plant species, a working hypothesis could be that host-plant preference exists. In support of this hypothesis, Bever *et al*. (1996), Del Val *et al*. (1999) and Eom *et al*. (2000) have suggested the possibility of a host-plant species effect on AM communities, based on the abundance of spore types.

Three questions are addressed in this paper. First, what is the AM fungal diversity in plants from a grassland ecosystem? Second, does the pattern of AM fungal community composition in the plant roots exhibit temporal changes? To answer this question, the fungal community composition has been analysed independently for 12 plants on four sampling dates of a field experiment. Third, does the fungal community differ among host-plant species? We assessed this question by comparing the patterns of AM fungal community diversity in the roots of two co-occurring species, *Agrostis capillaris* (Poaceae) and *Trifolium repens* (Fabaceae), sampled simultaneously from a seminatural grassland ecosystem.

Materials and methods

Sampling and total DNA extraction from roots

Replicate cores samples (6.3 cm diameter) were collected to a depth of 20 cm from the NERC Soil Biodiversity field experiment at the Macaulay Institute's Sourhope field station near Kelso (Scotland), a seminatural grassland ecosystem. The field experiment was divided into plots that received different treatments. Each plot is divided into two subplots. A map of the field experiment is available (http://www.nmw.ac.uk/soilbio/Sourhope_Design.htm). Here we have focused on the five control plots that received no chemical inputs. Sampling was carried out in September 1999, November 1999, May 2000 and October 2000. On each sampling date, samples were taken from the same 0.75 m^2 (0.5 m × 1.5 m) of each subplot (10 core samples altogether). From each core sample, plants were identified, separated and washed thoroughly, and the roots were collected and frozen. Total DNA was extracted from the roots according to Edwards *et al*. (1997). An additional step of DNA purification was performed using the Concert Rapid Purification kit (Gibco BRL Life Technologies, Paisley, UK) following the manufacturer's recommendations.

PCR amplification, cloning and sequencing

A partial sequence of the SSU rRNA gene was amplified (550 bp) using a universal eukaryotic primer NS31 (Simon *et al*. 1992) and an AM fungal-specific primer AM1 (Helgason *et al*. 1998). The reaction mixture contained typically 0.2 mM dNTPs, 10 pmol of each primer, 0.725 U of the proof-reading *Pfu* DNA polymerase (Promega, Southampton, UK), in the manufacturer's reaction buffer. The cycling regime used was the same as published previously (Helgason *et al*. 1999) on a PTC100 machine (MJ Research, Boston, MA, USA). Before cloning, the blunt-ended PCR products were modified using an A-overhanging post-amplification procedure. To the amplification mixture, 1 U of *Taq* DNA polymerase (Gibco BRL Life Technologies) and 0.1 mM of dATP was added and incubated for 15 min at 72 °C. A phenol–chloroform extraction was performed immediately to prevent the *Pfu* exonuclease activity. The PCR products were precipitated using 1/10 volume of 3 M sodium acetate and 2 volumes of absolute alcohol. The air-dry pellet was re-suspended in 50 μL of ultrapure water (Merck, Poole, UK). For ligation, 1.5 μL of 3'A-tailed PCR products, 1.0 μL of pGEM-T vector (Promega), 5.0 μL of rapid pGEM-T buffer and 1 μL of T4-DNA ligase (Promega) in a final volume of 10 μL were incubated at 4 °C overnight. Transformation of DH5α competent cells (Gibco BRL Life Technologies) was performed following the manufacturer's instructions. Within each resulting SSU rRNA gene library, up to 56 cloned fragments (48 as a minimum) were re-amplified and analysed. Each cloned SSU rDNA fragment was digested with the restriction enzymes *Hin*fI and *Hsp92II* (isoschizomer of *Nla*III) according to the manufacturer's advice (Promega) and analysed using 2% MetaPhor® agarose (BMA, Vallensbaek Strand, Denmark) gel electrophoresis. Representatives of each RFLP pattern detected were sequenced on an ABI377 automated sequencer (Perkin Elmer, Foster City, CA, USA) using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA polymerase FS (Perkin Elmer). Overall, 121 cloned DNA fragments were sequenced on both strands using AM1 or NS31 as primer. Then the two sequences were aligned using the program AUTOASSEMBLER (Perkin Elmer).

Sequence data analyses

A search for chimeric sequences was performed using the program CHIMERA_CHECK 2.7 of the Ribosomal Database Project (http://rdp.cme.msu.edu/html/analyses.html) (Maidak *et al.* 2001). A total of 33 sequences was clearly chimeric or

suspected being chimeric on the basis of this analysis and also because the phylotype they represented was found only among the clones obtained from a single rRNA gene library (i.e. a single plant). The other 88 sequences were registered in GenBank under the accession nos AF437637–AF437723.

A multiple alignment was performed using ClustalX 1.81 (Thompson *et al*. 1997) and refined by eye. Phylogenetic relationships among 56 known AM fungal species sequences and the 88 environmental phylotypes were established using the neighbour joining (NJ) algorithm (Saitou & Nei 1987) and the maximum parsimony (MP) procedure (Swofford 1993) with ClustalX 1.81 and PAUP 3.1.1, respectively. Distances for the NJ tree were computed with a correction for multiple substitution using a Kimura 2-parameter model. For the MP analysis, a heuristic tree search with tree bisection and reconnection (TBR) as swapping algorithm and 500 replicates with random addition order of sequences was used to find the shortest tree. Bootstrap support values for branches were estimated from 500 replicates for the MP phylogeny and 1000 replicates for the NJ tree. The putative choanozoan *Corallochytrium limacisporum*, a close relative of fungi (Cavalier-Smith & Allsopp 1996), was chosen as outgroup and used to root the trees on the basis of the widely accepted phylogenetic hypothesis that the fungal radiation is contemporary with the choanozoan radiation (Cavalier-Smith & Allsopp 1996).

Patterns of AM fungal community composition

The overall experimental design allows the comparison of variation in the AM fungal community composition among plants of a given species, between plant species and among sampling dates. The data were analysed using multidimensional scaling (MDS) implemented in SPSS 10.0. MDS is designed to analyse distance-like data, representing here the degree of dissimilarity of two AM fungal communities. To model dissimilarities, Euclidean distances are computed. This approach requires no strong assumption such as a normal distribution of error terms (Young & Harris 1994).

According to Losos (1996), one of the best ways to compare community composition and to assess community structure and evolution is to use a phylogenetic-based model (particularly MP). We therefore coded the presence or absence (1/0, respectively) of each AM fungal species as a discrete variable, in order to undertake a comparison of community composition under the MP procedure. The shortest topology found was calculated using a heuristic tree search with 100 replicates of random addition and using the TBR swapping algorithm.

Hierarchical log-linear modelling

To examine the relationship among variables (i.e. interactions), a saturated model that contains all the independent variables as well as combinations of the variables was constructed. The saturated model reproduces exactly the observed data. The relative importance of a given interaction term is estimated after having removed this term from the saturated model. The overall goodness-of-fit statistics are then calculated in SPSS 10.0 using likelihood ratio χ^2 to determine the significance levels and deduce whether this new unsaturated model fits the data. Here, the host plant, the sampling date and the AM fungal species (i.e. RFLP pattern) count are included in the model. The rarest 50% of species were not taken into account in this analysis in order to limit a possible bias in likelihood ratio estimates.

Results

AM fungal diversity is high

Overall, 2288 clones were screened by PCR, of which 2001 contained the SSU rRNA gene fragment (Table 1). On average, 43 positive clones were analysed per plant. The RFLP analyses differentiate 18 patterns (Table 2). Depending on the RFLP type frequencies, up to 20 cloned DNA fragments of each RFLP type were sequenced. BLAST searches in the GenBank sequence database show that the 88 sequences amplified using NS31-AM1 belong to the Glomales. Helgason *et al*. (1999) have estimated that, given the error rate of the proof-reading *Pfu* DNA polymerase, an expected 2.1% of the PCR products may contain a single PCR error. That corresponds to only about two of the 88 sequences, so we are confident of the phylogenetic information contained in the tree.

The phylogenetic tree topology (Fig. 1) is largely in agreement with previously published phylogenies (Simon *et al*. 1993; Gehrig *et al*. 1996; Helgason *et al*. 1998, 1999; Redecker *et al*. 2000; Tehler *et al*. 2000; Schüßler *et al*. 2001a). The phylogenetic tree supports groups of taxa corresponding to the Glomaceae, the Acaulosporaceae and the Gigasporaceae. The position of the Gigasporaceae is unclear as it is not congruent between the NJ and MP trees (Fig. 1), but all sequences can be assigned unambiguously to a family.

The phylogenetic relationships among the sequences clearly reveal discrete sequence groups (Fig. 1). Our 88 SSU rRNA gene sequences are partitioned in a minimum of 24 clusters, and hence potentially 24 taxonomic units (each with bootstrap support > 75%, Fig. 1). The sequence identity within the clusters ranges from 97.5 to 100%. The AM fungi colonizing *A. capillaris* and *T. repens* were found within the Glomaceae, Acaulosporaceae and Gigasporaceae. No sequences were detected within the Archaeosporaceae or Paraglomaceae, but this was expected because the AM1 primer does not match the highly divergent sequences of these newly defined taxa (Redecker *et al*. 2000). Among the 24 phylotypes, 19 belong to the Glomaceae, three to the Acaulosporaceae

Table 1 Number of arbuscular mycorrhizal fungal ribosomal small subunit RNA (SSU) gene clones analysed for each sampling date

Time point	Number of plants analysed	Total clones examined	Number of AM SSU gene clones
September 1999	9 *A. capillaris*	432	414
	3 *T. repens*	144	138
Σx_1		576	552
October 1999	8 *A. capillaris*	384	362
	3 *T. repens*	144	135
Σx_2		528	497
May 2000	9 *A. capillaris*	432	373
	3 *T. repens*	144	127
Σx_3		576	500
October 2000	9 *A. capillaris*	448	345
	3 *T. repens*	160	107
Σx_4		608	452
$\Sigma\Sigma x_n$		2288	2001

Table 2 RFLP patterns of AM fungal SSU rRNA PCR products. Fragment sizes are given in base pairs. The order of the fragments reflects the position of the restriction sites in the PCR product

RFLP pattern	Phylotype	*Hin*fI digestions	*Hsp92II* digestions
a	Glo3	131, 49, 334, 15	245, 284
b	Glo23	132, 383, 19	104, 142, 288
c	Glo25	132, 302, 81, 19	105, 142, 290
d	Glo24	135, 49, 339, 20	107, 142, 294
e	Glo2	179, 332, 15	154, 90, 282
f	Glo22, Glo9	516, 19	157, 90, 288
g	Glo1A, Glo1B	274, 244, 20	106, 143, 289
h	Glo21	514, 18	174, 71, 287
i	Glo13, Glo14, Glo15	131, 49, 90, 241, 15	245, 281
j	Glo16	181, 354	246, 289
k	Glo19	178, 81, 244, 15	150, 22, 62, 284
l	Glo18	181, 90, 244, 18	153, 22, 71, 287
m	Glo17	172, 90, 244, 15	87, 57, 93, 284
n	Glo20	182, 334, 20	247, 289
o	Acau3	134, 385, 18	158, 90, 23, 266
p	Acau6, Acau7	132, 384, 15	156, 90, 285
q	Glo12	134, 141, 263	159, 90, 289
r	Scut2, Scut3	292, 221, 18	157, 90, 284

and two to the Gigasporaceae. Only one corresponds to a morphologically defined species: Glo1B is *G. mosseae*.

The high number of TUs found in this study was unexpected given previously published work (Helgason *et al*. 1998, 1999; Daniell *et al*. 2001), especially as the AM fungal community has been examined in the roots of only two plant species at a single site. However, the number of clones analysed is higher than in those studies. An analysis of the effect of sampling effort on observed diversity (not shown) suggests that more sampling in those previous studies would have led to a modest increase in the number of phylotypes found, whereas in our study the sampling effort was more than sufficient to characterize the diversity. The phylogenetic analysis also reveals that just eight (one-third) of these AM fungal phylotypes have been observed previously: Acau3, Glo1A, Glo1B, Glo2, Glo3, Glo9, Glo23 and Glo24 (Fig. 1). The names Glo23 and Glo24 refer to types that were found in arable crops by Daniell *et al*. (2001), for example HW17 and HB6, respectively, but were not distinguished previously from Glo8 and Glo10, with which they share RFLP patterns.

AM fungal communities vary with time and host

Using the selected restriction enzymes, five RFLP patterns of 18 are shared by more than one AM fungal phylotype found in this study. *Scutellospora* spp. Scut2 and Scut3, *Acaulospora* spp. Acau6 and Acau7, *Glomus* spp. Glo1A and Glo1B, *Glomus* spp. Glo13, Glo14 and Glo15, *Glomus* spp. Glo22 and Glo9 are not distinguishable (Table 2). Overall, these phylotypes account for less than 8% of the total

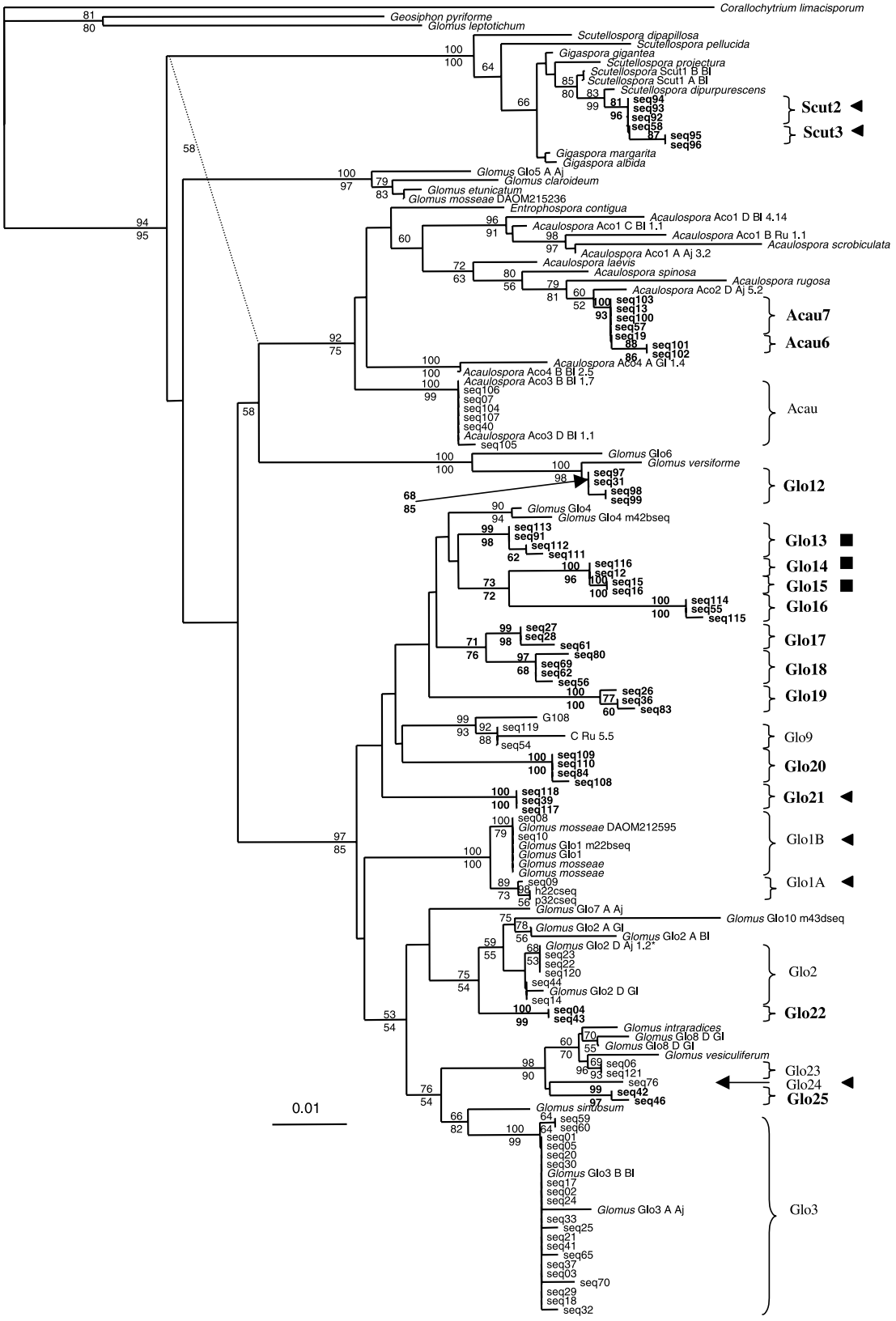

Fig. 1 Phylogeny of Glomales. The tree shown here represents the Neighbour Joining (NJ) analysis. Dashed lines represent the differences from the maximum parsimony (MP) tree. Bootstrap values > 50% for NJ and MP estimates are shown above and below the lines, respectively. New phylotypes are highlighted in bold. The three phylotypes found only in *T. repens* roots are marked with squares, and the six found only in the *A. capillaris* roots with triangles. *Corallochytrium limacisporum*, a putative choanoflagellate (Cavalier-Smith & Allsopp 1996), was used as outgroup.

General log-linear model: interaction removed from the saturated model	LR	Degree of freedom	*P*
AM species × plant removed	273	32	5. E-40
AM species × time removed	852	48	8. E-148
AM species × plant × time removed	107	24	2. E-12

Table 3 *P* values and likelihood ratios (LR) computed by general log-linear model. The *P* and LR estimates in the table are those found when the interaction term shown is removed from the saturated hierarchical log-linear model

number of clones. The phylotypes that shared an RFLP pattern are related phylogenetically, except *Glomus* spp. Glo22 and Glo9. For the community comparisons, we considered the RFLP patterns knowing that the complexity of the diversity within the AM fungal communities is slightly underestimated.

Whatever the term removed from the log-linear saturated model, the likelihood ratio χ^2 is very high and goodness-of-fit probability very low, indicating that the unsaturated models do not fit the data (Table 3). This implies that AM fungal diversity varies with sampling date and with host species and these two factors are interacting, with a very highly statistical confidence (Table 3). Along with the log-linear analysis, the Euclidean-based MDS analysis highlights differences in community composition through time (Fig. 2A). The AM fungal communities in the roots of the plants sampled in September 1999 and November 1999 are very similar (Fig. 2A). Conversely, the AM fungal communities in the roots of the sampled plants is different for the May 2000 and October 2000 samplings (Fig. 2A). It seems that a shift in the pattern of AM fungal community has occurred after a situation of stability.

In addition to modification of the AM fungal community over time, our data clearly demonstrate a host-plant effect. The communities on the two host species are not clearly separated in the first two dimensions of the MDS analysis (Fig. 2B), but the host difference is clearer in the third dimension (Fig. 2C). The host effect is revealed very clearly in a parsimony analysis that arranges the communities on a tree in which branch lengths represent differences in the presence or absence of taxa (Fig. 3).

Discussion

The interpretation of SSU rRNA phylotypes

For our molecular studies, we have used a phylogenetic approach to classification, identifying as 'phylotypes' those clades of closely related sequences that have high statistical support by bootstrap analysis. Phylotypes defined in this way have been used as the basis of species definitions (Mishler & Donoghue 1982; Mishler & Brandon 1987), although we are not equating our phylotypes directly with species. In most cases, they can be distinguished by restriction site differences, which is convenient operationally.

The formal classification of the Glomales is based on morphospecies, defined by phenotypic characters of spores, and molecular studies of cultured fungi have shown that the three morphologically defined families form distinct branches in the SSU phylogeny. However, it is not possible to extend this correspondence down to the species level, for a number of reasons. First, many of the phylotypes that we find in the field (Fig. 1) represent fungi that are not in culture or, at any rate, not in cultures for which sequence data are available. Second, distinct morphospecies have sometimes been defined for isolates that, by molecular criteria, are more closely related than cultures of a single species (Lloyd-MacGilp *et al*. 1996). Thirdly, single spores, and cultures derived from them, frequently contain a mixture of different ribosomal gene sequences. This was first observed for the ribosomal internal transcribed spacer, ITS (Sanders 1995; Lloyd MacGilp *et al*. 1996; Kuhn *et al*. 2001), but is also true for SSU (Clapp *et al*. 1999; Schüßler *et al*. 2001a). Single spores contain hundreds of nuclei, so they should be envisaged as populations or communities rather than individuals. The consequence as far as our study is concerned is that we cannot assume that each phylotype in a root is necessarily derived from an independent colonization event, but this does not invalidate our data on the distribution of phylotypes.

The early radiation of the Order Glomales (Simon *et al*. 1993; Redecker *et al*. 2000), or the Phylum Glomeromycota as proposed by Schüßler *et al*. (2001b), means that there is sufficient variation in the rather conserved SSU gene to give well-resolved phylogenies (e.g. Simon *et al*. 1992, 1993; Helgason *et al*. 1998, 1999; Vandenkoornhuyse *et al*. 1998; Redecker *et al*. 2000; Schüßler *et al*. 2001a, 2001b), while variation in the rapidly evolving ITS approaches saturation (Clapp *et al*. 2002). The NS31-AM1 primer combination, as used here, amplifies a region of the SSU that includes many phylogenetically informative positions.

AM fungal diversity is high

The richness of AM fungi colonizing the roots of *A. capillaris* and *T. repens* is unexpectedly high given our knowledge to date (Helgason *et al*. 1998, 1999; Daniell *et al*. 2001). The Shannon index (*H*) was 1.71 for this Sourhope grassland ecosystem, compared to 1.44 and 0.39 for the old woodland soil and arable soil, respectively (data from

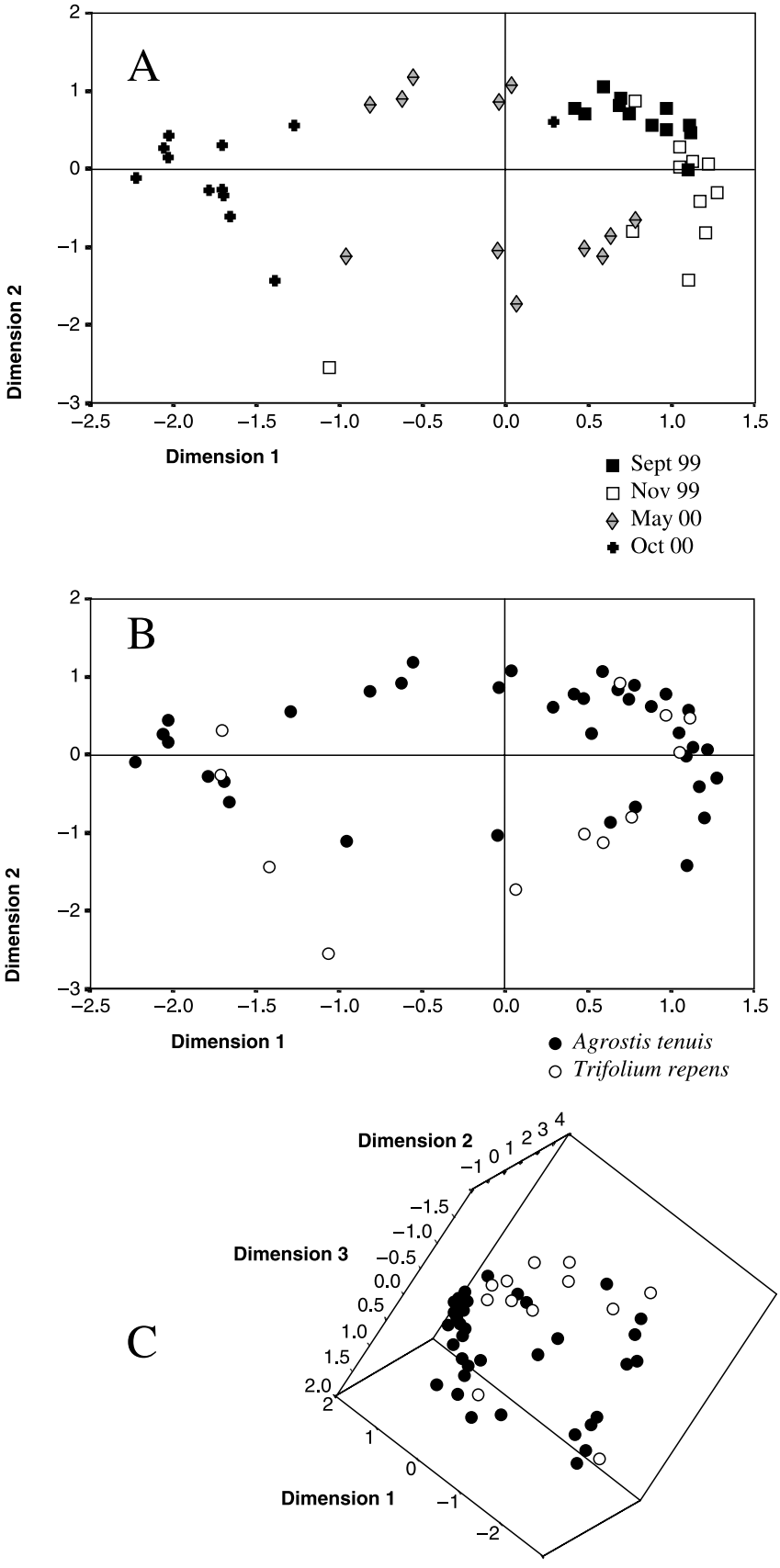

Fig. 2 Euclidean-based multidimensional scaling (MDS) analysis emphasizing changes in the patterns of AM fungal community composition through time (A) and between host plants (B and C). Each point represents an AM fungal root community, with communities of similar composition being located close together. (B) is identical to (A) except that communities are identified by host rather than date. A third dimension in this MDS analysis is plotted in (C) to highlight the difference in patterns of AM fungal community colonizing the two host plants.

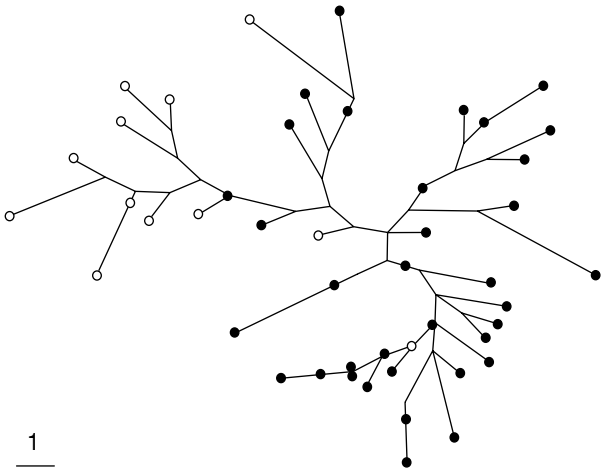

Fig. 3 Unrooted maximum parsimony tree showing differences in the composition of AM fungal communities associated with roots of *Trifolium repens* (white) and *Agrostis capillaris* (black). Communities on close branches share most of the same AM fungal phylotypes. Scale bar corresponds to gain/loss of one informative taxon.

Helgason *et al*. 1998). Although it is not possible to equate phylotypes directly with morphospecies, an inspection of Fig. 1 shows that the amount of sequence divergence between morphologically defined species is often similar to, or less than, the divergence between related phylotypes. Hence the number of phylotypes should be a conservative estimate of the number of AM fungal morphospecies present. The total number of 24 phylogenetic species or TUs is far greater than our expectation. Given the fact that just over 150 AM fungal species have been described so far (Walker & Trappe 1993), these 24 TUs, from the roots of a total of 47 individuals of two plant species sampled from a single site, would account for about 15% of the known species. Of these TUs, only 62.5% (15/24) were found on both host species: six TUs were found only within the *A. capillaris* roots (*Scutellospora* Scut2, Scut3; *Glomus* Glo1A, Glo1B, Glo21, Glo24) and three only within *T. repens* (*Glomus* Glo13, Glo14, Glo15). These results suggest that the AM fungal diversity on land has been massively underestimated. Alternatively, a possible hypothesis is that in seminatural grassland ecosystems the AM fungal diversity is particularly high. By contrast, soils heavily impacted by human exploitation, such as arable soils, contain a lower AM fungal diversity (Helgason *et al*. 1998; Daniell *et al*. 2001). In addition, the woodland and arable sites had far fewer potential AM fungal hosts; only the understorey plants are AM mycorrhizal in the woodland (except *Acer pseudoplatanus*). Van der Heijden *et al*. (1998a) found a positive correlation between AM fungal diversity and plant diversity in a grassland ecosystem. In the Sourhope grassland ecosystem field experiment, the plant species richness, like that of the AM fungi, was moderately high, reaching 25 different species (Kenny 2000).

Patterns of AM fungal community composition

A widely accepted hypothesis about the evolutionary strategy of the AM symbiosis is that a given AM fungus should colonize multiple plant partners to maximize its acquisition of C compounds (e.g. Bever 1999). At the same time, it has been shown in laboratory conditions that most culturable species of AM fungi are able to colonize almost any plant species, suggesting a lack of host specificity. On the basis of these widely accepted ideas, we expected to find a similar pattern of AM fungal community composition in the roots of the co-occurring *A. capillaris* and *T. repens*. By contrast, our results clearly demonstrate that the AM fungal community composition is host-plant dependent.

On the basis of a microcosm experiment, an alternative AM fungal colonization strategy has been proposed: the fungus promotes an existing colonized host plant (Hodge *et al*. 2001). This would fit better with our results, although they indicate that at least some AM fungi do express a host-plant preference. Biochemical mechanisms for this preference are completely unknown. Wilkinson (1997) noted that many plants reproduce vegetatively and also that most plant seed disperses over a short distance; he suggested that this pattern would allow seedlings or plant clones to come into contact with 'genetically identical' fungi to those associated with their parent plant. This corresponds to a 'pseudo-vertical' transmission that makes mutualism likely to evolve (Wilkinson 1997). In such a model, the mycorrhizal fungi could became adapted to both host plant and surrounding environment. One of the important underlying ideas is a stability of the fungus–host-plant partnership. There was little difference in the AM fungal community composition between the samplings of September 1999 and November 1999 (Fig. 2A), but major changes occurred in the May 2000 and October 2000 samplings. A possible explanation of this change is that a shift in the pattern of AM fungal community composition occurred because of a modification of the field management, which shifted from grazing to mowing in 1999. The consequence is a decreasing input of organic matter, which would alter both nutrient cycling and physical conditions. The intuitive idea that depletion or modification of resources can radically alter or modify fitnesses of different phenotypes has been modelled, and the models predict important consequences on the inter- and intraspecific competition within a community (Schluter 2000). It is therefore possible that the observed changes in the patterns of AM fungal community colonizing *A. capillaris* or *T. repens* result from this change in management. We need to determine whether the community trajectory will stabilize in future.

Community differences imply functional differences among AM fungi

The evolutionary and ecological processes that have led to the complexity of plant ecosystems are woven inextricably with the AM communities and evolution (van der Heijden *et al*. 1998b). Plants and AM fungi have coevolved over hundreds of millions of years. This long coevolution could have resulted in the AM fungal host-plant preference. In his theory of the ecology of adaptive radiation, Schluter (2000) states that 'there can be little doubt that competition for resources is an important process in many adaptive radiations'. Competition is an interaction in which neither party benefits and is an important motor for ecological speciation (Schluter 2000). In nature, it is likely that co-occurring AM fungi are competitors within the same niche to obtain carbon compounds from a host plant. Ecological speciation or ecological specialization of AM fungi should therefore lead to a fitness gain in a given environment. This hypothesis would explain the observed differences in composition among AM fungal communities. Future experiments will determine whether an AM fungal community colonizing a plant is an assemblage of different individuals driven by a 'host-plant preference' with an underlying difference in fitness, and also whether antagonistic or co-operative interactions exist among fungi.

Within the AM fungal community colonizing a plant, the biological activity and function of the AM species or genotype ranges from mutualism (i.e. a positive effect on plant growth) to a 'parasitism-like' relationship i.e. a negative effect on plant growth (Johnson *et al*. 1997; Van der Heijden *et al*. 1998a, 1998b). Some may colonize the plant more intensively than others (e.g. Helgason *et al*. 1999) and may receive more assimilates from the plant, while some are able to provide more phosphorus to the plant. It is almost inconceivable that all these AM fungi found within the roots have identical efficiency and functionality. A key issue will be to assess the functional diversity of AM fungi colonizing the roots of a plant to determine their relative contribution to different mycorrhizal functions.

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