Mycorrhiza (2007) 17:175–183
DOI 10.1007/s00572-006-0089-y

ORIGINAL PAPER

Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community

Benjamin E. Wolfe · Daniel L. Mummey · Matthias C. Rillig · John N. Klironomos

Received: 20 December 2005 / Accepted: 19 October 2006 / Published online: 21 December 2006

Abstract Although it has become increasingly clear that arbuscular mycorrhizal fungi (AMF) play important roles in population, community, and ecosystem ecology, there is limited information on the spatial structure of the community composition of AMF in the field. We assessed small-scale spatial variation in the abundance and molecular diversity of AMF in a calcareous fen, where strong underlying environmental gradients such as depth to water table may influence AMF. Throughout an intensively sampled 2×2 m plot, we assessed AMF inoculum potential at a depth of 0–6 and 6–12 cm and molecular diversity of the AMF community using terminal restriction fragment length polymorphism of 18S rDNA. Inoculum potential was only significantly spatially autocorrelated at a depth of 6–12 cm and was significantly positively correlated with depth to water table at both depths. Molecular diversity of the AMF community was highly variable within the plot, ranging from 2–14 terminal restriction fragments (T-RFs) per core, but the number of T-RFs did not relate to water table or plant species richness. Plant community composition was spatially autocorrelated at small scales, but AMF community composition showed no significant spatial autocorrelation. Saturated soils of calcareous fens contain many infective AMF propagules and the abundance and diversity of AMF inoculum is patchy over small spatial scales.

Keywords Spatial heterogeneity · Arbuscular mycorrhizal fungi · Wetland · Calcareous fen · T-RFLP

B. E. Wolfe (✉) · J. N. Klironomos
Department of Integrative Biology, University of Guelph, Guelph, ON N1G 2W1, Canada
e-mail: bewolfe@fas.harvard.edu

Present address:
B. E. Wolfe
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02130, USA

D. L. Mummey · M. C. Rillig
Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA

Introduction

Arbuscular mycorrhizal fungi (AMF) are widespread constituents of soil communities that can influence aboveground processes and properties including insect herbivory (Gange et al. 2005), plant fitness (Koide and Dickie 2002), and plant community diversity and productivity (van der Heijden et al. 1998). Although a comprehensive understanding of the population and community dynamics and potential function of these fungi has begun to emerge (van der Heijden and Sanders 2002), this knowledge is largely based on studies with homogenized soil samples or from studies that minimize spatial variation through statistical analysis and design approaches. Due to this sampling and analytical homogenization, our understanding of the spatial template on which plants and AMF interact in the field is limited.

Many soil organisms show distinct spatial patterns in both composition and abundance (as reviewed in Ettema and Wardle 2002), and some work has been conducted showing that AMF are also spatially heterogeneous in the field. For example, in old-field ecosystems, AMF can be spatially patchy in both abundance (Boerner et al. 1996) and composition (Hart and Klironomos 2002; Pringle and Bever 2002) at small spatial scales. In marquis and salt marsh plant communities in Portugal, AMF spore abundance was related to environmental variables and proximity to mycotrophic

hosts (Carvalho et al. 2003). Most of the work on AMF spatial patterns thus far has looked at the spatial patterns of spores of AMF and may not represent the spatial patterns of the community of AMF that might colonize roots of seedlings as they establish in these distinct patches over space (i.e., the AMF inoculum potential). Moreover, none of these studies have used DNA-based techniques to assess community composition of AM fungi over space. Because DNA-based techniques detect AMF species that may not sporulate or species that sporulate at a different time of the year from the time of sampling (Clapp et al. 2002), these molecular methods generally provide a more complete estimate of the composition of AMF communities.

Wetlands are one ecosystem type in which spatial heterogeneity of abiotic soil factors might lead to spatial heterogeneity of AM fungal communities. Although the importance of AMF in wetlands has been dismissed (Keddy 2000), AMF have been shown to be present in a range of wetland types (Rickerl et al. 1994; Turner et al. 2000; Cornwell et al. 2001; Carvalho et al. 2001) and have been shown to be functionally important for the growth of wetland plant species (Miller and Sharitz 2000; Stevens et al. 2002; Dunham et al. 2003). The soils of wetlands are often saturated and subsequently low in available oxygen for aerobic soil organisms, such as AMF. However, this soil saturation can be spatially variable over larger spatial scales due to bryophyte microtopographic features within wetlands. Over time, bryophytes create hummocks that raise the surface of the soil from the water table, creating areas that are not as saturated as surrounding hollows (Mitsch and Gosselink 2000). Anoxia in the soil environment might also be variable at smaller spatial scales due to aerobic microsites in the rhizosphere created from radial oxygen loss from the roots of aerenchymatous wetland plants (Colmer 2003).

In this study, we assessed the small-scale spatial heterogeneity of both the abundance and molecular diversity of AMF in a calcareous fen in Central New York State. Calcareous fens are globally rare groundwater-fed peatlands that are generally low in plant available phosphorus and have a high diversity of flora and fauna (Bedford and Godwin 2003). AMF have been shown to be widely distributed among plant species in these wetlands, despite the fact that fens soils are continuously saturated. AMF are functionally important in these systems and can increase the growth and tissue nutrient concentrations of host plants (Weishampel 2005; Wolfe et al., 2006). In these wetlands, there is significant underlying variation in depth to water table (soil saturation) due to bryophyte microtopography, although there are also gradients in soil pH and nutrient availability associated with fen microtopography (Weishampel 2005). Given that the abundance and composition of AMF can be affected by soil saturation (Cantelmo and Ehrenfeld 1999; Miller and Bever 1999; Miller 2000), this underlying abiotic heterogeneity might contribute to spatial heterogeneity of AMF communities and associated aboveground plant communities.

The specific questions we asked in this study were: (1) What is the spatial structure of the abundance and molecular diversity of AMF communities in a calcareous fen? (2) Are patterns in the abundance and diversity of AM fungal inoculum potential related to water table or local plant diversity?

Materials and methods

Field sampling

This study was conducted at Belle School Fen, a calcareous fen located outside of Ithaca, New York, USA Soils, hydrology, and vegetation information for this site are given in Cornwell et al. (2001) and Weishampel (2005).

A 2×2 m plot was established in the last week of May 2004. The plot was located in an area with a significant amount of hummocks created by the presence of bryophytes (mostly *Sphagnum warnstorfii*). This area is typical of the microtopography of many calcareous fens.

We only chose to sample one plot for this preliminary study within the site for two reasons. First, we wanted to focus our efforts on intensively sampling small-scale spatial patterns so we took many samples from a confined area as opposed to few samples from a range of areas. Second, this type of intensive sampling imposes significant trampling on the fen community. Because it takes many years for the peat substrate to form in calcareous fens and because of the high conservation priority of these globally rare communities, we attempted to minimize potential impacts to one intensively sampled area.

The 2×2 m plot was divided into 100, 20×20 cm cells. From the center of each cell, a 5-cm diameter soil core was taken to a depth of 12 cm and was immediately placed in a 5×18 cm Deepot (Steuwe and Sons, Corvallis, OR, USA) and then placed in a sterile plastic bag in a cooler.

Immediately adjacent to where the soil core was taken, the depth to water table was measured by inserting a perforated 12.8-mm (ID) PVC pipe into the peat and measuring the depth of the water table from the top of the soil surface using a section of nylon tubing inserted into the PVC pipe as a water level indicator. As is common with most undisturbed calcareous fens in North America (Bedford and Godwin 2003), depth to water table at Belle School Fen is temporally stable during the plant growing season (Cornwell et al. 2001). Therefore, we only measured water table at the time of soil sampling.

The species richness of the vascular plant community in each 20×20 cm cell was recorded. Nomenclature follows USDA NRCS (2004).

Inoculum potential assay

Cores were stored at 4°C for 2 days and were then transported to the University of Guelph for conducting the inoculum potential and molecular diversity assays. Each Deepot was placed in a rack on a greenhouse bench in a randomized order. Plants were grown without supplemental light in the greenhouse.

To assess inoculum potential, we used the bait plant approach described by Brundrett and Abbott (1995) and Johnson et al. (1999). Our approach to inoculum potential estimates propagules of AMF in intact soil cores, including spores, hyphal fragments, and colonized roots, that are able to colonize a new host plant in a standardized greenhouse environment. This method provides a relative measurement of the ability of AMF to infect new plant roots and does not specifically quantify the abundance of spores, hyphae, or other AMF propagules. Two corn (*Zea mays* L.) seeds were sown on the surface of the peat of each core and covered with a thin layer of AMF free growing media (Pro-Mix, Premier Tech, Rivière-du-Loup Québec, Canada). Corn seedlings were thinned to one per Deepot after 1 week of growth.

After 1 month of growth, fine roots of the corn seedling were selected from throughout the core with as minimal disturbance possible to the other plant growing in the core (see next section). We split the cores into two depths, 0–6 and 6–12 cm, and treated them separately in sample processing and analysis. Roots were stored in 70% ethyl alcohol until they were stained with Chlorazol Black E (Brundrett et al. 1984). Percent colonization of AMF structures was determined by examining the randomly selected root segments under 400× light microscopy as described in McGonigle et al. (1990).

Molecular diversity of AMF

When the corn seeds were planted for the inoculum potential assay (described above), five to ten seeds of *Solidago patula* Muhl. ex Willd., a plant species commonly found in calcareous fens (Godwin et al. 2002) and highly colonized by AMF in the field (Cornwell et al. 2001) were sown in each Deepot. Seeds used in the study were collected from a *S. patula* population at a calcareous fen within the same watershed as Belle School Fen in 2003. Seeds were surface sterilized for 5 min with 5% sodium hypochlorite and then rinsed with sterile deionized water before being placed on the soil surface. Two weeks after germination, seedlings were thinned to one seedling per pot. After 3 months, the entire root system of each plant was harvested, rinsed with deionized water, and frozen at −20°C.

Molecular diversity of AM fungi was measured in this study using terminal restriction fragment length polymorphism (T-RFLP) of a 550-bp length section of 18S ribosomal DNA. This method has been successfully used

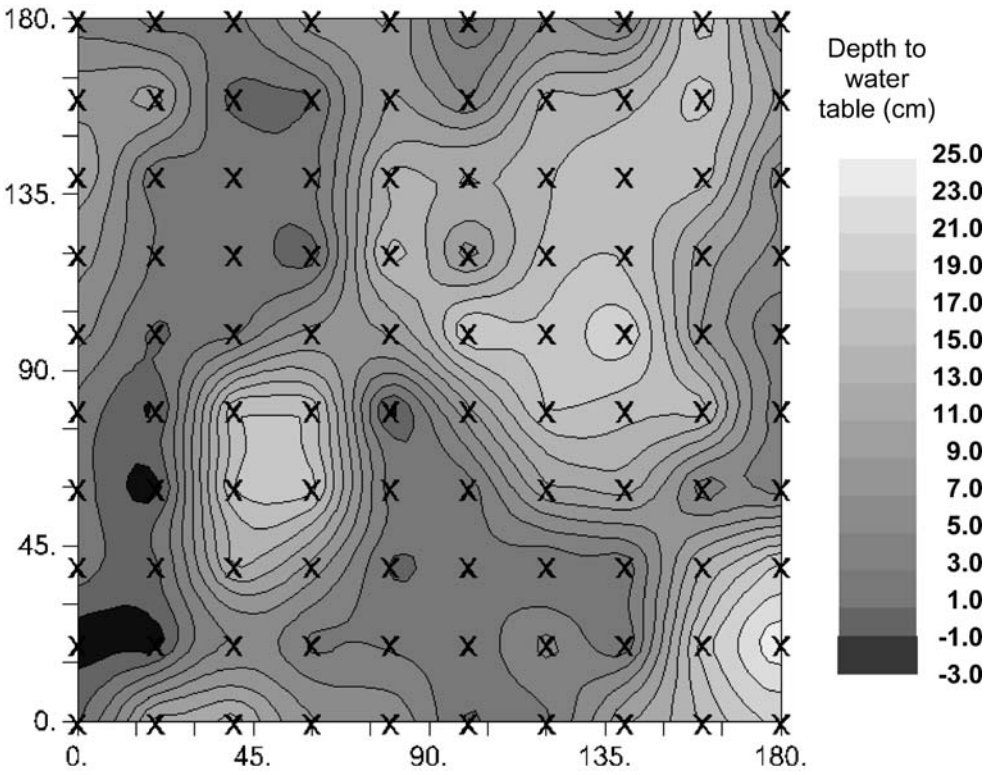

Fig. 1 Krigged map showing depth to water table in the intensively sampled plot. Each *X* represents a sampling point where a soil core was taken to assess inoculum potential and diversity of the AMF community as well as depth to water table and plant species richness. The entire plot area was 200× 200 cm, but because the sampling points were centered within the 100, 20×20 cm cells, the distance between the furthest sampling points along an edge was 180 cm, as indicated in the diagram. The krigged map was created based on semivariance analysis of depth to water table using an exponential model with a lag distance of 20 cm (nugget = 0.10, total = 49.53, structural = 0.998, fit = 0.88)

in several studies of AMF communities to detect fine-scale differences between community composition of AMF of different samples (Vandenkoornhuyse et al. 2003; Johnson et al. 2004; Mummey et al. 2005). Total genomic DNA was extracted from the *S. patula* root systems using the DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA). About 50 mg of 2- to 5-cm segments of each root system from each plant was placed in a screwtop cryovial, with two 0.635-cm sterile ceramic beads (Bio101, Carlsbad, CA, USA) and the AP1 buffer of the Qiagen kit and run on a GenoGrinder 2000 (BT&C/OPS Diagnostics, Bridgewater, NJ, USA) for three, 2-min cycles of 500 strokes per minute until roots were pulverized. We followed the manufacturer's instructions for the rest of the DNA extraction protocol.

AMF DNA was amplified from the total genomic DNA using the primer pair AM1-NS31 (Helgason et al. 1999). Two microliters of genomic DNA extract was added to a 50-μl reaction mixture containing: 10 pmol each of HEX labeled AM1 and FAM labeled NS31, 5 μl 10X iTaq buffer (Bio-Rad, Hercules, CA, USA), 0.25 μl iTaq DNA polymerase, 1.5 μl $MgCl_2$ (1.5 mM), 100 pmol of each deoxyribonucleotide triphosphate, and 0.5 μl of 0.1% bovine serum albumin. The polymerase chain reaction (PCR) program comprised of: 1 cycle at 95°C for 5 min; 10 cycles of 95°C for 0.5 min, 58°C for 1 min, and 72°C for 2 min; 19 cycles of 95°C for 0.5 min, 58°C for 1 min, and 72°C for 3 min; and 1 cycle of 95°C for 0.5 min, 58°C for 1 min, and 72°C for 10 min. PCR amplification was conducted on a PTC-100 thermocycler (MJ Research, Waltham, MA, USA). Roots of *S. patula* plants free of AMF growing in sterile soil were used to confirm that plant DNA was not being amplified from the genomic DNA extract. Amplification products were visualized using 1% agarose gels stained with ethidium bromide and viewed under UV light. Amplification products were purified using the Mo-Bio UltraClean-htp 96 Well PCR Clean-up Kit (Mo Bio Laboratories, Carlsbad, CA, USA).

PCR amplicons were digested using the restriction enzymes *Mbo*I and *Hin*fI, which have been used in the past with T-RFLP with AMF to successfully discriminate between AMF communities (Vandenkoornhuyse et al. 2003; Johnson et al. 2004; Mummey et al. 2005). Two units of each enzyme and 12 μl of PCR product were digested in the manufacturer's recommended buffer and incubated for 4 h at 37°C. Digestion products were purified by passing the products through Sephadex G-50 Superfine resin loaded in Millipore MultiScreen HV Plates (Millipore, Bedford, MA, USA). Terminal restriction fragment (T-RF) sizes from each sample were determined using the 3100 automated capillary DNA sequencer (Applied Biosystems, Foster City, CA) with ROX-500 (Applied Biosystems) as the size standard at the Murdoch Sequencing Facility of The University of Montana.

Data analysis

The size and peak height determination of T-RFs was performed using Genemapper software (Applied Biosystems). Differences between profiles in the amount of total fluorescence were standardized according to the methods outlined in Dunbar et al. (2001). Peaks that only occurred once throughout all samples were removed from the analysis. Only the presence or absence of peaks was considered in this study, not the height or area of peaks, because the latter parameters may not be indicative of relative abundance of ribotypes.

To visualize the sampled area, a krigged map of depth to water table was created based on measured autocorrelation between samples using semivariance analysis. The semivariance analysis and krigging were performed with GS+ v. 5 (Gamma Design Software, Plainwell, MI).

To test for spatial autocorrelation across multiple spatial scales for the point data in this study (water table and inoculum potential), we used Moran's *I* as an indicator of autocorrelation. Moran's *I* is commonly used in ecology to examine patterns of spatial autocorrelation and is analogous to Pearson's correlation coefficient; positive spatial autocorrelation is indicated by positive values and negative spatial autocorrelation is indicated by negative values (Fortin and Dale 2005). Using the program PASSaGE (Dr. Michael S. Rosenberg, http://www.passagesoftware.net), we created even interval distance classes to create a Moran's autocorrelogram and to test for the significance of spatial autocorrelation within each distance class.

To determine if a significant amount of the variation in AMF community composition, as indicated by the T-RFLP profiles, could be explained by distance between samples, Mantel's correlograms were used with the data broken into distance classes with equal numbers of samples within each distance class. A matrix of Sorensen similarity of each sample was created, and a Mantel's test comparing this matrix and a matrix of Euclidean distances between samples was run using PASSaGE. The same analysis was also conducted on plant species richness to assess spatial structure of plant community composition.

To test for relationships between the AMF T-RF richness, plant species richness, and water table, we used a modified *t*-test with correction for spatial autocorrelation. This test calculates Pearson's product–moment correlation between two variables that have some degree of spatial autocorrelation using the Clifford, Richardson, and Hemon (CRH) correction procedure (Clifford et al. 1989). Using this correction method, the effective sampling size of each variable is adjusted based on the level of spatial autocorrelation between samples. This test was performed using the "modified *t*-test for correlation" procedure of PASSaGE.

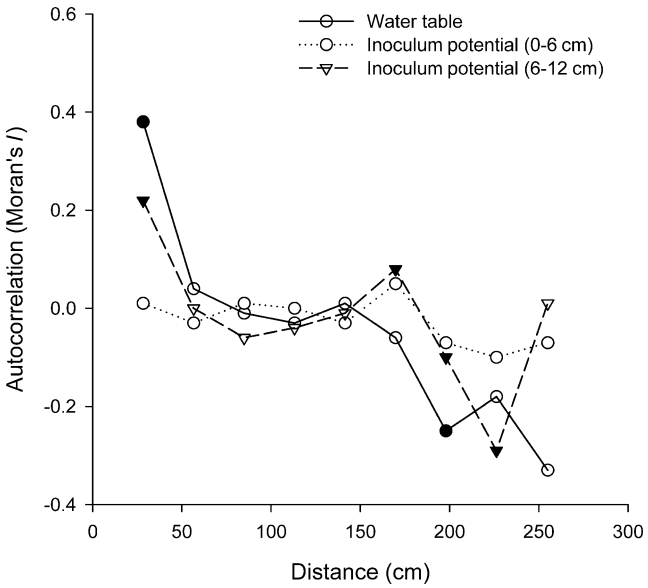

Fig. 2 Correlogram for spatial autocorrelation of depth to water table and inoculum potential of AMF in a calcareous fen. *Solid symbols* represent significant autocorrelation between samples within a certain distance class as measured by Moran's *I*

Results

Throughout the sampling area, there was considerable variation in depth to water table, ranging from 2.6 cm above the soil surface to 25.3 cm below the surface of the soil (8.02±0.69 SE; Fig. 1). Depth to water table showed significant positive spatial autocorrelation at small scales (0–50 cm) as indicated by significant values for Moran's *I* in these distance classes (Fig. 2).

All inoculum potential bioassay plants were colonized by AMF arbuscules, vesicles, and/or hyphae indicating that there was some form of AMF propagules across all patches of the area sampled. Mean colonization was higher for the 0–6 cm depth (58.2±2.60) compared to the 6–12 cm depth (29.56±2.56). Cores with low levels of colonization were more frequently encountered in the lower depth (Fig. 3). The Moran's correlogram for inoculum potential at 0–6 cm was flat across increasing distance classes, indicating no spatial autocorrelation across all spatial scales. However, there was significant positive spatial autocorrelation for inoculum potential at the 6–12 cm depth within the first distance class and significant negative spatial autocorrelation at larger spatial scales (Fig. 2). Depth to water table and AMF inoculum potential were positively correlated at 0–6cm (CRH corrected r=0.45, p<0.0001) and 6–12 cm (CRH corrected r=0.53, p<0.0001).

Throughout the 2×2 m area, 25 vascular plant species were encountered (Table 1). The number of plant species per 20×20 cm cell ranged from 2–9 species (5.44±0.16). There was significant spatial autocorrelation of plant community composition as indicated by significant positive autocorrelation in the smallest distance class and negative spatial autocorrelation in larger distance classes (Fig. 4). Plant species richness was not significantly correlated with depth to water table (CRH corrected r=−0.13, p=0.22).

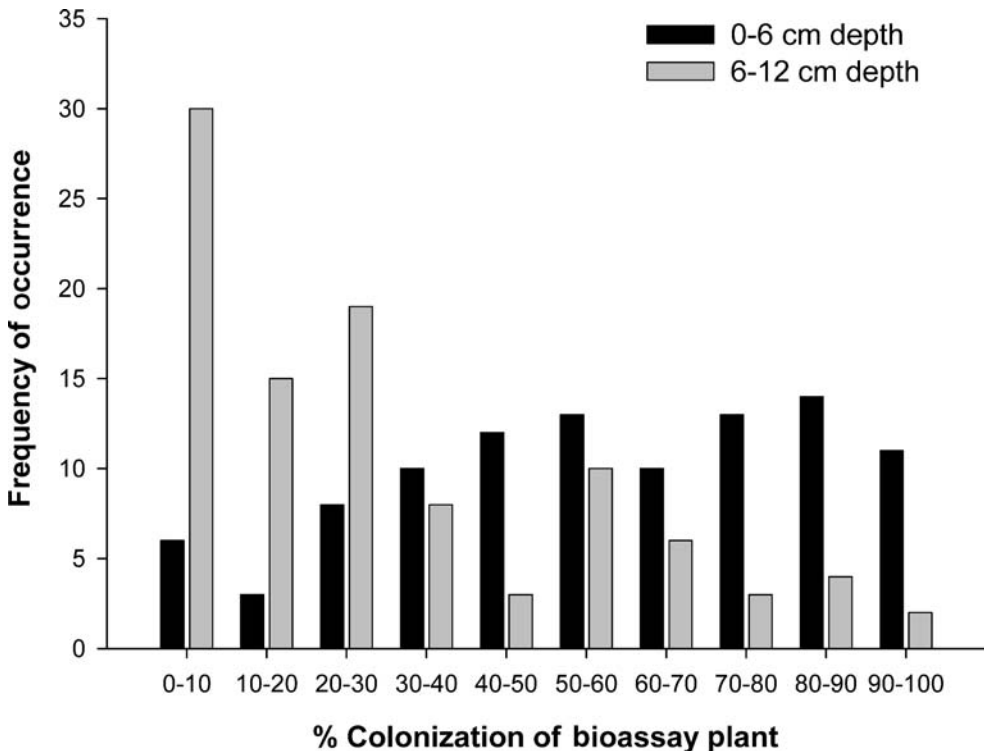

Fig. 3 Frequency histogram of percent colonization of corn bioassay plant as an indicator of inoculum potential. *Black bars* represent inoculum potential at a depth of 0–6 cm, and *gray bars* represent inoculum potential at 6–12 cm depth

A total of 33 T-RFs were observed for the restriction enzyme *Hin*fI and 24 T-RFs for the restriction enzyme *Mbo*I (Table 2). The number of T-RFs per core ranged from 2–14 T-RFs for *Hinf* (5.28±0.23) and from 2–10 T-RFs for *Mbo*I (6.25±0.22; Fig. 5). There was no relationship between either *Hin*fI AMF richness or *Mbo*I AMF richness per core and plant richness (*Hin*fI: CRH corrected r=0.16, p=0.12; *Mbo*I: CRH corrected r=0.06, p=0.55) or depth to water table (*Hin*fI: CRH corrected r=−0.13, p=0.21; *Mbo*I: CRH corrected r=−0.17, p=0.01). There was a significant positive relationship between the number of *Hin*fI T-RFs per core and the number of *Mbo*I T-RFs per core (CRH corrected r=0.49, p<0.0001). AMF community composition was not significantly spatially autocorrelated for either restriction enzyme as indicated by the flat lines for *Hin*fI and *Mbo*I in the Mantel's correlogram across all spatial scales (Fig. 4).

Table 1 Frequency of plant species encountered within the sampled 2×2 m plot

Plant species	Frequency of occurrence	Mycorrhizal status[a]
Acer rubrum	12	AMF
Alnus incana	18	AMF/EMF
Amelanchier arborea	3	X
Symphyotrichum puniceum	1	AMF
Carex flava	25	NM
Carex hystericina	8	NM
Carex scoparia	3	NM
Carex sterilis	88	NM
Chelone glabra	1	AMF
Clematis virginiana	8	AMF
Eleocharis elliptica	60	NM
Equisetum arvense	23	Low AMF
Eupatorium maculatum	5	AMF
Fragaria virginiana	3	AMF
Fraxinus americana	1	X
Heuchera sp.	8	X
Platanthera psycodes	7	Orchid
Rhubus pubescens	57	AMF
Packera aurea	6	AMF
Solidago patula	85	AMF
Solidago rugosa	6	AMF
Thelypteris palustris	81	AMF
Toxicodendron vernix	6	AMF
Typha latifolia	28	NM
Viburnum dentatum	1	AMF

AMF arbuscular mycorrhizal fungi, *Low AMF* low arbuscular mycorrhizal fungal colonization, *EMF* ectomycorrhizal fungal colonization, *NM* nonmycorrhizal, *X* not determined.

[a] Based on mycorrhizal survey data published in Cornwell et al. 2001 and Weishampel 2005. *P. psycodes* roots at this site are colonized by orchid mycorrhizal fungi as indicated by pelotons in the roots (B.E. Wolfe, personal observation).

Discussion

We found considerable spatial variation in the number of T-RFs per core within the sampled area. The mean richness of T-RFs per sample measured in this study falls within values observed for AMF in upland plant root systems (Vandenkoornhuyse et al. 2003; Johnson et al. 2004; Mummey et al. 2005). Combined with a previous assessment of molecular diversity in a disturbed wetland system in Europe (Wirsel 2004), this suggests that the molecular diversity of saturated soils in wetlands might be comparable to that of upland systems. Further studies of the molecular diversity of AMF communities from a range of wetland plant communities are needed to make any generalizations about wetland versus upland AMF community diversity.

Several studies with AMF and plant communities have demonstrated positive relationships between AMF species richness and plant species richness by either manipulating AMF species richness (van der Heijden et al. 1998) or manipulating plant species richness (Burrows and Pfleger 2002). Previous work in a grassland system using T-RFLP to assess AMF diversity has shown that T-RF richness is related to AMF function, with higher concentrations of tissue nutrients at higher numbers of AMF T-RFs (Johnson et al. 2004). Although we did not attempt to measure such a functional relationship in this study, this previous work suggests that the observed variation in AMF composition over space might lead to functional spatial variation of the AMF communities. However, the strong underlying gradients in environmental factors such as depth to water table may overwhelm any functional diversity effect of the AMF and mediate the outcome of the plant–AMF interactions.

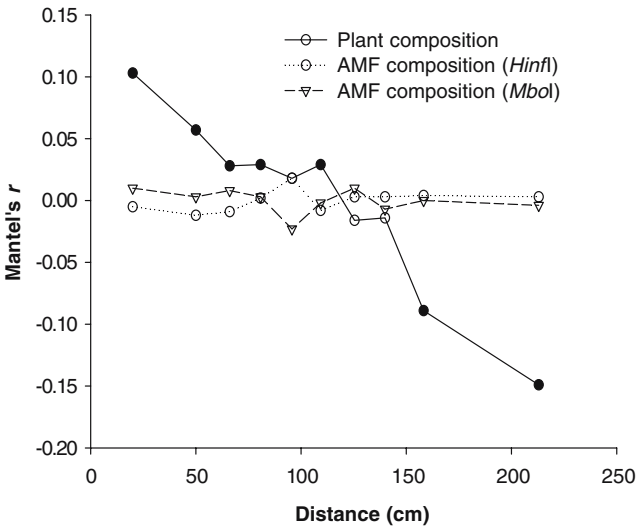

Fig. 4 Mantel's correlogram of plant community composition and AMF community composition. AMF community composition was based on T-RFs produced with two restriction enzymes, *Hinf*I and *Mbo*I. *Solid symbols* are significant Mantel's r values (p<0.05)

Table 2 Frequency of T-RFs encountered in the sampled 2×2 m plot

*Hin*fI		*Mbo*I	
T-RF length (bp)	Frequency of occurrence	T-RF length (bp)	Frequency of occurrence
139	81	94	76
190	52	279	76
138	44	444	74
192	37	302	56
174	31	443	41
278	30	303	38
141	25	453	38
94	24	301	29
189	21	87	23
173	19	102	17
279	19	196	15
500	17	174	12
102	14	197	12
158	12	305	12
191	11	304	11
290	7	452	10
140	6	445	9
145	6	454	9
146	6	442	7
497	6	278	6
113	5	446	3
197	5	93	2
282	5	188	2
498	5	280	2
90	4		
214	4		
124	3		
321	3		
151	2		
176	2		
198	2		
277	2		
499	2		

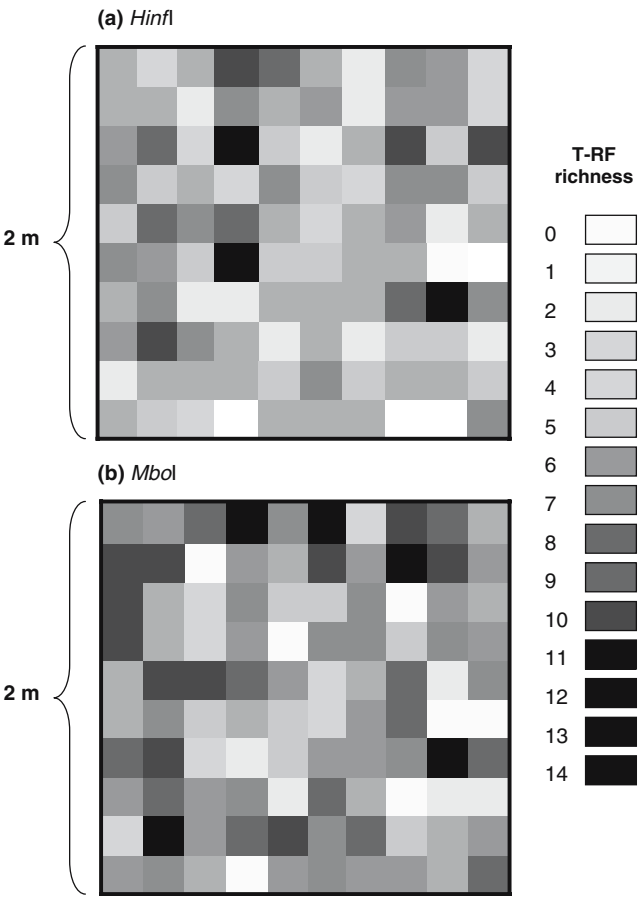

Fig. 5 Spatial variation in the number of AMF T-RFs throughout the intensively sampled 2×2 m plot. **a** Variation observed with T-RFs generated with the restriction enzyme *Hinf*I. **b** Variation observed with T-RFs generated with the restriction enzyme *Mbo*I. Cells where *0* restriction fragments are indicated are plots where we were unable to obtain a PCR product

Plant community composition was patchy across the sampled plot, with significant positive Mantel's *r* values at small spatial scales. In contrast, although the composition of AMF was variable over small spatial scales, we did not observe significant spatial structure in this study based on the spatial analyses conducted. The lack of association in spatial structure between aboveground and belowground communities suggests that the mechanisms that lead to spatial structuring are operating differently for plant and AMF communities. The mechanisms that lead to spatial structure are similar for both plant and AMF communities and include dispersal of propagules of different species, interactions between species, local disturbances, and underlying environmental gradients, but the scales at which these mechanisms act are most likely quite different for the two community types. For example, many of the plant species in this study that reproduce sexually disperse propagules over much larger distances than AMF propagules travel. In contrast, the size of an individual AMF may be much larger than the size of an individual plant, (Rosendahl and Stukenbrock 2004). Other studies simultaneously assessing spatial structure of plant and soil communities have also shown a similar lack of association between the spatial structure of aboveground and belowground community composition (Horner-Devine et al. 2004; Ritz et al. 2004).

The lack of spatial structure detected in the AMF community might be related to limitations of the molecular method used. It is still unclear what level of resolution T-RFLP provides for AMF communities. Preliminary work suggests that the restriction enzymes commonly used for T-RFLP with AMF, including *Hinf*I and *Mbo*I used in this study, can separate different closely related species of AMF (B.E. Wolfe, unpublished data). However, in some cases, the enzymes used may group different species of the same genera together depending on differences in sequences, leading to an underestimate of the actual richness.

It is also important to note that the "baiting" approach used in this study may have also led to an inaccurate representation of the spatial patterns of AMF in the field. We were concerned with measuring the diversity of AMF that would colonize plant seedlings establishing in discrete patches in the field and therefore used a bioassay approach with soil cores and sterile seedlings. More importantly, it would have been very difficult if not impossible to collect a sufficient amount of roots in the field of a plant of the same species and age from each 20× 20 cm plot. However, this approach may have shifted the composition of AMF at each sampling point from what existed in the field, as the AMF present in the soil inoculum colonized the bioassay plant roots. Depending on the life history strategies of the fungi present in each core, quick colonizers may have dominated the roots, whereas those fungi that are slow to colonize roots may have been excluded. Other studies comparing field roots and baited plant roots have found differences in the AMF community composition between the two approaches (Opik et al. 2003).

Recent research suggests that due to non-specific amplification of other fungi with the primers NS31 and AM1, T-RFLP of AMF communities may not provide an accurate representation of AMF community structure (Douhan et al. 2005). Although we cannot say exactly what species of AMF relate to all of the observed T-RF peaks, preliminary cloning and sequencing has demonstrated that the most abundant T-RFs are AMF fungal sequences and have the greatest sequence similarity to various *Glomus* spp. in basic local alignment search tool searches (B. E. Wolfe, unpublished data). Additionally, much of the non-specific amplification observed by Douhan et al. (2005) was in roots of plant species that are typically highly colonized by many other fungi. Subsamples of the roots of *S. patula* that we extracted DNA from in this study were highly colonized by hyphae, arbuscules, and vesicles of AMF (B.E. Wolfe, personal observation). Therefore, we assert that PCR amplification of non-AMF fungi was probably limited in this study.

Many studies have recently reported on patterns of colonization of roots in wetland plant species (Stevens and Peterson 1996; Cornwell et al. 2001; Carvalho et al. 2001), but there is very little work examining the abundance of AMF outside of the root such as inoculum potential as was measured in this study. It is somewhat surprising that although some of the samples in this study were collected where the water table was several centimeters above the soil surface, there were still some viable propagules in the soil of all cores at both the 0–6 and 6–12 cm depths. We did not determine what types of AMF propagules were infecting the bioassay plants, but it has been shown that hyphae, spores, and colonized root fragments can all serve as infective propagules of AMF in wetland soils (Carvalho et al. 2004). To make the environmental conditions similar across all cores for the bioassay, we did not completely saturate the soil cores, which may have made the soils more infective than they would be in the field. Regardless, AMF propagules are able to persist in the saturated fen soils and can serve as inoculum for establishing seedlings and uncolonized roots.

Previous work in calcareous fens has shown a limited effect of water table on internal root colonization of AMF (Weishampel 2005). In this study, inoculum potential was positively related to depth to water table, with greater levels of AMF colonization of bioassay plants in cores from areas where the depth to water table was greater (i.e., the soils were less saturated with water). Contrary to internal root colonization where oxygen in the roots of the plant host may buffer the anoxic effects of soil saturation, it appears that external forms of AMF such as hyphae and spores are sensitive to soil saturation. Some ribotypes of AMF may be more tolerant of flooding as indicated by the widespread occurrence of certain T-RFs throughout the plot, such as the T-RFS *Hin*fI 139 and *Mbo*I 94, 279, and 444. Future work examining modes of infection in saturated soils and mechanisms that AMF propagules might use to tolerate to anoxia will further elucidate how wetland soils can contain infective propagules of AMF and species-specific sensitivities to soil saturation.

In summary, we found that the community composition of AMF in a calcareous fen was variable over small spatial scales but found no significant associations between the diversity of the AMF community and soil saturation or plant diversity. Plant community composition and AMF community composition appear to be spatially structured at different scales in calcareous fens. Spatial variation in the composition of AMF or interactions between AMF and water table may lead to functional spatial heterogeneity of AMF in fens.

Acknowledgements Hafiz Maherali, Doug Larson, and Peter Weishampel provided useful comments on earlier versions of this manuscript. This work was funded by the US National Science Foundation and the Natural Sciences and Engineering Research Council of Canada.

References

Bedford BL, Godwin KS (2003) Fens of the United States: distribution, characteristics, and scientific connection versus legal isolation. Wetlands 23:608–629

Boerner REJ, DeMars BG, Leicht PN (1996) Spatial patterns of mycorrhizal infectiveness of soils along a successional chronosequence. Mycorrhiza 6:79–90

Brundrett MC, Abbott LK (1995) Mycorrhizal fungus propagules in the jarrah forest. 2. Spatial variability in inoculum levels. New Phytol 131:461–469

Brundrett MC, Piche Y, Peterson RL (1984) A new method for observing the morphology of vesicular–arbuscular mycorrhizae. Can J Bot 62:2128–2134

Burrows RL, Pfleger FL (2002) Arbuscular mycorrhizal fungi respond to increasing plant diversity. Can J Bot 80:120–130

Cantelmo AJ, Ehrenfeld JG (1999) Effects of microtopography on mycorrhizal infection in Atlantic white cedar (*Chamaecyparis thyoides* (L.) Mills.). Mycorrhiza 8:175–180

Carvalho LM, Cacador I, Martins-Loucao MA (2001) Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). Mycorrhiza 11:303–309

Carvalho LM, Correia PM, Ryel RJ, Martins-Loucao MA (2003) Spatial variability of arbuscular mycorrhizal fungal spores in two natural plant communities. Plant Soil 251:227–236

Carvalho LM, Correia PM, Martins-Loucao MA (2004) Arbuscular mycorrhizal fungal propagules in a salt marsh. Mycorrhiza 14:165–170

Clapp JP, Helgason T, Daniell TJ, Young JPW (2002) Genetic studies of the structure and diversity of arbuscular mycorrhizal fungal communities. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer, Berlin Heidelberg New York, pp 201–221

Clifford P, Richardson S, Hemon D (1989) Assessing the significance of the correlation between two spatial processes. Biometrics 45:123–134

Colmer TD (2003) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell Environ 26:17–36

Cornwell WK, Bedford BL, Chapin CT (2001) Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. Am J Bot 88:1824–1829

Douhan GW, Peterson C, Bledsoe C, Rizzo DM (2005) Contrasting root associated fungi of three common oak-woodland plant species based on molecular identification: host specificity or non-specific amplification? Mycorrhiza 15:365–372

Dunbar J, Ticknor LO, Kuske CR (2001) Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Appl Environ Microbiol 67:190–197

Dunham RM, Ray AM, Inouye RS (2003) Growth, physiology, and chemistry of mycorrhizal and nonmycorrhizal *Typha latifolia* seedlings. Wetlands 23:890–896

Ettema CH, Wardle DA (2002) Spatial soil ecology. Trends Ecol Evol 17:177–183

Fortin MJ, Dale MRT (2005) Spatial analysis: a guide for ecologists. Cambridge University Press, New York

Gange AC, Brown VK, Aplin DM (2005) Ecological specificity of arbuscular mycorrhizae: evidence from foliar- and seed-feeding insects. Ecology 86:603–611

Godwin KS, Shallenberger JP, Leopold DJ, Bedford BL (2002) Linking landscape properties to local hydrogeologic gradients and plant species occurrence in minerotrophic fens of New York State, USA: a hydrogeologic setting (HGS) framework. Wetlands 22:722–737

Hart M, Klironomos JN (2002) Diversity of arbuscular mycorrhizal fungi and ecosystem functioning. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer, Berlin Heidelberg New York, pp 225–239

Helgason T, Fitter AH, Young JPW (1999) Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. Mol Ecol 8:659–666

Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM (2004) A taxa–area relationship for bacteria. Nature 432:750–753

Johnson NC, O'Dell T, Bledsoe C (1999) Methods for ecological studies of mycorrhizae. In: Robertson GP, Bledsoe C, Coleman DC, Sollins P (eds) Standard soil methods for long-term ecological research. Oxford University Press, New York, pp 378–412

Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Booth RE, Grime JP, Young JPW, Read DJ (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytol 161:503–515

Keddy P (2000) Wetland ecology: principles and conservation. Cambridge University Press, New York

Koide RT, Dickie IA (2002) Effects of mycorrhizal fungi on plant populations. Plant Soil 244:307–317

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol 115:495–501

Miller SP (2000) Arbuscular mycorrhizal colonization of semi-aquatic grasses along a wide hydrologic gradient. New Phytol 145:145–155

Miller SP, Bever JD (1999) Distribution of arbuscular mycorrhizal fungi in stands of the wetland grass *Panicum hemitomon* along a wide hydrologic gradient. Oecologia 119:586–592

Miller SP, Sharitz RR (2000) Manipulation of flooding and arbuscular mycorrhiza formation influences growth and nutrition of two semiaquatic grass species. Funct Ecol 14:738–748

Mitsch WJ, Gosselink JG (2000) Wetlands. Wiley, New York

Mummey DL, Rillig MC, Holben WE (2005) Neighboring plant influences on AMF community composition: a potential mechanism for invasive plant success. Plant Soil 271:83–90

Opik M, Moora M, Liira J, Koljalg U, Zobel M, Sen R (2003) Divergent arbuscular mycorrhizal fungal communities colonize roots of *Pulsatilla* spp. in boreal Scots pine forest and grassland soils. New Phytol 160:581–593

Pringle A, Bever JD (2002) Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. Am J Bot 89:1439–1446

Rickerl DH, Sancho FO, Ananth S (1994) Vesicular–arbuscular endomycorrhizal colonization of wetland plants. J Environ Qual 23:913–916

Ritz K, McNicol W, Nunan N, Grayston S, Millard P, Atkinson D, Gollotte A, Habeshaw D, Boag B, Clegg CD, Griffiths BS, Wheatley RE, Glover LA, McCaig AE, Prosser JI (2004) Spatial structure in soil chemical and microbiological properties in an upland grassland. FEMS Microbiol Ecol 49:191–205

Rosendahl S, Stukenbrock EH (2004) Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. Mol Ecol 13:3179–3186

Stevens KJ, Peterson RL (1996) The effect of a water gradient on the vesicular–arbuscular mycorrhizal status of *Lythrum salicaria* L (purple loosestrife). Mycorrhiza 6:99–104

Stevens KJ, Spender SW, Peterson RL (2002) Phosphorus, arbuscular mycorrhizal fungi and performance of the wetland plant *Lythrum salicaria* L. under inundated conditions. Mycorrhiza 12:277–283

Turner SD, Amon JP, Schneble RM, Friese CF (2000) Mycorrhizal fungi associated with plants in ground-water fed wetlands. Wetlands 20:200–204

USDA NRCS (2004) The PLANTS Database, Version 3.5 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874–4490 USA

van der Heijden MGA, Sanders IR (eds) (2002) Mycorrhizal ecology. Springer, Berlin Heidelberg New York

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol Ecol 12:3085–3095

Weishampel PA (2005) Distribution and function of arbuscular mycorrhizal fungi in calcareous fen plant communities. PhD Dissertation. Cornell University, Ithaca, NY

Wirsel SGR (2004) Homogenous stands of a wetland grass harbour diverse consortia of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 48:129–138

Wolfe BE, Weishampel PA, Klironomos JN (2006) Arbuscular mycorrhizal fungi and water table affect wetland plant community. J of Eco 94:905–914