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Citation: This is a pre-copyedited, author-produced version of an article accepted for publication in FEMS Microbiology Ecology following peer review. The version of record:
Mummey, D. L., & Rillig, M. C. (2008). Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland. FEMS Microbiology Ecology, 64(2), 260–270. https://doi.org/10.1111/j.1574-6941.2008.00475.x

is available online at: https://doi.org/10.1111/j.1574-6941.2008.00475.x.

FEMS MICROBIOLOGY ECOLOGY

http://mc.manuscriptcentral.com/fems

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Journal:	*FEMS Microbiology Ecology*
Manuscript ID:	FEMSEC-07-11-0512.R2
Manuscript Type:	Research Paper
Date Submitted by the Author:	18-Jan-2008
Complete List of Authors:	Mummey, Daniel; University of Montana, Division of Biological Sciences Rillig, Matthias; Freie Universitaet Berlin, Plant Ecology
Keywords:	arbuscular mycorrhizal fungi, spatial variability, T-RFLP, AMF

Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the sub-metre scale in a temperate grassland.

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Key words: arbuscular mycorrhizal fungi, spatial variability, spatial autocorrelation, T-RFLP

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Abstract

Although arbuscular mycorrhizal fungi form spatially complex communities in terrestrial ecosystems, the scales at which this diversity manifests itself is poorly understood. This information is critical to the understanding of the role of AMF in plant community composition.

We examined small-scale (sub-metre) variability of AMF community composition (T-RFLP fingerprinting) and abundance (extraradical hyphal lengths) in two 1 m^2 plots situated in a native grassland ecosystem of western Montana. Extraradical AMF hyphal lengths varied greatly between samples (14 to 89 m g $soil^{-1}$) and exhibited spatial structure at scales < 30 cm. The composition of AMF communities was also found to exhibit significant spatial autocorrelation, with correlogram analyses suggesting patchiness at scales < 50 cm. Supportive of overall AMF community composition analyses, individual AMF ribotypes corresponding to specific phylogenetic groups exhibited distinct spatial autocorrelation. Our results demonstrate that AMF diversity and abundance can be spatially structured at scales of less than 1 m. Such small-scale heterogeneity in the soil suggests that establishing seedlings may be exposed to very different, location dependent AMF communities. Our results also have direct implications for representative sampling of AMF communities in the field.

Introduction

Spatial distribution is one of the most fundamental ecological parameters for any group of organisms. Community composition or structure is driven by a range of different biotic and abiotic factors that can exert influences at broadly different spatial and temporal scales (Levin, 1992). Disentangling these interactions is a major challenge to community ecology, requiring linkage of scales at which communities are measured with scales at which factors hypothesized to control their composition actually operate (Huston, 1999).

Amongst biotic interactions influencing plant community composition and ecosystem processes, the importance of mycorrhizae is becoming increasingly apparent and recent evidence suggests that interrelationships exist between plant and mycorrhizal fungal communities (Harnett & Wilson, 2002; Hart *et al*., 2003; Leake *et al*., 2004). Mycorrhizal fungi can influence plant

communities via a variety of mechanisms, including differential provision of benefits to different plant community members, and linking plants within a fungal network that allows for interplant trafficking of nutrient resources (Simard & Durall, 2004).

While arbuscular mycorrhizal fungal (AMF) communities have been shown to differ between sites, hosting plant species, habitats and ecosystem types (e.g. Helgason *et al*., 1999, 2002; Daniell *et al*., 2001; Husband *et al*., 2002), surprisingly little attention has been given to their community distribution at small spatial scales. Recent evidence indicating significant differences between AMF communities of co-occurring plants (Vandenkoornhuyse *et al*., 2002, 2003) would suggest host preferences as a potential mechanism promoting small-scale spatial structure. There is also a growing literature describing differences in life-history strategies among AMF taxa (e.g. Hart & Reader, 2002; Maherali & Klironomos, 2007). Interspecific differences in reproduction, dispersal, growth and environmental tolerances would be expected to result in formation of a spatial structure. Clearly, interspecific interactions are factors contributing strongly to the assembly of ectomycorrhizal fungal communities (Koide *et al*., 2004; Kennedy *et al*., 2007) and model AMF communities (Maherali & Klironomos, 2007). Specific species responses to physico-chemical gradients (e.g. nutrients, pH, soil texture and structure) have also been documented (e.g. Lekberg *et al*., 2007) and could influence both large and small-scale spatial stratification of AMF community composition.

Knowledge of how AMF are distributed in the environment is critical for determining spatial scales relevant for understanding relationships between AMF communities, plant communities and ecosystem processes. Such knowledge is also central to elucidation of belowground interactions that drive variance in plant community assemblages and to determine

what scales are most appropriate for capturing the effects of AMF community compositional changes.

Many different factors can potentially influence seedling establishment in terrestrial ecosystems, including the presence or composition of AMF communities (van der Heijden, 2004). If AMF communities are spatially homogeneous over short distances, relatively similar host/mutualist relationships would be expected. On the other hand, if small-scale spatial heterogeneity is pronounced, then at least the potential for differential benefit due to specific AMF community differences exists.

In this study we wished to examine the distribution of AMF in a grassland ecosystem at relatively small spatial scales relevant to individual plants from the perspective of seedling recruitment. Specifically, we asked the following questions: (a) On what scales is spatial structure in AMF abundance and community composition apparent? (b) Is soil AMF community spatial structure significantly explained by knowledge of plant species identity and aboveground location? (c) Is AMF community composition a function of AMF abundance?

Methods

Study site, sample strategy and plant measures

Two sites, located 5 km N of Missoula, MT (USA) and separated by a distance of approximately 25 m, were randomly selected on a gentle slope dominated by native grass species *Festuca idahoensis* Elmer, *Koeleria cristata* (Ledeb.) Schult, *Pseudoroegneria spicata* (Pursh) A. Löve ssp. *spicata* and *Hesperostipa comata* (Trin. & Rupr.) Barkworth ssp. *comata* (for a more detailed site description see: Lutgen *et al*., 2003; Mummey *et al*., 2006). Although minor forb cover is present on areas surrounding our study plots, no non-mycorrhizal plant species were identified within our plots that would serve to disrupt AMF community or abundance measures.

On each site a 1 m^2 frame was placed with sides corresponding to eastern and northern directions. Thirty-three soil samples (5 cm diameter, 10 cm depth) were collected from each plot at coordinates designed to facilitate spatial analyses (Halvorson *et al*., 1994; Fig. 1a). The spatial coordinates and species identity of all plants within each plot was carefully documented. For each plant individual, aboveground growth was harvested to ground level, dried (60^{o}C for 24 h) and weighed to determine its aboveground biomass.

Extraradical hyphal length analyses

Extraradical AMF hyphal lengths were measured in each soil core according to Rillig *et al*. (1999). This technique involves aqueous extraction of hyphae from soil samples (4 g) and subsequent determination of AMF hyphae at 200x magnification. Hyphal lengths were estimated using the line intersect method as described in Tennant (1975) and Jakobsen *et al*. (1992).

AMF community analyses: T-RFLP analysis optimization and validation

Prior to conducting T-RFLP analysis of samples, T-RFLP analysis methods were optimized and validated as described in Mummey and Rillig (2007). Briefly, DNA was extracted from plant roots and soils near our study plots and a portion of the AMF ribosomal large subunit gene amplified via polymerase chain reaction (PCR) using unlabeled primers in reactions as described below. These products were cloned and sequenced, as described previously (Mummey & Rillig, 2007). The computer program TRFSEQ (available from corresponding author upon request) was used to conduct simulated digestion of cloned sequences (GenBank accession numbers DQ468685 to DQ468824) with a broad range of restriction enzymes. This allowed for selection of restriction enzymes optimal for T-RFLP-based discrimination of the AMF diversity in our study plots. We used the following criteria to select restriction enzymes: (1) Overall ability to discriminate AMF diversity; (2) yielding unique terminal restriction fragment sizes (T-RF) for

each phylogenetic group that were as pronounced as possible; (3) T-RF sizes were greater than 40 bp to allow for optimal size calling. These analyses indicated that the reaction conditions were highly specific to Glomeromycota of our study sites. Of the restriction enzymes examined, two (*Taq I* and *Alu I*) were found to best meet the above criteria and were used in all subsequent analyses.

AMF community analyses: procedures

For analysis of samples from our study sites, each soil core was homogenized by manual kneading and shaking in plastic bags. Whole community DNA was extracted from subsamples (0.4 g wet weight) using the PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA). Bead-beating utilized a Geno/Grinder™ 2000 (SPEX CentriPrep, Inc.). To consistently obtain sufficient amounts of PCR product for analysis, PCR amplification of soil DNA extracts consisted of two PCR rounds, the first employing the "general fungal" primer pair LR1 and FLR2 (Trouvelot *et al*., 1999; van Tuinen *et al*., 1998) and the second using AMF-specific primers FLR3 and FLR4 (Gollotte *et al*., 2004). The 25 µl reaction mixtures included 1 µl soil extracted template DNA or product from the previous PCR, 100 pmol of each deoxynucleoside triphosphate, 10 pmol of each primer, 2 U HotMaster™ Taq DNA polymerase (Eppendorf, Hamburg, Germany) and 0.6 M betaine. Thermal cycling for all reactions included an initial denaturing step of 95^{o} C for 5 minutes, 25 cycles (primer pair LR1 and FLR2) or 30 cycles (primer pair FLR3 and FLR4) consisting of 1 min at 95^{o} C, 1 min at 58^{o} C and 1 min at 65^{o} C, followed by a final extension step of 65^{o} C for 10 min.

Products of two separate reactions were combined and purified using the GenCatch™ PCR cleanup kit (Epoch Biolabs, Inc. Sugar Land, TX) and subsequently quantified by image analysis of agarose gels following electrophoresis with Low DNA Mass Ladder (Invitrogen,

Carlsbad, CA) as the size standard. The purified PCR products were then digested in separate reactions with restriction enzymes *TaqI* (Fermentas, Hanover, MD) and *AluI* (New England Biolabs, Beverly, MA). Each digestion, containing 12 µl PCR product and 3 U *Alu I* or Taq *I* in the manufacturer's recommended buffer, was incubated for 4 h at either 37° C (*Alu I*) or 65 ° C (*Taq I*), followed by enzyme heat inactivation at 94 ° C for 10 minutes. All reactions were then treated with 3 U Mung Bean endonuclease (New England Biolabs) to remove single-stranded "pseudo-T-RFs" (Egert & Friedrich, 2003).

For each sample T-RF size distributions were determined using an ABI 3100 automated capillary DNA sequencer (Applied Biosystems, Foster City, CA) with ROX-500 (Applied Biosystems) as the size standard. Fragment size determination and quantification was performed using Genemapper software (Applied Biosystems). We used the Microsoft-Excel macro Treeflap (Rees *et al*., 2004; http://www.wsc.monash.edu.au/~cwalsh/treeflap.xls) to convert each fragment size present in T-RFLP profiles to the nearest integer value and to subsequently align peaks against rounded sizes of the fragments. Total relative fluorescence of T-RFLP profiles derived from each sample was standardized to 4000 relative fluorescence units with a minimum peak height threshold of 40 fluorescence units.

Data analyses

Correlations between extraradical hyphal lengths and T-RF size numbers were examined using SPSS software (ver. 15.0). Relationships between AMF community similarity measures and extraradical hyphal lengths were examined using the Mantel test (zt software; Bonnet & Van de Peer, 2002).

Spatial structure of hyphal lengths and T-RF size numbers were evaluated using Moran's *I* (Moran 1950) correlogram analyses (Cliff & Ord, 1981). Spatial structure of AMF

communities, including testing for the presence of autocorrelation, was specifically examined using Mantel correlogram (Oden & Sokal, 1986) analysis. Mantel correlograms were produced by calculating normalized r_M values for AMF community Bray-Curtis distances (Bray & Curtis, 1957) within different geographic distance classes. The degree of autocorrelation within each distance class was tested to determine whether it is greater or less than the overall mean autocorrelation between sites. The software program PASSaGE (ver 1.1; Rosenberg, 2001) was used to conduct these analyses.

Variance partitioning (Borcard *et al*., 1992; Borcard & Legendre, 1994; Legendre & Legendre, 1998) of AMF community data was used to examine variation explained by plant species identity and location and spatial structure. Spatial models for these analyses included both euclidean distance (planar) and principle coordinates of neighbour matrices (PCNM). PCNM spatial filtering [for detailed description of this method see Borcard & Legendre (2002) and Borcard *et al*. (2004)] involves eigenfunction decomposition of a truncated matrix of the geographical distances between sample locations. Eigenvectors corresponding to positive eigenvalues are then used as spatial descriptors. For each analysis, a number of different truncation distances were examined by forward selection, compared in terms of the total amount of variance explained, and the best models (axes found to account for a significant fraction of the overall variance) retained for subsequent analyses.

The computer program PASSaGE (Rosenberg, 2001) was employed to determine sample locations within 10, 15 and 20 cm from the base of each plant species. For both spatial models, forward selection with Monte-Carlo permutation testing (Canoco software; ter Braak & Smilauer, 1998) was used to determine plant and distance classes potentially exerting a

significant influence on AMF community composition. Insignificant variables ($P > 0.05$) were eliminated from further analyses.

Distance-based redundancy analysis (db-RDA; Legendre & Anderson, 1999) was then used to obtain ordinations of T-RFLP AMF community data constrained by spatial distance models and plant influences. Monte Carlo permutation tests were performed to assess the significance of canonical axes showing relationships between AMF community data of each sample and environmental factors.

We also examined the spatial distributions of T-RF sizes indicated by previous analyses (Mummey & Rillig, 2007) to be diagnostic for specific AMF groups present on our study plots. For these analyses spatial autocorrelation was examined using binary indicator correlogram analysis (Isaaks & Srivastava, 1989) in which samples yielding multiple T-RF sizes matching specific AMF ribotypes were assigned a value of 1 and samples lacking these T-RF sizes were assigned a value of 0.

Results

Plant aboveground measures

Although most plant species present in Plot 1 were also present in Plot 2, their relative numbers and contribution to total aboveground biomass differed (Fig. 1). *Koeleria cristata*, *P. spicata* and *F. idahoensis* accounted for 31, 58 and 10% of the aboveground biomass of Plot 1, respectively (Fig. 1a). *Koeleria cristata*, *P. spicata*, *F. idahoensis* and *H. comata* accounted for 10, 28, 56 and 5% of the aboveground biomass of Plot 2, respectively (Fig. 1b). Total aboveground biomass also differed substantially between plots (303 g and 225 g for Plots 1 and 2, respectively). Mean distance between nearest neighbouring plants was found to be 10.9 and 9.7 cm for Plots 1 and 2, respectively.

Abundance and distribution of AMF extraradical hyphae

Despite plant biomass and species compositional differences, overall hyphal lengths did not differ significantly between plots ($F = 0.84$, $P = 0.36$), averaging 43 and 41 m g soil^{-1} for Plot 1 and 2, respectively (Fig. 2a, b). However, hyphal lengths varied greatly between samples, ranging from 14 to 89 m g soil^{-1} for Plot 1 and 15 to 73 m g soil^{-1} for Plot 2.

Correlogram analysis indicated that hyphal lengths exhibited significant positive spatial autocorrelation (Moran's I) in the first distance class of each plot, while spatial autocorrelation was insignificant for all other distance classes (Fig. 3a, b). Since patch size is indicated by the distance at which the first near maximum negative autocorrelation value is found (Legendre & Legendre, 1998), these results would suggest patch size less than 30 cm for both plots.

AMF community composition analyses

Average T-RF numbers found per sample differed significantly between plots (Fig. 4a, b; ANOVA; $P < 0.005$). The number of T-RF sizes found for Plot 1 after combining all four T-RFLP profiles for each sample ranged from 34 to 56 and averaged 45. For Plot 2 the number of different T-RF sizes found per sample ranged from 22 to 46 and averaged 32.

Correlogram analysis of T-RF numbers indicated an insignificant trend towards increased spatial autocorrelation at the shorter distance classes for both plots (Fig. 3c, d). Plot 1, similar to what was found for hyphal lengths, exhibited maximum negative spatial autocorrelation near 30 cm, suggesting a trend towards patchiness at relatively small scales. In contrast, Plot 2 exhibited maximal negative spatial autocorrelation at near 60 cm, suggesting more of a gradient structure or a trend towards patchiness at a relatively greater scale (Legendre & Legendre, 1998).

Mantel correlograms indicated significant spatial autocorrelation for AMF community composition at the shorter distance classes (Fig. 3e, f). However, positive spatial autocorrelation

extended to greater distances than was apparent for hyphal lengths or T-RF numbers. Maximum negative autocorrelation occurred at relatively greater distances (45 cm and 55 cm for Plots 1 and 2, respectively), suggesting patchiness at a relatively larger scale.

Variance due to plants

Partitioning the variation in community similarity measures yielded similar results for both planar and PCNM models. For Plot 1, removal of spatial variability with either model indicated that a small but significant amount of the overall variance in AMF community composition data could be attributed to samples collected within 20 cm of *P. spicata* and 15 cm of *K. cristata* plants [8.6% (P = 0.013) and 9.1% (P = 0.04) of the variance in AMF community data explained by planar and PCNM models, respectively]. Neither extraradical hyphal length nor T-RF size numbers were found to be significantly related to plant location and identity after removal of spatial structure with either model.

Removal of spatial structure in AMF community data of Plot 2 using either model indicated that plant location and identity also accounted for a small but significant amount of the variance, although, unlike Plot 1, samples < 15 cm from the base of *K. cristata* and *F. idahoensis* significantly influenced AMF community composition [8.2% (P = 0.03) and 8.2% (P = 0.02) of the variance in AMF community data explained by planar and PCNM models, respectively].

Our previous cloning and sequencing efforts for this system were not exhaustive and focused on root samples obtained near the study site which, due to differences in functional traits between phylogenetic groups, may differ substantially in AMF species composition compared to that of soils (Hart & Reader, 2002; Maherali & Klironomos, 2007). Despite this, we did find a number of matches with T-RF sizes anticipated from analyses of site-specific sequence data that are thought to be diagnostic for specific AMF lineages (Mummey & Rillig, 2007). Spatial

dependence was apparent for a number of ribotypes when data for the presence or absence of diagnostic T-RF sizes in each sample is plotted graphically (Fig. 5). Indicator correlogram analyses confirmed spatial dependence for all of the specific ribotypes depicted (Fig. 5).

Relationships between AMF community abundance and composition

Examination of correlations between T-RF size numbers and hyphal lengths indicated an insignificant trend towards increased hyphal lengths with T-RF numbers for Plot 1 ($r = 0.31$; $P = 0.08$). No relationship between these variables was detected for Plot 2 ($r = -0.04$; $P = 0.83$). Similarly, Mantel tests comparing AMF community similarity with hyphal lengths indicated a positive, significant relationship between these variables for Plot 1 ($r = 0.16$; $P = 0.014$), but not for Plot 2 ($r = 0.01$; $P = 0.427$).

Discussion

A number of studies suggest that arbuscular mycorrhizal fungal communities are not homogeneously distributed in the environment, but vary both spatially (e.g. Rosendahl & Stukenbrock, 2004) and temporally (e.g. Husband *et al*., 2002). While individual plants and plant communities can contain distinct AMF communities that vary from each other in their composition and species number (e.g. Helgason *et al*., 2002; Husband *et al*., 2002), how these relationships manifest at small spatial scales in soil under the influence of plant communities is poorly defined.

Our results, taken individually or as a whole, demonstrate that AMF abundance and community composition can be definably spatially structured at scales of less than 1 m. From the perspective of establishing plants, these results suggest that small-scale spatial differences could strongly influence which subsets of the AMF community seedlings are exposed. Since it has been repeatedly demonstrated that plant response can vary significantly between different AMF

communities or species (e.g. Klironomos, 2003; Moora *et al*., 2004), such small-scale spatial heterogeneity could have important implications for plant establishment (van der Heijden, 2004).

While our results indicate spatial structure in small-scale AMF abundance and community composition, factors influencing this spatial heterogeneity are less clear. The results of variance partitioning analyses indicated that very little of the variation in our AMF abundance or community composition data could be accounted for by plant species identity and aboveground location. While these results appear to contrast with studies indicating significant differences between AMF communities associated with neighbouring plant species (e.g. Vandenkoornhuyse *et al*., 2002; 2003), molecular analyses of soil and root AMF communities can yield very different results (Hempel *et al*., 2007). This may be due to differences in life history strategies amongst AMF and host-specific growth responses (e.g. Bever, 2002; Maherali & Klironomos, 2007). Differences resulting in alteration of sporulation times (Pringle & Bever, 2002), for example, may be more apparent in analyses of soil, rather than root-inhabiting, AMF communities. Additionally, it has been suggested that some AMF may also access C in soil from sources other than host plants (Hempel *et al*., 2007), a hypothesis that remains to be tested.

Our results, contrary to what was hypothesized, did not indicate a relationship between AMF diversity, as indicated by T-RF size numbers, and extraradical hyphal abundance. However, Mantel test comparison of AMF community composition with abundance measures indicated a significant relationship for Plot 1, suggesting that AMF species composition had some influence on soil exploration by hyphae, as also found in mesocosm inoculation studies (van der Heijden *et al*., 1998). However, this relationship was not significant for Plot 2

A number of previous studies have employed molecular tools for spatial analysis of AMF communities, although typically at scales quite different from the work presented here (e.g.

Husband *et al*., 2002; Öpik *et al*., 2006). For example, Rosendahl & Stukenbrock (2004) examined spatial extents of *Glomus* within roots in undisturbed sand dunes at 5 m intervals and obtained evidence that *Glomus* species can be patchily distributed at scales approaching 10 m. Wolfe *et al*. (2007) used a T-RFLP-based method to assess spatial variation in AMF diversity within a 2 x 2 m plot situated on a calcareous fen having high plant species diversity at relatively small scales (2-9 species per 20 x 20 cm cell). Although AMF communities appeared to be quite complex, no significant spatial autocorrelation in AMF abundance or diversity was found at the scales measured.

Our results correspond with studies that have found AMF spores to be patchily distributed in both abundance and morphology at multiple scales (Boerner *et al*., 1996; Klironomos *et al*., 1999; Pringle & Bever, 2002; Carvalho *et al*., 2003). Two separate studies (Klironomos *et al*., 1999; Carvalho *et al*., 2003) used geostatistical methods to analyze spatial distributions of spores at scales approaching those examined in our study and found strong spatial autocorrelation in total spore numbers and morphologies associated with different AMF groups (*Glomus, Acaulospora, Scutellospora*) over relatively small distances. These studies, although both were conducted in shrublands, differed greatly in plant community composition and soil conditions (maquis, dense scrub, clay-loam, Carvalho *et al*., 2003; chaparral, sandy-loam, Klironomos *et al*., 1999). The proportion of variance in total spore numbers that could be accounted for by spatial characteristics was high for both systems (99.7 and 86.7% for Carvalho and Klironomos, respectively). Moreover, the percent of variance in family-specific spore numbers (*Glomus, Acaulospora, Scutellospora*) accounted for by spatial autocorrelation was high for both studies (63.8 to 99.9%, Carvalho *et al*., 2003; 22 to 95.7%, Klironomos *et al*., 1999). Similar to what we found for AMF community composition, spatial dependence ranged

from 30 cm to 120 cm for family-specific groups in the Klironomos study and from 54 to 300 cm in the Carvalho study.

Our results have important implications for representative sampling. The range of values obtained for hyphal lengths (Fig. 2), T-RF numbers (Fig. 4) and AMF community composition found at these small scales suggest that single samples would very poorly capture the variability present in a given site. For example, correlogram analysis suggests that in order to capture extraradical hyphal lengths representative of this ecosystem at this specific time, multiple samples would need to be collected at distance scales less than 30 cm (Fig. 3). Capturing all ribotype diversity would likely require a much larger sampling effort, similar to what was conducted here. However, if the goal of analysis is to determine spatial distributions of the dominant AMF ribotypes at the field scale, sampling at or below the scale of overall AMF community patch sizes indicated by our analyses (45 to 55 cm) may be warranted.

Since very little of the variance in the AMF community data could be accounted for by plant species identity and location, our results suggest that sampling soil AMF communities on the basis of plant species aboveground location may not be optimal for small-scale analyses. While AMF and host taxa preferences may occur, and may even be pronounced, aboveground plant measures can poorly reflect root spatial structure or distributions (e.g. Hutchings & John, 2003) potentially obscuring these relationships. Additionally, distances hyphae grow away from roots can vary with AMF species (Klironomos & Moutoglis, 1999) and these relationships would be expected to be influenced by host identity. Species-specific differences in external mycelium architecture and root vs. soil colonization rates (Hart & Reader, 2004) would also be expected to influence species-specific patch size.

Methodological considerations

Although we used restriction enzymes shown to allow for optimal discrimination of AMF species or phylotypes (Mummey & Rillig, 2007), it is still unclear to what extent these analyses are suitable for evaluation of species richness. There were T-RF sizes present in sample T-RFLP profiles which did not match sizes anticipated from simulated digestion of sequences cloned from plant roots near the study plots (data not shown). Additionally, in some cases T-RF sizes can overlap for different phylogenetic groups (Mummey & Rillig, 2007), which would lead to underestimation of diversity. The extent to which this occurred in this study is not known. Also, since no single current analysis method would be expected to capture all AMF diversity (Redecker *et al.,* 2003), including analyses employing the PCR primers used in this study (Mummey & Rillig, 2007), it cannot be ruled out that AMF species of importance were missed by our analyses.

A drawback to analysis of soil extracted whole-community DNA is that it is not possible to determine which fungal structures (such as spores or extraradical hyphae) the detected rRNA genes are predominantly derived. Thus, it is possible that these analyses predominantly capture LSU rRNA genes from active structures, such as hyphae, and inactive structures, such as spores, in different areas. Nevertheless, calculations in Hempel *et al*. (2007) indicate that spores probably contributed fairly little to the DNA isolated from soil compared to extraradical hyphae.

Conclusions

Microbial eukaryotes clearly exhibit spatial structure at a variety of scales (Green *et al*., 2004), and AMF are no exception. Given the tight links of AMF communities with plant community composition (Hart *et al*., 2003) and ecosystem functioning (Rillig, 2004; Rillig & Mummey, 2006) it is particularly important to know about the spatial heterogeneity of AMF. Here we demonstrate that AMF community spatial structure is detectable at small scales (<1 m) in a

seemingly homogenous grassland, using sample sizes (5 cm diameter) adequately representing the recruitment 'neighbourhood' of seedlings; clearly, this phenomenon needs to be examined in other ecosystems and over several scales.

Acknowledgements

This work was supported by grants from NSF Ecology (0515904) and USDA-CSREES (2005-35320-16267).

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Legends for Figures

Fig. 1. Sample strategy and plant locations, species identities and aboveground biomass (dry weight, g) for Plots 1 and 2. Symbols represent locations for *Koeleria cristata* (■), *Pseudoroegneria spicata* (●), *Festuca idahoensis* (▲), *Stipa comata* (∗), *Poa* species, (○), and mixed weedy grasses (▒).

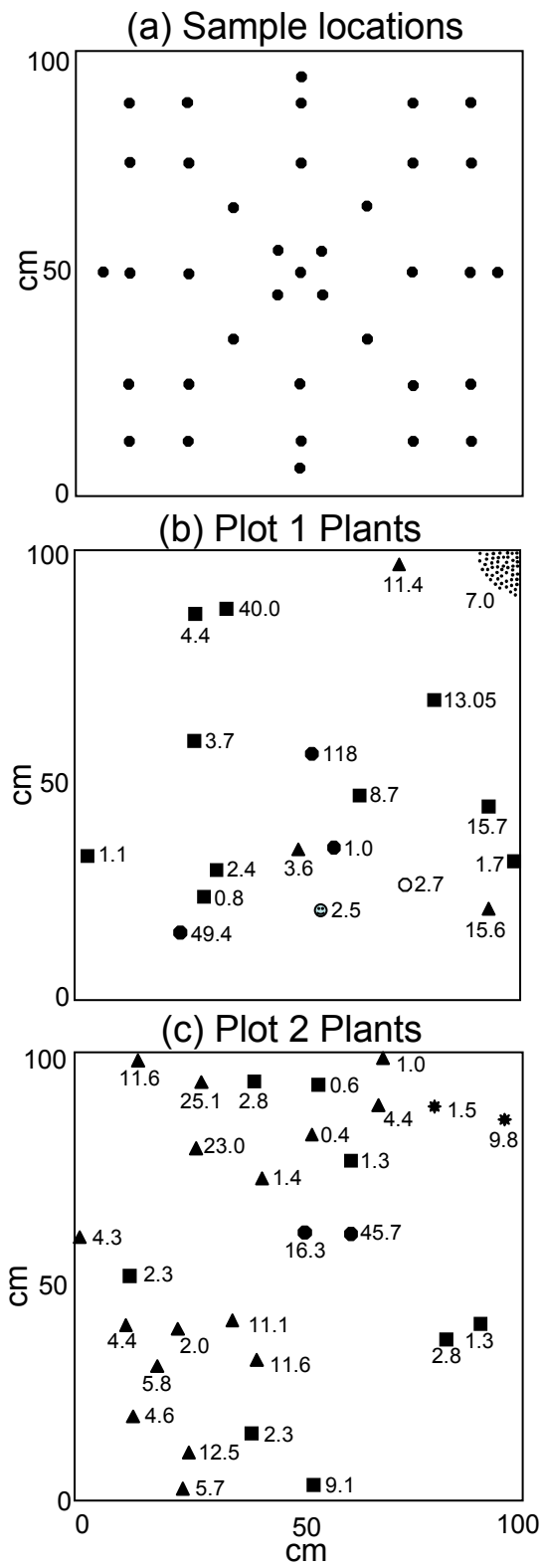
Fig. 2. Upper panels depict histograms representing AMF extraradical hyphal lengths estimated for samples of Plots 1 and 2. Lower panels depict sample location on each plot with symbol size corresponding to extraradical AMF hyphal length estimated for each sample.

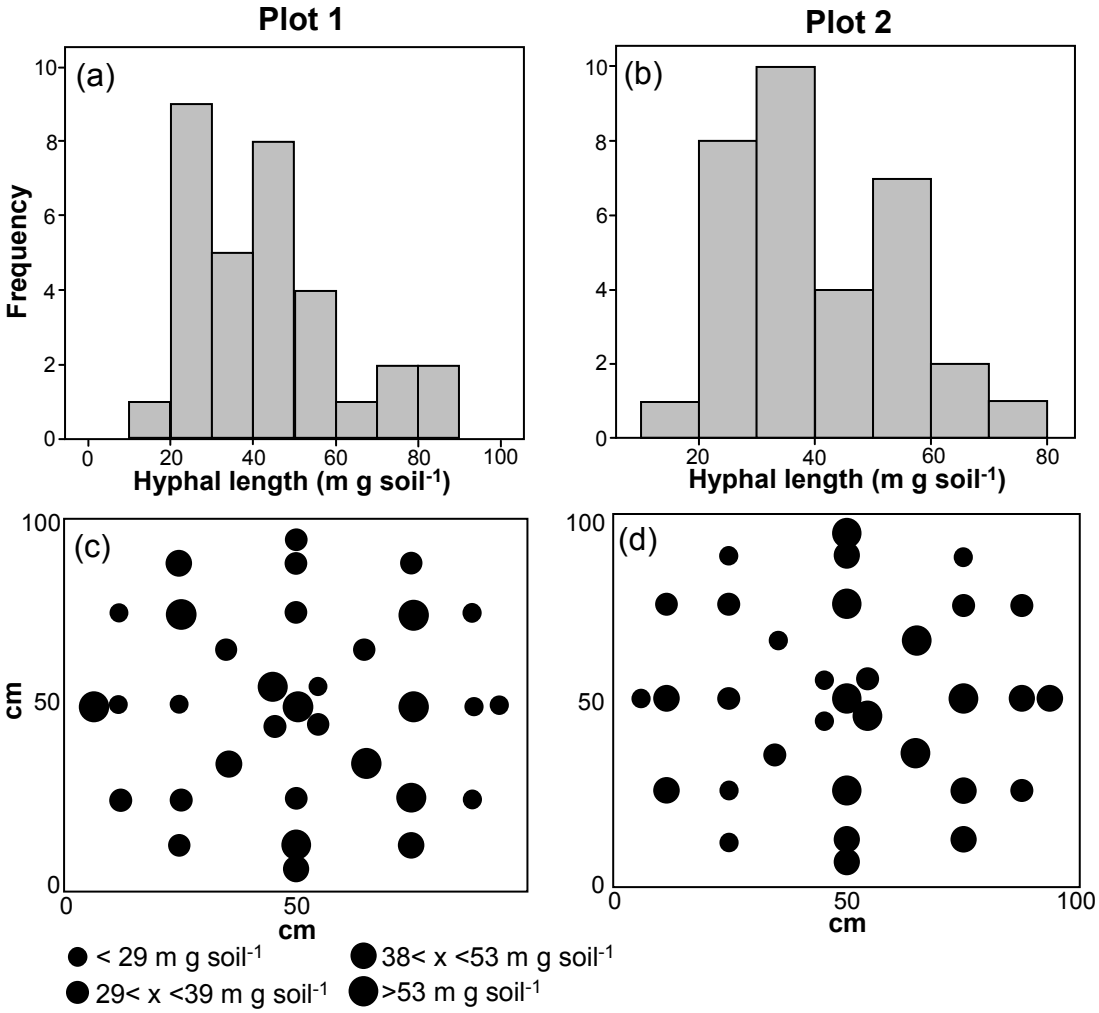
Fig. 3. Correlograms depicting spatial autocorrelation for extraradical AMF hyphal lengths, numbers of T-RF sizes detected, and AMF community composition for each plot. Solid symbols indicate significant spatial autocorrelation for samples within a distance class. A minimum of 60 sample pairs were analyzed in each distance class for each plot.

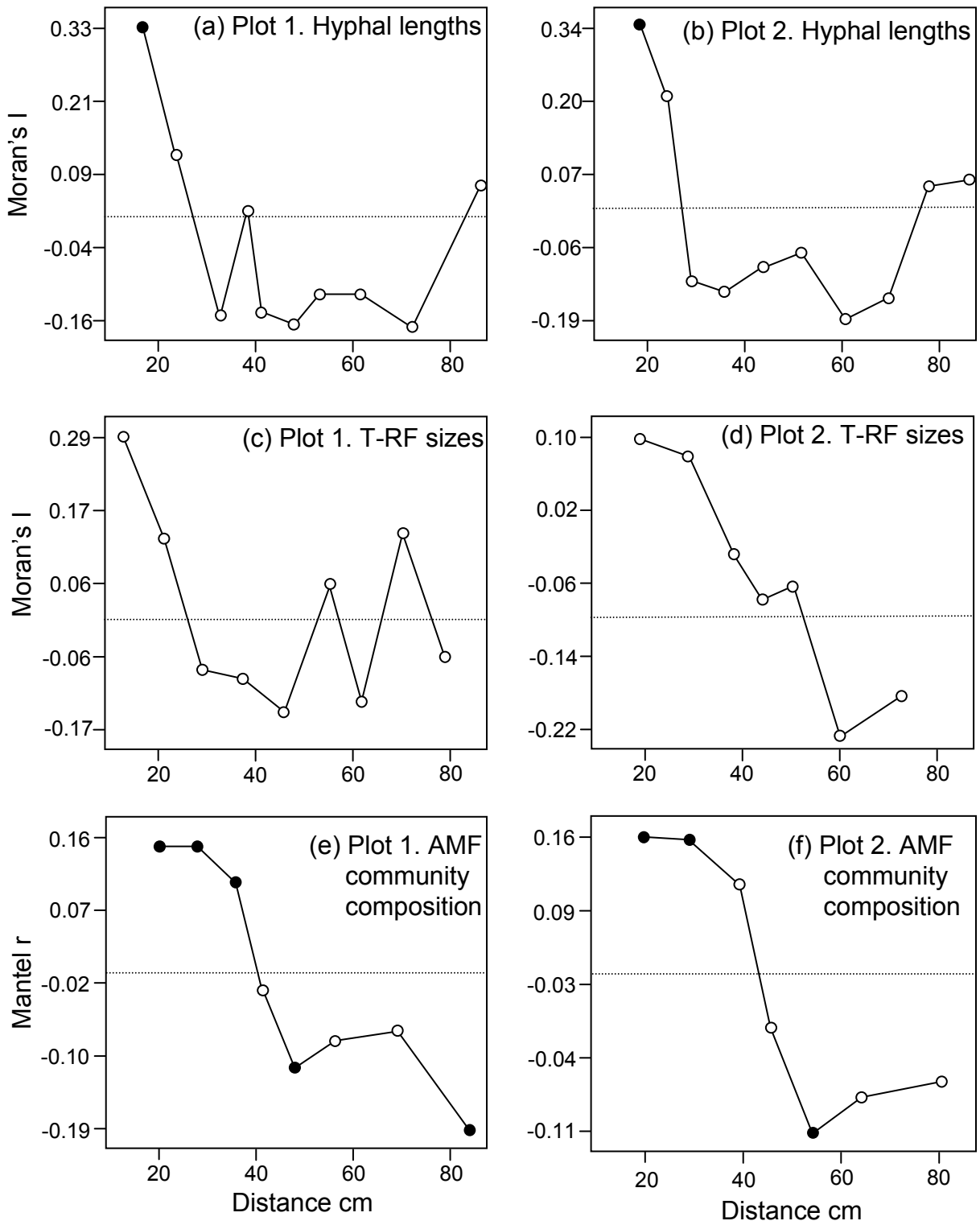
Fig. 4. Upper panels depict histograms representing the frequency at which specific numbers of different T-RF sizes were detected in samples of Plots 1 and 2. Lower panels depict sample locations on each plot with symbol size corresponding with the number of T-RF sizes detected in each sample.

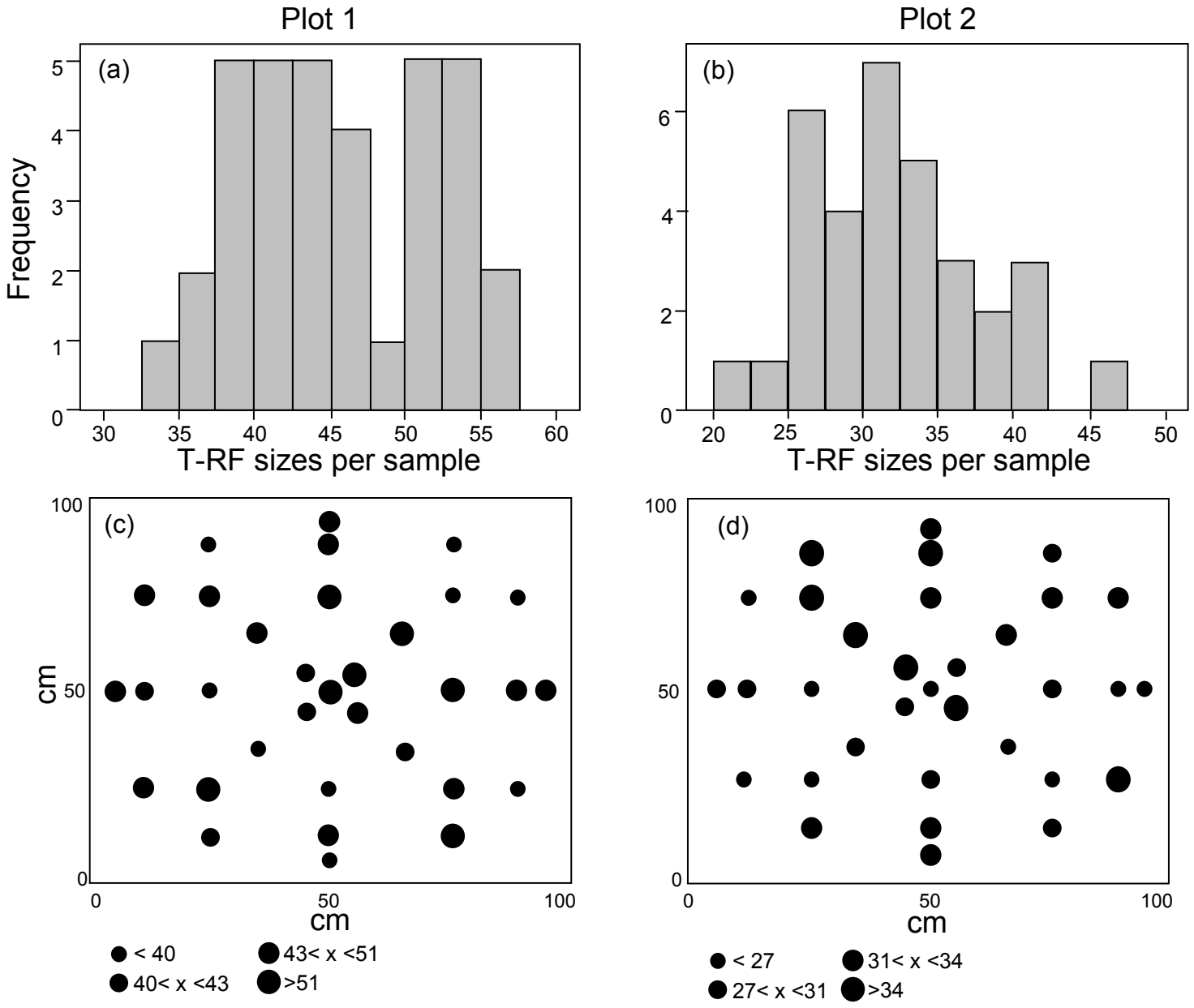
Fig. 5. (A) Plots depicting the spatial location of soil samples. Large symbols indicate the presence of T-RF sizes matching a specific AMF phylogenetic group for which site-specific sequence data is available. Open symbols indicate samples for which such T-RF sizes were not detected. (B) Correlograms depicting spatial autocorrelation with lag distance. Information pertaining to plot identity, phylogenetic affiliation of the ribotype being examined and GenBank accession number for a representative sequence cloned from near the study site are depicted on the right. *As defined in Mummey and Rillig (2007).

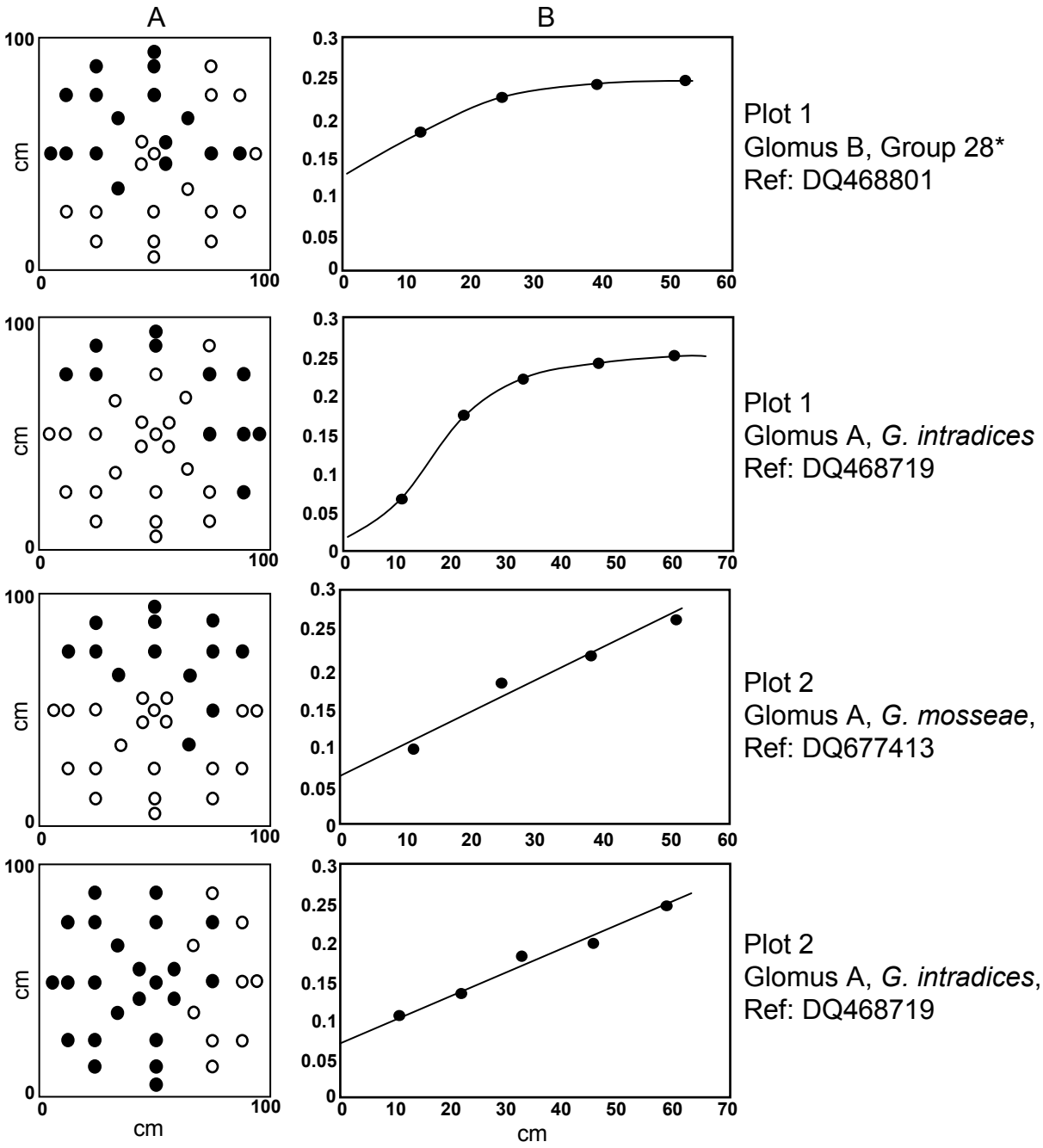
1


(a) Sample locations
100
50
0
cm
(b) Plot 1 Plants
11.4
7.0
40.0
4.4
13.05
3.7
118
8.7
15.7
1.1
2.4
3.6
1.0
1.7
2.7
0.8
2.5
15.6
49.4
(c) Plot 2 Plants
11.6
1.0
25.1
2.8
0.6
4.4
1.5
9.8
23.0
0.4
1.3
1.4
4.3
16.3
45.7
2.3
11.1
4.4
2.0
1.3
2.8
11.6
5.8
4.6
2.3
12.5
5.7
9.1
0
50
100
cm


Plot 1
Plot 2
(a)
(b)
(c)
(d)
Frequency
Hyphal length (m g soil-1)
cm
< 29 m g soil-1
29< x <39 m g soil-1
38< x <53 m g soil-1
>53 m g soil-1


(a) Plot 1. Hyphal lengths
(b) Plot 2. Hyphal lengths
(c) Plot 1. T-RF sizes
(d) Plot 2. T-RF sizes
(e) Plot 1. AMF community composition
(f) Plot 2. AMF community composition
Moran's I
Mantel r
Distance cm
0.33
0.21
0.09
-0.04
-0.16
0.34
0.20
0.07
-0.06
-0.19
0.29
0.17
0.06
-0.06
-0.17
0.10
0.02
-0.06
-0.14
-0.22
0.16
0.07
-0.02
-0.10
-0.19
0.16
0.09
-0.03
-0.04
-0.11
20
40
60
80


Plot 1
(a)
Frequency
T-RF sizes per sample
Plot 2
(b)
T-RF sizes per sample
(c)
cm
cm
< 40
40< x <43
43< x <51
>51
(d)
cm
< 27
27< x <31
31< x <34
>34


A
B
cm
cm
cm
cm
Plot 1
Glomus B, Group 28*
Ref: DQ468801
Plot 1
Glomus A, G. intradices
Ref: DQ468719
Plot 2
Glomus A, G. mosseae,
Ref: DQ677413
Plot 2
Glomus A, G. intradices,
Ref: DQ468719