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

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4 1 **Spatial characterization of arbuscular mycorrhizal fungal molecular diversity**  
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6 2 **at the sub-metre scale in a temperate grassland.**  
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19 **Abstract**

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

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

### 13 **Introduction**

14 Spatial distribution is one of the most fundamental ecological parameters for any group of  
15 organisms. Community composition or structure is driven by a range of different biotic and  
16 abiotic factors that can exert influences at broadly different spatial and temporal scales (Levin,  
17 1992). Disentangling these interactions is a major challenge to community ecology, requiring  
18 linkage of scales at which communities are measured with scales at which factors hypothesized  
19 to control their composition actually operate (Huston, 1999).

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

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

4 While arbuscular mycorrhizal fungal (AMF) communities have been shown to differ  
5 between sites, hosting plant species, habitats and ecosystem types (e.g. Helgason *et al.*, 1999,  
6 2002; Daniell *et al.*, 2001; Husband *et al.*, 2002), surprisingly little attention has been given to  
7 their community distribution at small spatial scales. Recent evidence indicating significant  
8 differences between AMF communities of co-occurring plants (Vandenkoornhuysen *et al.*, 2002,  
9 2003) would suggest host preferences as a potential mechanism promoting small-scale spatial  
10 structure. There is also a growing literature describing differences in life-history strategies  
11 among AMF taxa (e.g. Hart & Reader, 2002; Maherali & Klironomos, 2007). Interspecific  
12 differences in reproduction, dispersal, growth and environmental tolerances would be expected  
13 to result in formation of a spatial structure. Clearly, interspecific interactions are factors  
14 contributing strongly to the assembly of ectomycorrhizal fungal communities (Koide *et al.*, 2004;  
15 Kennedy *et al.*, 2007) and model AMF communities (Maherali & Klironomos, 2007). Specific  
16 species responses to physico-chemical gradients (e.g. nutrients, pH, soil texture and structure)  
17 have also been documented (e.g. Lekberg *et al.*, 2007) and could influence both large and small-  
18 scale spatial stratification of AMF community composition.

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

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

3 Many different factors can potentially influence seedling establishment in terrestrial  
4 ecosystems, including the presence or composition of AMF communities (van der Heijden,  
5 2004). If AMF communities are spatially homogeneous over short distances, relatively similar  
6 host/mutualist relationships would be expected. On the other hand, if small-scale spatial  
7 heterogeneity is pronounced, then at least the potential for differential benefit due to specific  
8 AMF community differences exists.

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

## 15 **Methods**

### 16 **Study site, sample strategy and plant measures**

17 Two sites, located 5 km N of Missoula, MT (USA) and separated by a distance of approximately  
18 25 m, were randomly selected on a gentle slope dominated by native grass species *Festuca*  
19 *idahoensis* Elmer, *Koeleria cristata* (Ledeb.) Schult, *Pseudoroegneria spicata* (Pursh) A. Löve  
20 ssp. *spicata* and *Hesperostipa comata* (Trin. & Rupr.) Barkworth ssp. *comata* (for a more  
21 detailed site description see: Lutgen *et al.*, 2003; Mummey *et al.*, 2006). Although minor forb  
22 cover is present on areas surrounding our study plots, no non-mycorrhizal plant species were  
23 identified within our plots that would serve to disrupt AMF community or abundance measures.

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

### 7 **Extraradical hyphal length analyses**

8 Extraradical AMF hyphal lengths were measured in each soil core according to Rillig *et al.*  
9 (1999). This technique involves aqueous extraction of hyphae from soil samples (4 g) and  
10 subsequent determination of AMF hyphae at 200x magnification. Hyphal lengths were estimated  
11 using the line intersect method as described in Tennant (1975) and Jakobsen *et al.* (1992).

### 12 **AMF community analyses: T-RFLP analysis optimization and validation**

13 Prior to conducting T-RFLP analysis of samples, T-RFLP analysis methods were optimized and  
14 validated as described in Mummey and Rillig (2007). Briefly, DNA was extracted from plant  
15 roots and soils near our study plots and a portion of the AMF ribosomal large subunit gene  
16 amplified via polymerase chain reaction (PCR) using unlabeled primers in reactions as described  
17 below. These products were cloned and sequenced, as described previously (Mummey & Rillig,  
18 2007). The computer program TRFSEQ (available from corresponding author upon request) was  
19 used to conduct simulated digestion of cloned sequences (GenBank accession numbers  
20 DQ468685 to DQ468824) with a broad range of restriction enzymes. This allowed for selection  
21 of restriction enzymes optimal for T-RFLP-based discrimination of the AMF diversity in our  
22 study plots. We used the following criteria to select restriction enzymes: (1) Overall ability to  
23 discriminate AMF diversity; (2) yielding unique terminal restriction fragment sizes (T-RF) for

1 each phylogenetic group that were as pronounced as possible; (3) T-RF sizes were greater than  
2 40 bp to allow for optimal size calling. These analyses indicated that the reaction conditions were  
3 highly specific to Glomeromycota of our study sites. Of the restriction enzymes examined, two  
4 (*Taq I* and *Alu I*) were found to best meet the above criteria and were used in all subsequent  
5 analyses.

### 6 **AMF community analyses: procedures**

7 For analysis of samples from our study sites, each soil core was homogenized by manual  
8 kneading and shaking in plastic bags. Whole community DNA was extracted from subsamples  
9 (0.4 g wet weight) using the PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA).  
10 Bead-beating utilized a Geno/Grinder™ 2000 (SPEX CentriPrep, Inc.). To consistently obtain  
11 sufficient amounts of PCR product for analysis, PCR amplification of soil DNA extracts  
12 consisted of two PCR rounds, the first employing the “general fungal” primer pair LR1 and  
13 FLR2 (Trouvelot *et al.*, 1999; van Tuinen *et al.*, 1998) and the second using AMF-specific  
14 primers FLR3 and FLR4 (Gollotte *et al.*, 2004). The 25 µl reaction mixtures included 1 µl soil  
15 extracted template DNA or product from the previous PCR, 100 pmol of each deoxynucleoside  
16 triphosphate, 10 pmol of each primer, 2 U HotMaster™ Taq DNA polymerase (Eppendorf,  
17 Hamburg, Germany) and 0.6 M betaine. Thermal cycling for all reactions included an initial  
18 denaturing step of 95° C for 5 minutes, 25 cycles (primer pair LR1 and FLR2) or 30 cycles  
19 (primer pair FLR3 and FLR4) consisting of 1 min at 95° C, 1 min at 58° C and 1 min at 65° C,  
20 followed by a final extension step of 65° C for 10 min.

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



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

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

### 17 **Data analyses**

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

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

1 communities, including testing for the presence of autocorrelation, was specifically examined  
2 using Mantel correlogram (Oden & Sokal, 1986) analysis. Mantel correlograms were produced  
3 by calculating normalized  $r_M$  values for AMF community Bray-Curtis distances (Bray & Curtis,  
4 1957) within different geographic distance classes. The degree of autocorrelation within each  
5 distance class was tested to determine whether it is greater or less than the overall mean  
6 autocorrelation between sites. The software program PASSaGE (ver 1.1; Rosenberg, 2001) was  
7 used to conduct these analyses.

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

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

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

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

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

## 14 **Results**

### 15 **Plant aboveground measures**

16 Although most plant species present in Plot 1 were also present in Plot 2, their relative numbers  
17 and contribution to total aboveground biomass differed (Fig. 1). *Koeleria cristata*, *P. spicata*  
18 and *F. idahoensis* accounted for 31, 58 and 10% of the aboveground biomass of Plot 1,  
19 respectively (Fig. 1a). *Koeleria cristata*, *P. spicata*, *F. idahoensis* and *H. comata* accounted for  
20 10, 28, 56 and 5% of the aboveground biomass of Plot 2, respectively (Fig. 1b). Total  
21 aboveground biomass also differed substantially between plots (303 g and 225 g for Plots 1 and  
22 2, respectively). Mean distance between nearest neighbouring plants was found to be 10.9 and  
23 9.7 cm for Plots 1 and 2, respectively.

## 1 **Abundance and distribution of AMF extraradical hyphae**

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

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

## 11 **AMF community composition analyses**

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

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

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

1 extended to greater distances than was apparent for hyphal lengths or T-RF numbers. Maximum  
2 negative autocorrelation occurred at relatively greater distances (45 cm and 55 cm for Plots 1 and  
3 2, respectively), suggesting patchiness at a relatively larger scale.

#### 4 **Variance due to plants**

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

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

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

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

#### 4 **Relationships between AMF community abundance and composition**

5 Examination of correlations between T-RF size numbers and hyphal lengths indicated an  
6 insignificant trend towards increased hyphal lengths with T-RF numbers for Plot 1 ( $r = 0.31$ ;  $P =$   
7  $0.08$ ). No relationship between these variables was detected for Plot 2 ( $r = -0.04$ ;  $P = 0.83$ ).  
8 Similarly, Mantel tests comparing AMF community similarity with hyphal lengths indicated a  
9 positive, significant relationship between these variables for Plot 1 ( $r = 0.16$ ;  $P = 0.014$ ), but not  
10 for Plot 2 ( $r = 0.01$ ;  $P = 0.427$ ).

#### 11 **Discussion**

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

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

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

3         While our results indicate spatial structure in small-scale AMF abundance and  
4 community composition, factors influencing this spatial heterogeneity are less clear. The results  
5 of variance partitioning analyses indicated that very little of the variation in our AMF abundance  
6 or community composition data could be accounted for by plant species identity and  
7 aboveground location. While these results appear to contrast with studies indicating significant  
8 differences between AMF communities associated with neighbouring plant species (e.g.  
9 Vandenkoornhuyse *et al.*, 2002; 2003), molecular analyses of soil and root AMF communities  
10 can yield very different results (Hempel *et al.*, 2007). This may be due to differences in life  
11 history strategies amongst AMF and host-specific growth responses (e.g. Bever, 2002; Maherali  
12 & Klironomos, 2007). Differences resulting in alteration of sporulation times (Pringle & Bever,  
13 2002), for example, may be more apparent in analyses of soil, rather than root-inhabiting, AMF  
14 communities. Additionally, it has been suggested that some AMF may also access C in soil from  
15 sources other than host plants (Hempel *et al.*, 2007), a hypothesis that remains to be tested.

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

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

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

9         Our results correspond with studies that have found AMF spores to be patchily  
10 distributed in both abundance and morphology at multiple scales (Boerner *et al.*, 1996;  
11 Klironomos *et al.*, 1999; Pringle & Bever, 2002; Carvalho *et al.*, 2003). Two separate studies  
12 (Klironomos *et al.*, 1999; Carvalho *et al.*, 2003) used geostatistical methods to analyze spatial  
13 distributions of spores at scales approaching those examined in our study and found strong  
14 spatial autocorrelation in total spore numbers and morphologies associated with different AMF  
15 groups (*Glomus*, *Acaulospora*, *Scutellospora*) over relatively small distances. These studies,  
16 although both were conducted in shrublands, differed greatly in plant community composition  
17 and soil conditions (maquis, dense scrub, clay-loam, Carvalho *et al.*, 2003; chaparral, sandy-  
18 loam, Klironomos *et al.*, 1999). The proportion of variance in total spore numbers that could be  
19 accounted for by spatial characteristics was high for both systems (99.7 and 86.7% for Carvalho  
20 and Klironomos, respectively). Moreover, the percent of variance in family-specific spore  
21 numbers (*Glomus*, *Acaulospora*, *Scutellospora*) accounted for by spatial autocorrelation was  
22 high for both studies (63.8 to 99.9%, Carvalho *et al.*, 2003; 22 to 95.7%, Klironomos *et al.*,  
23 1999). Similar to what we found for AMF community composition, spatial dependence ranged



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

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

13 Since very little of the variance in the AMF community data could be accounted for by  
14 plant species identity and location, our results suggest that sampling soil AMF communities on  
15 the basis of plant species aboveground location may not be optimal for small-scale analyses.  
16 While AMF and host taxa preferences may occur, and may even be pronounced, aboveground  
17 plant measures can poorly reflect root spatial structure or distributions (e.g. Hutchings & John,  
18 2003) potentially obscuring these relationships. Additionally, distances hyphae grow away from  
19 roots can vary with AMF species (Klironomos & Moutoglis, 1999) and these relationships would  
20 be expected to be influenced by host identity. Species-specific differences in external mycelium  
21 architecture and root vs. soil colonization rates (Hart & Reader, 2004) would also be expected to  
22 influence species-specific patch size.

### 23 **Methodological considerations**

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

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

## 18 **Conclusions**

19 Microbial eukaryotes clearly exhibit spatial structure at a variety of scales (Green *et al.*, 2004),  
20 and AMF are no exception. Given the tight links of AMF communities with plant community  
21 composition (Hart *et al.*, 2003) and ecosystem functioning (Rillig, 2004; Rillig & Mummey,  
22 2006) it is particularly important to know about the spatial heterogeneity of AMF. Here we  
23 demonstrate that AMF community spatial structure is detectable at small scales (<1 m) in a

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

## 5 **Acknowledgements**

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## 1 **Legends for Figures**

2 **Fig. 1.** Sample strategy and plant locations, species identities and aboveground biomass (dry

3 weight, g) for Plots 1 and 2. Symbols represent locations for *Koeleria cristata* (■),

4 *Pseudoroegneria spicata* (●), *Festuca idahoensis* (▲), *Stipa comata* (\*), *Poa* species, (○), and

5 mixed weedy grasses (⊙).

6 **Fig. 2.** Upper panels depict histograms representing AMF extraradical hyphal lengths estimated

7 for samples of Plots 1 and 2. Lower panels depict sample location on each plot with symbol size

8 corresponding to extraradical AMF hyphal length estimated for each sample.

9 **Fig. 3.** Correlograms depicting spatial autocorrelation for extraradical AMF hyphal lengths,

10 numbers of T-RF sizes detected, and AMF community composition for each plot. Solid symbols

11 indicate significant spatial autocorrelation for samples within a distance class. A minimum of 60

12 sample pairs were analyzed in each distance class for each plot.

13 **Fig. 4.** Upper panels depict histograms representing the frequency at which specific numbers of

14 different T-RF sizes were detected in samples of Plots 1 and 2. Lower panels depict sample

15 locations on each plot with symbol size corresponding with the number of T-RF sizes detected in

16 each sample.

17 **Fig. 5.** (A) Plots depicting the spatial location of soil samples. Large symbols indicate the

18 presence of T-RF sizes matching a specific AMF phylogenetic group for which site-specific

19 sequence data is available. Open symbols indicate samples for which such T-RF sizes were not

20 detected. (B) Correlograms depicting spatial autocorrelation with lag distance. Information

21 pertaining to plot identity, phylogenetic affiliation of the ribotype being examined and GenBank

22 accession number for a representative sequence cloned from near the study site are depicted on

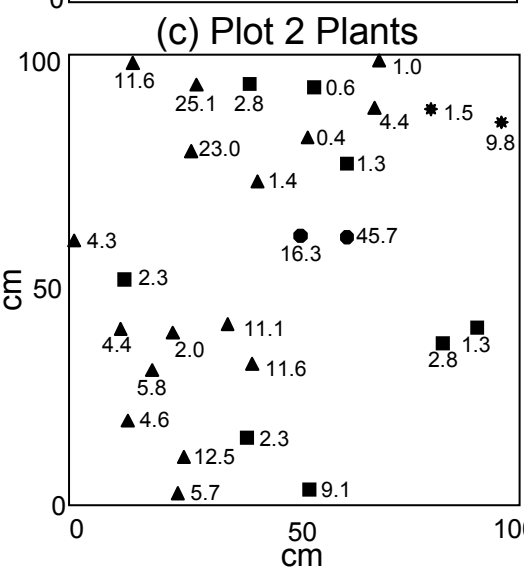
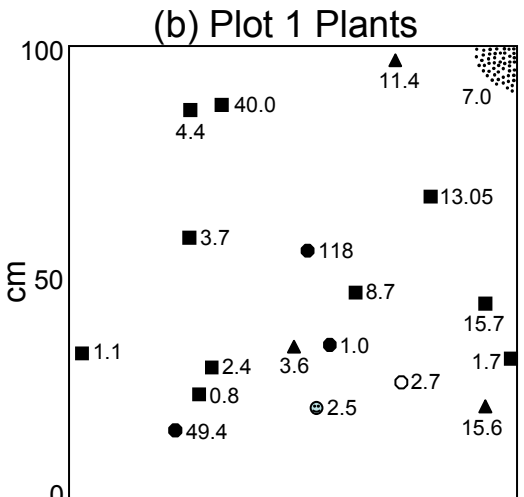
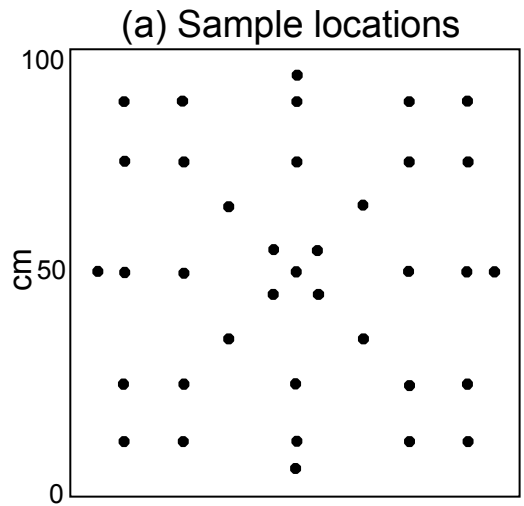
23 the right. \*As defined in Mummey and Rillig (2007).

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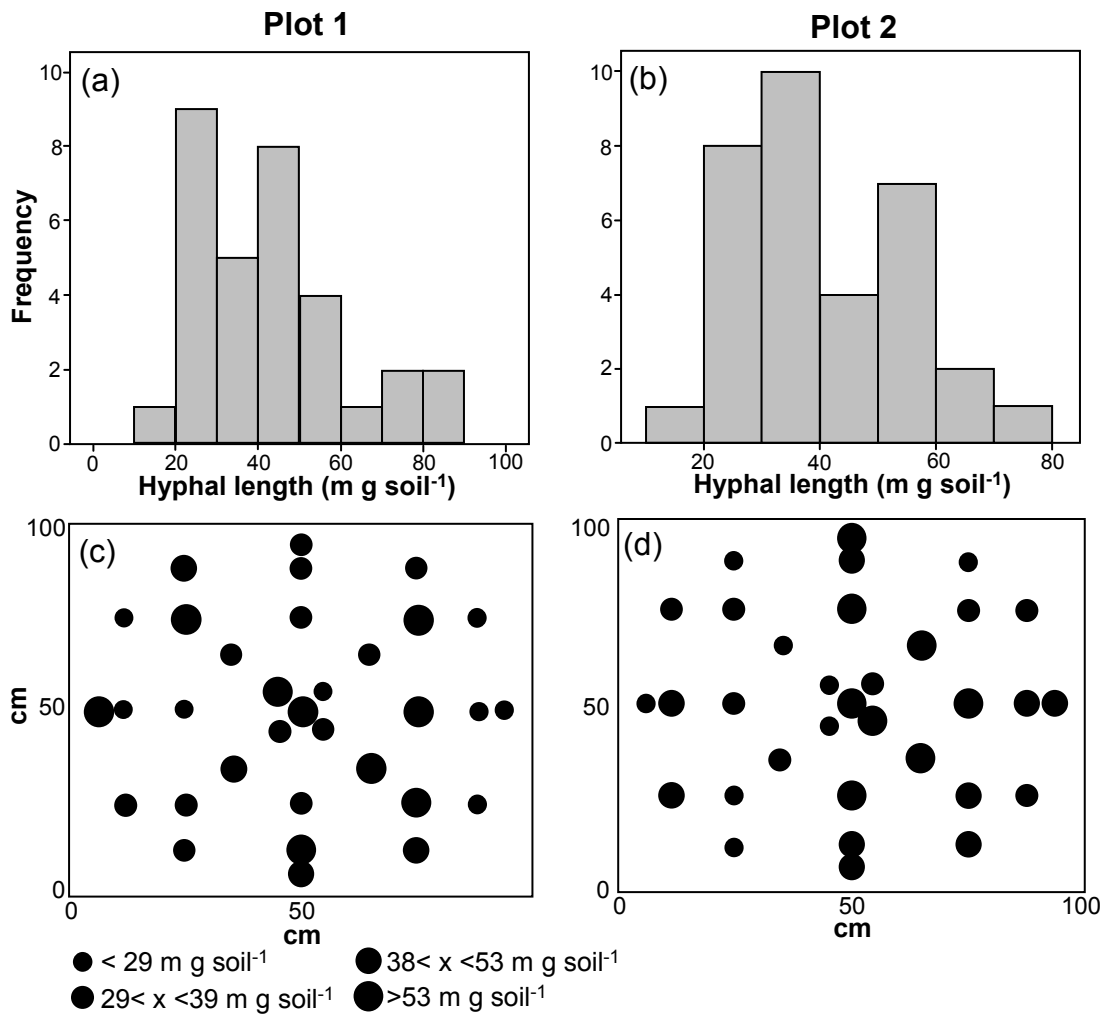
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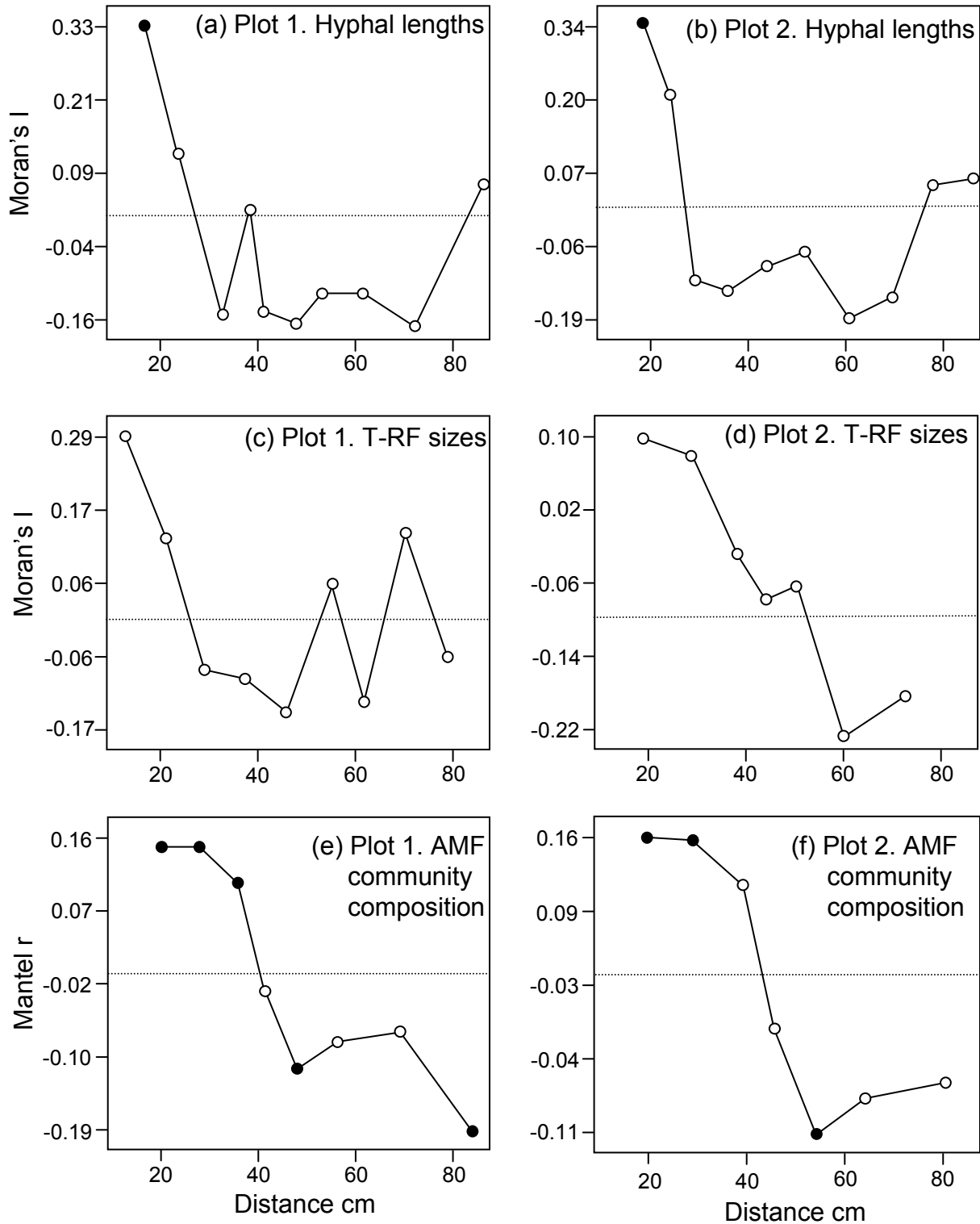
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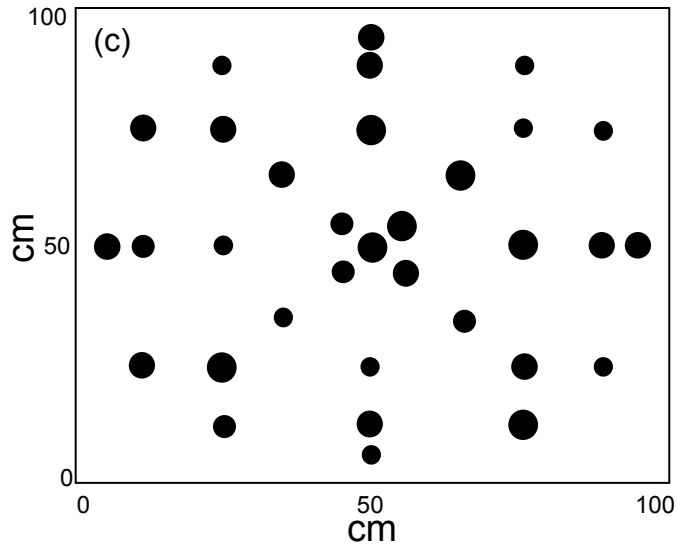
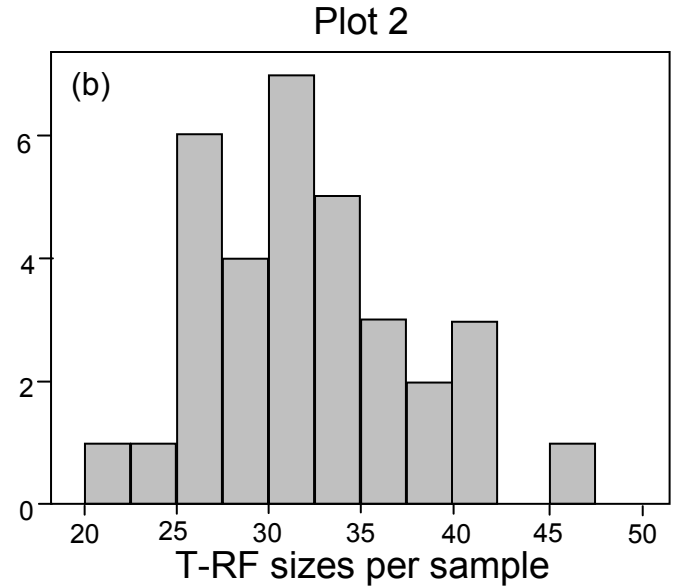
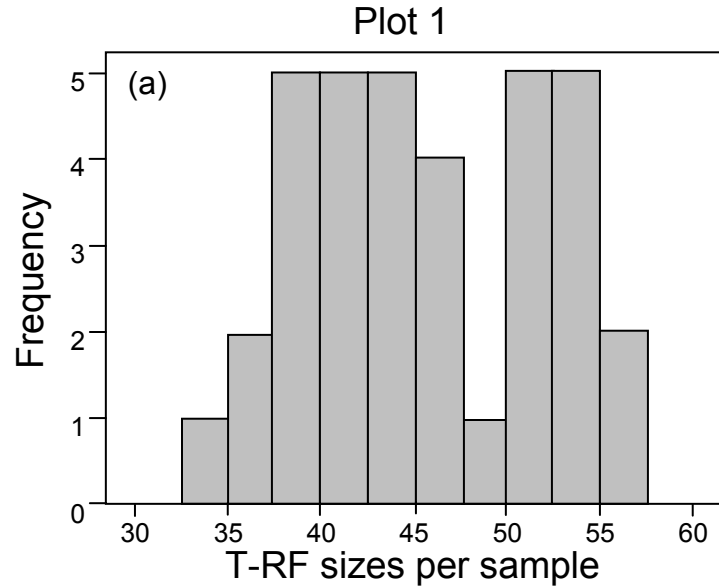


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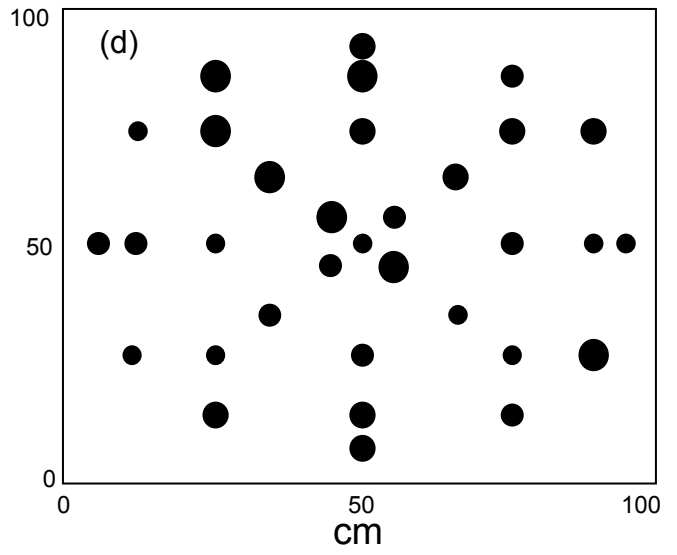




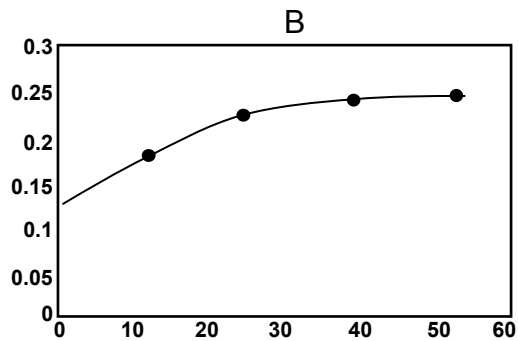
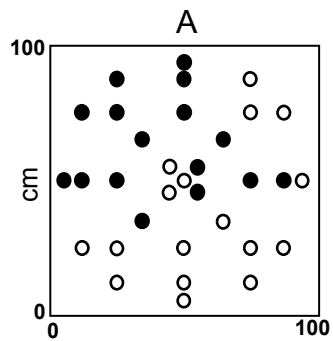
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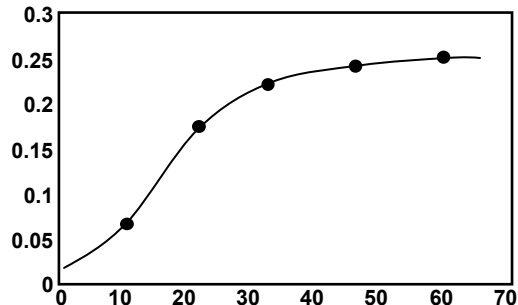
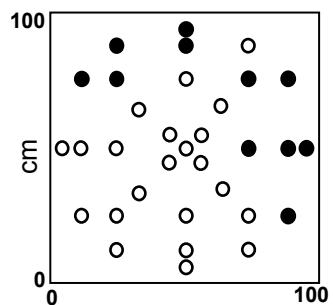
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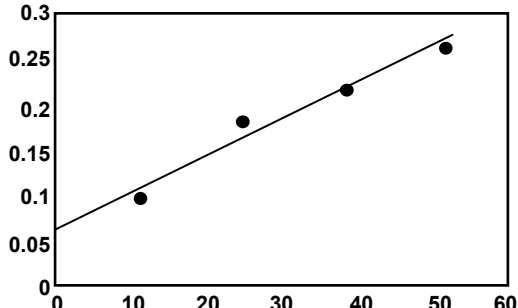
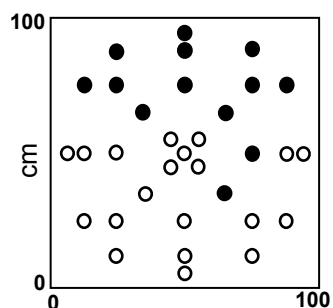
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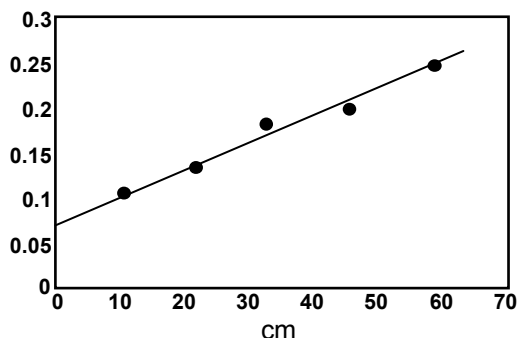
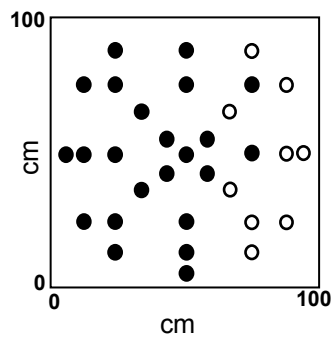
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Glomus B, Group 28\*  
Ref: DQ468801



Plot 1  
Glomus A, *G. intradices*  
Ref: DQ468719



Plot 2  
Glomus A, *G. mosseae*,  
Ref: DQ677413



Plot 2  
Glomus A, *G. intradices*,  
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