FUNGAL ECOLOGY XXX (2014) 1–12

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Multiscale patterns of arbuscular mycorrhizal fungal abundance and diversity in semiarid shrublands

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ARTICLE INFO

Article history:
Received 6 August 2013
Revision received 28 March 2014
Accepted 17 April 2014
Available online ■
Corresponding editor:
Kabir G Peay

Keywords:
Arbuscular mycorrhizal fungi
Arid ecosystems
Artemisia
Community ecology
Conservation
Distribution
Diversity
Glomeromycota
Scale

ABSTRACT

The distribution of arbuscular mycorrhizal (AM) fungal abundance and diversity across multiple scales, and the factors that drive spatial patterns, remains largely unknown in arid ecosystems. We examined multiple measures of AM fungal abundance, as well as spore diversity and community composition, at microsite (1 m^2), local (1 ha), and regional (5000 ha) scales in semiarid shrublands. At the microsite scale, hyphae, spores, and glomalin-related soil protein were more abundant underneath shrub canopies, but unvegetated shrub interspaces had similar amounts of viable propagules, spore diversity, and spore community composition compared to canopies. Significant local and regional scale variation in abundance, diversity, and community composition were correlated with variation in soil organic matter, climate, and soil phosphorus concentration. We observed high alpha, beta, and gamma spore diversity and significant spatial autocorrelation of communities. This study demonstrates how multiple indicators of Glomeromycotan abundance and diversity vary differentially in natural systems and how soil and climate factors are important drivers of spatial patterns.

Introduction

Arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota) form symbioses with the majority of plants in arid ecosystems (Miller, 1979; Allen et al., 1995; Tao and Zhiwei, 2005). These obligate root symbionts improve plant host access to limited soil resources (e.g. phosphorus, nitrogen, water) in exchange for photosynthates. Many factors, such as dominant vegetation type, climate, and edaphic properties, influence the abundance and distribution of AM fungi across multiple

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http://dx.doi.org/10.1016/j.funeco.2014.06.003
1754-5048/

spatial scales (Abbott and Robson, 1991). Abiotic factors (e.g. climate, edaphic properties), biotic factors (e.g. host plant community, interspecific interactions) and intrinsic properties of particular AM fungal species (e.g. dispersal capabilities, extinction rates) are predicted to influence AM fungal community structure at multiple scales as well (reviewed in Chaudhary et al., 2008; Fitzsimons et al., 2008; Caruso et al., 2012). Recent work examines the factors that influence Glomeromycotan distributions, focusing on biogeography at global scales, and highlights the paucity of AM fungal distributional data, particularly in arid ecosystems (Öpik et al., 2010; Turrini and Giovannetti, 2012). In many drylands, at smaller spatial scales, dominant perennial plants are distributed discontinuously within a matrix of unvegetated interspaces forming "islands of fertility" where soil nutrients and biotic activity are concentrated (Schlesinger et al., 1996). Because AM fungi are obligate plant symbionts, there is reason to think that their spatial distributions should mimic the patchiness of plants in drylands, but this hypothesis has not been thoroughly tested. Drylands comprise 41 % of terrestrial biomes (Reynolds et al., 2007); an improved understanding of Glomeromycotan abundance and diversity at multiple spatial scales will advance efforts to conserve fungal biodiversity in arid ecosystems.

Belowground, an AM fungus exists in several quantifiable forms and could exhibit different distributional patterns depending on spatial scale. Hyphae connect plant roots with the soil matrix, residing inside roots and extending into rhizosphere soil, where they absorb resources and deliver them to host plants. Hyphae can be long-lived or ephemeral (Hernandez and Allen, 2013) and also act to enmesh soil aggregates, promoting soil stability (reviewed in Rillig and Mummey, 2006). Therefore, assessments of extramatrical hyphae can indicate the potential of AM fungi to acquire resources and stabilize soil. Glomeromycota produce asexual spores which indicate reproductive output and variation in potential for dispersal and dormancy of different species. Although the community structure of AM fungi can be inferred from spore communities, it should not be assumed that the species of AM fungi inside plant roots determined using DNA-based techniques will exhibit the same patterns of occurrence as the spore communities in the surrounding soil (Bever et al., 2001; Liu et al., 2012). The phenology of AM fungi may generate distinct root and spore communities, and this may help partition fungal niches in space and time (Pringle and Bever, 2002). Relatively large AM fungal spores are rich in lipids and are likely to be an important food source for microscopic soil organisms, particularly in arid soils with low organic matter content. Living AM fungi, as well as decomposing hyphae and spores, enrich soil with organic compounds including glomalin, a highly recalcitrant glycoprotein (Rillig and Mummey, 2006). While glomalin is a putative gene product of AM fungi, glomalin-related soil protein (GRSP) is considered operationally distinct because it is the measureable quantity in soils that is deposited by a consortium of microorganisms in addition to AM fungi (Gillespie et al., 2011). Soil aggregation is strongly correlated with GRSP and its quantity in the soils reflects the potential for AM fungi to stabilize soil and store carbon. Glomeromycota propagate from spores, hyphal fragments, and colonized segments of plant roots (Jasper et al., 1989). It is extremely difficult to visually determine the viability of spores and hyphae in the soil but the mycorrhizal infection potential (MIP) bioassay offers an integrated method to measure the abundance of all viable propagules (Moorman and Reeves, 1979). Because hyphae, spores, GRSP, and MIP play different roles in the life history of AM fungi and ecosystem function, it is insightful to examine their spatial patterns individually.

Previous studies of AM fungi in arid ecosystems have shown that spore density and GRSP concentration are higher underneath shrub canopies and that hyphal density and GRSP differ across sites (Klironomos et al., 1999; Bird et al., 2002; Rillig et al., 2003). A regional scale study of Glomeromycota in *Artemisia tridentata* shrubs showed that AM spore communities varied both spatially and temporally with latitude, and that local environment likely influenced species distributional patterns (Allen et al., 1995). Previous cross-habitat comparisons have noted that the AM fungal spores of the order Glomerales (e.g. *Glomus*) tend to dominate arid environments, while the relative abundance of spores of the family Gigasporaceae (e.g. *Scutellospora*, *Gigaspora)* increase in more mesic environments (Stutz et al., 2000; Egerton-Warburton et al., 2007). No prior studies have simultaneously examined the distributions of multiple indicators of Glomeromycotan abundance, diversity and community composition, across a range of spatial scales. Our study is unique because we examine variation in four measures of AM fungal abundance as well as spore diversity and community composition across three scales ranging from 1 m^2 to 5,000 ha. We specifically test the following hypotheses:

H_1: Glomeromycota will be more abundant and diverse underneath shrub canopies compared to interspaces between shrubs.
H_2: Glomeromycota will exhibit spatial autocorrelation such that more similar communities are located closer to each other in space. In other words, AM fungal communities are expected to differ more at the regional scale than at the site scale.
H_3: Soil and climate variables will help explain variation in AM fungal distributions across multiple scales of observation.
H_4: Particular AM fungal species may be indicators of particular microsites, sites, or regions. Species in the Glomerales will be relatively less abundant, while Gigasporaceae species will be relatively more abundant, in regions with higher annual precipitation.

Materials and methods

Sampling design

The abundance, diversity, and community composition of Glomeromycota was examined at three spatial scales within the 769,000 ha Grand Staircase-Escalante National Monument in southern Utah, USA (37°24′N, 111°41′W): microsite (1 m^2), site (1 ha), and region (5 000 ha). The geologic and topographic environment of this large national monument is very heterogeneous, with 136 distinct soil types and 50 distinct ecological

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community types. We measured AM fungal variables at four different regions ranging in elevation from 1250 to 2030 m and ranging in annual precipitation from 16.8 to 24.2 cm (Table 1). Three sites in each of the four regions for a total of 12 study sites were sampled. Within each site, soil sampling was stratified at the microsite scale; 9 samples were collected from the rhizosphere of randomly selected shrubs (referred to as "canopy") and 9 from the adjacent interspaces at least 1 m away from shrub islands (referred to as "interspace"). In entirety, the study contained 216 total observations (4 regions × 3 sites × 2 microsites × 9 samples). The sampling design is illustrated and a detailed account of sampling protocol is given in Chaudhary et al. (2009). There is good evidence that certain AM fungal species preferentially associate with particular plant species (Johnson et al., 1992; Yang et al., 2012 Hazard et al., 2013). Consequently, to best focus our study on abiotic forces (e.g. climate, edaphic properties) and minimize variation caused by biotic forces (e.g. host plant community, interspecific interactions), canopy soil samples were always collected from the rhizosphere of shrubs belonging to a single plant genus. *Artemisia* was chosen as the target shrub because it dominates the canopy at all sites and has a wide distribution across the western U.S. In the Boulder, Cannonville, and Escalante regions *A. tridentata* was sampled and in the Big Water region *A. filifolia* was sampled. Soil samples were collected to a depth of 15 cm at the beginning of the dry season in May, air-dried and stored at 4 °C until processed. Plant cover was also visually quantified within a 1 m^2 quadrat surrounding each soil sampling location.

Assessment of AM fungi

AM fungal abundance was quantified using four different methods: hyphal density, spore abundance, GRSP concentration, and MIP. Multiple methods were used to capture the broadest possible range of AM fungal structures and potential functions. Hyphal density was quantified by agitating 5 g of soil in a blender, siphoning the suspension with a pipette, and then collecting hyphal fragments on a membrane filter (modified from Jakobsen et al., 1992). Hyphae were preserved on permanent slides and measured using a compound microscope (200 × magnification) based on morphology that is characteristic of AM fungi (e.g. absence of regular septae), and length per gram of soil was calculated using a grid-line intersection method. Spore abundance was quantified by extracting spores from a 30 g subsample of field soil by wet-sieving and density centrifugation (McKenney and Lindsey, 1987). Spores were also used to assess AM fungal diversity according to morphological characteristics. Spores were collected by suction filtration onto gridded membrane filters and mounted on glass microscope slides for identification and enumeration using a compound microscope (200 − 1,000 × magnification). Morphospecies were identified according to information from original taxonomic descriptions and recent phylogenetic reclassifications (Redecker et al., 2013). Multiple cycles of greenhouse trap cultures (Stutz and Morton, 1996), using soils collected from each site, were also conducted to attempt to reveal AM fungal species not present as spores in field soils, but no additional species were detected. Morphospecies diversity was assessed by both spore species richness and Shannon–Wiener evenness (H'/ln S). The easily extractable soil protein fraction of GRSP (EE-GRSP) was quantified using the Bradford colorimetric protein assay (Bradford, 1976). To assess MIP, root colonization of bait plants was compared among samples. Our previous studies have shown that organic corn (*Zea mays*) provides a uniform host for MIP bioassays, and is preferable to slow-growing native plants (Johnson et al., 1999). In Jul-2004, organic corn seeds were sown in 150 ml of each soil sample in 3.8 cm diameter Conetainers™ (Stuewe and Sons Inc.) in a greenhouse. After 6 weeks, plants were harvested and a 0.25 g root subsample was cleared, stained, and examined using a compound microscope (200× magnification) for the presence of AM fungal structures (e.g. arbuscules, vesicles, aseptate hyphae) (McGonigle et al., 1990). We did not measure percent colonization of field collected roots because fine roots were rare in most of our samples.

Soil and climate properties

Five abiotic soil characteristics were measured to explore their potential relationships with AM fungal abundance and

Table 1 – Physical, climatic, biological, and soil characteristics of the four regions: Big Water (Bw), Escalante (Es), Cannonville (Ca), and Boulder (Bo). Values are means with standard errors in parentheses. Letters indicate statistically significant differences according to Tukey's HSD multiple comparisons test

	Regions			
	Bw	Es	Ca	Bo
Elevation (m)	1250	1770	1800	2030
MAP (cm)	16.8	26.6	29.6	24.2
MAT (°C)	12.5	9.20	10.0	9.44
Dominant plant	*A. filifolia*	*A. tridentata*	*A. tridentata*	*A. tridentata*
Soil texture	Fine sand	Fine sandy loam	Very gravelly loam	Fine sand
Avail P (ppm)	20.55 (1.39)a	31.31 (7.73)a	10.71 (3.01)a	31.71 (3.68)a
Avail N (ppm)	3.95 (0.36)a	5.01 (0.75)a	2.86 (0.26)a	3.70 (0.66)a
%SOM	0.652 (0.06)a	4.62 (0.21)b	1.43 (0.28)c	1.17 (0.12)d
pH	7.63 (0.11)ab	7.66 (0.11)ab	7.97 (0.11)a	7.05 (0.18)b
EC	43.9 (6.86)a	101.2 (5.72)b	44.7 (5.29)a	32.1 (4.84)a

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Table 2 – Summary of observed variation in hyphal density, spore density, easily extractable glomalin related soil protein (EE-GRSP), percent mycorrhizal infection potential (%MIP), spore richness, and spore evenness across three spatial scales. Values are ANOVA F-statistics with *p*-values in parentheses. For spore community, values are MRPP A-statistics with *P*-values in parentheses. NS = not significant. NA = not applicable

	Microsite	M × R	Site	Region
Hyphal density	11.7 (0.009)	7.36 (0.011)	7.85 (0.004)	20.6 (0.0004)
Spores/g	20.2 (0.002)	NS	4.25 (0.028)	NS
EE-GRSP	42.7 (0.0002)	NS	10.5 (0.0016)	NS
%MIP	NS	NS	NS	6.13 (0.018)
Spore richness	NS	NS	5.61 (0.013)	NS
Spore evenness	5.84 (0.042)	8.24 (0.008)	NS	NS
Spore community	NS	NA	0.139 (0.0001)	0.072 (0.0001)

diversity. Soil organic matter was measured by percent mass loss after ashing for 24 h at 550 °C. Prior to ashing, carbonates were removed by evolving with 6 M HCl. Soil pH and electrical conductivity (EC) were determined by creating a soil-water slurry and measuring with a glass electrode pH meter (Maizeing Inc.) and a table-top EC meter (YSI Inc.). Available nitrogen (N) concentrations were determined by summing ammonium and nitrate concentrations as measured by KCl extraction. Available phosphorus (P) concentration was determined using the Melich III extraction procedure. Available N and P analyses were conducted at the Kansas State University Soil Testing Laboratory. Mean annual temperature (MAT) and mean annual precipitation (MAP) data were obtained from Bureau of Land Management weather stations.

Analyses

Differences in four abundance measures and two diversity measures of AM fungi were assessed across spatial scales by conducting mixed model ANOVAs to evaluate the fixed effects of microsites (M), regions (R) and region by microsite interaction (R × M) and the random effect of site nested within regions (S(R)) on all AM fungal response variables (Neter et al., 1996). To satisfy the criteria for ANOVA, tests were conducted on mean canopy and interspace values such that sites were replicates and $N = 24$. Tukey's HSD multiple comparison tests with $\alpha^* = 0.05$ were used to determine differences between groups of means. ANOVA was also used to examine differences in all abiotic soil characteristics across regions, microsites, and sites. All ANOVAs and multiple comparison analyses were performed using JMP 4.0.4 statistical software. To evaluate the importance of abiotic environmental variables in predicting AM fungal abundance and diversity, multimodel inference and AIC was used (Burnham and Anderson, 2002). For each AM fungal response variable a global model that included 7 predictor variables was constructed (see Table 3), which was deemed appropriate to include in the analyses because of *a priori* knowledge of AM fungi and the ecological system. Then, the global model was compared to all possible combinations of models with various subsets of predictors using the dredge function in the R package MuMIn (Barton, 2013). Further analyses were conducted on a subset of models considered most informative and best supported by the data with ΔAIC values less than 4 (Burnham and Anderson, 2002). Using this subset of informative models, a summed Akaike weight (w_i) was determined for each predictor variable using the importance function, which indicates relative importance in predicting the response. Predictors with w_i greater than 0.50 were considered important variables (Burnham and Anderson, 2002).

Alpha diversity was calculated as the number of spore morphospecies detected per soil sample. Beta diversity was calculated by dividing landscape-level, or gamma diversity (number of species detected across entire study), by average alpha diversity (McCune and Grace, 2002). Spore morphospecies community data were relativized by species maximum and ordinated using nonmetric multidimensional scaling (NMS) with the Sorensen (Bray–Curtis) distance measure. Final ordinations were produced using 40 runs of real data and a 3-dimensional solution, as was suggested by a plot of final stress versus number of dimensions (1 through 6). NMS

Table 3 – Summed Akaike weights (w_i) of explanatory variables for each of the six AM fungal abundance and diversity measures. Bold values are greater than 0.50 and represent important explanatory environmental variables for the AM fungal responses

	Hyphal density	Spore density	EE-GRSP	MIP % colonization	Spore richness	Spore evenness
%SOM	**1.00**	**0.54**	**0.83**	**0.99**	0.29	0.32
Avail P (ppm)	0.28	**1.00**	**1.00**	**1.00**	**0.82**	**0.50**
Avail N (ppm)	**0.92**	0.30	**0.62**	0.34	0.27	0.33
EC	0.38	0.40	0.41	0.42	**0.97**	0.28
pH	**0.72**	0.37	0.42	0.36	**0.58**	0.35
MAT	0.47	0.43	0.49	**0.80**	**0.96**	0.46
MAP	0.35	**0.77**	**0.78**	0.45	0.45	0.33

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ordinations were also conducted using presence-absence data to determine if patterns were due simply to spore abundances or entire community structure. Differences between *a priori* groups (i.e. microsites, sites, and regions) were assessed using multi-response permutation procedure (MRPP) with the Sorensen (Bray–Curtis) distance measure. MRPP produces two statistics: a *P*-value, the probability that the observed difference between groups is due to chance alone, and the agreement statistic A, within-group homogeneity compared to the random expectation. An A-statistic of 0.10 or greater generally indicates good within-group homogeneity (McCune and Grace, 2002). To identify the AM fungal species and genera driving differences between groups, indicator species analysis (ISA) was conducted for microsites, sites, and regions (Dufrene and Legendre, 1997). ISA calculates an Indicator Value (IV) by multiplying the relative abundance and relative frequency of a species; we used a Monte Carlo test with 1000 randomizations to obtain a *P*-value which indicates the probability that a species has a greater IV within a particular group of interest compared to chance alone. All NMS ordinations, MRPPs, and ISAs were performed in PC-ORD Statistical Software. To examine relationships between AM fungal communities and environmental variables, we conducted distance-based redundancy analysis, which can be used to examine the correlations between environmental variables and NMDS ordination axes using non-Euclidean distance measures (Legendre and Anderson, 1999). Distance-based redundancy analyses were conducted using the capscale function in the R package vegan (Oksanen et al., 2007). The majority of AM fungal community variance was explained by a single constrained axis; biplot scores for constraining variables on this axis are reported. To assess spatial autocorrelation, we used the Mantel test, a standard method for analyzing spatial patterns in ecological data that tests the relationship between distance and similarity among samples (Martiny et al., 2006; Borcard and Legendre, 2012). We examined the relationship between AM fungal community dissimilarity and spatial dissimilarity, as well as the correlation between AM fungal community dissimilarity and climate and soil dissimilarity. For AM fungal communities, dissimilarity matrices were calculated using the Sorensen (Bray–Curtis) distance measure while dissimilarity matrices for spatial, climate, and soil properties were calculated using Euclidean distance. Results of the Mantel test are interpreted by two test statistics: the *P*-value, based on a 1000 iteration Monte Carlo test, indicates the probability that the observed difference between the two dissimilarity matrices is due to chance alone, and the standardized Mantel statistic (*r*) indicates the strength of the correlation between the two dissimilarity matrices. To visualize the results of the Mantel tests, we constructed Mantel correlograms, which plot Mantel correlation statistics by distance on an ordinate scale (Borcard and Legendre, 2012). Correlograms are interpreted by examining the statistical significance of the points, the shape of the plot, and the Mantel statistic values on the *y*-axis. Strong spatial autocorrelation is indicated by a negative linear gradient with mostly statistically significant points and high Mantel *r* *y*-axis values (close to 1.0). Mantel tests were conducted using the mantel function and correlograms were constructed using the mantel.correlog function in the R Package vegan (Oksanen et al., 2007).

Results

Glomeromycota abundance across scales

Patterns in the different AM fungal abundance measurements varied depending on spatial scale (Table 2). Hyphal density was more than twice as high in the Escalante region compared to all other regions (Fig 1A). There was a significant microsite by region interaction such that density was higher in canopies in the Big Water, Escalante, and Cannonville regions but higher in interspaces in the Boulder region. In general, hyphal density differed among sites nested within regions (Table 2). Differences among the 3 sites nested within each region are not shown graphically. Spore abundance did not differ among regions, but was twice as high underneath shrub canopies compared to interspaces (Fig 1B). Spore abundance also varied among sites (Table 2). For EE-GRSP, differences were observed at the site and microsite scales; certain sites had higher EE-GRSP concentrations than others and, across all sites and regions, nearly twice as much EE-GRSP was found underneath shrub canopies compared to interspaces (Fig 1C). Percent MIP colonization differed only on a regional scale such that the relative amount of infective AM fungal propagules was highest in the Escalante region, followed by Cannonville, Boulder, and then Big Water (Fig 1D). In all regions, the relative amount of infective AM fungal propagules did not differ between canopies and interspaces or among sites.

AIC analyses revealed several important abiotic predictors for AM fungal abundance and diversity measures (Table 3). The most important predictor of hyphal density was soil organic matter ($w_i = 1.00$) followed by soil N ($w_i = 0.92$) and soil pH ($w_i = 0.72$). Spore density was best predicted by soil P ($w_i = 1.00$), MAP ($w_i = 0.77$), and organic matter ($w_i = 0.54$). The most important predictors of EE-GRSP concentration were P ($w_i = 1.00$), organic matter ($w_i = 0.83$), precipitation ($w_i = 0.78$), and N ($w_i = 0.62$). Soil P ($w_i = 1.00$), organic matter ($w_i = 0.99$), and MAT ($w_i = 0.80$) were the best abiotic environmental predictors of viable AM fungal propagules as measured by the MIP bioassay. Four abiotic variables were important predictors of spore species richness, EC ($w_i = 0.97$), MAT ($w_i = 0.96$), soil P ($w_i = 0.82$), and pH ($w_i = 0.58$), while only soil P was an important predictor of spore evenness ($w_i = 0.50$).

Glomeromycota diversity and community patterns

A total of 42 AM fungal spore species (gamma diversity) among 12 known genera were observed across the entire study. The species versus effort curve exhibited a long asymptote, which suggests an adequate amount of sampling to capture diversity beneath *Artemisia* shrublands across the entire study area (Appendix 1). An average of 9.8 spore species was detected per sample (alpha diversity), with a maximum of 20 and minimum of 4 spore species per sample. Overall beta diversity was 4.3, suggesting high landscape-level species turnover across the entire study area (McCune and Grace, 2002). Spore species richness did not vary at the regional scale and a similar number of spore species was found between canopy and interspaces at the microsite scale (Fig 1E, Table 2). However, spore species richness differed among sites

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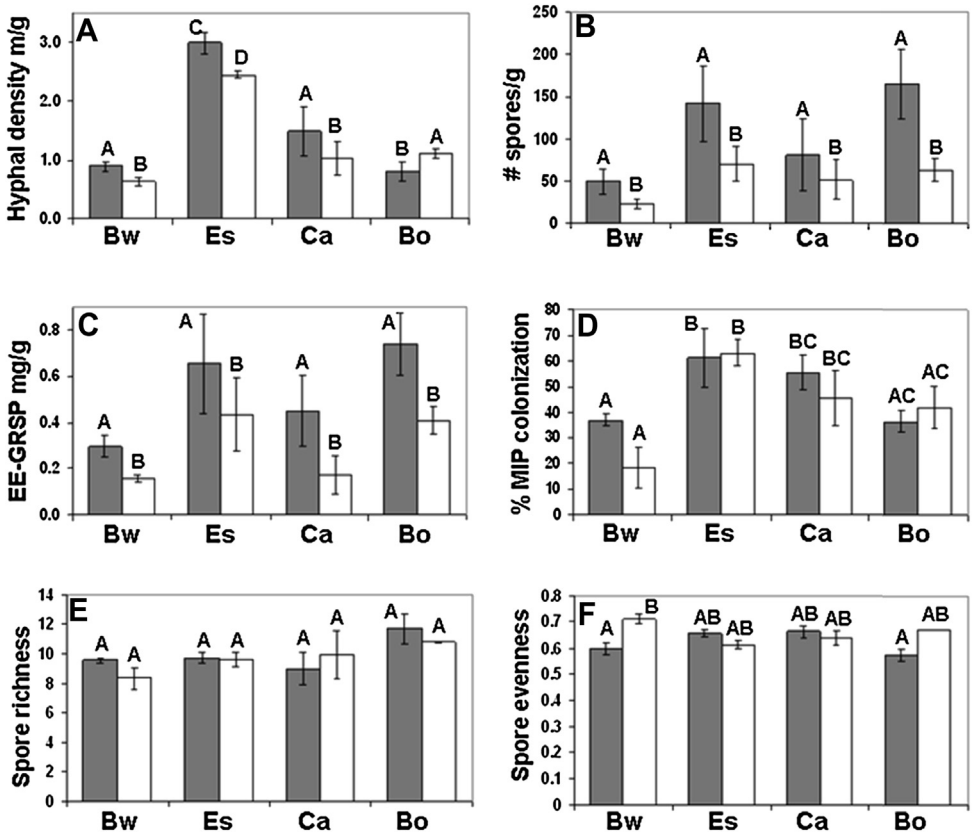

Fig 1 – Differences in AM fungal abundance and diversity among regions and microsites. Bw = Big Water, Es = Escalante, Ca = Cannonville, and Bo = Boulder. Shaded bars represent canopy and open bars represent interspaces. Letters indicate significant differences according to Tukey's HSD test and error bars represent standard errors.

such that certain sites had more species than others (Table 2). Spore species evenness did not differ among regions or sites, but there was a region by microsite interaction such that evenness was higher in interspaces than canopies only in the Big Water region (Fig 1F). In all other regions, there was no difference in spore evenness between shrub canopies and interspaces (Table 2).

NMS ordination and MRPP demonstrate that AM fungal spore communities were similar between canopy and interspace microsites (Fig 2; Table 2). Glomeromycotan spore communities differed among sites and also among regions, though the within group heterogeneity was higher for regions (Fig 3; Table 2). Our analyses included spore abundance and the same patterns were maintained when analyses were conducted using presence-absence data (results not shown). Pairwise comparisons revealed that the AM fungal communities of each region were distinct, with the greatest difference occurring between the Escalante and Big Water regions (Fig 3; $p < 0.0001$, $A = 0.102$). As shown by overlain vectors, the environmental variables that significantly ($p < 0.001$) explained AM fungal community variation were latitude ($r = 0.84$), soil P ($r = 0.61$), MAT ($r = 0.41$), elevation ($r = 0.39$), and MAP ($r = 0.16$).

Spatial autocorrelation of AM fungal spore communities was observed such that communities that were more similar to each other were also located closer to each other in space

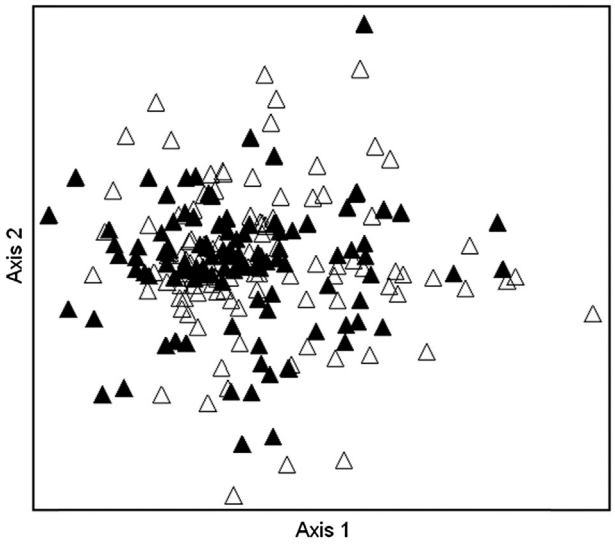

Fig 2 – NMDS ordination of AM fungal communities by microsite. Each point represents the spore community found within a single sample. Filled triangle = Canopy, Open triangle = Interspace.

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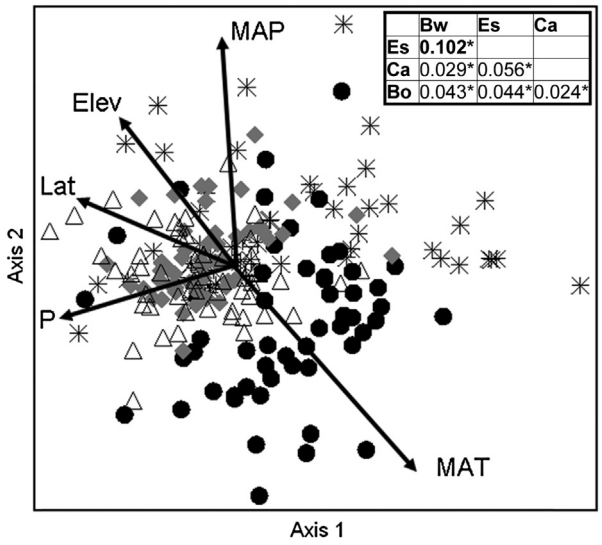

Fig 3 – NMDS ordination of AM fungal communities by region. Each point represents the spore community found within a single sample. Bw = circles, Es = diamonds, Ca = asterisks, Bo = triangles. Vectors indicate strongest environmental variables correlated with communities: mean annual precipitation (MAP; $r = 0.500$), soil phosphorus (P; $r = 0.461$), latitude (Lat; $r = 0.390$), mean annual temperature (MAT; $r = 0.388$) and elevation (Elev; $r = 0.348$). Figure inset shows MRPP A-values for pairwise comparisons between regions. * indicates $p < 0.0001$ and bold values indicate high within-group similarity ($A > 0.10$).

(Fig 4A; $p = 0.001$, Mantel $r = 0.486$). Furthermore, samples with similar spore communities also come from areas with similar climates (Fig 4B; $p = 0.001$, Mantel $r = 0.505$) but did not share similar soil properties (Fig 4C; $p = 0.179$, Mantel $r = 0.185$).

Species patterns

Several spore species were indicators of specific microsites, sites, and regions (Table 4). For example, *Archaeospora trappei*, *Diversispora spurca*, and *Pacispora* species 2 were significant indicators of shrub canopies because of their high relative abundance and frequency in these samples. There were no significant indicators for interspaces, likely because of the low relative abundance of spores in these samples. Thirteen species were significant indicators for both a single site within a region and the entire region, while 4 species were indicators for only a single site. The Boulder region had the most indicator species of all the regions. Of all Glomeromycota, most spore species were detected from the order Glomerales, including 1 *Claroideoglomus*,1 *Funneliformis*, 7 *Glomus*, 3 *Rhizophagus*, 1 *Septoglomus*, and 9 unknown Glomerales species (22 total). Spores from eight different species were observed within the family Gigasporaceae, including 1 *Gigaspora*, 2 *Scutellospora*, and 6 unknown Gigasporaceae spore species. The only *Gigaspora* species observed, *Gigaspora rosea*, was found in all regions except Escalante. *Rhizophagus irregularis* (formerly known as *Glomus intraradices*) was the most common spore species, present in 100 % of samples; the rarest spore species, *Gigasporaceae* species 1 and Glomerales species 8, were each only observed in single samples from shrub canopies of the Cannonville region.

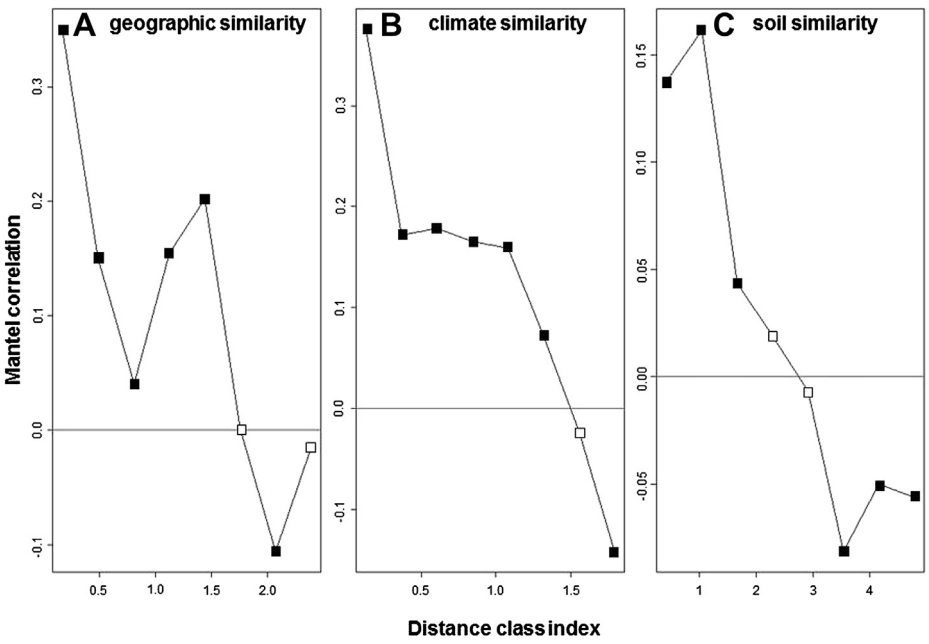

Fig 4 – Mantel correlograms for geographic (A), climate (B), and soil (C) similarity. Mantel r values are plotted on the y-axes (note different value ranges for each plot) as a function of geographic, climate, and soil distance class indices. Points that are statistically significant are black ($p < 0.05$).

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Table 4 – Species list with relative abundances (% of perfect indication) across microsites and regions

Species name	Microsite		Region			
	Canopy	Interspace	Bw	Es	Ca	Bo
Acaulosporaceae species 1	55	45	0	0	64[c]	36
Acaulosporaceae species 2	97	3	52	0	0	48
Acaulosporaceae species 3	90	10	1	0	1	98[c]
Acaulosporaceae species 4	43	57	1	11	10	78[c]
Archaeospora trappei	78[a]	22	18	22	17	44[c]
Claroideoglomus etunicatum	57	43	9	46[c]	15	30
Diversispora spurca	83[a]	17	9	31	21	39
Entrophospora infrequens	44	56	26	11	6	57[c]
Funneliformis mosseae	72	28	4	17	22	57
Gigaspora rosea	62	38	25	0	63	13
Glomerales species 1	57	43	0	89[c]	7	4
Glomerales species 2	42	58	35[b]	6	14	45[a]
Glomerales species 3	62	38	1	0	49[a]	50
Glomerales species 4	40	60	36	4	28	32
Glomerales species 5	96	4	0	4	0	96
Glomerales species 6	40	60	50	0	0	50
Glomerales species 7	50	50	100	0	0	0
Glomerales species 8	100	0	0	0	100	0
Glomerales species 9	61	39	73[c]	17	0	10
Glomus ambisporum	51	49	10	42[c]	22	25
Glomus deserticola	55	45	6	50[c]	15	29
Glomus hoi	45	55	9	0	45	45
Glomus macrocarpum	49	51	12	1	59[c]	28
Glomus microaggregatum	41	59	43	0	25	32
Glomus microcarpum	52	48	22	20	21[b]	36
Glomus tortuosum	36	64	14	0	14	71[b]
Gigasporaceae species 1	100	0	0	0	100	0
Gigasporaceae species 2	68	32	7	48	3	41
Gigasporaceae species 3	63	37	38	25	12	25
Gigasporaceae species 4	0	100	70	0	30	0
Gigasporaceae species 5	33	67	0	0	0	100[c]
Gigasporaceae species 6	0	100	50	0	0	50
Pacispora species 1	63	37	42[b]	0	16	42
Pacispora species 2	100[a]	0	50	12	25	13
Paraglomus occultum	52	48	23	22	30	26
Rhizophagus clarus	60	40	12	40[c]	16	31
Rhizophagus diaphanus	21	79	0	2	98[c]	0
Rhizophagus fasciculatus	34	66	11	8	41[b]	40
Rhizophagus intraradices	51	49	6	45[c]	25	24
Scutellospora calospora	71	29	0	0	0	100[a]
Scutellospora pellucida	16	84	84[c]	0	0	16
Septoglomus constrictum	53	47	8	31	23	39

a Indicates a significant indicator species ($p < 0.05$) for a particular group
b Indicates a significant indicator species for a particular site within a region
c Indicates a species was a significant indicator of both the region and a site within that region

Discussion

This study revealed that spatial patterns of AM fungal abundance and diversity vary with the indicator measured and with scale in semiarid shrublands. We hypothesized that Glomeromycota should be more abundant and diverse under perennial *Artemisia* shrubs compared to unvegetated spaces between shrubs (H_1). Surprisingly, although AM fungal hyphae, spores and EE-GRSP were more abundant under shrubs, we found no difference in the density of infective AM fungal propagules (MIP), spore species richness, and spore community structure between canopy and interspace microsites. It is notable that we sampled during the dry season of a record drought year (Cook et al., 2004); average plant cover in interspaces was only 5.6 % and no roots were observed in the interspace soil samples. Shrub canopies in arid ecosystems are often referred to as islands of fertility where nutrients and organic matter are enriched compared to the surroundings (Schlesinger et al., 1996). Indeed, we found soil organic matter, P and N to be higher underneath shrubs but there was no reduction in viable AM fungal inoculum in unvegetated interspaces more than a meter away from living shrubs. Furthermore, we observed the same number and identity of spore species in interspaces as under *Artemisia* canopies. Thus, our findings do not support the prediction of

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H_1 that unvegetated interspaces are relatively devoid of microbial life (Schlesinger et al., 1996); but rather that Glomeromycota are not restricted to life in the rhizosphere, and that diverse and viable AM fungal communities exist in unvegetated interspaces. This conclusion is supported by previous findings (Allen and MacMahon, 1985; Friese and Koske, 1991; Rillig et al., 2003) and suggests that hyphae could be exploring the soil matrix to mine nutrients and water from interspaces and deliver them to host shrubs. Additionally, it is possible that interspace hyphae are remnants of mycorrhizas from ephemeral annual plants that occupied interspaces in the past. Recent work indicating rapid hyphal turnover in semi-arid ecosystems (Hernandez and Allen, 2013), however, suggests that hyphae in canopies and interspaces are physiologically active. Furthermore, the best predictor of viable AM fungal propagule density (MIP) was hyphal density, suggesting that hyphal fragments are viable propagules, and important dispersal agents, even more so than spores, of AM fungi in arid ecosystems.

Our study revealed high morphological species richness, with 42 total spore species and an average of 10 species per sample. Compare these results to 37 spore species detected over several seasons and years in a mesic, albeit much smaller, area in North Carolina, USA (Bever et al., 2001). The diversity we found is particularly high considering we sampled at a single time point and from a single host genus; sampling other plant hosts or seasons would have likely revealed even more spore species. As spores represent the dormant stage of AM fungi, their production in natural systems varies seasonally (Bever et al., 2001) and likely impacted the number of species detected in our study. No studies of seasonal variation in spore production have been conducted in semi-arid environments with biannual precipitation patterns (i.e. wet winter with a late summer monsoon). We sampled during the summer dry season; it is possible that we did not capture certain AM fungal taxa because they preferentially sporulate during the summer monsoon, the dry autumn, the wet winter, or do not sporulate at all. Our results were similar, with respect to gamma diversity but not alpha diversity, to a large-scale survey of *Artemisia* shrublands in the western U.S.A where 48 spore species were found at 68 sites, with most sites containing less than 4 species (Allen et al., 1995). In a comparison of deserts in the U.S.A and Namibia, 21 AM fungal spore species were found across 16 sites (Stutz et al., 2000), while 47 total spore species with an average of 10 species per sample were detected within a 40 km^2 region in an arid savannah in southwestern China (Li et al., 2007). These studies and ours suggest that although AM fungal biomass is relatively low in arid systems, diversity is quite high.

Our results support H_2 and corroborate previous studies that have demonstrated spatial autocorrelation in AM fungal communities (Carvalho et al., 2003; Mummey and Rillig, 2008; Hazard et al., 2013). Across all sampling locations, we detected strong positive spatial autocorrelation, such that samples located closer to each other in space also had more similar AM fungal spore communities. Spatial autocorrelation of AM fungal communities was detected in 5 m × 5 m plots in Portugal but not in a 2 m × 2 m plot in the U.S.A (Carvalho et al., 2003; Wolfe et al., 2007). Our observation of spatial autocorrelation at site and regional scales, but not the microsite scale, indicate some degree of limitation for dispersal by Glomeromycota in our system. Certain AM fungal spores have demonstrated dispersal of up to 2 km in arid shrublands (Warner et al., 1987), but spores vary substantially in size, and dispersal capabilities likely depend on environmental conditions, interactions with animal vectors, and the particular AM fungal species in question (Chaudhary et al., 2008). Our results indicate that AM fungal dispersal within the range of 1 m is more common than at ranges of hectares or thousands of hectares. For certain microbes it has been argued that dispersal is essentially unlimited, contributing to the "everything is everywhere" hypothesis in microbial biogeography (reviewed in Martiny et al., 2006); our data corroborate those of Öpik et al., 2010 and suggest that AM fungal dispersal, at least for certain species, is not global in nature.

Our results also support H_3; soil properties and climate variables help explain variation in AM fungal distributions across multiple scales of observation. Hyphal density, relative amount of infective propagules (MIP), and spore community composition varied across regions and the four regions differed considerably with respect to soil parent material, texture, and chemistry, as well as elevation and climate (Table 1). The Big Water region is at the lowest elevation, nearly 800 m lower than the highest region; it is also the hottest and driest of the regions. The Boulder region is at the highest elevation, while the Cannonville region received the most precipitation and the Escalante region had the coolest average temperature. Although the influences of soil and climate cannot be uncoupled in this study, the stark environmental differences between regions can provide some insight into why AM fungal abundance and community composition varies among regions. Hyphal density and also the relative amount of infective propagules were highest in the Escalante region. It is noteworthy that the organic matter content of soils in the Escalante region was more than four times higher than all other regions and organic matter was the most important predictor of hyphal density (Table 3). Previous work has demonstrated a positive relationship between Glomeromycota and soil organic matter and that the addition of organic matter to soils stimulates AM fungal hyphal production (St. John et al., 1983; Johnson, 1998; but see Hu et al., 2013).

Although species richness did not vary among regions, spore community composition was distinct among all regions, with climate and soil properties contributing to regional Glomeromycota patterns. The biggest pairwise difference in AM fungal communities was between the Escalante and Big Water regions. Host species identity may have contributed to this pattern as *A. filifolia* was sampled in Big Water, while *A. tridentata* was sampled in Escalante. However, other regions where *A. tridentata* was the dominant shrub had AM fungal communities that were more similar to that of Big Water suggesting that, across large spatial scales, soil properties and climate contribute more to differences in Glomeromycotan community structure than plant host identity. MAT was highest in the Big Water region and lowest in the Escalante region, and the two regions differed in MAP by nearly 10 cm. The hypothesis that climate is a factor that shapes AM fungal communities in our study is corroborated by the strong correlation between AM fungal community dissimilarity and

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climate dissimilarity of samples. Previous studies have demonstrated that both temperature and precipitation, which are coupled with elevation and latitude, can influence AM fungal community composition (Egerton-Warburton et al., 2007; Lovelock et al., 2003).

In support of H_4, several indicator species of particular microsites, sites, and regions were observed, contributing knowledge to the autecology of AM fungal species. Across our study, *A. trappei* and *D. spurca* were common but their relative abundance and relative frequency was greater underneath shrub canopies compared to interspaces. It is possible that nutrient or microclimate conditions are driving this pattern; *A. trappei* spores were more prevalent in the wettest soils along a hydrologic gradient (Miller and Bever, 1999) but occurrence of *D. spurca* has been reported in Arizona, Texas, South Carolina, Hawaii, Cuba and Namibia from a range of different environments and vegetation types (Kennedy et al., 1999; Stutz et al., 2000). At the regional scale, Gigasporaceae species 5, *S. pellucida* and *S. calospora* were significant indicators of Big Water and Boulder, two regions with sandy soils. These results corroborate previous studies that have found members of the Gigasporaceae family, which includes *Scutellospora*, to be more prevalent in sandy soils (Koske and Tews, 1987; Lekberg et al., 2007). We observed many undescribed Gigasporaceae spore species in our study; this corroborates findings in Venezuela (Cuenca and Lovera, 2010) and suggests that *Scutellospora* may be an important genus in hot shrublands. Distributional patterns for many of the AM fungal species detected in our study have not been reported previously and our findings add to a growing body of literature on AM fungal biogeography in natural systems (Öpik et al., 2013; Turrini and Giovannetti, 2012).

Finally, most studies examine only one or two metrics of AM fungi. We demonstrate that different measures of AM fungal abundance and diversity, which have different biological functions for the organism, do not vary spatially in the same manner. Furthermore, AM fungal communities vary more at large spatial scales than small spatial scales. An improved understanding of how the abundance and diversity of Glomeromycotan fungi vary across spatial scales will improve ecosystem-scale predictions and extrapolations. Due to lack of data, small scale measurements have been used to make biome-level predictions about AM fungal biomass (Treseder and Cross, 2006), but assuming that different metrics of Glomeromycotan abundance and diversity vary equally across all scales could lead to spurious predictions. A better understanding of the range in variability of different AM fungal structures across multiple scales will enhance our ability to predict distributions, aiding efforts to conserve the biodiversity and ecosystem functions of this important group of fungi.

Acknowledgments

This research was funded by the Cooperative Ecosystems Studies Program of the Bureau of Land Management (JSA990018), the National Science Foundation (grants DGE-0549505, DGE-0742483, and DEB-0842327) and the ARCS Foundation. Laboratory and field assistance was provided by A. Redman, T. Baker, M. Halldorson, T. Irving, and R. Rieder. We are grateful to C. Gehring and two anonymous reviewers for valuable comments on the manuscript.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funeco.2014.06.003.

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Please cite this article in press as: Chaudhary VB, et al., Multiscale patterns of arbuscular mycorrhizal fungal abundance and diversity in semiarid shrublands, Fungal Ecology (2014), http://dx.doi.org/10.1016/j.funeco.2014.06.003