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Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities

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Summary

1 Metacommunity and neutral theory have reinvigorated the study of 'niches' and have emphasized the importance of understanding the influences of competition, abiotic factors and regional spatial processes in shaping communities.
2 We conducted a field survey to examine the effects of soil characteristics and distance on arbuscular mycorrhizal (AM) fungal communities of maize (*Zea mays*) in sand and clay soils. To address whether the field distributions of AM fungal species represented their fundamental or realized niches, we grew representative species of the two dominant genera, *Glomus* and *Gigaspora*, alone or together on *Sorghum bicolor* plants in sand, clay or a sand/clay mixture in the glasshouse.
3 In the field, soil characteristics and spatial structure accounted for significant proportions of the variation in community composition among sites, suggesting that both environmental variables and dispersal were important factors shaping AM fungal communities.
4 AM fungi in the family Glomeraceae occurred predominately in clay soils, whereas AM fungi in the family Gigasporaceae dominated in sand soils. Niche space of Glomeraceae was further partitioned by levels of soil organic carbon and nitrogen.
5 In the glasshouse, root colonization by *Glomus* was high in all three soils when grown in the absence of *Gigaspora*, indicating a broad fundamental niche. Root colonization by *Gigaspora* was negatively correlated with percentage clay when grown in the absence of *Glomus*, consistent with the low abundance of this family in clay soils in the field. When grown together, spore production of both *Glomus* and *Gigaspora* was significantly reduced only in the sand soil, indicating that competition could limit niches of both families in certain soil environments.
6 Our results suggest that AM fungal distributions are the product of environment, interspecific competition and regional spatial dynamics, emphasizing the importance of using a metacommunity perspective in community ecology.

Key-words: arbuscular mycorrhizal fungi, community composition, dispersal, fundamental and realized niche, internal transcribed spacer, metacommunity, soil characteristics, competition, T-RFLP, *Zea mays*.

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Introduction

A central goal of ecology is to understand the underlying mechanisms that generate differences in species composition among communities. The classical paradigm for explaining diversity and coexistence is based on niche theory. This theory is founded on two

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fundamental concepts. The first is the competitive exclusion principle, which essentially states that no two species with identical niches can coexist indefinitely (Gause 1934). The second is that species may differ in a potentially unlimited numbers of niche dimensions (Hutchinson 1957).

Neutral theory, in contrast to niche theory, initially assumes that trophically similar species have functionally equivalent vital rates (i.e. birth, death, dispersal and spec0iation rates) and that their distributions are predominantly influenced by spatial processes rather than species–environment relationships (Hubbell 2001). Proponents of neutral theory have criticized traditional niche theory for its lack of dispersal limitations (Hubbell 2001). Consequently, a more synthetic view that incorporates metacommunities, or local communities linked by the dispersal of multiple species, has been encouraged (Leibold *et al*. 2004; Chase 2005). Here we integrate classical niche theory and spatial processes under the metacommunity framework by examining the roles of abiotic factors, competition and spatial structure (a surrogate for dispersal; *sensu* Chase 2005 and Cottenie 2005) in determining the distribution of arbuscular mycorrhizal (AM) fungi, a ubiquitous but understudied terrestrial taxon.

AM fungi are common components of most natural and agricultural ecosystems. The fungi are members of the phylum Glomeromycota, and many species appear to have worldwide distributions (Morton *et al*. 1995). AM fungi colonize roots and form a symbiosis, arbuscular mycorrhiza, with most terrestrial plant families (Smith & Read 1997). This symbiosis entails the exchange of materials between the host plant and fungus, with the fungus receiving carbon and the host plant receiving phosphorus and other nutrients of poor mobility in soils (Fitter 2005). AM fungal species were considered to be more or less functionally equivalent, but recent studies have indicated that AM fungal community composition may influence plant species composition and productivity (van der Heijden *et al*. 1998; Bever *et al*. 2002; Hart & Klironomos 2002; Helgason *et al*. 2002; Stampe & Daehler 2003), as well as ecosystem properties such as soil aggregation and carbon and nitrogen storage (Miller & Jastrow 2000). AM fungal communities, in turn, have been shown to vary with plant community (Helgason *et al*. 1998; Öpik *et al*. 2003), season (Daniell *et al*. 2001; Klironomos *et al*. 2001; Pringle & Bever 2002) and soil type (Johnson *et al*. 1992; Landis *et al*. 2004). However, the mechanisms generating differences among AM fungal communities are largely unknown (but see Klironomos *et al*. 2001).

The objectives of this study were to elucidate the relationships among soil characteristics, spatial structure and AM fungal community composition. We used a molecular identification technique to survey AM fungal communities in maize (*Zea mays* L.) roots from sand and clay soils in a subsistence farming region of south-west Zimbabwe. Spatial structure was used as a surrogate for dispersal because AM fungi have been shown to be dispersed by wind (Warner *et al*. 1987) and animals (Mangan & Adler 2000), both of which are distance-dependent processes. Following the field survey, a glasshouse experiment was designed to address whether the effects of soil characteristics on the field distributions of AM fungi were due to disparities among AM fungi species in their tolerances to abiotic factors alone (fundamental niche) or were also mediated by competition (realized niche).

Materials and methods

DESCRIPTION OF FIELD SITES AND SAMPLING

Ten fields, five on sand and five on black clay, were selected within the Tsholotsho communal area (27°49.73′E, 19°51.07′S) of Zimbabwe. The climate is semi-arid, with a mean annual rainfall of 540 mm concentrated in the growing season from November to April. Average yearly maximum and minimum air temperatures are 26 and 11 °C, respectively (ICRISAT, unpublished data). All fields had been converted during the past 10–15 years to agriculture from native bush vegetation (based on interviews with farmers). Since then, common subsistence crops such as maize, sorghum (*Sorghum bicolor* L.), groundnut (*Arachis hypogaea* L.) and cowpea [*Vigna unguiculata* (L.) Walp.] have been cultivated on all fields. Maize was grown the previous year without any fertilizer on each experimental plot. All 10 fields were located within 25 km of each other (Fig. 1). Soil samples were taken randomly at a depth of 0–15 cm from five locations per field on 5 December 2002 and pooled by field. Soil characteristics were analysed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Zimbabwe and are presented in Table 1.

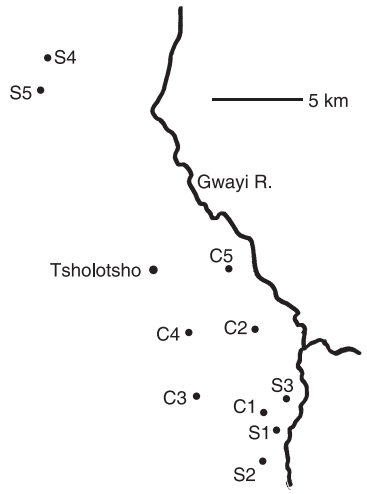

Fig. 1 Map of Tsholotsho communal area in Zimbabwe indicating locations of the 10 study fields. C, clay soil; S, sand soil.

Table 1 Mean (SE) of selected soil characteristics of the two soils included in the field experiment. *P*-values were calculated based on a two-sample *t*-test, $n = 5$ (significant values in bold type)

	Sand soil	Clay soil	*P*
Texture (%)			
Clay	4.8 (0.9)	48.4 (3.3)	**< 0.001**
Silt	8.2 (1.3)	14.7 (1.4)	**0.011**
Sand	87.0 (1.9)	36.9 (4.1)	**< 0.001**
Moisture (%)*	4.4 (0.5)	15.1 (1.2)	**< 0.001**
Organic C (g C kg^{-1} soil)	3.9 (0.5)	9.0 (0.8)	**< 0.001**
pH ($CaCl_2$)	5.2 (0.6)	7.1 (0.2)	0.053
Olsen P (mg P kg^{-1} soil)	6.2 (3.0)	1.3 (0.3)	0.093†
Total P (g P kg^{-1} soil)	0.37 (0.1)	0.63 (0.1)	0.067†
N available (mg NO_3^- kg^{-1} soil)	3.2 (1.2)	5.0 (1.5)	0.380
Total N (g N kg^{-1} soil)	0.26 (0.04)	0.61 (0.1)	**0.016**

*These values were determined on the same day.
†Data were log-transformed.

Maize seeds (SC 401, a common short-duration variety sold in Zimbabwe) were planted on a 0.5-ha ploughed section of each field between 7 and 19 December 2002. Twenty randomly selected maize plants per field were harvested 7 weeks after planting, allowing time for any slow colonizing AM fungal species to be detected in the roots (Hart & Reader 2002). Root subsamples from each plant were washed, blotted dry, further dried with a desiccant (Drierite, W.A. Hammond Drierite Company Ltd, Xenia, OH, USA), and shipped to The Pennsylvania State University, Pennsylvania, USA, where they were stored at –20 °C. Shoots and remaining roots were dried at 65 °C to constant mass, and root colonization by AM fungi was assessed by the line intersect method (Koide & Mooney 1987) after staining with trypan blue.

T-RFLP ANALYSIS AND SPECIES IDENTIFICATION

We characterized the AM fungal community in maize roots in each field using terminal restriction fragment length polymorphisms (T-RFLP) of the internal transcribed spacer (ITS) region within the ribosomal gene complex. DNA was extracted from a single, 3-cm-long, root segment per plant using the Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA, USA) according to Koide *et al*. (2005), and amplified using a nested polymerase chain reaction (PCR) protocol (Renker *et al*. 2003). Our PCR mixture was according to Koide *et al*. (2005), with the primer set for the second PCR (PCR2) labelled with fluorescent tags. DNA was purified following PCR2 using a commercially available kit (UltraClean™ PCR Cleanup Kit, Mo Bio).

The PCR2 product was digested with the restriction enzyme *Hinf*I (New England Biolabs, Beverly, MA, USA) as in Koide *et al*. (2005). GeneScan analysis was performed on a nine-fold dilution of *Hinf*I digestion fragments and on an 18-fold dilution of the PCR2 product according to Koide *et al*. (2005). To compensate for differences in DNA concentration across samples, the GeneScan output was standardized so that the tallest peak in each sample had a height of 1000; no peak with a height of < 50 after standardization was recorded. T-RFLP analyses were also performed on spores that had been isolated and identified from all 10 fields using trap culture techniques (Morton *et al*. 2004). The PCR protocol for spores was the same as for roots, except that DNA was extracted by crushing the spore in the PCR tube immediately prior to the first PCR.

Each T-RFLP profile comprised three data points: the length of the PCR2 product and the lengths of the two terminal restriction fragments (T-RFs) from the PCR product. A profile from a root sample was considered to match a spore profile if corresponding lengths of the PCR2 product and T-RFs were within 2 bp. This criterion was based on the precision of the ABI sequence analyser (our personal observations). Each known T-RFLP profile is hereafter referred to as a ribotype.

Profiles that did not match those of spores were compared with ribotypes in two other databases: (i) T-RFLP profiles from spores collected in Costa Rica, and (ii) virtual profiles determined for 435 ITS sequences from spores of 37 species published in GenBank (Aldrich-Wolfe, in press). Frequently encountered T-RFLP profiles that did not match ribotypes in our three databases (Zimbabwe spores, Costa Rican spores and spore virtual database from GenBank) were cloned and sequenced at the Nucleic Acid Facility at The Pennsylvania State University. PCR2 products were purified from agarose gels, ligated into the plasmid pGEM®-T Easy (Promega, Madison, WI, USA) and electroporated into *Escherichia coli* DH10. Transformants were amplified directly from colonies using the PCR protocol described previously. One transformant per sample (verified as producing the correct profile) was sequenced and matched to sequences previously reported using the BLAST function in GenBank (Altschul *et al*. 1997). Our sequences have been submitted to GenBank under accession numbers DQ294944–DQ294955. The PCR2 products from roots were also sequenced directly without the cloning step in a limited number of cases. The names of our ribotypes, their respective T-RFLP

profiles and their matches with spores or GenBank sequences are listed in Supplementary Appendix S1.

Several ribotypes matched *Glomus intraradices* sequences from GenBank. *Gl. intraradices* strains have been documented to contain considerable genetic diversity in the ITS region (Jansa *et al*. 2002), which could be one potential explanation for this result. In other cases, ribotypes from morphologically different spores were within 2 bp of each other and therefore these were grouped within a single ribotype according to the criteria we set. It is possible that *Hinf*I was not sufficient to detect sequence variability among species. Consequently, our various ribotypes may span taxonomic levels and comprise isolates, single species or species groups. We did not consider use of our ribotype designations to be a major limitation in our study for two main reasons: (i) the species concept in this seemingly asexual organism may be of questionable relevance given the considerable genetic and ecological variability that has been observed within a species (Munkvold *et al*. 2004; Koch *et al*. 2006); and (ii) our main objective was to compare differences in communities between two soil types. Any shortcomings of our ribotype designations would apply to both soil types.

DNA was successfully amplified from all root samples, with a majority of the PCR2 product lengths within a range (500–620 bp) typical for AM fungi (Aldrich-Wolfe, in press). Of all T-RFLP profiles, 62% matched AM fungal ribotypes, 13% matched non-AM fungal ribotypes and 25% could not be placed in either group with confidence. The great majority of the unmatched profiles (92%) were detected only once, and the remaining 8% (comprising three profiles) occurred between two and four times over several sites. The PCR2 product lengths of unmatched profiles did not differ between the two soil types ($F_{1,143} = 1.86$, $P = 0.18$). Because PCR product length alone appears to be a good indicator of AM fungal family (Appendix S1), our inability to identify all profiles to ribotypes probably did not affect our comparisons between soil types. All unmatched profiles and non-AM fungi were removed prior to subsequent analyses. We considered only members of the Gigasporaceae and Glomeraceae because AM fungi of other families were rarely detected.

GLASSHOUSE NICHE SPACE EXPERIMENT

Seeds of *Sorghum bicolor* L. were planted in 164-mL Cone-tainer tubes (Stuewe and Sons Inc., Corvallis, OR, USA) on 14 February 2004. Sorghum was chosen as a host plant for the glasshouse experiment as in our experience it is better suited for growth in Cone-tainers than maize. Each tube contained either sand (3 : 7 : 90 of clay, silt and sand; pH = 6.0), clay (41 : 11 : 48 of clay, silt and sand; pH = 7.8), or a sand/clay mixture (4 : 1, v/v) from one randomly selected clay and sand field from Tsholotsho. The sand/clay mixture resulted in a textural combination of the two soil types (18 : 9 : 73 of clay, silt and sand), with a pH more similar to that of the clay soil (7.6). All three soils were autoclaved for 2 h on two consecutive days before use in the experiment. Based on the family-level differences in the T-RFLP analyses, we inoculated all three soils with either 2.5 mL of a Glomeraceae inoculum (*Gl. etunicatum* and *Gl. mosseae*, originally isolated from trap cultures of the sand and clay soils in Tsholotsho) or 17.5 mL of a Gigasporaceae inoculum (*Gi. gigantea* and *Gi. rosea*, isolated from trap cultures of the sand soil in Tsholotsho), or a mixture of both inocula (2.5 mL Glomeraceae and 17.5 mL Gigasporaceae inocula). The difference in inoculum volume resulted from differences in spore densities and ensured that approximately 400 spores from each inoculum type were inoculated into each Cone-tainer. Sand was added to each inoculum for Cone-tainers receiving a single inoculum type to bring the total volume added to 20 mL for each Cone-tainer. The AM fungal species were chosen based on their field distributions and ability to sporulate under glasshouse conditions.

Each treatment was replicated 20 times. Half of the replicates were harvested for shoot dry weight and mycorrhizal colonization at 54 days, and half were harvested for spore production at 89 days. However, as a result of poor seed germination in some tubes, the number of replicates harvested varied from 8 to 10. Plants were thinned to a single individual after emergence and grown at 21 °C (± 3 °C) air temperature with 100 μmol m^{-2} s^{-1} additional photosynthetically active radiation from supplemental lighting for 16 h per day. Plants were fertilized with 20 mL of a quarter-strength Hoagland solution (Machlis & Torrey 1956) containing 1.5 mg P L^{-1} at weekly intervals for 7 weeks and twice weekly thereafter. Cone-tainers were arranged randomly with respect to treatment on the glasshouse bench. At harvest, shoots were cut at the soil surface and dried at 65 °C to constant mass. Root colonization by AM fungi was assessed as before and spores were counted under an 80× dissecting microscope following wet sieving (Brundrett *et al*. 1996).

STATISTICAL ANALYSES

To compare the occurrences of Glomeraceae and Gigasporaceae in the two soil types in the field, we counted the number of root samples in the five sand fields and the five clay fields for which we detected members of the two families and conducted a chi-squared test of independence with a Yates correction (Zar 1999). A second chi-squared test of independence was conducted to determine if ribotype distribution within a family differed among the five fields in either soil type (Zar 1999). We did not analyse Glomeraceae ribotype distribution in sand or Gigasporaceae ribotype distribution in clay, because their relative frequencies were too low.

To determine the relationship between environmental variables and ribotypes, we first conducted a principal component analysis (PCA), which sequentially

identifies hypothetical environmental gradients that account for the greatest remaining variation in taxa dispersion among samples. Only ribotypes with an incidence ≥ 3 was used in these analyses because a majority of T-RFLP profiles with an incidence of < 3 occurred only once and could not be matched with known ribotypes. We then used redundancy analysis (RDA), which constrains the taxa and samples to axes determined by supplied environmental variables (in this case, soil variable; ter Braak & Prentice 1988). PCA and RDA were selected because the average ribotype response to the hypothetical gradient was linear (2.15 standard deviations) rather than unimodal, and because they allow for straightforward variance partitioning, unlike non-metric multidimensional scaling (NMDS). Results generated from NMDS (conducted using Sørensen distances in PC-ORD v. 5.01; McCune & Mefford 1999) were consistent with those generated from the PCA and RDA and therefore are not presented.

The eight soil variables measured (Table 1) were passively projected *post-hoc* onto the PCA diagram, whereas the RDA was constrained to environmental variables identified as significant by Monte Carlo permutations under the full model ($P < 0.05$; using a forward stepwise selection procedure with 999 iterations). Ordination analyses and hypothesis testing were conducted using CANOCO 4.5 (ter Braak & Šmilauer 2002) with log-transformed ribotype data and a focus on interspecies correlations. Biplots were created using CanoDraw 4.12 to display ordination results (ter Braak & Šmilauer 2002). The ribotype scores were post-transformed so that correlations of the ribotypes and soil variables with the ordination axes could be inferred by perpendicular projection.

We conducted a one-tailed Mantel's test to determine whether there was a relationship between distance among sites (based on Euclidean distance) and the similarity of their ribotypes (based on a Sørensen index) and soil characteristics (based on the relative Euclidean index because soil characteristics were measured in different units). The *a priori* hypothesis was that both ribotype and soil dissimilarity would increase with distance.

We used the methods of Borcard *et al.* (1992) to determine how much of the ribotype variation was accounted for by (i) spatial variables alone, (ii) soil variables alone and (iii) how much was shared by the two. Spatial predictors were the nine terms of a cubic trend-surface equation based on the *x*- and *y*-coordinates of each site (Borcard *et al.* 1992). This variance partitioning procedure extracts these three variance components and residual variation by first identifying minimal subsets of environmental and spatial predictors (using stepwise forward selection), and then using RDA, partial RDA and subtraction to calculate the components. These analyses were conducted on Hellinger-transformed data (see Legendre & Gallagher 2001), and probability values were calculated using Monte Carlo tests (999 iterations).

Finally, mycorrhizal colonization, spore numbers and shoot dry mass from the glasshouse experiment were analysed separately using the general linear model in Minitab Release 11 (Minitab Inc., State College, PA, USA) with soil type, fungal treatment (grown with a member of the same family only, or with members of the other family) and AM fungal species as factors. As the spores of our four AM fungal species differ in size, we also analysed spore production based on spore biovolumes using average spore diameters for each species given on the INVAM website (http://invam.caf.wvu.edu). Results of these analyses were similar to those based on spore numbers and therefore are not presented.

Transformations were performed, when necessary, to fulfil the underlying assumptions of normality and equal variance. Two means were compared using a two-sample *t*-test, and three or more means were compared using Tukey's HSD at $\alpha = 0.05$.

Results

AM FUNGAL COMMUNITY STRUCTURE IN SAND AND CLAY FIELDS

Seven weeks after planting, maize seedlings were highly colonized in both soil types, averaging 52 ± 5.2% (± SE) in the clay soil and 63 ± 7.6% in the sand soil, and there was no significant difference in shoot dry weight between the two soil types ($F_{1,9} = 0.05$, $P = 0.83$).

Gigasporaceae AM fungi were detected more frequently in root samples from sand fields (73 in sand vs. 18 in clay out of 100 in total), whereas Glomeraceae AM fungi occurred more frequently in root samples from clay fields (87 in clay vs. 33 in sand out of 100 in total). This resulted in a significant soil type × AM fungal family interaction ($\chi^2 = 55.5$, $P < 0.001$) and a negative correlation between Gigasporaceae and Glomeraceae abundances across all fields ($n = 10$, $r = -0.87$, $P = 0.001$). This pattern was reflected in the PCA analysis (Fig. 2). Members of the Gigasporaceae were found predominantly in the more acidic, sandy soils with low moisture and organic content (left side of Fig. 2) while members of the Glomeraceae were more abundant in neutral to alkaline clay soils with high moisture and organic content (right side of Fig. 2). In the RDA, percentage clay accounted for the greatest variation in ribotype dispersion (Monte Carlo simulation, $P = 0.001$, but note the high correlation among most soil variables in Fig. 2). Organic carbon content was the only remaining significant environmental variable (Monte Carlo simulation, $P = 0.019$) after controlling for percentage clay. The RDA constrained to these two environmental variables accounted for 42.8% of the ribotype variation (eigenvalues for first and second axes are 0.266 and 0.163). Consistency between the PCA and RDA suggests that important environmental variables were quantified.

Compared with sand fields, clay fields had more than twice the variation in organic carbon and more than

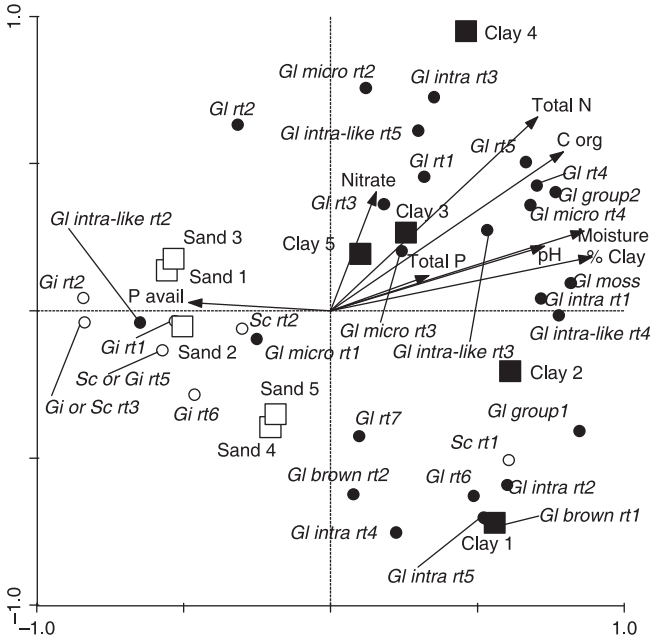

Fig. 2 Correlation biplot based on principal component analysis of AM fungal ribotype distribution in 10 fields. Percentage clay, moisture, organic carbon, nitrate, total nitrogen, pH, available phosphorus and total phosphorus (arrows) were passively (posthoc) projected into the ordination space. The biplot displays ribotypes in the Gigasporaceae (○) and Glomeraceae (●), and sandy (□) and clay fields (■). The distance of ribotypes and soil variables from the origin indicates their relative importance in the biplot. Perpendicularly projecting the soil variables to the axes provides an estimate of the correlation coefficient of that variable with that axis. The angle between soil variables is proportional to the correlation of those variables. Distance among samples approximates the dissimilarity of their taxa. The first and second axes account for 30.3% and 20.2% of the variance in ribotype frequencies, respectively. The eigenvalues for the first three canonical axes are 0.303 (*x*-axis), 0.203 (*y*-axis) and 0.138 (not displayed). Fields were multiplied by 0.44 to fit in the coordinate system.

four times the variation in total nitrogen (ribotypes within Glomeraceae were considerably more dispersed along the second PCA axis compared with those within Gigasporaceae). This may indicate an interaction between soil type and organic carbon and/or total nitrogen, so we re-computed the RDA to include all two-way interactions with percentage clay. The interaction between percentage clay and total nitrogen and between percentage clay and organic carbon had the largest marginal effects and were the only two significant interactions in the model (% clay × total nitrogen: $P = 0.001$; % clay × organic carbon: $P = 0.001$ when excluding the clay × total nitrogen interaction). This suggests that while percentage clay appeared to separate the niches of the two families, organic carbon and/or total nitrogen were important factors partitioning niches within the Glomeraceae.

Our spatial analyses revealed that distance among sites was positively correlated with AM fungal community dissimilarity (clay: standardized Mantel $r = 0.68$, $P = 0.021$; sand: standardized Mantel $r = 0.64$, $P = 0.039$), but was not correlated with soil dissimilarity (clay: standardized Mantel $r = -0.01$, $P = 0.482$; sand: standardized Mantel $r = -0.23$, $P = 0.252$). Variance partitioning showed that the environmental and geographical variables were not confounded (shared variation was minimal; Table 2). Soil characteristics alone and spatial structure alone both accounted for significant proportions of the explained variation in ribotype dispersion (Table 2). Ribotypes within Glomeraceae were not evenly distributed among the five clay fields and ribotypes within Gigasporaceae were not evenly distributed among the five sand fields (Fig. 3a,b), resulting in a significant ribotype × field interaction for both clay ($\chi^2 = 66.5$, $P < 0.001$) and sand ($\chi^2 = 48.9$, $P < 0.001$).

Table 2 Quantifying the influences of environmental variables and spatial structure on the dispersion of arbuscular mycorrhizal fungal ribotypes by multivariate variance partitioning. Significant *P*-values in bold type

Source*	Variation (%)	*P*
Soil alone	38.6	**0.002**
Shared	2.0	–
Space alone	23.5	**0.034**
Total	64.1	**0.001**
Residual	35.9	–

*Equal numbers of environmental and geographical variables were used.

NICHE SPACE EXPERIMENT IN GLASSHOUSE

In the absence of interfamilial competition, there was a significant AM fungal family × soil type interaction for both colonization ($F_{2,48} = 20.67$, $P < 0.001$) and shoot dry weight ($F_{2,48} = 15.30$, $P < 0.001$). *Gigaspora* colonization and shoot dry weight of *Gigaspora*-inoculated

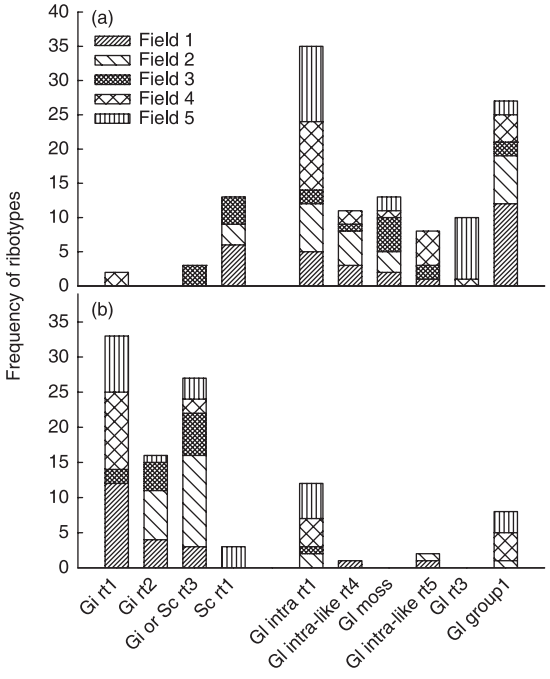

Fig. 3 Distribution of the 10 most common ribotypes in five clay fields (a) and five sand fields (b), where the field number refers to the replicate field within each soil type. The ribotype abbreviations are listed in Appendix S1.

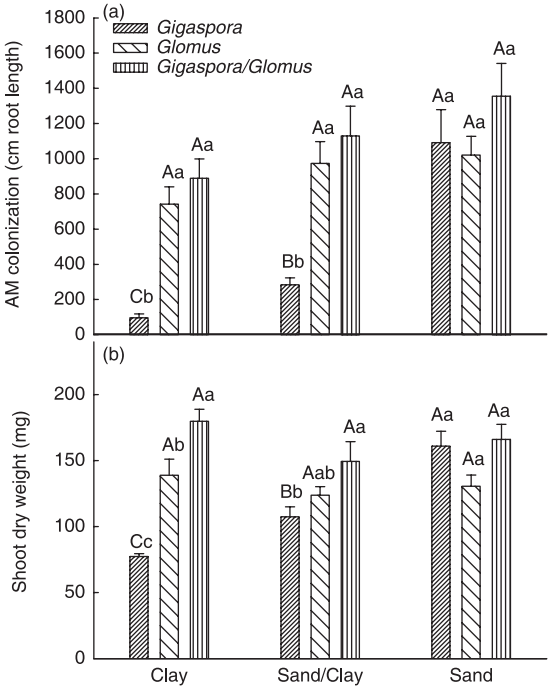

Fig. 4 Means (+ 1 SE) of mycorrhizal colonization (a) and shoot dry weight (b) of sorghum inoculated with either *Gi. rosea* and *Gi. gigantea* (*Gigaspora*), *Gl. etunicatum* and *Gl. mosseae* (*Glomus*), or all four species (*Gigaspora*/*Glomus*), and grown in pots containing either clay, sand/clay or sand for 56 days. Different upper-case letters indicate differences within AM fungal treatment among soil types, and different lower-case letters indicate differences within soil type among AM fungal treatments using Tukey's HSD at $\alpha = 0.05$.

plants were negatively correlated with percentage clay, while *Glomus* colonization and shoot dry weight were not influenced by soil clay content (Fig. 4a,b). Sporulation by the two *Gigaspora* species and the two *Glomus* species was significantly higher in sand soil than in the clay soil (Table 3). Furthermore, vesicle formation in *Glomus*-inoculated plants (only measured in *Glomus*, as Gigasporaceae species do not form vesicles) was affected by soil type ($F_{2,49} = 3.64$, $P = 0.033$) and was negatively correlated with percentage clay (data not shown), which could indicate that less carbon was allocated below-ground to *Glomus* in soils with higher clay content.

In the presence of interfamilial competition (realized niche), there was a significant AM fungal family × soil type interaction ($F_{4,73} = 2.89$, $P = 0.028$) with less than additive colonization levels in the sand (Fig. 4a), suggesting that competition existed among members of the two families in this soil. Spore production offered additional insight into the nature of this competition. We found that spore numbers were drastically reduced for all four species in the dual family inoculated treatments relative to the single family inoculated treatments in the sand, but not in the sand/clay and clay (Table 3). This resulted in a significant AM fungal treatment (grown alone or together with members of the other family) × soil type interaction for the two *Glomus* species ($F_{2,105} = 7.72$, $P = 0.001$, Table 3), as well as the two *Gigaspora* species ($F_{2,102} = 9.22$, $P < 0.001$, Table 3). Furthermore, there was a significant AM fungal species × soil type interaction ($F_{2,105} = 10.76$, $P < 0.001$) for the two *Glomus* species resulting from the greater reduction of *Gl. etunicatum* spores with increasing clay content compared with *Gl. mosseae*. There was also a significant AM fungal species × AM fungal treatment interaction for the *Gigaspora* species ($F_{1,102} = 4.86$, $P = 0.030$). *Gigaspora rosea* appeared to be more affected by competition than *Gi. gigantea* ($F_{2,51} = 9.47$, $P < 0.001$, Table 3). The percentage vesicular colonization, which may be a reflection of the carbon allocated to the *Glomus* species, was reduced significantly ($P = 0.032$) in the dual family inoculated treatment relative to the single family inoculated treatment only in the sand.

Discussion

DIFFERENCES IN AM FUNGAL COMMUNITY STRUCTURE IN CLAY AND SAND SOILS

AM fungal community composition differed greatly between soil types in the field, with Gigasporaceae predominating in sand soil and Glomeraceae predominating in clay soil (Figs 2 & 4). Because these soil types co-occur within an otherwise fairly homogeneous area, potentially confounding factors such as climate, host plant (Schenck & Kinloch 1980; Johnson *et al*. 1992; Bever *et al*. 1996), management practices (Boddington & Dodd 2000; Jansa *et al*. 2002, 2003; Oehl *et al*. 2004) and season (Daniell *et al*. 2001; Klironomos *et al*. 2001; Pringle & Bever 2002) were minimized. Previous

Table 3 Mean spore abundance (± 1 SE) in 2 mL of soil after colonization of sorghum in the absence of interfamilial competition ('*Glomus* only' or '*Gigaspora* only') or in the presence of interfamilial competition ('With *Gigaspora*' or 'With *Glomus*') in either clay, sand/clay or sand, 89 days after planting. Species belonging to the same genus were always grown together. Mean separations were conducted separately for each fungal species. Different upper-case letters indicate differences within AM fungal treatment among soil types using Tukey's HSD, and different lower-case letters indicate differences within soil type when the AM fungal species was grown with a member of the same genus only, or together with members of the other genus, using a two sample *t*-test at $\alpha = 0.05$

		Soil type		
Fungal species	Fungal treatment	Clay	Sand/clay	Sand
Gl. etunicatum	*Glomus* only	39.1 (12.8)^{Ba}	35.8 (6.8)^{Ba}	235.7 (27.0)^{Aa}
	With *Gigaspora*	44.0 (16.1)^{Ba}	36.7 (7.2)^{Ba}	97.2 (22.8)^{Ab}
Gl. mosseae	*Glomus* only	10.3 (1.6)^{Bb}	18.1 (9.3)^{Ba}	31.6 (3.8)^{Aa}
	With *Gigaspora*	21.4 (4.3)^{Aa}	17.3 (1.7)^{Aa}	12.6 (3.0)^{Ab}
Gi. gigantea	*Gigaspora* only	0.4 (0.3)^{Ba}	3.4 (0.7)^{Ba}	14.3 (3.2)^{Aa}
	With *Glomus*	0.8 (0.2)^{Aa}	2.0 (0.6)^{Aa}	2.7 (0.7)^{Ab}
Gi. rosea	*Gigaspora* only	0.2 (0.1)^{Ba}	0.6 (0.2)^{ABa}	3.2 (1.2)^{Aa}
	With *Glomus*	0.1 (0.1)^{Aa}	0.1 (0.1)^{Aa}	0.1 (0.1)^{Ab}

studies have shown that AM fungal α-diversity can be maintained through seasonal differences, differentiation among host plant species (see references above) and spatial separation (Rosendahl & Stukenbrock 2004). The observed divergence of AM fungal communities between sand and clay in this study suggests that a regional mosaic of soil types may be important for maintaining high AM fungal β-diversity.

AM fungal generalists and specialists have been identified before (Oehl *et al*. 2003), and our study also suggests that this group of fungi differ in niche breadth. For example, a *Gl. intraradices* ribotype (*Gl. intra rt1*) occurred in 9 out of the 10 fields, suggesting that it is widely distributed in the study area and is tolerant of a broad range of soil conditions (Fig. 3). Conversely, the distribution of *Gl. mosseae* was restricted to clay soil (Figs 2 & 3). This species has been found predominately in finer textured soils in other studies (Johnson *et al*. 1992; Landis *et al*. 2004), suggesting some degree of either specialization or restriction.

FUNDAMENTAL NICHES OF GIGASPORACEAE AND GLOMERACEAE

In the field, ribotypes that match with *Gi. gigantea* and *Gi. rosea* were very common and occurred predominately in sandy soils (Fig. 3, Appendix S1). In the glasshouse, overall colonization and sporulation by both *Gi. rosea* and *Gi. gigantea* were negatively correlated with percentage clay when grown in the absence of *Gl. etunicatum* and *Gl. mosseae* (Fig. 4a). This behaviour suggests that Gigasporaceae distribution may be influenced by soil characteristics independent of interactions with other AM fungi. Our results correspond with previous studies that have shown that Gigasporaceae dominate in sandy soils (Koske 1987; Duponnois *et al*. 2001) and are reduced in abundance in finer textured soils (Landis *et al*. 2004) based on sporulation patterns. In contrast to the *Gigaspora* species, the two *Glomus* species colonized roots well in all three experimental soils in the glasshouse (Fig. 4a), and thus appeared to have a broader fundamental niche than the two *Gigaspora* species. This was not the result of complementary niche space between the two *Glomus* species, because they responded similarly to increased clay content and they both sporulated successfully in all three soils (Table 3). This is in agreement with previous work by Duponnois *et al*. (2001) and Johnson *et al*. (1992) that indicate that many Glomeraceae do not show any strong dependence on soil characteristics.

What may cause this disparity between the two AM fungal families cannot be deduced from our study, as clay and sand differ in many variables (Table 1) that do not act independently (as is evident by their close correlation in Fig. 2). Soil fertility can impact AM fungal community structure, as indicated by shifts in AM fungal communities along gradients of N deposition (Egerton-Warburton & Allen 2000), soil organic carbon (Johnson *et al*. 1991), or N and P additions (Johnson 1993). Indeed, organic carbon and total nitrogen appear to have been important factors partitioning niche space within the Glomeraceae in the current study (Fig. 2). However, the RDA indicated that the separation on the family level was driven by percentage clay, but why clay has a negative effect on Gigasporaceae remains unknown. Possibly, differential biomass allocation and growth patterns of the external hyphae between Gigasporaceae and Glomeraceae, as detected by Hart & Reader (2002) and Dodd *et al*. (2000), are affected by texture. Additionally, our glasshouse study indicated that sporulation may be impaired in clay (Table 3). This may have a greater negative effect on Gigasporaceae than on Glomeraceae, as spores are the main propagules for many Gigasporaceae AM fungi (Klironomos & Hart 2002). Further comparative studies with *Scutellospora cerradensis* (*Sc. rt1*), the only member of the Gigasporaceae that occurred predominately in clay soil in the field (Fig. 2), could be informative. Overall, to understand the underlying mechanisms for the adverse effects of clay on most Gigasporaceae, more basic ecological experiments on AM fungi clearly are needed.

REALIZED NICHES OF GIGASPORACEAE AND GLOMERACEAE AM FUNGI

Glomeraceae were predominately found in clay soils in Tsholotsho (Figs 2 & 3). This family contains many species that are likely to differ from each other in their colonization pattern, growth rate, host specificity and tolerance to disturbance, which makes generalizations regarding underlying mechanisms for their restricted field distribution difficult. Our two representative *Glomus* species were exclusively found in clay soils in the field study and although *Gl. etunicatum* was rarely detected in roots (twice in total), *Gl. mosseae* was relatively common (Fig. 3). However, in spite of their restricted field distribution, both species were able to colonize roots well in all three soils in the glasshouse (Fig. 4a). In fact, spore production (Table 3) and vesicle abundance were higher in the sand than in the clay soil, except when the two *Gigaspora* species were present. This behaviour is consistent with competition for host carbon, as suggested in previous work (Pearson *et al*. 1993). Even though the percentage colonization of *Glomus* and *Gigaspora* could not be determined separately in the dual family inoculation treatment, comparisons of the overall mycorrhizal colonization in the single and dual family inoculations suggest that the fungi may also have competed for space within the root cortex (Fig. 4a). The absence of competition in the sand/clay and clay soils in the glasshouse could have been the result of such low abundance of *Gigaspora* that competition with *Glomus* could not occur.

The observed dominance of Gigasporaceae in sand soils in the field might be explained if members of the Gigasporaceae were better competitors in sand than members of the Glomeraceae. Whereas asymmetric competition has been documented before between Gigasporaceae and Glomeraceae (Wilson 1984; but see van Tuinen *et al*. 1998), the competitive response was not asymmetric in our glasshouse study. We found that the presence of *Glomus* reduced spore production by *Gigaspora* at least as much as the presence of *Gigaspora* reduced spore production by *Glomus* (Table 3). The lack of asymmetric competition in our study could be due to the particular combination of AM fungal species (Sainz *et al*. 1989), the inoculum potential, the short duration of the study (spanning only a single plant generation) or disparity between glasshouse and field conditions, including the difference in host plants. The degree of host specificity of AM fungi is still uncertain and may differ between plant communities (Vandenkoornhuyse *et al*. 2003; Stukenbrock & Rosendahl 2005). One may speculate that agricultural systems harbour AM fungi that are less host-specific owing to yearly crop rotations, but the effect of using sorghum rather than maize as host plants in the glasshouse study is unknown. Nevertheless, if the species we chose for our glasshouse experiment are representative of their respective families, our results indicate that competitive interactions between co-occurring AM fungi could be one mechanism that affects the relative success of these two families.

THE ROLE OF DISPERSAL

The negative correlation between distance among samples and ribotype similarity within soil types indicates that spatial structure may indeed be a good surrogate for dispersal limitation, as suggested by others (e.g. Chase 2005; Cottenie 2005). The variance partitioning procedure allowed us to evaluate the independent contributions of spatial structure and environmental variables to the observed AM fungal ribotype distribution. These analyses indicated that both the environmental variables and the geographical variables explained substantial portions of the variability in AM fungal community composition, but environmental variables had a slightly stronger influence (Table 2). We found no evidence that the AM fungal communities were structured by neutral processes alone. However, some of the fields in our study may harbour competitively inferior species simply because the best competitors have not arrived or because the competitive inferiors are rescued from competitive exclusion by immigration from communities where they are good competitors. This is consistent with the results of Cottenie (2005), who conducted a meta-analysis on 158 community-level studies using the same variance partitioning approach as we took here and discovered that a majority of communities were structured by environmental variables while very few were structured by neutral processes alone.

Conclusion

Our goal was to understand the underlying mechanisms that generate differences in species composition among AM fungal communities. The results indicate that species traits, local environmental factors and, potentially, regional dispersal dynamics may all be important in structuring AM fungal communities, emphasizing the value of a metacommunity perspective.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1 T-RFLP profiles of AMF ribotypes and their occurrences in maize roots

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2745.2006.01193.x

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