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ORIGINAL ARTICLE

Relative roles of niche and neutral processes in structuring a soil microbial community

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Most attempts to identify the processes that structure natural communities have focused on conspicuous macroorganisms whereas the processes responsible for structuring microbial communities remain relatively unknown. Two main theories explaining these processes have emerged; niche theory, which highlights the importance of deterministic processes, and neutral theory, which focuses on stochastic processes. We examined whether neutral or niche-based mechanisms best explain the composition and structure of communities of a functionally important soil microbe, the arbuscular mycorrhizal (AM) fungi. Using molecular techniques, we surveyed AM fungi from 425 individual plants of 28 plant species along a soil pH gradient. There was evidence that both niche and neutral processes structured this community. Species abundances fitted the zero-sum multinomial distribution and there was evidence of dispersal limitation, both indicators of neutral processes. However, we found stronger support that niche differentiation based on abiotic soil factors, primarily pH, was structuring the AM fungal community. Host plant species affected AM fungal community composition negligibly compared to soil pH. We conclude that although niche partitioning was the primary mechanism regulating the composition and diversity of natural AM fungal communities, these communities are also influenced by stochastic-neutral processes. This study represents one of the most comprehensive investigations of community-level processes acting on soil microbes; revealing a community that although influenced by stochastic processes, still responded in a predictable manner to a major abiotic niche axis, soil pH. The strong response to environmental factors of this community highlights the susceptibility of soil microbes to environmental change.
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Introduction

Understanding the processes that structure ecological communities has long been a central aim in ecological research (Preston, 1948). Traditionally, the diversity, structure and composition of natural communities are explained through 'niche-based' mechanisms (Tokeshi, 1990). However, competing, yet not mutually exclusive (Chave, 2004), 'neutral theories' have gained recent interest (McGill *et al.*, 2006). Niche-based theories suggest that inter-specific variation in species' ecologies allows the partitioning of limited resources between competing species and the differentiation of niche space across all species within a community (Leibold and McPeek, 2006). Thus, the fundamental premise of niche theory, as an explanation for the coexistence of species and maintenance of biodiversity, is that ecological traits differ among species within a community (Leibold and McPeek, 2006). In contrast, neutral theories assume all species to be ecologically equivalent and to have the same demographic rates (birth, death, dispersal and speciation rates; Chave, 2004). Under these assumptions, the structure of communities comes solely from stochastic processes and dispersal limitation (Hubbell, 2001).

Although details of niche or neutral theory may differ among studies (McGill *et al.*, 2006), a general set of testable predictions has arisen from each. Niche theories predict that changes in species composition will be related to changes in environmental variables (Jongman *et al.*, 1995) and that species abundances will follow a log-normal distribution, although this is not universally accepted (Williamson and Gaston, 2005). Neutral theories predict that changes in species composition will be related to geographic distance between samples as a result of dispersal limitation and that species abundances will follow a zero-sum multinomial (ZSM) distribution (McGill *et al.*, 2006), which is the unique species abundance distribution predicted by neutral theory (Hubbell, 2001). These theories, although often viewed as contradictory, are

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not mutually exclusive; deterministic niche-based processes and stochastic-neutral processes are probably jointly responsible for structuring ecological communities, yet few studies examine their relative importance (Chave, 2004).

In order to examine the relative importance of niche and neutral mechanisms in structuring natural communities, an ideal study community would be relatively species rich, functionally important and show *a priori* evidence of both niche and neutral processes. The arbuscular mycorrhizal (AM) fungi (Phylum: Glomeromycota) fit these criteria. AM fungi are obligate plant-root endosymbionts that colonize approximately 2/3 of terrestrial plant species, acquire all their carbon from the host plant and deliver to the plant a range of beneficial impacts, notably increased phosphorus uptake, and thus have profound effects on plant community dynamics and diversity (Fitter, 2005; Rosendahl, 2008). AM fungal species cannot be identified morphologically in roots (Merryweather and Fitter, 1998) and to overcome these problems DNA-based techniques have been developed that quantify fungi from plant roots collected from natural systems (Helgason *et al.*, 1998). However, most DNA-based studies have either compared AM fungal diversity between habitats or simply listed species (see Öpik *et al.*, 2006 for a review). The few attempts to understand processes structuring these communities have been based on spore identification (Fitzsimons *et al.*, 2008), which does not reveal processes regulating fungal communities *in planta*; or on fungi sampled from a small selection of plant species (Santos *et al.*, 2006; Lekberg *et al.*, 2007; Schechter and Bruns, 2008) or from an environment with minimal variance in soil chemistry (Singh *et al.*, 2008), which will not fully sample all the potential habitats that AM fungi might occupy.

Experimental studies indicate that AM fungi have low functional diversity and, in particular, that the ability to colonize any host plant is equal across AM fungal species (Smith and Read, 2008), suggesting that natural communities are structured entirely by stochastic processes (as assumed by neutral theory). However, these studies were based on host range tests using isolates amenable to culture, a group that subsequent field studies have shown to be unrepresentative of AM fungal species in natural systems (Helgason *et al.*, 2002). In contrast, studies using molecular techniques to identify fungi *in planta* have revealed large differences among the communities of fungi colonizing the roots of coexisting plant species (Vandenkoornhuyse *et al.*, 2003). In addition to host plant effects, soil physical and chemical properties may affect AM fungal communities (Fitzsimons *et al.*, 2008; Liu *et al.*, 2009), implying that AM fungal communities are actually structured by deterministic processes (niche theory). However, the relative importance of these niche and neutral processes in structuring AM fungal communities has yet to be established.

In this study, we examine whether neutral or classic niche-based mechanisms best explain the composition and structure of natural AM fungal communities. We compile the largest data set to date examining AM fungi from natural soil systems and investigate the relative importance of these niche and neutral processes by examining species abundance distributions, dispersal limitation, and potential soil and plant niche axes. If neutral processes dominate we predict that:

1. AM fungal species abundances will fit the ZSM distribution.
2. Changes in AM fungal community composition will be related to distance between samples, indicating the effects of dispersal limitation.
3. There will be no relationship between AM fungal communities and either host plant or soil properties.

However, if niche-based mechanisms dominate we predict that:

1. AM fungal species abundances will fit a log-normal or other niche-based species abundance distribution.
2. Changes in AM fungal community composition will be related to changes in either host plant or soil properties between samples.
3. AM fungal communities will not be structured by isolation by distance effects.

Materials and methods

Study site

Fieldwork was conducted during July 2007 at Hetchell Wood near Leeds, UK (53°52′36.54″N, 1°25′45.94″W). Hetchell Wood is a Site of Special Scientific Interest comprising 14 ha mosaic of grassland, wood and heath on a steep escarpment where magnesian limestone overlays millstone grit causing a severe variation in soil pH (pH 3.72–8.04) and a marked transition in vegetation over a few metres. Samples in this study were taken at regular intervals along six transects that lay along the pH gradient, taking 40 mm diameter soil cores at each point (Figure 1). From each soil core plant roots and soil were separated, plant roots were then washed, and both roots and soil were then oven-dried (70 °C) for approximately 1 week. Plant roots were then stored in airtight containers for downstream DNA processing and dried soil was analysed for soil physicochemical properties (Supplementary Table S1). These data were then used as environmental variables in the subsequent analyses.

Molecular methods

We extracted DNA from 2–4 cm long pieces of individual plant roots (20 root fragments per soil core) using MoBio PowerPlant DNA isolation kits

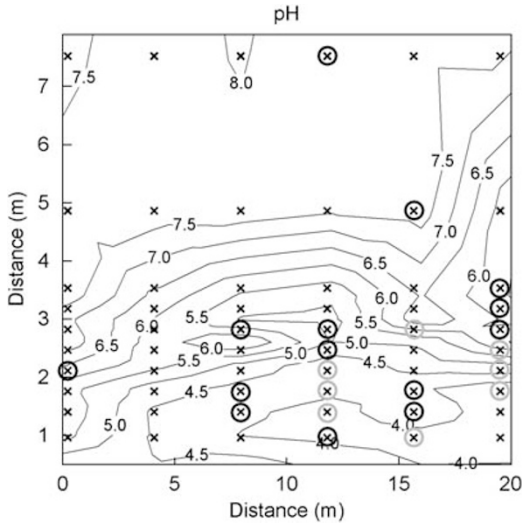

Figure 1 Contour plot of soil pH across the study site, contour lines represent changes in soil pH across the study site to the nearest 0.5 pH unit. Crosses mark locations of sampled soil cores from the six transects. Grey circles represent soil cores that failed to produce arbuscular mycorrhizal (AM) fungal terminal restriction fragment length polymorphism (TRFLP) profiles and black circles represent soil cores excluded from analyses because <5 plant roots produce AM fungal TRFLP profiles.

(Mo Bio Laboratories Inc., Carlsbad, CA, USA). To identify the plant species of individual roots, we amplified the plastid *trn*L intron (*ca.* 550–650 bp) from root DNA using *Taq* DNA polymerase (Invitrogen Co., Carlsbad, CA, USA) in a semi-nested PCR with the universal chloroplast primers C and F for the initial amplification followed by primers C and D (Taberlet *et al.*, 1991). PCR reactions were carried out in the presence of 2 mM dNTPs, 10 pmol of each primer and the manufacturer's reaction buffer in 25 μl reactions (PCR conditions: 95 °C for 2 min; 30 cycles at 94 °C for 0.5 min; 53 °C for 0.5 min and 72 °C for 1 min; and 72 °C for 10 min) on a Techne TC-512 (Techne Co., Staffs, UK). PCR products were purified using QIAquick PCR Purification Kit (Qiagen Ltd, W Sussex, UK) and sequenced on an ABI 3730xl (Macrogen Inc., Seoul, Korea). DNA sequences were checked and compared against reference sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the Blast algorithm (Altschul *et al.*, 1990; for further details see Supplementary Materials and methods).

To quantify the AM fungal community from individual plant roots, we used terminal restriction fragment length polymorphisms (TRFLP) of small subunit (SSU) rDNA. Analysis of TRFLP is a culture-independent community profiling technique that has been shown to produce robust estimates of microbial taxon richness and abundance (Lueders and Friedrich, 2003), and TRFLP-produced estimates of AM fungal communities have been shown to be consistent with findings from studies based on clone libraries (see Vandenkoornhuyse *et al.*, 2003). However, as with any PCR-based technique there is potential for bias, to minimize the risk of PCR bias we used conserved primers, focused on a taxa with minimum GC variance between sequence types, and kept amplification cycles to a minimum (Kanagawa, 2003). Thus, we are satisfied that any effects of PCR bias would be consistent across samples and unlikely to quantitatively affect results.

In order to design a suitable TRFLP protocol and choose appropriate enzymes for restriction digests of the AM fungal communities at our study site, we first conducted a broad screening of the AM fungal community using a standard protocol of sequencing and RFLP typing of SSU rDNA (for full details see Supplementary Materials and methods). For TRFLP analysis of the AM fungal community a 550 bp partial fragment of SSU rDNA was amplified by PCR using *Taq* DNA polymerase (Invitrogen) and the universal eukaryotic primer NS31 (Simon *et al.*, 1992) and the AM fungal primer AM1 (Helgason *et al.*, 1998). These primers were labelled with the fluorescent markers HEX (NS31) and FAM (AM1) on the 5′ end. PCR was carried out in a 25 μl reaction volume with 1 μl of DNA template, 2 mM dNTPs, 10 pmol of each primer (PCR conditions: 95 °C for 2 min; 30 cycles at 94 °C for 0.5 min, 63 °C for 0.5 min and 72 °C for 1 min; and 72 °C for 10 min) on a Techne TC-512. To remove humic-acid-based PCR inhibitors, 0.125 μl of T4 gene 32 protein (Roche Diagnostics Ltd, W Sussex, UK) was added to all PCR reactions. Labelled PCR products were purified using QIAquick PCR Purification Kit (Qiagen).

Fluorescently labelled PCR amplicons were digested separately with the enzymes *Hsp*92II, *Hin*fI and *Rsa*I (10 μl reactions, 0.5 μl enzyme, 0.2 μl bovine serum albumin) and purified using QIAquick PCR Purification Kit (Qiagen). The resulting clean product (5 μl) was loaded onto an ABI 3130 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA) to determine the sizes and quantities of terminal fragments (TFs) in each digest. TRFLP profiles were analysed using GeneMapper (Applied Biosystems Inc.). Peaks between 50 and 8000 fluorescent units in height, representing TFs longer than 85 bp were analysed using a bin width of 2 bp and the local southern method of peak calling (see Osborn *et al.*, 2000). Peak area was used to measure TF frequency rather than peak height to avoid down-weighting the relative importance of longer fragments (Dickie and FitzJohn, 2007). Raw peak area data were transformed into proportional abundances to account for differences in the total quantity of DNA between samples (Culman *et al.*, 2008). All singletons (across all samples) and peaks representing less than 5% of the total abundance on average across all samples were excluded to ensure the removal of all background noise (Culman *et al.*, 2008).

Data analysis

For all data analyses the number of distinct TFs was used as an estimate of species richness and the relative abundance of each TF was treated as the

proportion of that 'species' within a sample; this is not a taxonomic measure of the true number of species within a sample, and we use the term TF-species in recognition of that fact. Species accumulation curves were computed using rarefaction in the R statistical language (R Development Core Team, 2007) following methods in Magurran (2004). Data were only included from soil cores from which $\geqslant 5$ of the 20 plant roots examined yielded AM fungal TRFLP profiles. The excluded soil cores were taken from across the pH gradient and there was no effect of soil pH on the proportion of roots that yielded AM fungal TRFLP profiles.

TF-species abundance data were fitted to theoretical species abundance distributions. Two purely statistical models (log-normal and log-series), two niche-based models (geometric series and broken stick) and neutral theory's ZSM were compared to observed data (see Hubbell, 2001; Magurran, 2004). χ^2 goodness of fit tests were initially used to examine whether the data fitted the purely statistical and niche-based models. Models that did not fit the data were then rejected. We used the exact likelihood function of Etienne (2005) to estimate the parameters θ, the fundamental biodiversity number and m, the probability of immigration for the ZSM (Etienne, 2005; Chave *et al.*, 2006), using the program TeTame (http://www.edb.ups-tlse.fr/equipe1/chave/tetame.htm). Maximum likelihood was then used to fit the remaining species abundance models that were not rejected following χ^2-analysis. The fit of the models to our observed data was compared using AIC (defined as $2(p–L_i)$ where p is the number of parameters and L_i is the maximum likelihood estimate of the ith model) and Akaike weights following Chave *et al.* (2006). Akaike weights were computed as $w_i = \exp(-\Delta_i/2)/[1+\exp(-\Delta_i/2)]$, where Δ_i is the difference in AIC between the model with lowest AIC and model i.

Two separate methods were used to examine the relative importance of spatial processes (for example, dispersal limitation) and environmental factors on regulating AM fungal community composition and structure; partitioning of β-diversity based on raw species abundance data and Mantel tests based on distance dissimilarity matrices (Legendre *et al.*, 2005; Tuomisto and Ruokolainen, 2006). Partitioning of β-diversity into spatial and environmental components followed recommendations by Legendre *et al.* (2005, 2009), and a brief description of their methods follows. Principal coordinates of neighbour matrices (PCNM) eigenfunctions were computed across the sampling locations. PCNM eigenfunctions represent a 'spectral decomposition of the spatial relationship among sampling locations' (Legendre *et al.*, 2009) and can be considered as purely spatial variables in an ordination-based analysis. Forward selection of PCNM variables based on permutation tests (10 000 randomizations) was used to identify 7 of the 19 extracted PCNM variables that significantly ($\alpha = 0.05$) explained the spatial structure of the TF-species abundance data. The variation of the community composition data was then partitioned between the extracted PCNM spatial variables and the soil environmental variables (Supplementary Table S1) using a canonical redundancy analysis (RDA). Variation partitioning analysis was carried out in the R statistical language (R Development Core Team, 2007) using the 'vegan', 'spacemakeR' and 'packfor' libraries following methods outlined in Legendre *et al.* (2009).

In order to examine the relative importance of potential niche axes and dispersal limitation on AM fungal β-diversity, AM fungal β-diversity between sampled cores was correlated with changes in soil variables (Supplementary Table S1), host plant β-diversity and distance between samples using Mantel tests based on 10 000 randomizations of the original data. AM fungal β-diversity was measured by the complement of Morisita–Horn's index. Host plant β-diversity was calculated using the complement of Jaccard's index, a qualitative measure, as sample size was too low to use the quantitative Morisita–Horn's index. Computation of diversity indices followed recommendations by Magurran (2004).

In order to examine the relationship between environmental variables and AM fungal community composition, we used both indirect (detrended correspondence analysis, DCA) and direct (canonical correspondence analysis, CCA) ordination techniques (Jongman *et al.*, 1995). However, not all roots examined yielded AM fungal TRFLP profiles. To account for unequal sample sizes between soil cores, we weighted all ordination analyses by the number of plant roots that yielded positive TRFLP profiles in that sample. Ordination analysis of TRFLP data followed recommendations by Culman *et al.* (2008), which included down-weighting of rare species (see Jongman *et al.*, 1995). DCA was used to indirectly relate the species composition of each sample to underlying environmental variables by comparing DCA axes against environmental variables using step-wise multiple regressions. CCA uses a direct ordination method where environmental variables are incorporated in a linear combination that best separates samples in terms of species composition. The significance of each environmental variable was assessed using a distribution free Monte Carlo randomization test. Ordination analysis was conducted using the computer package Canoco 4.5.

Results

We sampled the TRFLP profiles of AM fungi from 51 soil cores across an intense soil pH gradient (Figure 1). Of the 51 soil cores sampled, 14 produced AM fungal TRFLP profiles from fewer than 5 separate plant roots and were excluded from further analysis, leaving 425 roots from 37 soil cores in the subsequent analyses. Analysis of plant DNA

sequences revealed that 28 different species of plant were sampled. TRFLP analysis detected 68 TFs ranging from 109 to 550 bp. Visual inspection of rarefied TF-species accumulation curves (Supplementary Figure S1) showed TF-species accumulation had begun to asymptote when data were analysed per root (Supplementary Figure S1a) and per soil core (Supplementary Figure S1b). Thus, further sampling would have added few additional data and would be unlikely to qualitatively affect the results.

Arbuscular mycorrhizal fungal TF-species abundance distributions (Supplementary Figure S2) were shown to fit both log-normal and broken stick models (broken stick: $\chi^2 = 11.8$, $P = 0.69$; log-normal: $\chi^2 = 21.1$, $P = 0.14$) and were significantly different to those predicted by the log-series and geometric series ($\chi^2 > 44$, $P < 0.001$ in both the cases). Maximum likelihood estimates showed a close fit of the data to the ZSM ($L = -109.73$, estimated parameters $\theta = 28.49$, $m = 0.03$). Comparisons between the log-normal, broken stick and ZSM, based on Akaike weights, showed that the probability that either the log-normal or the broken stick could outperform the ZSM at predicting these data was less than 1%. Thus the AM fungal TF-species abundance distribution had a closer fit to the ZSM than any other model (ZSM: AIC = 223.46; broken stick: AIC = 392.11; log-normal: AIC = 468.04).

Analysis of variation partitioning between spatial (PCNM eigenfunctions) and environmental variables (Supplementary Table S1) was used to examine the relative influence of spatial processes (for example, dispersal limitation) and environmental factors on AM fungal community composition. This analysis partitioned the data between four fractions; (a) variation explained by environmental variables with no spatial structure, (b) variation explained by environmental variables with spatial structure, (c) spatially structured variation not explained by environmental variables and (d) residual variation. Analysis of variation partitioning explained 27% of the variation, based on adjusted R^2 values (R^2_a), in AM fungal community composition between samples (permutation test based on 10 000 randomizations, of fraction (a + b + c); F = 1.82; $P < 0.001$). Just over half of the explained variation in community composition was attributed to environmental factors (a + b; $R^2_a = 0.15$) and just under half to spatially structured variation (c; $R^2_a = 0.12$). The fractions (a) and (b) showed that the majority of variation explained by environmental variables was spatially structured (b; $R^2_a = 0.08$) reflecting the strong spatial pH gradient.

Potential niche axes that may explain the observed distribution, abundance and diversity of AM fungi were investigated. Mantel tests showed that β-diversity was positively correlated primarily with change in pH, then distance (m), C/N ratio, host plant composition and soil phosphorus between samples (Figure 2; Mantel test, using 10 000 randomizations; pH: $r = 0.26$, $P = 0.0001$; distance

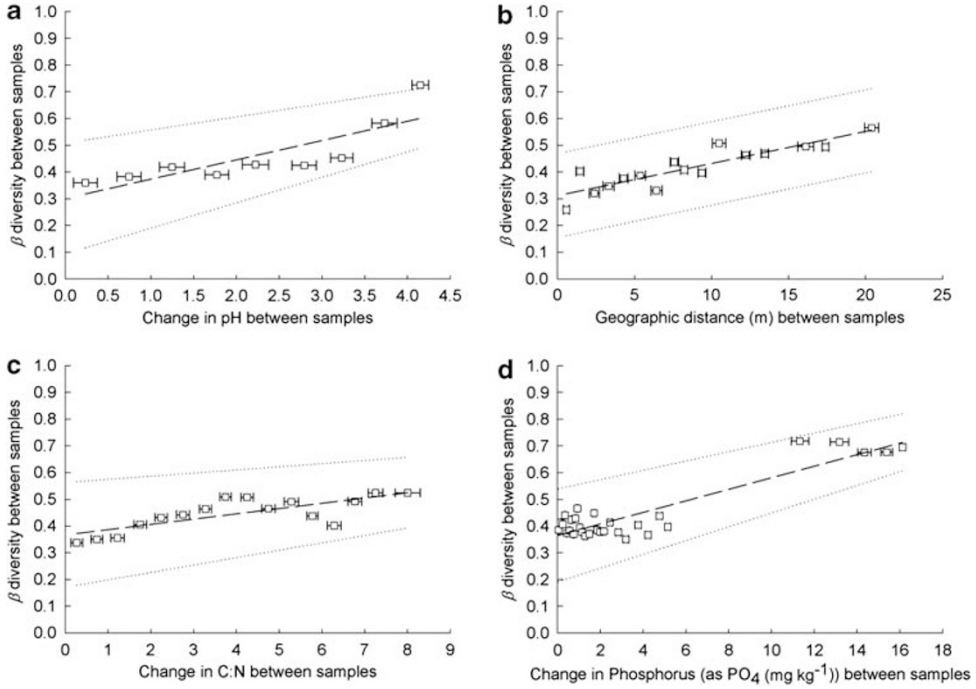

Figure 2 Arbuscular mycorrhizal (AM) fungal β-diversity relationships. Mean AM fungal β-diversity (± s.d. represented by dotted lines) between samples in relation to changes between samples in the four main abiotic variables explaining AM fungal β-diversity; soil pH (**a**), geographic distance (**b**), soil C/N ratio (**c**) and soil phosphorus (**d**). Error bars represent ± s.d. based on binning explanatory variables to aid clarity. β-diversity is measured by the complement of Morisita–Horn's index. The dashed line indicates a significant relationship using Mantel tests.

(m): $r=0.32$, $P=0.0001$; C/N: $r=0.31$, $P=0.0006$; host plant: $r=0.19$, $P=0.004$; phosphorus: $r=0.20$, $P=0.04$). There was no evidence that β-diversity was correlated with the remaining soil variables ($P>0.05$ in all the cases). It was difficult to separate the relative effects of changes in distance and soil pH between samples in relation to β-diversity as these two variables were also correlated (Mantel test, using 10 000 randomizations: $r=0.19$, $P=0.002$). However, examining the effects of distance between samples within similar pH soils (change in pH between samples = 0–1; change in distance between samples = 0.4–16.7 m) revealed isolation by distance effects and dispersal limitation had minimal effects on β-diversity (mean Mantel test correlation, using 10 000 randomizations, between β-diversity and distance between samples within soils of similar pH: $r=0.50$, s.d. = 0.38; mean $P=0.112$, s.d. = 0.14).

Detrended correspondence analysis explained 42.2% of the variation within the TF-species abundance data (Figure 3). Step-wise multiple regression revealed the first DCA ordination axis to be significantly related to AM fungal TF-species richness in the sample ($R^2=0.58$, $F_{1,35}=48.25$, $P<0.001$) and the second DCA ordination axis was significantly related to soil pH ($R^2=0.47$, $F_{1,35}=31.5$, $P<0.001$). There was no significant relationship between any of the environmental variables and either DCA axis three or four. CCA significantly explained 68.5% of the species–environment relationship across the first two canonical axes under the full model that included all environmental variables (Monte Carlo permutation test; first axis: $F=4.54$, $P=0.02$; all axes: $F=1.97$, $P=0.0001$). After manual forward selection of environmental variables, and testing using Monte Carlo permutation tests, CCA revealed three soil variables, pH, C/N ratio and phosphorus, that were significantly related to AM fungal community composition (Monte Carlo permutation test: pH, $F=2.76$, $P=0.005$, C/N, $F=3.24$, $P=0.007$; phosphorus: $F=2.74$, $P=0.01$). Under this restricted model CCA showed that these variables explained 87.3% of the variation within the species–environment relationship across the first two canonical axes (Figure 3). No other environmental variable was significantly related to AM fungal community composition ($P>0.05$ in all the cases).

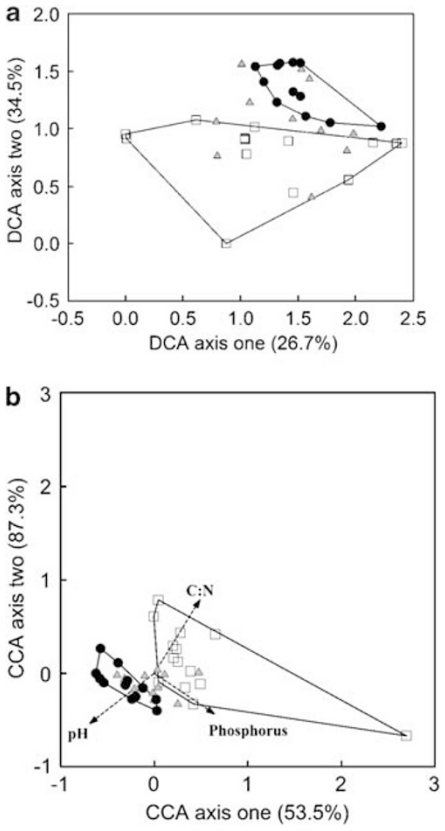

Figure 3 Sample-based ordination plots. Ordination plots of samples collected from low pH (<5; open squares), medium pH (5–7; open triangles) and high pH (>7; closed circles) soils using indirect (**a**; detrended correspondence analysis, DCA) and direct (**b**; canonical correspondence analysis, CCA) ordination techniques. Percentage values on axes represent cumulative percentage variation explained by consecutive axes. DCA axis 1 is significantly related to arbuscular mycorrhizal (AM) fungal species richness of each sample and DCA axis 2 is significantly related to soil pH (**a**). CCA revealed soil pH, C/N ratio and phosphorus significantly explained the species–environment relationship of AM fungi within these samples (**b**).

Discussion

Niche or neutral theory

Processes derived from niche theory played the major role in regulating the composition and structure of this AM fungal community but there was also a contribution from underlying neutral processes. The two main predictions of neutral theory are a close fit of observed species abundance distributions to a ZSM and dispersal limitation as a central mechanism structuring communities (McGill *et al*., 2006). The AM fungal community surveyed in this study closely fitted the ZSM species abundance distribution. However, although there was evidence of spatial structure unrelated to environmental pattern in the data, whether dispersal limitation is a central mechanism structuring this community is less clear. In contrast, abiotic variables, notably soil pH, C/N ratio and phosphorus, were the main factors determining the environmental niche of AM fungi and regulating the composition of these communities.

In this study TRFLP analysis revealed 68 distinct TFs, indicating a highly diverse community. However, levels of AM fungal richness per root were similar to those previously recorded when analysis was adjusted to account for differences in the number of enzymes used between studies (for example, Vandenkoornhuyse *et al*., 2003; see Supplementary Figure S1). AM fungal TF-species abundances most closely fitted the ZSM distribution

followed by the classic broken stick and log-normal distributions. One of the properties of the ZSM is that it predicts more rare species than the log-normal species abundance distribution (Olszewski and Erwin, 2004). Microbial communities are highly diverse and often contain many rare species irrespective of whether they are sampled to an asymptote (Hughes *et al.*, 2001). This high number of rare species in microbial communities results in a structure that is similar to the highly diverse communities typically found in tropical forests (Hughes *et al.*, 2001) and this is having a major influence on the ability of the ZSM to describe the AM fungal community in this study. This study represents one of the few attempts to fit the ZSM to microbial species abundance distributions, and shows that the ZSM describes the structure of AM fungal communities as well as, if not better than, tropical-tree communities (Volkov *et al.*, 2005; Chave *et al.*, 2006), suggesting that the neutral theory is an appropriate null model to be used to describe microbial communities. However, a good fit of species abundance distributions to a theoretical model does not distinguish process from pattern (Alonso *et al.*, 2006) and to understand fully the mechanisms regulating AM fungal communities the relative influences of processes derived from both niche and neutral theories must be deconstructed.

Analysis of variation partitioning revealed evidence of an underlying spatial structure in the AM fungal community that could have occurred from dispersal limitation as predicted by neutral theory (Hubbell, 2001). This was less apparent using Mantel tests based on distance dissimilarity matrices because of difficulty controlling for the spatial structure in the soil pH data. However, the analysis of variation partitioning successfully partitioned out environmental variables with spatial structure (Legendre *et al.*, 2005) and still showed a remaining but smaller response of the community data to pure spatial variables. It has been suggested that in some systems both deterministic (niche-based) and stochastic (neutral-based) processes are responsible for structuring ecological communities (Chave, 2004). In this system, it appears neutral processes affect AM fungal communities but secondarily to niche-based ones. Other studies have suggested that dispersal limitation may have a role in structuring AM fungal communities but that it is second to environmental effects (Lekberg *et al.*, 2007). Our data support this finding showing a clearly overarching response to environment driving AM fungal community patterns but with subtle underlying evidence of spatial processes such as dispersal limitation having a role. However, it should be noted that although both deterministic and stochastic processes are evident in structuring the AM fungal community examined here, outside such a highly patterned environment or acting at different spatial scales, the relative importance of these processes may change.

Environmental niche of AM fungi

Our results show that the main factors determining the composition of natural communities of AM fungi are soil pH, phosphorus and C/N ratio. This finding is supported universally across our analyses (Mantel tests, DCA and CCA). Mantel tests also showed that β-diversity was related to changes in host plant composition as well as soil variables. Soil C/N ratio and phosphorus levels are ultimately heavily influenced by pH because of its impact both on rates of decomposition and on the solubility of phosphate compounds. Host plant composition also reflects the response of plants to soil pH, a major determinant of plant community composition (Lee, 1999). Thus data in this study support the notion that soil pH is a major factor structuring natural AM fungal communities.

Arbuscular mycorrhizal fungi, although obligate plant symbionts, have extensive extra-radical mycelial networks that spread throughout the soil and it is likely that the effect of soil pH on mycelia produces strong selective pressures structuring AM fungal niche space (van Aarle *et al.*, 2002). However, previous studies on soils with a smaller range of pH have suggested that host plant species is an important factor structuring AM fungal communities (Helgason *et al.*, 2007). It is likely that it is the pronounced variability in soil pH in our study that makes pH the over-arching factor controlling AM fungal niche space. Although we found that both soil pH and host plant preference affected AM fungal communities, it is unclear whether the host plant effect is a direct influence on AM fungal communities or an indirect response arising from the plants' response to soil pH. Answering this question is beyond the scope of this study.

Detrended correspondence analysis supported Mantel tests and CCA in showing pH as the major determining factor structuring AM fungal communities, but also revealed a strong signal separating samples in ordination space by AM fungal richness of individual samples. This signal is unlikely to be a sampling artefact reflecting differences between samples in number of roots from which informative TRFLP profiles were gathered, because this was controlled for by weighting all ordination analyses by sample size. This result therefore indicates high β-diversity of AM fungi between roots from within the same soil core, suggesting that at a very small spatial scale within soils of the same pH, secondary effects regulate AM fungal communities. At this scale, host plant effects may be important (Vandenkoornhuyse *et al.*, 2003), offering a possible explanation for the observed high β-diversity between roots within a soil core.

Conclusions

To date this is the largest and most comprehensive study of AM fungal communities within a natural

system. Uniquely for a microbial community study, we determine the relative importance of abiotic, biotic and stochastic processes at this site. We can state firmly that AM fungal communities are strongly influenced by environmental factors and that they responded in a predictable and deterministic manner to changes in soil pH. However, in addition to the overarching effect of soil pH on regulating the AM fungal community there was also evidence of underlying stochastic community-level processes, such as dispersal limitation. Robust information on the mechanisms that regulate the diversity, structure and composition of natural communities is urgently needed to help conserve ecosystem functioning and mitigate biodiversity loss from current and future environmental change. The strong response to environmental factors and highly deterministic nature of this community highlight the susceptibility of soil microbes to environmental change.

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