The ISME Journal (2016) 10, 885–896
© 2016 International Society for Microbial Ecology All rights reserved 1751-7362/16
www.nature.com/ismej

ORIGINAL ARTICLE

Stochastic distribution of small soil eukaryotes resulting from high dispersal and drift in a local environment

Mohammad Bahram[1,2], Petr Kohout[1,3,4], Sten Anslan[1], Helery Harend[1], Kessy Abarenkov[5] and Leho Tedersoo[5]

[1]*Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia;* [2]*Department of Organismal Biology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden;* [3]*Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic;* [4]*Department of Experimental Plant Biology, Charles University in Prague, Prague, Czech Republic and* [5]*Natural History Museum, University of Tartu, Tartu, Estonia*

A central challenge in ecology is to understand the relative importance of processes that shape diversity patterns. Compared with aboveground biota, little is known about spatial patterns and processes in soil organisms. Here we examine the spatial structure of communities of small soil eukaryotes to elucidate the underlying stochastic and deterministic processes in the absence of environmental gradients at a local scale. Specifically, we focus on the fine-scale spatial autocorrelation of prominent taxonomic and functional groups of eukaryotic microbes. We collected 123 soil samples in a nested design at distances ranging from 0.01 to 64 m from three boreal forest sites and used 454 pyrosequencing analysis of Internal Transcribed Spacer for detecting Operational Taxonomic Units of major eukaryotic groups simultaneously. Among the main taxonomic groups, we found significant but weak spatial variability only in the communities of Fungi and Rhizaria. Within Fungi, ectomycorrhizas and pathogens exhibited stronger spatial structure compared with saprotrophs and corresponded to vegetation. For the groups with significant spatial structure, autocorrelation occurred at a very fine scale (<2 m). Both dispersal limitation and environmental selection had a weak effect on communities as reflected in negative or null deviation of communities, which was also supported by multivariate analysis, that is, environment, spatial processes and their shared effects explained on average <10% of variance. Taken together, these results indicate a random distribution of soil eukaryotes with respect to space and environment in the absence of environmental gradients at the local scale, reflecting the dominant role of drift and homogenizing dispersal.
The ISME Journal (2016) **10,** 885–896; doi:10.1038/ismej.2015.164; published online 22 September 2015

Introduction

Niche-based and niche-neutral models constitute two alternative but complementary paradigms for understanding diversity patterns (Hubbel, 2001; Chase and Leibold, 2003). Niche theory posits that communities are shaped by deterministic processes (that is, environmental selection and niche partitioning) owing to different habitat preferences and fitness of species. According to neutral theory, random fluctuations in species abundance (ecological drift) and limited dispersal shape the communities. Emerging evidence suggests that both groups of processes jointly regulate ecological communities (Chave, 2004) with varying relative effects depending on geographic scales and strength of environmental gradients (see Hanson *et al*., 2012) and type of organism (body size, dispersal mode, see Soininen *et al*., 2007). At smaller scales, habitat heterogeneity declines, which generally results in lower habitat preference and therefore greater importance of stochastic processes over deterministic factors (Legendre *et al*., 2009; Chase, 2014). However, the evolutionary and ecological factors that influence dispersal of organisms may also diminish with decreasing geographic scale (Warren *et al*., 2014). Thus reducing the study scale enables to control for both environmental filtering and dispersal limitation as a result of recruitment failure (Wang *et al*., 2013; Lowe and McPeek), which minimizes the confounding effects of unmeasured variables.

Diversity patterns provide evidence for the processes underlying community assembly (Vellend, 2010).

Correspondence: M Bahram, Institute of Ecology and Earth Sciences, University of Tartu, 40 Lai Street, Tartu 50411, Estonia.
E-mail: Bahram@ut.ee
Received 27 May 2015; revised 27 July 2015; accepted 30 July 2015; published online 22 September 2015

One of the most common patterns in ecological communities is the negative relationship between community similarity and spatial distance, known as distance–decay of similarity (DDS; Nekola and White, 1999; Poulin, 2003). DDS constitutes a general pattern across different groups of organisms, and it is widely employed to disentangle the relative importance of neutral and niche processes in maintaining biodiversity (Condit *et al.*, 2002; Cottenie, 2005; Hanson *et al.*, 2012; Stegen *et al.*, 2013). DDS results from the joint effects of environmental selection and dispersal limitation coupled with ecological (or evolutionary) drift. Drift acting alone and high dispersal rates homogenize the community and thus lead to weaker DDS patterns (Vellend, 2010). As a result, the rate and extent of DDS varies across different organism groups with different habitat preference and also organism type, that is, body size and dispersal mode (Soininen *et al.*, 2007).

In contrast to macro-organisms, microbes are dispersed more easily owing to their smaller size, which allows them to inhabit temporarily unsuitable habitats. There is an old assumption that free-living soil microbial eukaryotes do not face dispersal limitation (Finlay, 2002), but several molecular studies provide evidence for their biogeographic patterns (for example, Green *et al.*, 2004; Bahram *et al.*, 2013; Bates *et al.*, 2013; but see Queloz *et al.*, 2011). Recent findings also suggest that similarly to macro-organisms, microbes are influenced by neutral processes (for example, Cottenie 2005; Hájek *et al.*, 2011; Astorga *et al.*, 2012). Furthermore, some studies provide evidence for limited dispersal and biogeographic patterns in soil mesofauna (Wu *et al.*, 2011; Porco *et al.*, 2012). Greater dispersal limitation in soil matrix, compared with air, may lead to a stronger spatial structure in soil biota compared with aboveground organisms (Ettema and Wardle, 2002). Although dispersal limitation is commonly considered a neutral process, the overlap between dispersal and establishment success, which can result from environmental filtering, complicates accurate estimates of dispersal limitation *per se* (Lowe and McPeek, 2014). Besides, dispersal may not be neutral if species differ in traits related to dispersal abilities or when dispersal is density dependent.

Differential dispersal abilities may lead to contrasting DDS patterns in macro-organisms and microorganisms (Nekola and White, 1999; Soininen *et al.*, 2007; Hájek *et al.*, 2011; Farjalla *et al.*, 2012). These studies provide evidence that the effect of deterministic processes in community assembly increases with increasing body size, while stochastic processes are dominant in small organisms with passive dispersal. Most of our knowledge about DDS of various organisms is derived from meta-analyses (Nekola and White, 1999; Soininen *et al.*, 2007; Hanson *et al.*, 2012) that comprise studies with different sampling grain and geographic scale, which may strongly bias the understanding of DDS relationships (Steinbauer *et al.*, 2012). Thus simultaneous analyses of DDS in organisms with different dispersal traits (owing to their varying size and active vs passive motility) are needed for better understanding the relative contribution of deterministic and neutral processes (Soininen *et al.*, 2007; Hájek *et al.*, 2011; Bie *et al.*, 2012). Small-scale studies are subject to fewer confounding factors such as climate, soil type, random environmental fluctuations and history and thus provide a good basis to determine neutral stochasticity (drift and dispersal limitation *per se*; Vellend *et al.*, 2014).

Our knowledge about the spatial structure of eukaryotic microbes is very limited. Only a few studies have studied the DDS relationship at the fine scale (Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010). These studies were performed along an environmental gradient, which prevents assessing the effects of neutral processes in shaping communities. By using a spatially explicit experimental design, we examined DDS patterns in the communities of soil eukaryotic microbes and mesofauna that provide the key ecosystem services, such as plant nutrition, herbivory, decomposition and propagule dispersal (Wardle, 2002). Our main goal was to examine the stochastic component and thus to elucidate the relative importance of stochastic (dispersal and ecological drift) and deterministic factors (soil, vegetation parameters) in structuring communities of soil biota in the absence of environmental gradients. Our hypothesis was that microbial communities exhibit significant DDS due to their more pronounced intrinsic spatially aggregated distribution (Morlon *et al.*, 2008) as a result of the weak environmental variation and thus its weak confounding effect at homogeneous local habitats. Our second aim was to define the range of spatial aggregation in different microbial groups, with contrasting dispersal ability and body size. We simultaneously examined the spatial structure of protists, mesofauna and the major taxonomic as well as functional groups of fungi that differ in dispersal traits (for example, passive spore dispersal in protists and fungi vs mesofauna) and body size (1–10^3 µm in protists; 1 µm to 10^2 m in fungi; 10^2–10^4 µm in mesofauna). We carefully performed a neighbourhood analysis (Canham *et al.*, 2004) of the surrounding tree cover and examined soil nutrients properties to account for environmental effects.

Material and methods

Sampling and site characteristics

This study was carried out in three replicated sites in mixed temperate forests in southern Estonia. The Järvselja site (58°15' N, 27°19' E) represents an old-growth forest (130 years) on Luvisols that is dominated by *Picea abies* (L.) H.Karst. with abundant *Tilia cordata* Mill., understorey. The Maarjaküla site (58° 07' N, 27° 02' E) constitutes a mixed forest of *P. abies* and *Pinus sylvestris* L.

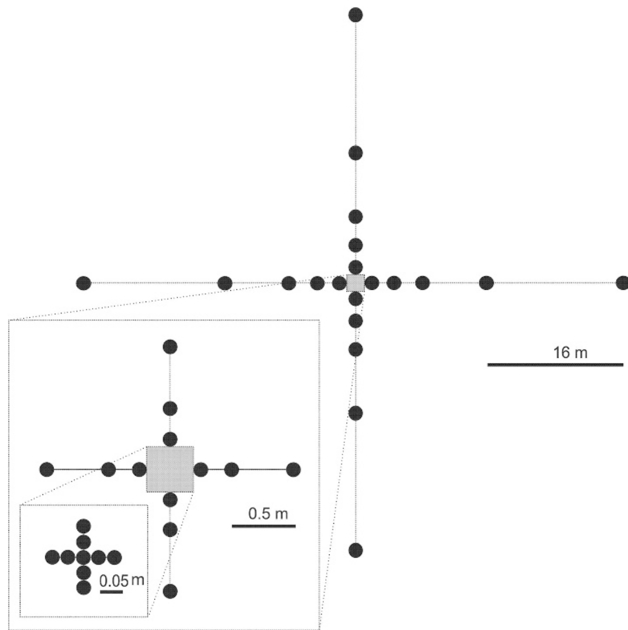

Figure 1 Schematic map of sampling design in each study site.

(age, 110 years) on podzols with abundant *Vaccinium myrtillus* L. shrubs and moss layer. The Elva site (58°11' N, 26°26' E) is a 100-year-old forest dominated by *P. sylvestris* with *P. abies* undergrowth, *V. myrtillus* shrub layer and mosses on podzols. In each site, we established a 64 × 64 m^2 plot in homogeneous areas in terms of vegetation (that is, tree basal area and plant community and coverage) and topography. There were no noticeable environmental gradients in these plots.

Within a single day in the summer of 2012, 41 soil cores (5 cm diameter to 5 cm depth) were taken from each plot in a nested design with base-2-logarithmically increasing distance between samples from 0.01 to 64 m (Figure 1). Such a sampling scheme enables to account for soil patchiness on a millimetre scale (Zhou *et al.*, 2004) and provides sufficient replication for distance classes from 0.01 to 32 m. The central sampling spot was randomly selected with the constraint that it had to comprise a uniform >0.25-m^2 patch of microtopography and ground vegetation and lie >1 m from any surrounding tree (>1 cm diameter at the base). Around each sample, a 0.5 × 0.5 m^2 quadrat was established, and the cover of understorey plant species was recorded. Precise location of all trees and their diameter at ~1.5 m height were determined to account for the potential neighbourhood effects on spatial distribution of pathogens and mycorrhizal fungi (Bahram *et al.*, 2011; Gómez-Aparicio *et al.*, 2012). Soils were kept at −30 °C for 24 h to suspend motile organisms before air-drying at 25 °C for 48 h. The soil samples were pulverized and 0.2 g of soil dust was taken to DNA extraction using the UltraClean 100 Kit (MoBio, Carlsbad, CA, USA), following the manufacturer's instructions. The remaining soil was used for chemical analysis of pH_{KCl} and concentrations of P_{total}, K, Ca, Mg, C, N, ^{15}N and ^{13}C (Supplementary Table S1) as described in Tedersoo *et al.*, (2012). In each soil sample, the proportion of O, A and E horizons were measured because of differences in functional composition of soil microbes (Zhou *et al.*, 2004; Lindahl *et al.*, 2007). There were only minor differences in the thickness of soil layers within each plot (mean ± s.d.: Organic layer, 3.40 ± 1.08 cm; Mineral layer, 1.60 ± 1.08 cm).

Molecular methods

We used the Internal Transcribed Spacer 2 (ITS2) as a locus for DNA metabarcoding, which offers higher resolution at species level compared with 18S rDNA (Bates *et al.*, 2013) and provides less primer bias compared with mitochondrial cytochrome oxidase I across multiple eukaryote kingdoms (Deagle *et al.*, 2014). PCR was carried out using a mixture of 11 modified ITS3ngs forward primers (in equimolar concentration) and a degenerate ITS4ngs reverse primer (Tedersoo *et al.*, 2016; Supplementary Table S2). PCR was performed with four replicates and programmed as follows: an initial 15 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final cycle of 10 min at 72 °C. These primers were modified to perfectly match the prominent soil eukaryotes, including Fungi, Viridiplantae, Ciliophora, Rhizaria, Straminipila and groups of mesofauna (such as Nematoda, Annelida and Collembola). Both forward and reverse primers were tagged with 1 of the 64 identifiers (10–12 bases). Negative (DNA extraction and PCR) and positive controls (single fungal specimens) were used throughout the experiment. The amplicons were purified using EXO-saprotrophic enzymes (Sigma, St Louis, MO, USA), normalized using a SequalPrep Normalization Kit (Invitrogen Inc., Carlsbad, CA, USA), pooled and subjected to 454 adaptor ligation, emulsion PCR and 454 pyrosequencing using the GS-FLX+ technology and Titanium chemistry. Sequences were submitted to Short Read Archive under accession number SRP045587.

Bioinformatics

Pyrosequencing reads were denoised by using the default settings (average quality cutoff = 30; significance level = −9) in Acacia (Bragg *et al.*, 2012). Reads were further trimmed using Mothur (Schloss *et al.*, 2009) by discarding regions with any ambiguous nucleotides and >10-bp homopolymers. Reads with mismatches to primer and tag combination and <150 bp were removed. Chimeras were checked in UCHIME (Edgar *et al.*, 2011). The full ITS2 region was extracted in the ITSx (Bengtsson-Palme *et al.*, 2013). The extracted sequences were assembled into Operational Taxonomic Units (OTUs) based on 97% pairwise similarity using CD-hit 4.6.1 (Fu *et al.*, 2012). We evaluated different clustering algorithms

(Uclust, Crop, CD-hit, Swarm and CAP3) by comparing the outcomes with phylogenetic trees of selected dominant genera (*Tomentella*, *Russula*). The phylogenetic trees were prepared under maximum likelihood model in RAxML 7.0.4 (Stamatakis *et al.*, 2008). Among available pairwise clustering methods, CD-hit gave very similar results to that deduced from the phylogenetic trees and resulted in the fewest OTUs. Singletons were removed from downstream analysis to eliminate potentially artefactual taxa (Tedersoo *et al.*, 2010). Blast searches were performed against both NCBI and UNITE databases. OTUs without any blast match or with $<60\%$ identity to best blast matches were removed from subsequent analyses. The taxonomy of OTUs was determined based on 10 best matches against these databases. The lifestyle of fungal OTUs was determined based on functional annotation of database sequences (Tedersoo and Smith, 2013; Tedersoo *et al.*, 2014). Because of high abundance of unidentified taxa in public databases, taxonomy of OTUs with blast score <100 and identity $<80\%$ were considered to be unknown.

Data analysis

Because of low representation in our data set, we excluded Straminipila, Alveolata and arbuscular mycorrhizal fungi from the analyses. We focussed not only on mesofauna, Rhizaria and Chlorophyta as well as major functional groups within Fungi such as saprotrophs, ectomycorrhizal (EcM) symbionts and plant pathogens but also on fungal taxonomic groups (Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota s.lato). We used a modified Raup–Crick dissimilarity metric that is independent of sample size (Chase *et al.*, 2011). This measure calculates the proportion of observed dissimilarities across samples that are higher than those estimated from the null model (1000 randomized matrices); it ranges from −1 to 1, with values near −1 (−0.95 to −1) indicating homogenizing dispersal (that is, mass effect), values near 1 (0.95 to 1) indicating environmental selection and other values (−0.95 to 0.95) indicating drift. Because beta diversity may result from both species replacement (species turnover component) and variation of species richness (nestedness component), we followed the approach of Baselga (2010) to address both processes in parallel. This approach enables partitioning variation that results from either nestedness or species turnover into individual distance matrices. Each group from each site was analysed individually; however, for simplicity, if possible, the average (±s.d. or confidence interval (CI)) statistics of the three sites are reported. Analyses were performed in Vegan, Ape, Betapart and Ecodist packages of R (R Development Core Team, 2007) and custom scripts in Python 2.7.

For each DDS relationship, we calculated the slope of relationship that indicates the rate of spatial turnover (Soininen *et al.*, 2007). The overall spatial structure was analysed by Mantel test. Using simple Mantel correlograms and semivariograms (based on the exponential fit of the relationship between dissimilarity of samples vs spatial distance), we calculated the spatial autocorrelation range, that is, the distance up to which the composition of samples is significantly more similar than expected (Robeson *et al.*, 2011). For each data set, we explored linear, exponential or no significant fit according to determination coefficients. For the groups with significant spatial autocorrelation, partial Mantel tests were performed to examine the correspondence of individual environmental variables with the observed spatial autocorrelation.

Principal Coordinates of Neighbouring Matrices vectors were calculated based on geographical distances among samples to represent different spatial scales (Borcard and Legendre, 2002). Variation partitioning was performed using the varpart function of the package Vegan (Oksanen *et al.*, 2013) to disentangle the effects of major component sources of variation, that is, vegetation, soil and space. Variables from each category (that is, vegetation, soil, spatial) were forward selected ($P<0.05$) before using in variation partitioning. For vegetation, both plant community and neighbourhood effects of surrounding trees were included. For soil variables, pH and concentrations of P, K, C and N and C:N ratio were included (Supplementary Table S1). Prior to the analysis, soil variables were log-transformed to approximate normal distribution. We tested the spatial autocorrelation in soil variables and vegetation (separately for trees and understorey vegetation) using Moran's I correlograms. We further explored the effect of individual environmental variables on the spatial distribution of belowground communities by incorporating biotic factors (neighbourhood effect, estimated herb and shrub cover and their community composition), and abiotic factors (soil pH, nutrients and depth of horizons) in partial Mantel correlograms as implemented in mpmcorrelogram package. Because of their potential influence on soil chemistry, neighbouring trees may have a great effect on spatial structure of soil communities. Using a neighbourhood analysis, we included the effect of conspecific and heterospecific neighbouring trees within 10 m radius based on the following equation:

$$\mathrm{NIF} = \sum_{i=0}^{n} \text{Area at breast height}_i \times \left(\frac{1}{\text{Distance}_i}\right),$$

where NIF is the neighbourhood effect. Distribution range of OTUs in different groups was calculated based on the average distance among samples where OTUs were present, and it was compared with the average distance among all samples as the expected null range. Further details on the Materials and Methods used in this study are provided in the Supplementary Methods.

Table 1 Results of spatial analysis across different taxonomic and functional groups of soil biota

	Site I			*Site II*			*Site III*		
	Mantel r	*Slope*	*SAR*	*Mantel r*	*Slope*	*SAR*	*Mantel r*	*Slope*	*SAR*
Metazoa	0.011	—[a]	—	− 0.1	—	—	0.083	—	—
Rhizaria	**0.338**[b]	**0.392**	**6**	**0.22**	**0.22**	—	0.102	—	**0.75**
Chlorophyta	− 0.24	—	—	0.13	—	—	0.124	—	—
Fungi	**0.171**	**0.032**	**0.25**	**0.18**	**0.011**	—	**0.294**	**0.039**	**1.25**
Fungal taxonomic groups									
Basidiomycota	**0.301**	**0.055**	**1.5**	0.101	—	**0.5**	**0.371**	**0.078**	**3**
Ascomycota	0.004	—	—	0.04	—	—	− 0.017	—	—
Chyteridiomycota	0.016	—		0.133	—	0.12	0.155	—	**0.7**
Zygomycota	0.153	—	0.25	0.181	—	—	**0.234**	**0.055**	**1.5**
Fungal functional groups									
Saprotrophic	0.137	—	—	**0.21**	**0.011**	—	**0.236**	**0.044**	**1.5**
Ectomycorrhizal	**0.203**	**0.063**	**2.5**	**0.242**	**0.028**	**0.5**	**0.365**	**0.326**	**3**
Pathogenic	− 0.147	—	—	−0.07	—	—	**0.216**	**0.260**	**3**

Abbreviation: SAR, spatial autocorrelation range (m).
[a]'—' indicates that a value was not measured owing to no significant relationship based on Mantel test ($P<0.05$).
[b]Values in bold indicates significant relationship.

Results

A total of 4131 OTUs (including 2282 singletons) were retrieved from 90 761 high-quality ITS2 sequences and 123 soil samples. Of the non-singleton OTUs, 1458 belonged to fungi (including 588 Basidiomycota, 243 Ascomycota, 476 Zygomycota and 69 Chytridiomycota), 70 to Metazoa, 66 to Rhizaria, 41 to Alveolata and 29 to Chlorophyta. Of the fungal OTUs, 197 were determined to be EcM, 818 saprotrophic, 38 pathogens and 5 arbuscular mycorrhizal symbionts (Supplementary Figure S1).

Mantel tests showed significant spatial turnover only in certain groups (Table 1). The rate of DDS was the greatest for Rhizaria, followed by fungal phyla Basidiomycota, Zygomycota and Chytridiomycota (Table 1). There was no significant DDS in the other taxonomic groups. EcM fungi showed the greatest DDS rate among the functional groups (mean ± s.d. of the slope of DDS in three sites: 0.139 ±0.133), followed by pathogens (0.087 ± 0.15) and saprotrophs (0.018 ± 0.023). The semivariogram analyses and Mantel correlograms revealed significant autocorrelation within smaller distance classes (< 3 m) in the majority of groups with significant autocorrelation (Table 1). In Basidiomycota and EcM fungi, significant autocorrelation was found in all three sites (mean ± s.d. of autocorrelation ranges in three sites: 1.33 ± 0.76 m; 1.16 ± 1.58 m; 2.50 ± 0.86 m, respectively). For Rhizaria, spatial autocorrelation range was significant in two sites (6 and 0.75 m). In Chlorophyta, Chytridiomycota, Zygomycota, saprotrophic and pathogenic fungi, significant autocorrelation was found only in one of the three sites (at 0.9, 0.7, 1.5, 1.5 and 3 m, respectively; Table 1). In site III, a larger proportion of groups (81%) showed significant spatial autocorrelation compared with the other sites (36% and 27% in sites II and I, respectively). There was no significant spatial autocorrelation in Ascomycota and Metazoa. Across all groups, the observed significant DDS was related to species replacement, whereas the nestedness component was not significant (Supplementary Table S3). A test of correspondence among different communities based on Mantel test revealed weak but in some cases significant relationships between the communities of different groups (Supplementary Table S4).

Community turnover was lower than expected by the null model (1000 randomized matrices) in the majority of organism groups (55% of communities; Figure 2). The average Raup–Crick distance was significantly lower than the null model in Basidiomycota (mean ± CI in three sites: − 0.406 ± 0.037), saprotrophic fungi (−0.755 ± 0.026), Zygomycota (−0.586 ± 0.031) and Rhizaria (−0.227 ± 0.041), but it was slightly greater than expected by the null model in EcM (0.018 ± 0.034) and pathogenic (0.044 ± 0.039) fungi. Similarly, the average Raup–Crick pairwise distances within the smallest distance class (0–6 m) was the highest in EcM fungi, followed by pathogenic and saprotrophic fungi. For all groups, environmental selection and dispersal limitation (calculated as ratio of dissimilarity values between 0.95 and 1) was negligible (< 0.01). In contrast, mass effect (dissimilarity values between − 0.95 and − 1) was significant in Basidiomycota (15.56%) and Zygomycota (28.4%) but not in Ascomycota (2.8%), Chytridiomycota (1.3%) or other eukaryotes (Supplementary Figure S2). The mass effect was significantly stronger in saprotrophic (51.87%) than in EcM (4.63%) and pathogenic (1.43%) fungi.

The average distribution range of OTUs of the majority of groups was significantly higher than predicted by the null model, that is, the average range and its confidence intervals were higher than average distance among samples (Figure 3).

Within plots, the average distribution range of OTUs in Metazoa (mean ± CI in three sites: 17.61 ± 2.87 m), Rhizaria (19.71 ± 3.49 m), Basidiomycota (17.42 ± 1.14 m), Ascomycota (14.29 ± 1.45 m), and Zygomycota (27.03 ± 1.25 m) were greater than the average distance among samples (11.10 ± 0.84 m). Across the taxonomic groups, the average distribution range of OTUs was the lowest in Chlorophyta and the highest in Zygomycota (Supplementary Figure S3). On average, saprotrophic fungi (22.50 ± 0.93 m) exhibited significantly greater distribution range compared with EcM (15.73 ± 1.77 m) and pathogenic (15.38 ± 4.91 m) fungi.

Variation partitioning analyses revealed that the neighbourhood effect, plant community and soil pH are the main determinants of community composition in most studied groups (Figure 4). Only a small proportion of variation was explained by space, environment and their shared effect (Figure 4) in the communities of Metazoa (mean ± s.d. in three sites: 6.00 ± 3.56%), Chlorophyta (6.00 ± 4.35%), Rhizaria (7.33 ± 4.02) and Fungi (4.34 ± 2.05). Similar results were obtained for the fungal groups: Ascomycota (4.67 ± 1.53%), Basidiomycota (8.0 ± 3.46%), Chytridiomycota (8.66 ± 0.58%), Zygomycota (9.33 ± 2.62%), saprotrophic fungi (6.34 ± 2.16%), pathogenic fungi (9.33 ± 1.52%), and EcM fungi (7.67 ± 3.26%). Pure spatial effect explained a small proportion of the variation in communities of Rhizaria (3.00 ± 2.65%), Chlorophyta (0%), Metazoa (2.67 ± 3.05%) and fungal groups, including Chytridiomycota (3.0 ± 1.0%), Ascomycota (1.33 ± 2.31%), Zygomycota (3.00 ± 1.0%), Basidiomycota (2.00 ± 2.64%), pathogenic fungi (1.67 ± 2.89%), saprotrophic fungi (2.67 ± 1.15%) and EcM fungi (3.5 ± 0.35%). Pathogenic and EcM fungal communities were significantly related to plant community (explaining 4.67 ± 1.7 and 5.66 ± 2.30% of EcM and pathogenic fungal communities, respectively), compared with saprotrophic fungal communities (Figure 4). Nonetheless, similarly to vegetation, soil variables had a weak relationship with the spatial variation of communities. At small distance classes (on average <2 m), soil and the neighbourhood component (significant in all sites: 2.50 ± 2.17 m), C:N (significant in site I and site III: 1.75 ± 1.76 m) and pH (significant in site I and site III: 1.75 ± 1.76 m) showed significant spatial structure which more or less corresponded to the observed autocorrelation in the communities (Table 1). There was also significant spatial structure in $\delta^{15}N$ or $\delta^{13}C$ at <1 m scale (Supplementary Figure S3). Partial Mantel correlograms further revealed significant correlation between the spatial autocorrelation of plant community and neighbourhood effect at the first distance class (on average <2 m) in the majority of groups, whereas soil parameters had a negligible effect (Figure 5; Supplementary Figure S3).

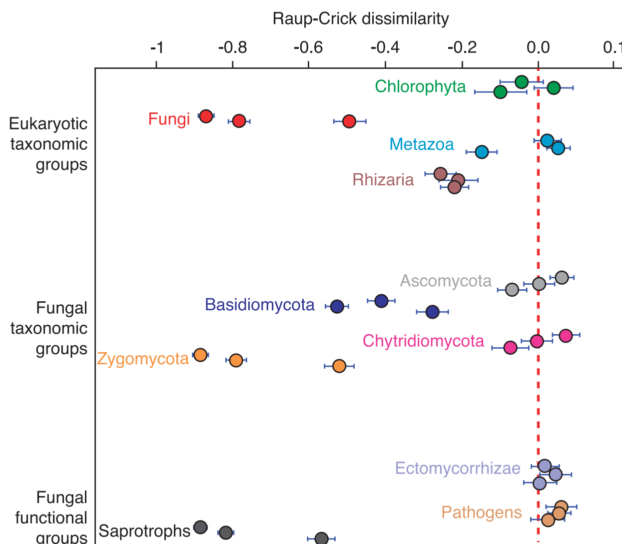

Figure 2 Deviations from the null-model expectations in the community turnover of soil biota, measured as the average Raup–Crick index of dissimilarity between samples and its confidence intervals. Positive and negative values are, respectively, higher and lower dissimilarities between samples compared with the null model, with values near − 1 (−0.95 to − 1) indicating mass effect, values near 1 (0.95 to 1) indicating environmental selection and values between − 0.95 and 0.95 indicating drift. The vertical red dashed line indicates zero dissimilarity.

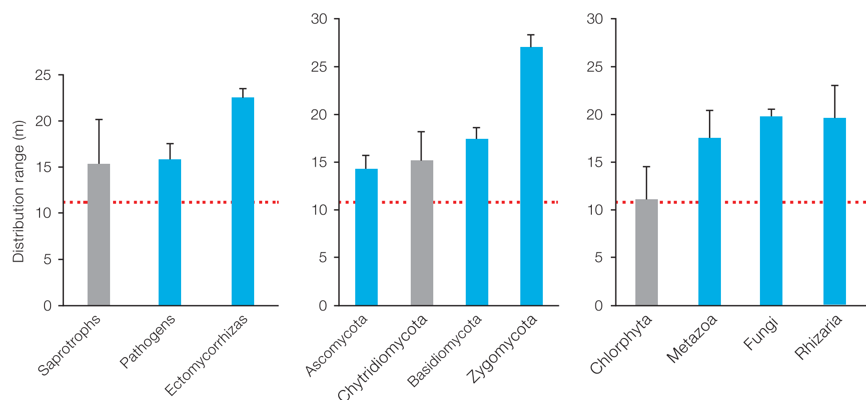

Figure 3 The average distribution range of OTUs across different taxonomic and functional groups within each plot. The range of each OTU was calculated based on the maximum distance between individuals of that OTU. Groups that are indicated with blue colour had significantly higher distribution range than average distance among samples (dotted red line).

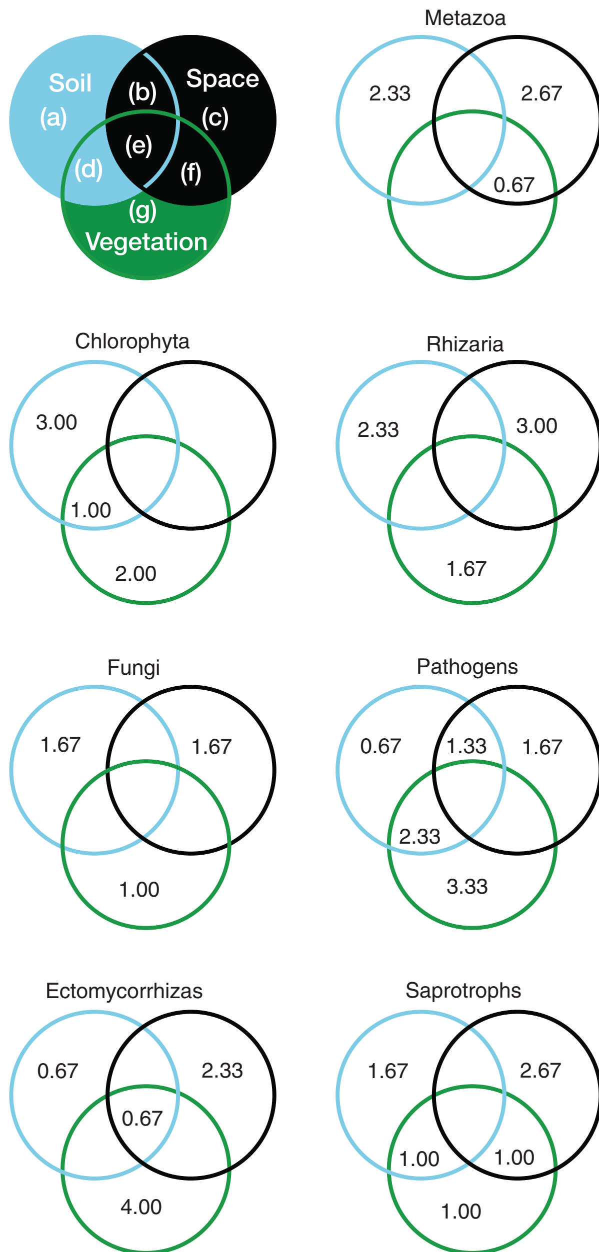

Figure 4 Venn diagram of variation partitioning analysis, illustrating the effects of soil, vegetation and spatial variables on the community structure of soil biota. Values show the percentage of variation explained by each fraction, including pure soil effect (**a**), shared effect between soil and space (**b**), pure spatial effect (**c**), shard effect of soil and vegetation (**d**), shared effect of soil, space and vegetation (**e**), shared effect of space and vegetation (**f**) and pure vegetation effect (**g**). Vegetation includes both plant community and spatial distribution of trees diameters at breast height (that is, neighbourhood component). Note that the fraction of unexplained variation and values <1% are not shown for simplicity.

Discussion

Small-scale biogeography of eukaryotic microbes has received little empirical attention so far, perhaps owing to the common assumption of no dispersal limitation in microbes (Finlay, 2002). Our study contributes to understanding the fine-scale spatial structure of different taxonomic and functional groups of soil eukaryotes and sheds light into the underlying processes. Small scale and homogeneous vegetation of our study sites enabled us to minimize the effects of environmental and historical factors. Our analysis revealed that most groups of the addressed soil biota exhibit negligible fine-scale spatial distribution patterns (Table 1; Figure 2). In spite of some level of heterogeneity in the soil variables (Supplementary Figure S3), tree neighbourhood and soil had a small effect on community composition of most groups of soil biota. Although our sampling addressed fine-scale spatial distances spanning three orders of magnitude, most of the soil biota was only marginally structured by the spatial framework, and generally >90% of community variation remained unexplained by space or the environment. This could be ascribed to stochasticity in these spatially homogeneous systems, unmeasured environmental variables or methodological issues (see below).

Our results support the prominent role of stochastic processes, that is, mass effect coupled with stochastic ecological drift (Chase *et al.*, 2011) in structuring microbial communities at the small scale. A large fraction of pairwise community comparisons did not deviate from the null model, that is, 1000 randomized matrices (Figure 2), indicating ecological drift acting alone as the main determinant of community structure (Stegen *et al.*, 2013). In the majority of groups, the average distribution range of OTUs was greater than expected by chance (Figure 3), reflecting homogenizing effect of dispersal (that is, mass effect). Together with the relatively minor spatial effect on communities, the wide distribution range of taxa suggests that dispersal limitation has only a weak influence on spatial turnover in soil biota at the local scale. A few previous studies on arbuscular mycorrhizal fungi over a similar geographical scale revealed significant DDS and the dominance of deterministic processes in structuring soil communities along strong environmental gradients (Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010). Focussing on a local scale in the absence of environmental gradients enabled us to reduce the effect of deterministic processes. High taxonomic richness coupled with short generation periods, functional redundancy and strong priority effects (Soininen *et al.*, 2007; Vellend, 2010) add to the stochasticity. This may override the weak effect of environmental factors as well as dispersal limitation and lead to weak microbial spatial structure in the absence of strong environmental heterogeneity at the local scale. Moreover, soil comprises resting propagules such as pollen, seeds, spores and eggs, the passive dispersal of which and detection along with the active community may reduce the resolution of environmental and spatial effects. Several studies at larger scales provide evidence for the

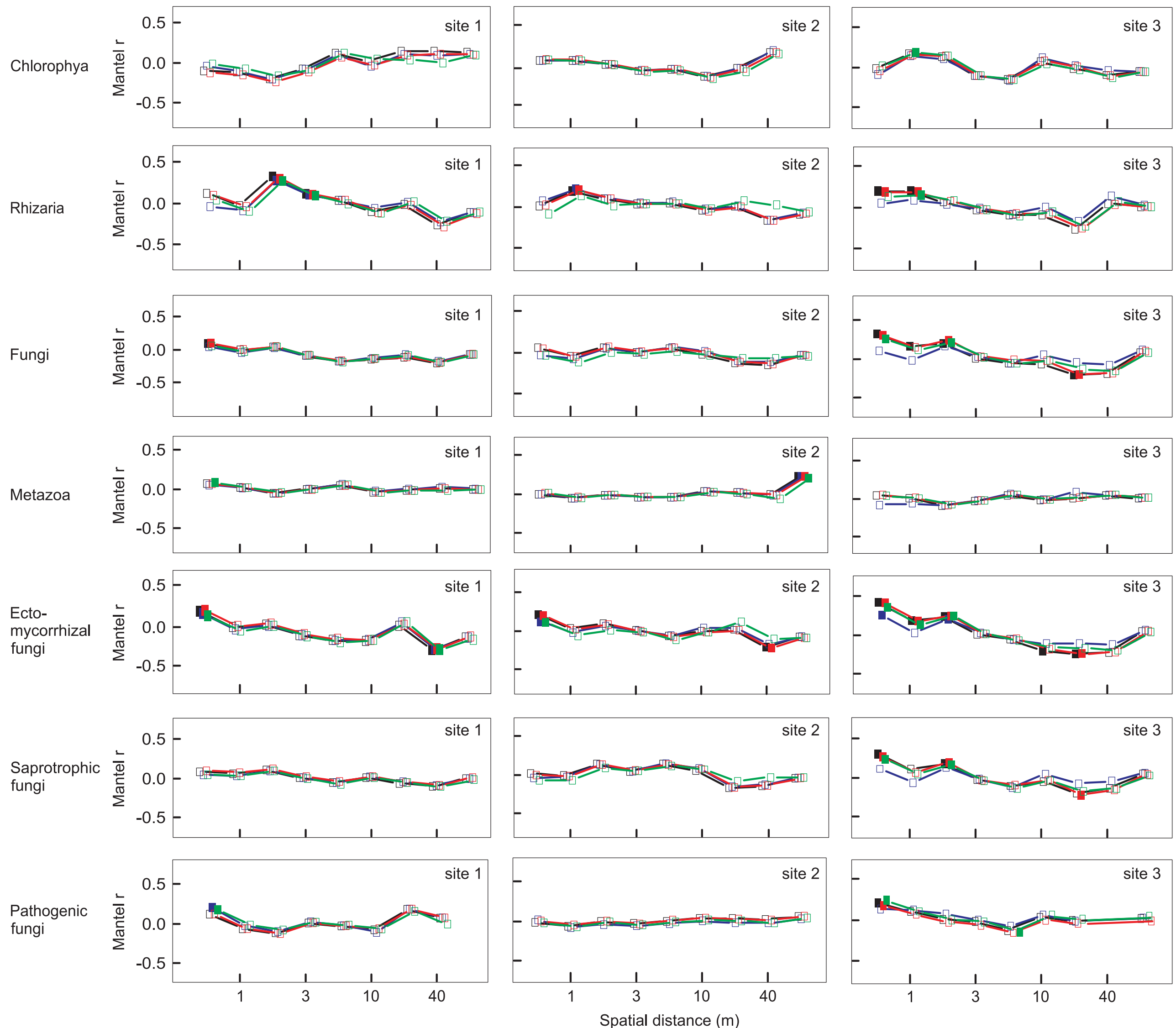

Figure 5 Spatial structure of the communities at various distance classes as revealed by Mantel and Partial Mantel correlograms in relation to biotic and abiotic factors, including pure spatial (black line and squares) plant community (blue line and squares), soil parameters (red line and squares) and neighbourhood effect (green line and squares). Distance classes were defined as follows (in m): 0–0.5, 0.5–1, 1–2.5, 2.5–3.5, 3.5–5, 5–11, 11–21, 21–41 and 41–64. Filled squares represent significant Mantel *r* after Bonferroni multiple test correction.

significant DDS in microbial communities independent of environmental heterogeneity (Martiny *et al.*, 2011; Robeson *et al.*, 2011; Talbot *et al.*, 2014), indicating substantial historical dispersal limitation and drift.

Communities of different taxonomic and functional groups may be structured by contrasting underlying factors. We found the weakest DDS rate in soil mesofauna that could relate to their high dispersal rate at the fine scale (Table 1). EcM and pathogenic fungi exhibited relatively higher spatial turnover than expected by the null model, whereas saprotrophic fungi and Rhizaria had significantly lower spatial turnover than expected (Figure 3). These results are consistent with random dispersal that has been reported for soil protozoa and mesofauna (Petersen and Luxton, 1982; Esteban *et al.*, 2006; Lara *et al.*, 2011). This can be attributed to their high dispersal rate, which overwhelms both environmental selection and ecological drift. The high dispersal in this group was also supported by their greater distribution range than the null model. In particular, the distribution range of saprotrophic fungal OTUs exceeded that of the other groups, for which we found the largest ratio of deviations from the null model (Figure 2). The weak DDS was observed across all taxonomic groups of saprotrophic fungi (that is, saprotrophic Basidiomycota, Ascomycota and Zygomycota). Spatial structure of saprotrophic fungi was mainly related to soil variables, whereas EcM and pathogenic fungi showed little correspondence with soil. This is

expected given that EcM and pathogenic fungi gain most of their C demand directly from host plants, while saprotrophic fungi rely on organic material of soil (Plett and Martin, 2011). Compared with the other studied groups, we detected relatively stronger spatial structure as well as greater vegetation and neighbourhood effect in EcM fungal communities. We also found a strong spatial structure in a pathogenic fungal community in one site, which was mainly related to plant community based on partial Mantel test, by testing the correlation between plant and fungal communities while controlling for soil and spatial components. These results indicate stronger spatial structuring of biotrophic groups that correspond to vegetation patchiness. The importance of aboveground interactions on these groups was also supported by the scale of the observed spatial autocorrelation range in EcM fungi, which nearly corresponded to that in the spatial distribution of trees in our study sites. Spatial distance has an important role in maintaining symbiotic (Bever *et al.*, 2009) and pathogenic relationships (Kerr *et al.*, 2006) through positive and negative soil feedbacks, respectively.

At very fine scales (<2 m), spatial autocorrelation resulting from aggregate growth of individuals affects community turnover in certain eukaryotic microbes. In aboveground macro-organisms, body size is strongly linked to dispersal limitation and thus spatial aggregation (Nekola and White, 1999). In fungi, most of their body comprises vegetatively spreading mycelium in soil, reaching up to 100 m in extreme cases (Douhan *et al.*, 2011). Therefore, in certain fungal groups, our sampling may capture the same individual multiple times, which less likely occurs in mesofauna or protists. This could result in spatial aggregation of species that may consequently affect DDS (Morlon *et al.*, 2008). The spatial structure in other groups may be detectable beyond the scale of our study. For example, spatial structure in nematode populations can be detected at the scale of more than tens of metres (Liang *et al.*, 2005), whereas bacterial communities shift within a few millimetres because of differences in soil microhabitats (Zhou *et al.*, 2004). Soil animals comprise organisms of very different size and mobility, which may blur their distribution patterns because of multiple conflicting signals. Because of the strong effect of grain size on the detection of spatial structure (Nekola and White, 1999), the resolution of our samples, including their lag distance (1 cm) and grain size (5 cm), could have been insufficient for detecting spatial aggregation in some of the groups.

Our data also indicate that heterogeneity of vegetation rather than edaphic variables may contribute to spatial patterning of soil microbes at the very fine scale, that is, <2 m (Figure 5). We found significant spatial autocorrelation in plant communities, neighbourhood effect as well as soil pH and C:N ratio at the scale of <2 m (Supplementary Figure S3). These factors exhibited relatively stronger correlation with community, and partial Mantel correlograms indicated that these could explain the observed spatial autocorrelation in certain studied groups, in particular Rhizaria, EcM, saprotrophic and pathogenic fungi. Previous studies have shown that soil chemistry, particularly pH, strongly impacts microbial communities (Franklin and Mills, 2003; Rousk *et al.*, 2010). Soil pH and N may be spatially autocorrelated up to 5 m (Ettema and Wardle, 2002; Pärtel *et al.*, 2008; Baldrian *et al.*, 2010). Besides the great spatial variability of soil nutrients and pH that is commonly observed in forest soil (Ettema and Wardle, 2002; Štursová and Baldrian, 2011), soil microbial biomass may also be spatially autocorrelated up to 10 m (Ritz *et al.*, 2004), which may affect soil spatial heterogeneity and in turn microbial communities (Lauber *et al.*, 2009). The neighbourhood effect of trees and influence of understorey vegetation were relatively more important in shaping communities of biotrophic fungi rather than free-living organisms.

Conclusions

Our study provides empirical support for the assumption of no dispersal limitation in eukaryotic microbial distribution at small scales. Communities of soil eukaryote groups with varying size and dispersal traits exhibit negligible DDS patterns and little correspondence to deterministic factors along a weak local environmental gradient, indicating stochastic distribution patterns consistent with neutral theory. For all groups, mass effect and ecological drift appear to be the main drivers of communities in the absence of environmental gradients. Nevertheless, we detected some level of spatial autocorrelation in certain groups, in particular EcM and pathogenic fungi, which mainly reflects their correspondence to the spatial structure of vegetation.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank M Mett for help during field sampling. M Nõukas and R Puusepp for sample preparation. We also thank Ari Jumpponen and five anonymous referees for their constructive comments on the earlier version of this manuscript. This study was funded by ESF grant nos. 9286, PUT0171, NPUI LO1417 MSMT and FIBIR.

References

Astorga A, Oksanen J, Luoto M, Soininen J, Virtanen R, Muotka T (2012). Distance decay of similarity in

freshwater communities: do macro-and microorganisms follow the same rules? *Glob Ecol Biogeogr* **21**: 365–375.

Bahram M, Põlme S, Kõljalg U, Tedersoo L (2011). A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiol Ecol* **75**: 313–320.

Bahram M, Koljalg U, Courty P, Diedhiou AG, Kjøller R, Polme S *et al.* (2013). The distance decay of similarity in communities of ectomycorrhizal fungi in different ecosystems and scales. *J Ecol* **101**: 1335–1344.

Baldrian P, Merhautová V, Cajthaml T, Petránková M, Šnajdr J (2010). Small-scale distribution of extracellular enzymes, fungal, and bacterial biomass in *Quercus petraea* forest topsoil. *Biol Fertil Soils* **46**: 717–726.

Baselga A (2010). Partitioning the turnover and nestedness components of beta diversity. *Glob Ecol Biogeogr* **19**: 134–143.

Bates ST, Clemente JC, Flores GE, Walters WA, Parfrey LW, Knight R *et al.* (2013). Global biogeography of highly diverse protistan communities in soil. *ISME J* **7**: 652–659.

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A *et al.* (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol Evol* **4**: 914–919.

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M (2009). Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecol Lett* **12**: 13–21.

Bie T, Meester L, Brendonck L, Martens K, Goddeeris B, Ercken D *et al.* (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecol Lett* **15**: 740–747.

Borcard D, Legendre P (2002). All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol Model* **153**: 51–68.

Bragg L, Stone G, Imelfort M, Hugenholtz P, Tyson GW (2012). Fast, accurate error-correction of amplicon pyrosequences using acacia. *Nat Methods* **9**: 425–426.

Canham CD, LePage PT, Coates KD (2004). A neighborhood analysis of canopy tree competition: effects of shading versus crowding. *Can J Forest Res* **34**: 778–787.

Chase JM, Kraft NJB, Smith KG, Vellend M, Inouye BD (2011). Using null models to disentangle variation in community dissimilarity from variation in α-diversity. *Ecosphere* **2**: 1–11.

Chase JM (2014). Spatial scale resolves the niche versus neutral theory debate. *J Veg Sci* **25**: 319–322.

Chase JM, Leibold MA (2003). *Ecological Niches: Linking Classical and Contemporary Approaches*. University of Chicago Press: Chicago, IL, USA.

Chave J (2004). Neutral theory and community ecology. *Ecol Lett* **7**: 241–253.

Condit R, Pitman N, Leigh EG Jr, Chave J, Terborgh J, Foster RB *et al.* (2002). Beta-diversity in tropical forest trees. *Science* **295**: 666–669.

Cottenie K (2005). Integrating environmental and spatial processes in ecological community dynamics. *Ecol Lett* **8**: 1175–1182.

Deagle BE, Jarman SN, Coissac E, Pompanon F, Taberlet P (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. *Biol Lett* **10**: 20140562.

Douhan GW, Vincenot L, Gryta H, Selosse MA (2011). Population genetics of ectomycorrhizal fungi: from current knowledge to emerging directions. *Fungal Biol* **115**: 569–597.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010). Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME J* **4**: 337–345.

Edgar RC, Haas BJ, Clemente J C, Quince C, Knight R (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Esteban GF, Clarke KJ, Olmo JL, Finlay BJ (2006). Soil protozoa—an intensive study of population dynamics and community structure in an upland grassland. *Appl Soil Ecol* **33**: 137–151.

Ettema CH, Wardle DA (2002). Spatial soil ecology. *Trends Ecol Evol* **17**: 177–183.

Farjalla VF, Srivastava DS, Marino NAC, Azevedo FD, Dib V, Lopes PM *et al.* (2012). Ecological determinism increases with organism size. *Ecology* **93**: 1752–1759.

Finlay BJ (2002). Global dispersal of free-living microbial eukaryote species. *Science* **296**: 1061–1063.

Franklin RB, Mills AL (2003). Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiol Ecol* **44**: 335–346.

Fu L, Niu B, Zhu Z, Wu S, Li W (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* **28**: 3150–3152.

Gómez-Aparicio L, Ibánez B, Serrano MS, De Vita P, Avila JM, Pérez-Ramos IM *et al.* (2012). Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. *New Phytol* **194**: 1014–1024.

Green JL, Holmes AJ, Westoby M, Oliver I, Briscoe D, Dangerfield M *et al.* (2004). Spatial scaling of microbial eukaryote diversity. *Nature* **432**: 747–750.

Hájek M, Roleček J, Cottenie K, Kintrová K, Horsák M, Poulíčková A *et al.* (2011). Environmental and spatial controls of biotic assemblages in a discrete semi-terrestrial habitat: comparison of organisms with different dispersal abilities sampled in the same plots. *J Biogeogr* **38**: 1683–1693.

Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JB (2012). Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat Rev Microbiol* **10**: 497–506.

Hubbell SP (2001). *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press: Princeton, NJ, USA.

Kerr B, Neuhauser C, Bohannan BJ, Dean AM (2006). Local migration promotes competitive restraint in a host–pathogen 'tragedy of the commons'. *Nature* **442**: 75–78.

Lara E, Heger TJ, Scheihing R, Mitchell EA (2011). COI gene and ecological data suggest size-dependent high dispersal and low intra-specific diversity in free-living terrestrial protists (Euglyphida: Assulina). *J Biogeogr* **38**: 640–650.

Lauber CL, Hamady M, Knight R, Fierer N (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* **75**: 5111–5120.

Legendre P, Mi X, Ren H, Ma K, Yu M, Sun I *et al.* (2009). Partitioning beta diversity in a subtropical broad-leaved forest of China. *Ecology* **90**: 663–674.

Lekberg Y, Koide R T, Rohr J R, Aldrich-Wolfe L, Morton JB (2007). Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *J Ecol* **95**: 95–105.

Liang W, Jiang Y, Li Q, Liu Y, Wen D (2005). Spatial distribution of bacterivorous nematodes in a Chinese Ecosystem Research Network (CERN) site. *Ecol Res* **20**: 481–486.

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Hogberg P, Stenlid J *et al.* (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol* **173**: 611–620.

Lowe WH, McPeek MA (2014). Is dispersal neutral? *Trends Ecol Evol* **29**: 444–450.

Martiny JB, Eisen JA, Penn K, Allison SD, Horner-Devine MC (2011). Drivers of bacterial beta-diversity depend on spatial scale. *Proc Natl Acad Sci USA* **108**: 7850–7854.

Morlon H, Chuyong G, Condit R, Hubbell S, Kenfack D, Thomas D *et al.* (2008). A general framework for the distance–decay of similarity in ecological communities. *Ecol Lett* **11**: 904–917.

Nekola JC, White PS (1999). The distance decay of similarity in biogeography and ecology. *J Biogeogr* **26**: 867–878.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara R *et al.* (2013). Package 'vegan'.

Pärtel M, Laanisto L, Wilson SD (2008). Soil nitrogen and carbon heterogeneity in woodlands and grasslands: contrasts between temperate and tropical regions. *Glob Ecol Biogeogr* **17**: 18–24.

Petersen H, Luxton M (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos* **39**: 288–388.

Plett JM, Martin F (2011). Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. *Trends Genet* **27**: 14–22.

Porco D, Potapov M, Bedos A, Busmachiu G, Weiner WM, Hamra-Kroua S *et al.* (2012). Cryptic diversity in the ubiquist species *Parisotoma notabilis* (Collembola, Isotomidae): a long-used chimeric species? *PLoS One* **7**: e46056.

Poulin R (2003). The decay of similarity with geographical distance in parasite communities of vertebrate hosts. *J Biogeogr* **30**: 1609–1615.

Queloz V, Sieber TN, Holdenrieder O, McDonald BA, Grünig C R (2011). No biogeographical pattern for a root-associated fungal species complex. *Glob Ecol Biogeogr* **20**: 160–169.

R Development Core Team (2007). R: A Language and Environment for Statistical Computing. R.F.f.S. Computing: Vienna, Austria.

Ritz K, McNicol J, Nunan N, Grayston S, Millard P, Atkinson D *et al.* (2004). Spatial structure in soil chemical and microbiological properties in an upland grassland. *FEMS Microbiol Ecol* **49**: 191–205.

Robeson MS, King AJ, Freeman KR, Birky CW Jr, Martin AP, Schmidt SK (2011). Soil rotifer communities are extremely diverse globally but spatially autocorrelated locally. *Proc Natl Acad Sci USA* **108**: 4406–4410.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG *et al.* (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* **4**: 1340–1351.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB *et al.* (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Soininen J, McDonald R, Hillebrand H (2007). The distance decay of similarity in ecological communities. *Ecography* **30**: 3–12.

Stamatakis A, Hoover P, Rougemont J (2008). A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* **57**: 758–771.

Stegen JC, Lin X, Fredrickson J K, Chen X, Kennedy DW, Murray CJ *et al.* (2013). Quantifying community assembly processes and identifying features that impose them. *ISME J* **7**: 2069–2079.

Steinbauer MJ, Dolos K, Reineking B, Beierkuhnlein C (2012). Current measures for distance decay in similarity of species composition are influenced by study extent and grain size. *Glob Ecol Biogeogr* **21**: 1203–1212.

Štursová M, Baldrian P (2011). Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. *Plant Soil* **338**: 99–110.

Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI *et al.* (2014). Endemism and functional convergence across the North American soil mycobiome. *Proc Natl Acad Sci USA* **111**: 6341–6346.

Tedersoo L, Bahram M, Cajthaml T, Põlme S, Hiiesalu I, Anslan S *et al.* (2016). Tree diversity and species identity effects on soil fungi, protists and animals are context-dependent. *ISME J* **10**: 346–362.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R *et al.* (2014). Global diversity and geography of soil fungi. *Science* **346**: 1256688.

Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, Saar I *et al.* (2010). 454 pyrosequencing and sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytol* **188**: 291–301.

Tedersoo L, Smith ME (2013). Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biol Rev* **27**: 83–99.

Tedersoo L, Naadel T, Bahram M, Pritsch K, Buegger F, Leal M *et al.* (2012). Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytol* **195**: 832–843.

Vellend M (2010). Conceptual synthesis in community ecology. *Q Rev Biol* **85**: 183–206.

Vellend M, Srivastava DS, Anderson KM, Brown CD, Jankowski JE, Kleynhans EJ *et al.* (2014). Assessing the relative importance of neutral stochasticity in ecological communities. *Oikos* **123**: 1420–1430.

Wang J, Shen J, Wu Y, Tu C, Soininen J, Stegen JC *et al.* (2013). Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. *ISME J* **7**: 1310–1321.

Wardle DA (2002). *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton University Press: Princeton, NJ, USA.

Warren DL, Cardillo M, Rosauer DF, Bolnick DI (2014). Mistaking geography for biology: inferring processes from species distributions. *Trends Ecol Evol* **29**: 572–580.
Wu T, Ayres E, Bardgett RD, Wall DH, Garey JR (2011). Molecular study of worldwide distribution and diversity of soil animals. *Proc Natl Acad Sci USA* **108**: 17720–17725.
Zhou J, Xia B, Huang H, Palumbo AV, Tiedje JM (2004). Microbial diversity and heterogeneity in sandy subsurface soils. *Appl Environ Microbiol* **70**: 1723–1734.

Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)