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Variation in forest soil fungal diversity along a latitudinal gradient

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Abstract In forest ecosystems, plant communities shape soil fungal communities through the provisioning of carbon. Although the variation in forest composition with latitude is well established, little is known about how soil fungal communities vary with latitude. We collected soil samples from 17 forests, along a latitudinal transect in western China. Forest types covered included boreal, temperate, subtropical and tropical forests. We used 454 pyrosequencing techniques to analyze the soil communities. These data were correlated with abiotic and biotic variables to determine which factors most strongly influenced fungal community composition. Our results indicated that temperature, latitude, and plant diversity most strongly influence soil fungal community composition. Fungal diversity patterns were unimodal, with temperate forests (mid latitude) exhibiting the greatest diversity. Furthermore, these diversity patterns indicate that fungal diversity was highest in the forest systems with the lowest tree diversity (temperate forests). Different forest systems were dominated by different fungal subgroups, ectomycorrhizal fungi dominated in boreal and temperate forests; endomycorrhizal fungi dominated in the tropical rainforests, and non-mycorrhizal fungi were best represented in subtropical forests. Our results suggest that soil fungal communities are strongly dependent on vegetation type, with fungal diversity displaying an inverse relationship to plant diversity.

Keywords Soil · Fungal communities · Latitudinal gradient · Abiotic factors · Biotic factors · Boreal, temperate, subtropical, and tropical forest · Biodiversity pattern

Introduction

Soil fungi are critical components of microbial communities in terrestrial ecosystems, where they are integral constituents

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of decomposition and nutrient cycles (Warcup 1951). Furthermore, there are many plant symbionts and pathogens within soil fungal communities, which greatly affect the health of forests (Maron et al. 2011). Considering the significant role that soil fungi play in forest health and productivity, it is critical to catalogue their diversity and composition, and better understand how these factors change according to biotic and abiotic variables. In the past, soil fungal diversity and composition have primarily been studied at a local scale, and are reportedly affected by a wide range of biotic and abiotic factors (Johnson et al. 2004; Lauber et al. 2008; Barcenas-Moreno et al. 2009; Hawkes et al. 2011). However, we lack a clear understanding of how soil fungal communities change over larger distances and to which variables these changes are most strongly related.

Given that soil fungi are best known for their relationships with plants, whether symbiotic, parasitic, or saprophytic, it is not surprising that current soil fungal biogeography is primarily defined by the global distribution of known plant hosts and plant-defined biomes (Berg and Smalla 2009). Several studies surveyed fungal diversity in different forest ecosystems, suggested a strong correlation between fungal and plant diversity, due to fungal host specificity (Peay et al 2013). The underline mechanism has been studied previously (Gao et al 2013). The C available to soil microorganisms is ultimately derived from plant photosynthesis, and there are two major ways for fungi to acquire this plant C: aboveground input from litter and belowground input through the roots (Högberg et al. 2001). Different fungal groups can access this C by different means: ectomycorrhizal and endomycorrhizal fungal source C directly from host roots while saprophytic fungi derive carbon from the decomposition of dead plant material (Hršelová et al. 1999). Therefore, the important mechanism underlying plant-fungal diversity interactions is the quantity and quality of carbon resources, which vary among plant species and ecosystems.

Temperature and precipitation are known to affect photosynthetic processes and, as a result, litter production (Boisvenue and Running 2006). In addition to these climate factors, photosynthetic C production is also affected by soil physical and chemical properties (Davidson and Janssens 2006). Photosynthetic C destined for the root systems, including mycorrhiza, is limited when the host roots are exposed to high levels of soil phosphate and nitrogen, due to regulatory feedbacks within the host (Treseder and Allen 2002; Mortimer et al. 2008, 2012). Saprophytic fungi are also sensitive to soil conditions, soil pH, texture, moisture and oxygen levels all impact fungal development and ultimately plays a determining role in fungal community composition (Setala and McLean 2004). Understanding the response of fungal communities to biotic and abiotic factors will contribute to our understanding of how environmental factors influence fungal diversity. A further factor that is known to influence fungal community composition is the competition between fungal groups. Studies have shown that mycorrhizal fungi outcompete saprophytic fungi in the organic and upper layers of the soil, which is likely due to the reliable source of host derived C supplied to the mycorrhizal fungi (Lindahl et al. 2007; Voříšková and Baldrian 2013).

In the past, surveys of soil fungal diversity have relied heavily on the identification of sporocarp morphology. However, such an approach is limited to the species that produce sporocarps and ignores the large number of soil fungi that do not and also depends on the quality of the sporocarp identifications (taxonomic impediment), as well on the environmental factors known to influence sporocarp production (Brock et al. 2009; Nouhra et al. 2012). In recent years, DNA sequence-based methods have been used to survey soil fungi from a range of forests and have revealed an extremely high fungal diversity in soils (Lim et al. 2010; Lumini et al. 2010; Tedersoo et al. 2010)). However, in contrast to bacterial studies, surveys of soil fungal diversity and composition across large geographic scales remain rare (Green et al. 2004; Öpik et al. 2006) even though molecular techniques now offer a unique possibility and potential to study large scale biogeographical patterns (Fierer and Jackson 2006). Our aim was to investigate the diversity of soil fungi along a latitudinal gradient, encompassing a broad range of forest types, soil types, and climatic zones. We selected an area in western China for the purposes of this study as we could access a large range of relatively undisturbed forests following such a gradient. Forest types included tropical, sub-tropical, temperate and boreal forests, all occurring within national forest reserves. Soil samples were taken from 17 forests within this latitudinal range and the soil fungal communities were determined using 454 pyrosequencing. We addressed three main questions in this study. 1) Is the diversity of soil fungal communities correlated to the diversity of the above ground vegetation? 2) How does fungal diversity vary along the latitudinal gradient? And 3), what are the primary factors affecting soil fungal community composition?

Materials and methods

Study sites

Seventeen study sites, consisting of relatively undisturbed forest systems, running along a latitudinal gradient in China, were selected. This gradient spans a latitudinal range from 21°27′ N to 48°45′ N. Five sites were located in tropical and sub-tropical forests in Xishuangbanna (BN1, BN2, BN3) and the Ailao Mountains (AL1 and AL2) of Yunnan province, both sites are managed by the Chinese Long-Term Ecological Research (CLTER) network. There were nine sites located in temperate forests, including two sites in Zhongdian (ZD1 and

ZD2), northern Yunnan; three sites (also CLTER managed) in the Minya Konka Mountains (GG1, GG2, GG3) of Sichuan province and four sites in the Qingling Mountains (QL1, QL2, QL3 and QL4) of Shanxi province. A further three sites were established in boreal forests (AT1, AT2, and TS1) in Tian and Altai Mountains of Xinjiang province (Table S1). All sites are in protected areas (Fig. 1).

For each site, information was gathered regarding geography (LAT: latitude, ELE: Elevation), climate, forest types (FT) and tree species diversity (H_{tree}) (Peet 1974) (Table S1). The climate data for the all sites, including mean annual temperature (MAT) and mean annual precipitation (MAP), were the values taken from 1971 to 2000 (Table S1). Annual variation in temperature (△T) was the difference between the maximum and the minimum mean-monthly temperature for a given year. All tree stems with a diameter at breast height (dbh) >2 cm were surveyed for diameter and relative abundances in the respective plots. We used Shannon-Weaver indexes to describe tree diversity (H_{tree}) based on the survey data (Hill et al. 2003).

Soil samples from AT1, AT2, TS1, QL1-4 were collected in June of 2009, from GG1-3 in August of 2009, AL1-2 and BN1-3 were collected in June of 2010, and samples from ZD1-2 were collected in September of 2010. A 1000 m^2 square plot was established in each of the seventeen forests, the plots were then divided into nine 10×10 m sub-plots. The sub-plots located in the four corners and the one in the center of the plot were used for soil sampling. The organic layer on the soil was first removed and the soil cores to the depth of 10 cm were taken from each subplot, these 5 cores were then bulked to represent 1 sample. All samples were transported to the laboratory on ice for analyses within one week The soil samples were first sieved through a 4-mm mesh to remove root and plant material. Each sample was then separated into two parts: one stored in a freezer at −80 °C before nucleic acid analysis, and the other air-dried for chemical analysis.

Soil analysis

Soil samples for chemical analysis were sieved through a 2 mm mesh in preparation for further analysis. After homogenization to a saturated colloid, pH was measured using a pH-meter, which was regularly calibrated during the sampling process (PHS-3C; Shanghai Precision & Scientific Instrument, Shanghai, China). Total C content (T.C) was analyzed based on the chromic acid wet oxidation method (Walkley and Black 1934). Total N was detected using an Auto Kjeldahl Unit model K370 (Jones and Bradshaw 1989). Soil exchangeable base cations (Ca^{2+}) were analyzed using hydride formation and measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using external calibration (Li et al. 1995). Soil characteristics for all sites are described in Table S1.

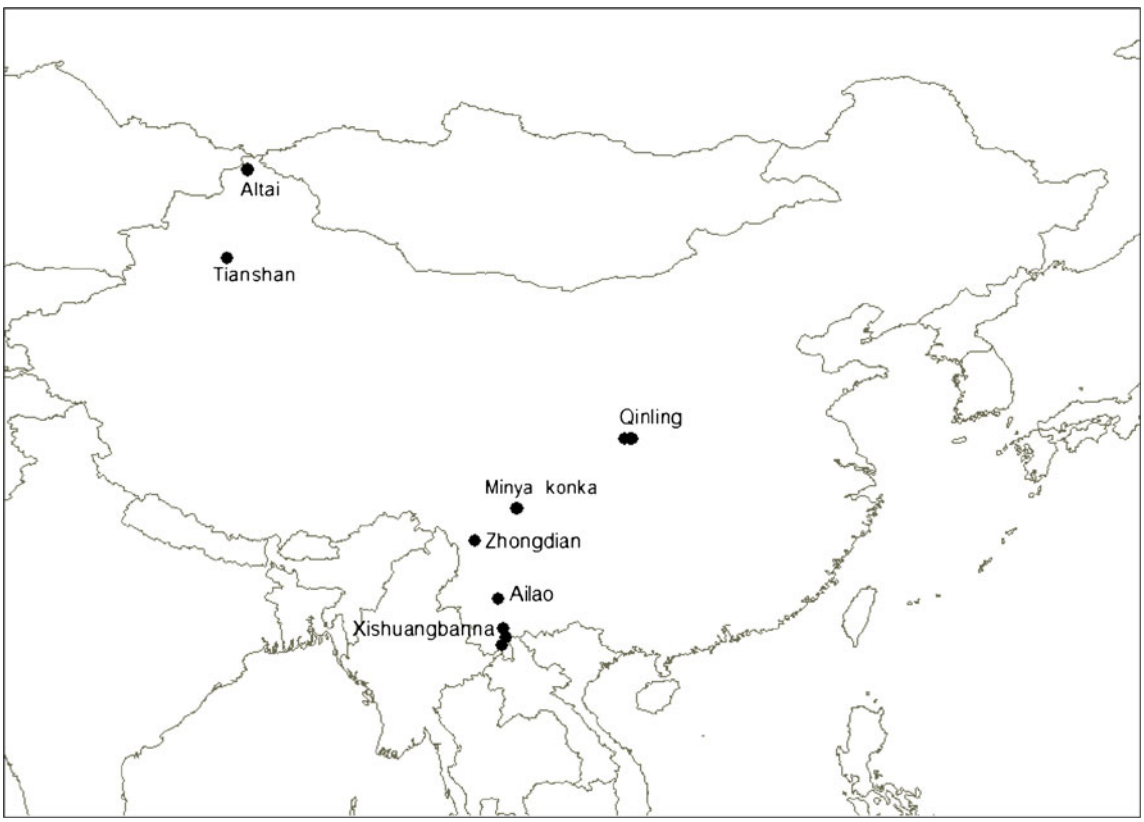

Fig. 1 Sampling site map

Amplification of ITS rRNA genes and bar-coded pyrosequencing

454 pyrosequencing was used to examine the fungal community composition from the soil samples collected in each forest system. Genomic DNA extracted from the samples was prepared for pyrosequencing according to the protocol of Lauber et al. (2008). Amplicon libraries were performed using a combination of tagged primers designed for the variable ITS-1 region, as recommended for the tag-encods 454 GS-FLX amplicons pyrosequencing method (Acosta-Martinez et al. 2008). The 17 genomic DNA samples were diluted to 1:5 and 1:100. The resulting 34 DNA samples were amplified separately using the fungal primer pair ITS1F (5′-ACTTGG TCATTTAGAGGAAGTAA-3′) and ITS2 (5′-BGCTGCGTTCTTCATCGATGC-3′) to generate PCR ITS rRNA fragments of ca. 400 bp in length, where A and B represent the two pyrosequencing primers (A: GCCTCCCT CGCGCCATCAG and B: GCCTTGCCAGCCCGCTCAG). The PCR reaction mixture (50 μL) contained 5 ng template DNA, 5 μL of 10×PCR buffer (100 mol Tris-HCl, 500 mol KCl, and 15 mol MgCl2), 5 μL of deoxyribonucleotide triphosphate mixture (2.5 mol of each dNTP), 1 μM of each primer, and 1 U of TaKaRa ExTaq (Takara Bio, Otsu, Japan). The PCR condition was modified several times until a clear and strong PCR product generated. The final PCR conditions used were 94 °C for 4 min, 30 cycles of 30 s at 94 °C (denaturation), 50 °C for 1 min (annealing) and 72 °C for 90 s (extension), followed by 10 min at 72 °C. The two sets of PCR products were purified using the Multiscreen-PCR plate system (Millipore Corporation, Billerica, MA, USA), and then pooled to obtain 17 amplicon libraries corresponding to the 17 different forest soils. The amplicons length and concentration were estimated, and an equimolar mix of all 17 amplicon libraries was established. Pyrosequencing of the 17 amplicon libraries (from the ITS1F priming site) were performed on the Genome Sequencer FLX 454 System at the Research and Testing Laboratory (Texas, USA).

Species identification and functional groups classification

The following quality filters were applied: sequences were denoised following the single-linkage preclustering (SLP) method (Huse et al. 2010); low quality scores (< 25) and short length of sequences (< 250 bp) were discarded. In order to retrieve only the ITS region, all remaining 18S or 5.8S regions were removed by Fungal ITS Extractor (Nilsson et al. 2011). Putative chimeric sequences were checked using de novo UCHIME algorithm (Edgar 2010). Quality-checked sequences were clustered into operational taxonomic units (OTUs) using USEARCH (Edgar et al. 2011) at a 97 % similarity cut-off. Taxonomic classification of each sequence was performed using BLASTn search against NCBI-nt database (April 2012) and only exact matches were considered accurate (*E*-values=0). Rarefaction statistics were calculated using *vegan* package in R (http://www.R-project.org/) (Dixon 2003). In the case of ITS genes belonging to ectomycorrhizal fungi, confirmation were achieved using the ectomycorrhizal fungal sequence database UNITE (Koljalg et al. 2005) (http://unite.ut.ee/index.php).

Diversity environment correlation analyses

The sequence sizes in the sample were unequal (ranging from 970 to 12157 sequences per sample). Therefore, we applied the rarefaction function in "vegan" of R to build 17 rarefied quantitative data sets of 970 sequences per sample. For 454 pyrosequencing, species richness within each community was quantified as the total number of OTUs within that community. Singleton sequences (i.e., reads that were not assembled into contigs) were retained on the basis that although some singletons might represent artifacts or contaminants, others are likely fragments of transcripts present at low levels in the original sample. OTUs observed after removing singleton OTUs. Furthermore, we used the Shannon index to estimate phylotype diversity, as recommended by Hill et al. (2003). These indices were calculated using the function *diversity* of the *vegan* package implemented in the software R. Multiple regression analyses with a Pearson correction was used to examine the correlations between fungal diversity (Shannon diversity) and each variable category (i.e., soil physical and chemical parameters, plant diversity, spatial variables, and relative abundance of predominate groups) using SPSS 13.0. In each case, we fitted a linear and a quadratic model; results are shown for the model with the lowest Akaike information criteria (AIC) (Sakamoto et al. 1986).

Community similarity analysis

For data from 454 pyrosequencing, a Bray-Curtis similarity index was calculated using a hellinger-transformed data matrix and overall community similarities were displayed using non-metric multidimensional scaling (NMDS) by function *metaMDS* in the *vegan* package in R (Beals 1984). An analysis of the correlation between the environmental variables and community composition was also conducted using function *envfit* in the *vegan* package in R. *P* values are based on 999 permutations. We then assessed the effect of site elevation on fungal assemblage structure, by analyzing the average Bray-Curtis dissimilarity index in permutational multivariate analyses of variance. These analyses were carried out with the *Adonis* function of the R *vegan* package, with 999 permutations. Sampling date and its interaction with site were also introduced in the analyses of variance, to investigate whether the effect of site was constant through time.

Results

Composition of soil fungi across 17 forests

From the 17 samples, we obtained 62839 quality fungal DNA sequences. On average, each individual sample was represented by 3600 classifiable sequences, with a range of 970 to 12157 sequences per sample. At this depth of sequencing, we have surveyed almost the full extent of taxonomic diversity within individual soils, at the 97 % similarity level of taxonomic resolution. This is evidenced by the trends of the rarefaction curves for the samples shown in Fig. 2. Samples have been classified into two categories according to climate zone. The rarefaction curves indicate that the species accumulation in tropical and subtropical forests is slower than in temperate forests (Fig. 2).

A total of 7630 OTUs (determined at >97 % similarity) were identified across the sample set, including representatives from four phyla: Ascomycota, Basidiomycota, Glomeromycota and Zygomycota (Table S2). These were further classed into the following subgroups: ectomycorrhiza (EcMF), endomycorrhiza (EMF) and non-mycorrhizal (NMF). The rarified sequence data suggested that soils from temperate forests were overwhelmingly dominated by taxa belonging to the EcMF group, especially in the cold temperate forests (more than 60 % of the community) (Fig. 3). Our findings show that the EcMF *Inocybe* (Agaricomycetes) was the most common fungal genus to occur in several of the samples (i.e. AT1, TS, QL1, QL4, GG1 and GG2) (Table S1). Aside from *Inocybe*, we also found that EcMF *Piloderma*, *Sebacina* and *Russula* dominated in temperate forests (AT2, QL2 and QL3). There is a general shift along the temperature gradient towards less EcMF in warmer climates,

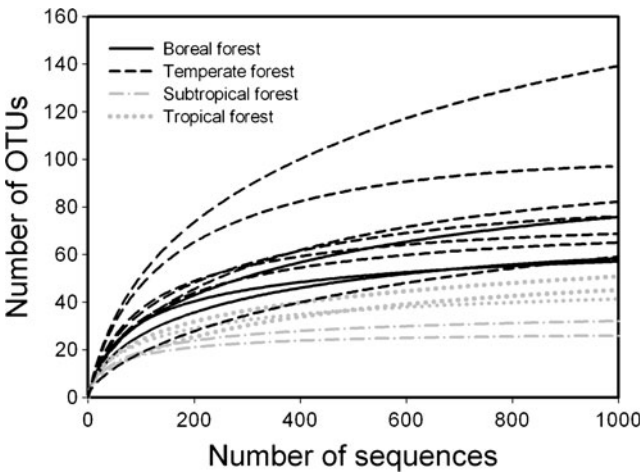

Fig. 2 Rarefaction curves results for soils classified into two categories according to climate zones. To make the patterns clear, we have shown rarefaction curves for only the first 1000 sequences per sample. Boreal forest: AT (1–2) and TS1; Temperate forest forests: QL (1–4), GG(1–3), ZD (1–2); Subtropical forest: AL (1–2); Tropical forest: BN (1–3)

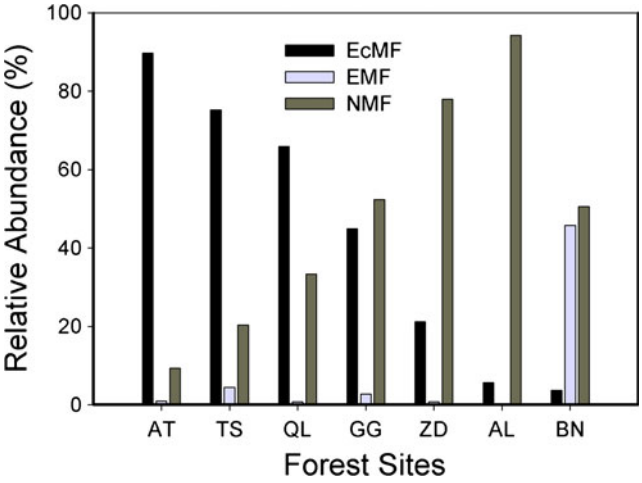

Fig. 3 Relative abundance of major soil fungal taxa in each forest (y axis is group percentage of the total number of ITS RNA gene sequences per sample), after rarefaction to correct for uneven sampling effort

while EMF and NMF displayed the reverse pattern of distribution (Fig. 3). The fungal compositions of subtropical or near subtropical forests were more complex and significantly different from other forests, in that they exhibited specific fungal groups. For example, the saprophytic fungi (placed in the NMF group), *Veluticeps* dominated in the GG3 sample. *Mortierella* and *Umbelopsis*, which belong to Zygomycota represent a large part of the fungal communities of ZD1 and ZD2 (Table S2). The yeast *Cyphellophora* dominated in AL1. *Leptodontidium*, a root endophyte, dominated in AL2. *Zopfiella* and *Stagonosporopsis* dominated in BN1. These fungi are unique in limestone forests (BN1) and rarely found in other forest types (Table S2). The tropical forests were similar to the cold temperate forests, and were dominated by a single EMF: *Paraglomus*, representing 90 % of the fungal sequences recovered (Table S2).

Variability in overall and functional fungal community diversity

The 454 pyrosequencing results showed that fungal diversity and species richness followed a unimodal pattern with a peak in the Shannon diversity index at mid-latitude (Fig. 4a, b). Furthermore, we found that the total community level diversity and richness was similar to that of the EcMF (Fig. 4a, b) ($P<0.05$), Correlation analysis showed a significantly positive relationship between diversity of total fungi and mycorrhizal fungi (the sum of EcMF and EMF) ($r^2=0.738, P<0.05$) (Fig. 4c).

Of the soil and site characteristics considered, fungal diversity was significantly correlated with LAT, MAT, FT and H_{tree}. Fungal diversity was most strongly correlated with LAT and least correlated with FT (Table 1). For the fungal subgroups, EMF diversity was significantly influenced by LAT, but was rarely affected by other environmental factors tested

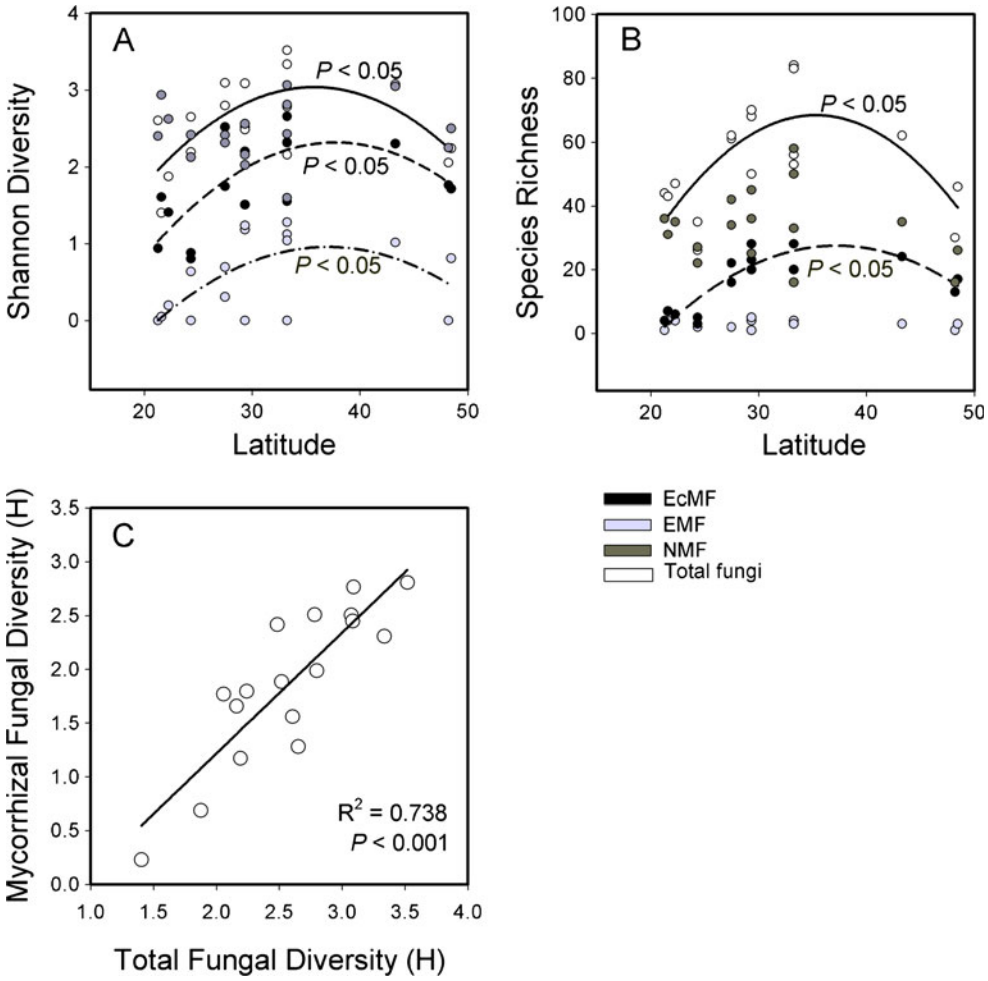

Fig. 4 The total and functional fungal diversity (**a**) and richness (**b**) calculated from 454 pyrosequencing correlated with latitudinal gradients. The total fungal diversity correlated with that of mycorrhizal fungi (**c**) calculated from 454 pyrosequencing. Diversity was estimated by using the Shannon index, a summary variable that incorporates the richness and evenness of phylotypes. Detailed information on the individual soils is provided in Table 3. Both quadratic regressions were statistically significant (P <0.05). *Solid line*: total fungi; *dashed line*: EcMF; *dotted line*: NMF

Table 1 Relationships between Shannon diversity (H) and measured climate, vegetation and soil characteristics for the full sequence set and the four most abundant phyla

	Total fungi					EcMF				EMF				NMF		
	M	AIC	r^2	P	M	AIC	r^2	P	M	AIC	r^2	P	M	AIC	r^2	P
LAT	Q	−27.463	**0.456**	**0.014**	Q	**−27.836**	**0.506**	**0.007**	**Q**	**−9.404**	**0.357**	**0.046**	Q	−30.288	0.018	0.820
ELE	Q	−19.691	0.140	0.347	Q	−16.124	0.015	0.897	Q	−21.537	0.107	0.454	Q	−34.182	0.219	0.177
MAT	Q	−26.912	**0.438**	**0.018**	Q	−16.124	0.015	0.897	Q	−24.039	0.229	0.162	Q	−31.311	0.075	0.580
MAP	Q	−24.175	0.340	0.055	Q	−20.649	0.245	0.139	Q	−23.516	0.205	0.201	Q	−30.994	0.058	0.659
▵T	Q	−23.360	0.304	0.077	Q	**−25.179**	**0.422**	**0.022**	Q	−25.019	0.272	0.108	Q	−30.630	0.037	0.766
FT	Q	−25.191	**0.378**	**0.036**	Q	−22.907	0.339	0.055	Q	−24.594	0.254	0.129	Q	−32.204	0.122	0.401
H_{Tree}	Q	−25.241	**0.388**	**0.032**	Q	−18.352	0.136	0.358	Q	−22.215	0.142	0.343	Q	−36.399	0.314	0.071
pH	Q	−23.375	0.308	0.076	Q	**−24.772**	**0.408**	**0.025**	Q	−23.724	0.215	0.184	Q	−31.796	0.101	0.475
T.C	Q	−17.263	0.008	0.944	Q	−20.103	0.221	0.174	Q	−21.699	0.115	0.425	Q	−34.768	0.245	0.140
C/N	Q	−23.068	0.295	0.086	Q	−17.459	0.09	0.517	Q	−24.201	0.236	0.152	Q	−34.487	0.233	0.157
ECa	Q	−19.471	0.129	0.380	Q	−16.498	0.037	0.768	Q	−20.24	0.036	0.774	Q	−33.111	0.168	0.276

In each case, we fitted a linear and a quadratic model; results are shown for the model with the lowest Akaike information criteria (AIC) value

LAT latitude; *ELE* elevation; *MAT* mean annual temperature; *MAP* mean annual precipitation; *Htree* tree species diversity; ΔT annual temperature range; *FT* forest type; *T.C* total organic carbon; *ECa* exchangeable calcium (cmol kg-1)

(Table 1). EcMF diversity was significantly correlated with LAT, △T, FT and soil pH, with LAT most strongly influencing EcMF diversity and FT having the lowest impact (Table 1). There were no significant correlations between environmental variables and the NMF group (Table 1). It is worth noting that certain of the environmental variables were correlated, as in the case of MAT and △T, with MAT being the most influencing of these variables.

Variability in fungal community composition

The composition of fungal communities was highly variable across the soils represented in this study (Fig. 5a). Visualization of the Bray-Curtis distances on nonmetric multidimensional scaling plots indicated significant variability across the biomes (Fig. 5a). Soils from similar climatic biomes harbored similar fungal communities, as the variability between climatic zones exceeded the variability within similar climatic zones. However, there was a significant correlation between Bray-Curtis distances (total fungi, EcMF and NMF) and the pairwise geographic distances between sampling locations, indicating that soils collected from distant locations did harbor more distinct communities than those collected in close proximity (Fig. 5b, c, d).

Climatic factors (MAT, MAP and △T) and tree diversity were most strongly correlated with the overall Bray-Curtis distances between soils (Table 2). Mantel tests of the remaining edaphic characteristics indicated no significant relationship with the overall Bray-Curtis distances between communities (Table 2). As with overall fungal community composition, if we examined the four subgroups of fungi individually, we find that the structure within these groups is most strongly correlated with temperature, followed by forest types (Table 2).

Permutation multivariate analysis of variance confirmed that sampling time was a significant factor influencing variation in the composition of total fungi, EcMF and NMF groups. Sampling site alone was not a significant factor. However, interaction between sampling date and sites had a strong effect on the total fungal and the NMF communities. In addition, we also found a significant interaction between sampling time and latitudinal gradient, in particular of NMF (Table 3).

Discussion

For most taxa of terrestrial macro-organisms, diversity peaks at the lower latitudes (Willig et al. 2003). However, our results show that fungal diversity patterns follow a different trend, with the greatest diversity in the middle latitudes. Furthermore, our findings indicate that total soil fungal diversity was strongly correlated with mycorrhizal fungi, across the entire latitudinal range.

Diversity and composition of soil fungi in forests

Soil fungal diversity was lowest in the subtropical and tropical forests, slightly higher in boreal forests, and highest in the temperate forests. This pattern of diversity, starting with lower diversity in tropical forests, may be a result of the high plant

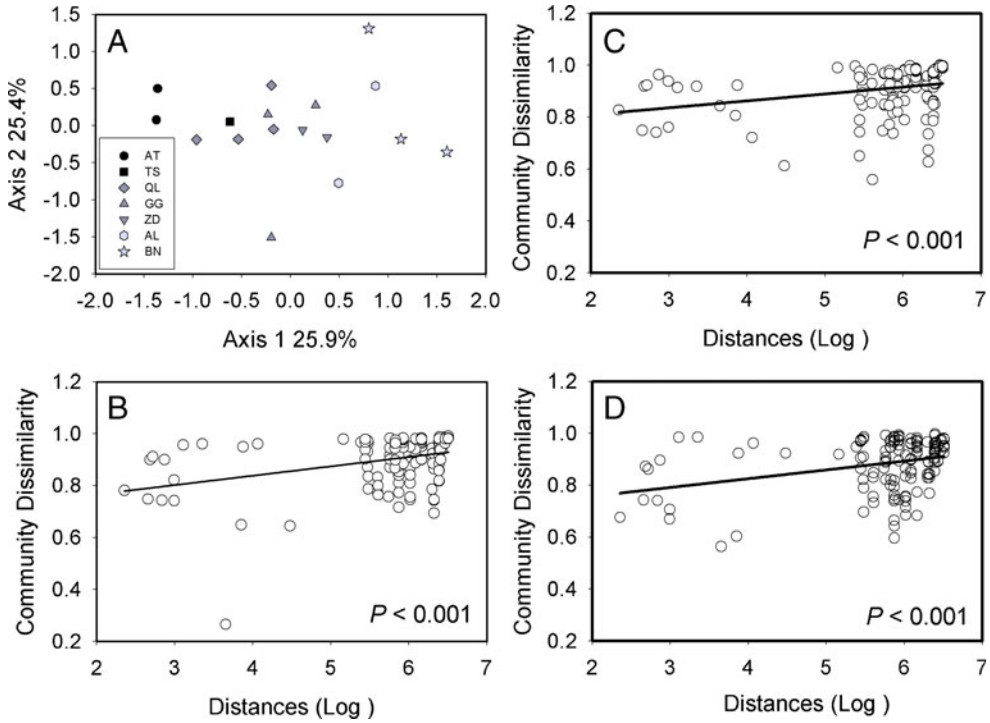

Fig. 5 Nonmetric multidimensional scaling plots of soil fungal communities (**a**), showing the relative differences in community composition. *Black*: cold temperate zone; *Dark gray*: warm temperate zone; *Light gray*: topical and subtropical zone. The Bray-Curtis distances metric was used to quantify the similarity between phylotype patterns. Relationship between geographical distance and percentage similarity index between each samples (**b**) Total fungi (**c**) EcMF. **d** NMF One plot represent the pairwise distances between two sample sites (x axis) and dissimilar of two community (y axis)

Table 2 Pearson correlations between the ordination score of the first axis of the nonmetric dimensional scaling ordination and key soil and site characteristics

Significant correlations are indicated in bold ($P<0.05$)

LAT latitude; *ELE* elevation; *MAT* mean annual temperature; *MAP* mean annual precipitation; H_{tree} tree species diversity; ΔT annual temperature range; *FT* forest type; *T.C* total organic carbon; *ECa* exchangeable calcium (cmol kg-1)

	Total fungi		EcMF		EMF		NMF	
	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
LAT	**0.764**	**0.001**	**0.775**	**0.001**	**0.523**	**0.020**	**0.700**	**0.001**
ELE	0.035	0.797	0.029	0.846	0.112	0.520	0.054	0.708
MAT	**0.444**	**0.008**	**0.462**	**0.011**	**0.391**	**0.047**	**0.374**	**0.038**
MAP	**0.603**	**0.001**	**0.622**	**0.001**	0.370	0.070	**0.525**	**0.001**
ΔT	**0.683**	**0.001**	**0.692**	**0.001**	**0.457**	**0.025**	**0.624**	**0.001**
FT	**0.393**	**0.020**	**0.417**	**0.02**	**0.373**	**0.045**	**0.314**	**0.044**
H_{tree}	0.139	0.310	0.155	0.271	0.272	0.145	0.084	0.448
pH	0.005	0.933	0.007	0.932	0.199	0.256	0.011	0.910
T.C	0.012	0.894	0.017	0.823	0.023	0.872	0.019	0.818
C/N	0.362	0.053	**0.366**	**0.042**	0.17	0.311	0.303	0.088
ECa	0.015	0.736	0.022	0.704	0.084	0.528	0.001	0.962

diversity found at these sites. Gilbert (2005) suggested that the high plant diversity of tropical systems results in a decrease in fungal specificity due to a lack of selective pressure for specialization, often resulting in low species diversity. Similarly, another study by Gilbert et al. (2002) found an inverse relationship between tree species diversity and saprophytic fungal diversity, showing that high tree host density, such as in temperate forests, led to greater fungal diversity than in forest systems with low host density.

The significant correlation between the diversity of the greater fungal community and mycorrhizal fungi is consistent with the work of Bueé et al. (2009) and Voříšková and Baldrian (2013), who showed that mycorrhizal fungi were prevalent in all the fungal communities sampled in their study. Based on our findings, most forest systems were dominated by mycorrhizal fungi: EcMF dominated in the temperate forests, and EMF in the tropical and subtropical forest systems. EcMF dominance in temperate forests has been shown in past studies (Bruns et al. 2002; Ishida et al. 2007). Tedersoo et al. (2012) reviewed data from 66 studies and showed that the general latitudinal gradient of biodiversity, which increases towards the tropics, is reversed in EcMF, as confirmed by our results. In addition, our results indicating the prevalence of EMF in tropical forests are in agreement with the findings of Rillig et al. (2001). However, it is surprising that such low EMF diversity was observed in the other forest systems investigated in this study, as EMF are considered to be ubiquitous throughout most ecosystems (Opik et al. 2010).

The observed shift in the fungal community composition of EcMF and EMF, between temperate and tropical forests, has not been found in previous studies. However, the majority of past studies have been based on the collection of sporocarps, which is not as accurate as high sequencing technologies for quantitative analyses, as sporocarp production can be variable and is dependent on a variety of factors, often giving an unreliable view of EcMF community structure (Dahlberg 2001; Taylor 2002; Tedersoo et al. 2012).

Our results suggest that there was competition for resources among the fungal groups. It appears that ectomycorrhizal fungi were the dominant group, outcompeting non-mycorrhizal fungi for C resources from the host tree, which is in agreement with the work of Fitter and Garbaye (1994).

Table 3 Permutational multivariate analyses of variance of the compositional dissimilarity between fungal assemblages along the latitudinal gradients

	Total fungi			EcMF			EMF			NMF		
	Df	Sq	F.Model	Df	Sq	F.Model	Df	Sq	F.Model	Df	Sq	F.Model
LAT	1.000	0.867	2.721***	1.000	1.004	2.818***	1.000	0.46	1.262	1.000	0.612	1.905***
Time	1.000	0.685	2.151***	1.000	0.576	1.616*	1.000	0.48	1.316	1.000	0.672	2.093***
Site	1.000	0.412	1.291	1.000	0.409	1.148	1.000	0.238	0.652	1.000	0.416	1.296
LAT×Time	1.000	0.641	2.011***	1.000	0.494	1.387	1.000	0.769	2.108*	1.000	0.610	1.900***
LAT×Site	1.000	0.330	1.036	1.000	0.293	0.823	1.000	0.225	0.616	1.000	0.374	1.165
Time×Site	1.000	0.568	1.783***	1.000	0.345	0.968	1.000	0.607	1.664	1.000	0.539	1.678***
LAT×Time×Site	1.000	0.193	0.605	1.000	0.332	0.933	1.000	0.076	0.207	1.000	0.207	0.644
Residuals	9.000	2.868	0.437	9.000	3.207	0.481	9.000	2.553	0.472	9.000	2.891	0.457

$P=0$ *** $P<0.001$ ** $P<0.01$ *

Evidence for this competition is provided by the observed increases of the relative abundance of non-mycorrhizal communities when ectomycorrhizal fungi were absent. These findings are confirmed by the work of Lindahl et al. (2010), who found that once ectomycorrhizal fungi dominance was removed from a fungal community, there were distinct increases in relative abundance of other free-living fungal groups, which had previously been suppressed by the ectomycorrhizal fungi.

Distance effect on community structure

Green et al. (2004) demonstrated that fungal communities become less similar with increasing geographic distance, a similar distance-decay pattern was observed within our study, showing a decline in community similarities as the spatial distance between communities increased. Our analyses took into account the geographic distance between sites (26 km between the lowest and highest sites) as a partial predictor of the spatial structure of fungal assemblages along the gradient. This result, along with those of other recent, sequence based, studies characterizing the diversity soil macro- and mesofauna (Robeson et al. 2011; Steel and Bert 2012), suggests that terrestrial fungi are much more diverse than previously thought, and that they are not cosmopolitan in their distribution patterns. The difference among the fungal communities from the spatially separated evergreen broad leaf forests of Zhongdian, Ailao Mountains, and Xishuangbanna provide confirmation of this theory, whereas the fungal communities for different sites within these forest systems were highly similar. In addition, dispersal constraints over this distance are unlikely to be a strong structuring factor because many fungi have a high capacity for dispersal and a cosmopolitan distribution (Levetin and Dorsey 2006). Our results therefore confirm that fungal assemblages have a spatial structure despite the high capacity for dispersal of the species. They suggest that the spatial structure at the regional scale might be shaped by variations in abiotic factors, especially temperatures.

Factors influencing fungal community composition

Mean annual temperature (MAT) and mean annual precipitation (MAP) are cited as being good predictors of plant and animal diversity at a continental scale (Hawkins et al. 2003). This is partially in agreement with our findings, showing that soil fungal diversity was strongly influenced by variations in soil temperature. However, in contrast to temperature, precipitation had only a minor effect on soil fungal diversity. The weak association between rainfall patterns and fungal diversity may raise the point that these studies are based on soil DNA, which is not indicative of which fungal species are fruiting. Traditional approaches to mycology have focused on the collection and sampling of sporocarps, which have been shown to be influenced by rainfall patterns, which can therefore lead to a misrepresentation of the true diversity of a forest system (Nouhra et al. 2012).

Of the soil variables tested, only soil pH had a close correlation with fungal diversity, particularly with regard to the diversity of mycorrhizal fungal groups. The reason for this may be related to the enzyme activity in fungal decomposition, which is limited to specific pH scales (Leprince and Quiquampoix 1996). However, there is conflicting evidence for the role of pH in determining fungal diversity patterns: A study conducted by Rousk et al. (2010) found that pH had a weak effect on fungal diversity, whereas the work of Bååth and Anderson (2003) found a strong correlation between soil fungal and bacterial diversity and an increasing pH gradient.

Conclusion

This study highlights the changes in fungal diversity along a latitudinal gradient, running from boreal forest systems, through temperate zones and into tropical rainforests. The results clearly show that fungal diversity does not follow the same distribution pattern as that of other macro- and meso-organisms, but rather it is dependent on vegetation type and forest composition. Furthermore, fungal diversity, as in the case of EcMF, was inversely correlated with plant diversity; this is attributed to the high level of fungal speciation occurring in less diverse forest systems. However, the most influential environmental factor affecting fungal diversity and distribution patterns was temperature.

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