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RESEARCH ARTICLE

Greater diversity of soil fungal communities and distinguishable seasonal variation in temperate deciduous forests compared with subtropical evergreen forests of eastern China

Jinhong He[1,2], Leho Tedersoo[3], Ang Hu[4], Conghai Han[4], Dan He[1], Hui Wei[5], Min Jiao[1], Sten Anslan[3], Yanxia Nie[1], Yongxia Jia[1], Gengxin Zhang[4], Guirui Yu[6], Shirong Liu[7] and Weijun Shen[1,*]

[1]Center for Ecological and Environmental Sciences, South China Botanical Garden, Chinese Academy of Sciences, 723 Xingke Rd, Tianhe District, Guangzhou 510650, China, [2]Department of Life Science, University of Chinese Academy of Sciences, Beijing 100049, China, [3]Natural History Museum, Tartu University, 14A Ravila, Tartu 50411, Estonia, [4]Key Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China, [5]Department of Ecology, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China, [6]Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China and [7]Laboratory of Forest Ecology and Environment of State Forestry Administration, Institute of Forest Ecology, Chinese Academy of Forestry, Beijing 100091, China

*Corresponding author: Center for Ecological and Environmental Sciences, South China Botanical Garden, Chinese Academy of Sciences, 723 Xingke Road, Tianhe District, Guangzhou 510650, China. Tel: +86-20-3725-2950; Fax: +86-20-3725-2950; E-mail: shenweij@scbg.ac.cn

One sentence summary: Soil fungal diversity increased linearly or parabolically with latitude, depending on the season, taxonomic and functional groups of analysis.

Editor: Petr Baldrian

ABSTRACT

Whether and how seasonality of environmental variables impacts the spatial variability of soil fungal communities remain poorly understood. We assessed soil fungal diversity and community composition of five Chinese zonal forests along a latitudinal gradient spanning 23°N to 42°N in three seasons to address these questions. We found that soil fungal diversity increased linearly or parabolically with latitude. The seasonal variations in fungal diversity were more distinguishable in three temperate deciduous forests than in two subtropical evergreen forests. Soil fungal diversity was mainly correlated with edaphic factors such as pH and nutrient contents. Both latitude and its interactions with season also imposed significant impacts on soil fungal community composition (FCC), but the effects of latitude were stronger than those of season. Vegetational properties such as plant diversity and forest age were the dominant factors affecting FCC in

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the subtropical evergreen forests while edaphic properties were the dominant ones in the temperate deciduous forests. Our results indicate that latitudinal variation patterns of soil fungal diversity and FCC may differ among seasons. The stronger effect of latitude relative to that of season suggests a more important influence by the spatial than temporal heterogeneity in shaping soil fungal communities across zonal forests.

Keywords: fungal diversity; fungal community composition; latitude; season; forest; high-throughput sequencing

INTRODUCTION

Soil fungi are critical components of microbial communities living in a dynamic and complex soil environment. They play crucial functional roles as decomposers, mutualists, parasites and/or pathogens to impact soil carbon (C) and nutrient cycling (Peay, Kennedy and Bruns 2008; Burke *et al.* 2011), and therefore soil fertility and forest productivity (van der Heijden *et al.* 1998; Maron *et al.* 2011). Relative to the functions that soil fungi perform, we have a very limited understanding of the spatial and temporal variations of soil fungal communities and their driving forces (Tedersoo *et al.* 2014; Liu *et al.* 2015). This is partly due to the limitations associated with the traditional culture-based approach and the identification methodology based on sporocarp morphology (Shi *et al.* 2014). It has been estimated that <2% of the global fungal species are described (Blackwell 2011) and that <5% of soil fungi are culturable (Giri *et al.* 2005). In recent years, DNA sequence-based methods (notably high-throughput sequencing) have been used to survey soil fungal diversity of various terrestrial ecosystems, including forests (Buée *et al.* 2009; Shi *et al.* 2014), grasslands (Dumbrell *et al.* 2011), and arable lands (Orgiazzi *et al.* 2012); unexpectedly high fungal diversity was uncovered in these soils. Even though sequencing-based methods provide a powerful tool to investigate fungal communities, surveys of soil fungi across large spatial and temporal scales are still rare in forests (Shi *et al.* 2014; Tedersoo *et al.* 2014).

As a general spatial distribution pattern, species diversity of plants, animals and aquatic microorganisms declines towards the poles or high latitudes (Hillebrand 2004; Qian and Ricklefs 2007; Fuhrman *et al.* 2008; Condamine *et al.* 2012). However, there is no such general spatial distribution pattern for fungal diversity, which has been found to decrease with increasing latitude (Tedersoo *et al.* 2014; Liu *et al.* 2015), or peak at mid latitudes and decline towards high and low latitudes (Shi *et al.* 2014). The lack of a consistent spatial distribution pattern for fungal diversity may be ascribed to various influential factors such as the environmental settings (Hawkes *et al.* 2011), ecosystem or land use types (Monard, Gantner and Stenlid 2013), spatial scales (Bahram, Peay and Tedersoo 2015) and organizational levels of interest (Liu *et al.* 2015). Surveys of soil fungal diversity under different environmental settings and spatial scales are needed to provide data for establishing natural distribution patterns and building species distribution models (Tedersoo *et al.* 2014), but focusing on one ecosystem type (e.g. forest) may facilitate the identification of dominating factors and underlying mechanisms.

Soil pH has been frequently reported as an overarching factor in determining the spatial distribution of soil bacterial communities (Fierer and Jackson 2006; Liu *et al.* 2014), but there is no such widely accepted factor governing the spatial distribution of soil fungal communities, and this is now being recognized as ecosystem-specific (Liu *et al.* 2015). In arable soils, soil fungal community composition was closely related to soil carbon content, C:N ratio or extractable phosphorus (P) content (Lauber *et al.* 2008; Rousk *et al.* 2010). In forest soils, soil fungal diversity was found to be strongly associated with plant diversity (Peay, Baraloto and Fine 2013). The determinants of fungal spatial distribution may also vary among different taxonomic (Liu *et al.* 2015) or functional groups (Shi *et al.* 2014). The richness of saprotrophic fungi was reported to be sensitive to wet conditions (Tedersoo *et al.* 2014), whereas mycorrhizal fungal diversity was indirectly affected by climate through the feedback loop of photosynthetic products and soil nutrition (Treseder and Allen 2002). Understanding the spatial variation patterns of fungal communities across environmental gradients is therefore thought to be helpful in unravelling the mechanisms underlying fungal spatial variation (Liu *et al.* 2015).

At the local scale, it has been well established that fungal diversity and community composition can vary across temporal scales, such as days (Ettema, Lowrance and Coleman 1999), months (Courty *et al.* 2008), seasons (Vargas-Gastélum *et al.* 2015) and years (Mundra *et al.* 2015). Some DNA-based studies have shown that the temporal variability of bacterial diversity and community composition may be masked by spatial variability across broader spatial gradients (Fierer and Jackson 2006), but no study, to our knowledge, has been devoted to assessing the temporal variation of fungal spatial distribution patterns.

Here we use high-throughput sequencing of the nuclear ribosomal internal transcribed spacer 1 (ITS1) rRNA genetic marker to examine the diversity and composition of forest soil fungal communities across five latitudinal forests in eastern China over two consecutive years. These forests span about 20 latitude degrees (23°N to 42°N) and cover the major forest types in the region. By analyzing the samples collected from such a relatively large spatial scale and multiple temporal scales, we aimed to address the following questions: (i) how do soil fungal diversity and community composition vary across the five latitudinal forests? (ii) do the latitudinal variation patterns differ across seasons? And (iii) what are the dominant environmental variables explaining the latitudinal and seasonal variation patterns?

MATERIALS AND METHODS

Study site and soil sampling

Five study sites were established in relatively undisturbed mature forests of eastern China. These sites were scattered along a latitudinal gradient ranging from 23°N to 42°N (Supplementary Fig. S1 and Table S1). The two southern sites, Dinghu Mountain (DHM) in Guangdong province and Jinggang Mountain (JGM) in Jiangxi province, are situated in the subtropical evergreen broadleaved forest zone. The two northern sites, Changbai Mountain (CBM) in Jilin province and Dongling Mountain (DLM) in Beijing City, are located in the temperate deciduous forest zone. The mid-latitude site, Baotianman Mountain (BTM) in Henan province, lies in a transitional zone between the subtropical evergreen forests and the temperate deciduous forests, but the dominant trees are deciduous (Supplementary Table S1). We therefore categorized the five forests into two types:

temperate deciduous forest (TDF) and subtropical evergreen forest (SEF). All these sites are located in national or provincial nature reserves and are associated to the National Ecosystem Research Network of China (CNERN). More detailed information on site locations, vegetation and climatic characteristics are given in Supplementary Table S1. The climatic data were obtained from the CNERN dataset (http://cerndis1.cern.ac.cn). Vegetation information was mainly adopted from previous studies (Supplementary Table S1).

Soil samples were collected at the five forest sites in April, July and October for two years (2013 and 2014). At each site, five 10 m × 15 m plots were established to collect independent replicate soil samples. Within each plot, topsoil samples (0–10 cm) were randomly taken using a soil auger (Φ 5 cm) at 12–18 spots to form one composite sample, which resulted in 30 soil samples for each forest site in the two years. Visible roots or plant residues were removed prior to homogenizing each sample. Each homogenized sample was sieved through a 2-mm mesh and subdivided into two subsamples. One subsample was used to determine soil properties and the other was kept at −80°C for subsequent DNA extraction and molecular analyses.

Soil chemical analysis

Soil pH was measured in a 1:2.5 air-dried soil/water suspension using a pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland). Soil water content (SWC) was measured gravimetrically by oven-drying 10 g of fresh soils at 105°C for 24 h. Soil total organic carbon (TOC) was determined by heating using potassium bichromate-concentrated sulphuric acid and titrating with ferrous sulfate. Total nitrogen (TN) and total phosphorus (TP) were determined by the indophenol blue colorimetric method and molybdenum antimony blue colorimetry, respectively, after digestion by concentrated sulphuric acid. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were measured by indophenol blue colorimetry and the cadmium reduction method with a modified Berthelot reaction, respectively, after filtration of a 2 M KCl extract of 20 g fresh soils. More detailed descriptions of the above methods can be found in Liu (1996). The contents of soil potassium ion (K^+), sodium ion (Na^+), calcium ion (Ca^{2+}), magnesium ion (Mg^{2+}), aluminum ion (Al^{3+}), iron ion (Fe^{3+}) and manganese ion (Mn^{2+}) were determined using the atomic absorption method (Chen *et al.* 2015). Soil pH ranged from 3.82 at DHM in the South to 6.15 at DLM in the North. Soil carbon and nutrient contents were 2–4 times higher at CBM than at the other sites. More detailed information on soil properties are given in Supplementary Tables S2 and S3.

Molecular analyses

In order to avoid DNA extraction bias, DNA was extracted in triplicate from each composite soil sample using the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. DNA quantity and quality were assessed using agarose gel electrophoresis and a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The resulting three DNA extractions per sample were pooled and stored at −20°C before further analysis. The polymerase chain reaction (PCR) was performed using the fungus-specific primers ITS5–1737F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2–2043R (5′-GCTGCGTTCTTCATCGATGC-3′) (White *et al.* 1990) to generate ITS1 fragments of *ca.* 400 bases in length. The PCR reaction system (30 μL) consisted of 10 μL DNA extract, 3 μL reverse and forward primers (2 μM), 15 μL 2× Phusion High-Fidelity PCR master mix with GC buffer (New England BioLabs Inc., Ipswich, MA, USA) and 3 μL double-distilled water. The PCR conditions were modified to generate a clear and strong PCR product according to the guidelines in Lindahl *et al.* (2013). The PCR was carried out in triplicate using the following thermocycling conditions: 98°C for 1 min, 30 cycles of 10 s at 98°C (denaturation), 50°C for 30 s (annealing) and 72°C for 30 s (extension), followed by 5 min at 72°C. PCR products were mixed in equidensity ratios, and purified with Qiagen Gel Extraction Kit (Qiagen, Dusseldorf, Germany). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Library Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations. Index codes were added. The library quality was assessed on the Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The library was sequenced on the Illumina HiSeq2500 platform and 250 bases paired-end reads were generated.

Bioinformatics

Paired-end reads of 143 samples from the Illumina platform were assembled and quality filtered with VSEARCH (Rognes *et al.* 2016; https://github.com/torognes/vsearch) (parameters: minimum overlength = 15 bases; maximum error rate = 1%; minimum quality per base = 20; no ambiguous nucleotides allowed) to exclude low-quality sequences. The reads were assigned to samples based on their unique barcode and truncated by trimming the barcode and primer sequences using mothur (v1.35.1; Schloss *et al.* 2009). We then used ITSx 1.0.11 (Bengtsson-Palme *et al.* 2013) to remove the flanking gene fragments for optimal resolution of ITS1 clustering. The sequences were filtered to exclude potential chimeric sequences using *de novo* (against UNITE v7 database; Abarenkov *et al.* 2010), which was implemented in VSEARCH. After these filtering steps, the non-chimeric reads were clustered into operational taxonomic units (OTUs; Blaxter *et al.* 2005) at a 97% similarity threshold using CD-HIT (Fu *et al.* 2012). Singletons (OTUs composed of a single sequence) were discarded from all further analyses. The most abundant sequence of each cluster was selected with mothur as the representative sequence for a BLASTn search against the UNITE database (version 7.0; 31 January 2016 release) (Kõljalg *et al.* 2013).

OTUs were further filtered manually based on the BLASTn values to remove contaminants and potential artefacts. All non-fungal OTUs were removed. The remaining fungal OTUs were then categorized into functional guilds such as saprotrophic fungi, plant pathogens, animal parasites and mycoparasites using FUNGuild (Nguyen *et al.* 2016; https://github.com/UMNFuN/FUNGuild). Ambiguous groups were compared with Tedersoo *et al.* (2014). One dominant OTU was removed due to a suspected sample contamination—the sequences of this OTU were assigned to the mycoparasite functional guild with an unreasonably high relative abundance (14.8%) compared with that of 0.0067% for forest soils reported in Nguyen *et al.* (2016). Taxa were considered to be ectomycorrhizal (EcM) fungi if they best matched any sequences of known EcM lineages (Tedersoo and Smith 2013). All Glomeromycota were considered to be arbuscular mycorrhizal fungi (AMF). To account for the unequal sequencing depth, we performed the subsampling procedure to the depth of 4652 sequences using mothur. The ITS1 sequence data were deposited in the NCBI Short Read Archive under Accession No. PRJNA360476.

Statistical analysis

We calculated the Shannon index (diversity) to estimate the phylotype diversity (Hill *et al.* 2003) using the *diversity* function of the VEGAN package (Oksanen *et al.* 2016) in R version 3.3.1 (R Core Development Team 2016). Repeated-measures analysis of variance (ANOVA) was used to detect the latitude (or site), season and their interactive effects on fungal diversity. Multiple regression was applied to derive the relationships between the fungal diversity and latitude. One-way ANOVA was used to test the significance of seasonal difference in fungal diversity followed by a Tukey's HSD *post hoc* test for a given forest type. Student's *t*-test was used to test the differences of fungal diversity between the two forest types (i.e. TDF *vs* SEF). These analyses were performed in SPSS Statistics 22.0 software (IBM Corp., Armonk, NY, USA). The Spearman correlation was used to identify the relationships between fungal diversity and other influential factors (i.e. soil properties, climate and vegetation) in R. Soil properties, climatic variables and forest age were log-transformed prior to analysis where necessary to approximate normal distribution.

Non-metric multidimensional scaling (NMDS) analysis was performed to analyze the differences in fungal community composition (FCC) among samples, as implemented in the VEGAN package in R. The significance of latitude, sampling season and their interactions on the FCC was assessed through the multivariate permutational analysis of variance (PERMANOVA) as implemented in the *adonis* function of the package VEGAN in R. The relationships between fungal community composition and influential factors were investigated using canonical correspondence analysis (CCA). Specifically, the variables were selected by the *vif* function of the VEGAN package in R. The significance of each environmental variable on the fungal community was performed using the *envfit* function of the VEGAN package in R with 999 random permutations. The variables that displayed significant effects ($P < 0.05$) were included in the ordination plot.

RESULTS

Composition of taxonomic groups and functional guilds

After the quality-filtering and subsampling procedures, we obtained in total 7501 fungal OTUs (665 236 reads), 95.6% of which were assigned to six fungal phyla: Ascomycota, Basidiomycota, former Zygomycota, Glomeromycota, Rozellomycota and Chytridiomycota (Fig. 1a; Supplementary Fig. S2a), among which Ascomycota and Basidiomycota were the dominant phyla accounting for 95.2% of sequences. Sordariomycetes was the most frequent class (23.1% of OTUs) in Ascomycota, whereas Agaricomycetes was the most abundant (59.2% of sequences) and frequent class (28% of OTUs) in Basidiomycota.

Most of the OTUs were further classed into the following ecological or functional guilds: EcM fungi (53.6% of sequences and 17.6% of OTUs), saprotrophs (34.8% of sequences and 43.4% of OTUs), plant pathogens (2.3% of sequences and 5.5% of OTUs), mycoparasites (0.6% of sequences and 1.5% of OTUs), animal parasites (1.3% of sequences and 2.1% of OTUs) and AMF (0.03% of sequences and 0.6% of OTUs). In summary, the saprotrophs had the highest species richness while the EcM fungi had the highest abundance (Fig. 1b; Supplementary Fig. S2b).

Latitudinal and seasonal variations in fungal diversity

The repeated-measures ANOVA showed that for the whole data set, latitude and its interaction with season had a significant influence on fungal diversity at the overall community level, the taxonomic group level and the functional guild level ($P < 0.01$; Table 1; Supplementary Table S4). Seasonal effects were weaker than the latitudinal effects and only had significant influence on the overall community level and some taxonomic groups or functional guilds ($P < 0.05$; Table 1; Supplementary Table S4). For the TDF data set, season was generally more important than latitude in affecting the Shannon diversity at different organizational levels, i.e. the overall fungal community level, the taxonomic group level, and the functional guild level. For the SEF data set, the effects of latitude and season on the diversity of overall fungal community were both insignificant, but latitude exerted a significant influence on some specific fungal groups (Table 1; Supplementary Table S4).

The diversity of the overall fungal community (Fig. 2a–c) and the major functional guilds (Fig. 2d–l) showed differentiated latitudinal variation patterns among seasons. In April, there was no explicit latitudinal pattern (Fig. 2 left column). In July and October, the fungal diversity increased linearly or parabolically with latitude (Fig. 2 middle and right columns). These latitudinal and seasonal variation patterns of fungal diversity also appeared for the major taxonomic groups (Supplementary Fig. S3). Nevertheless, the *t*-test showed that the diversity of the overall fungal community was higher in TDF than SEF (*t*-test, $t = 3.1$, $P < 0.01$). Similar patterns occurred for some taxonomic groups (e.g. Dothideomycetes and Eurotiomycetes; $t = 5.4$ and 9.7, respectively; $P < 0.001$) and functional guilds (e.g. EcM fungi and plant pathogens; $t = 5.9$ and 8.4, respectively, $P < 0.001$). The diversity of the overall fungal community from the whole data set was positively correlated with the contents of soil cations (mainly Mg^{2+}, Ca^{2+}, Mn^{2+} and Na^{+}), pH, TP, TOC and TN ($P < 0.01$; Table 2). The diversity of the main functional guilds was primarily correlated with soil nutrients (e.g. TP, TN and TOC) and pH ($P < 0.01$; Table 2). In addition to soil TN, TP and pH, some taxonomic groups (e.g. Eurotiomycetes and Tremellomycetes) were also strongly correlated with the contents of soil cations such as Mg^{2+}, Na^{+} and Ca^{2+} ($P < 0.01$; Supplementary Table S5). When focused on a given forest type, the relationships between environmental properties and fungal diversity were not all the same as that for the whole data set (Table 2; Supplementary Table S5).

The seasonal patterns of fungal diversity were indicated by the one-way ANOVA, which showed that the seasonal variation of fungal diversity was more distinguishable in TDF than in SEF, for the overall fungal community and some of the functional guilds (e.g. saprotrophs; Fig. 3) and taxonomic groups (e.g. Basidiomycota; Supplementary Fig. S4). This was also supported by the repeated-measures ANOVA on fungal diversity (Table 1; Supplementary Table S4). Specifically, the diversity of the overall fungal community was significantly higher in July than in other months in TDF ($P < 0.05$; Fig. 3a). Similar seasonal variation was found in the main functional guilds and taxonomic groups (Fig. 3b–d; Supplementary Fig. S4). However, the diversity of some functional guilds (e.g. plant pathogens) and taxonomic groups (e.g. Ascomycota) was higher in April than in July and October in SEF (Fig. 3; Supplementary Fig. S4).

Latitudinal and seasonal variations in fungal community composition

The NMDS plot showed that latitude had a greater effect on fungal community composition than had season (Fig. 4). The PERMANOVA analysis showed that latitude, season and their interactions all significantly affected the overall FCC for the whole

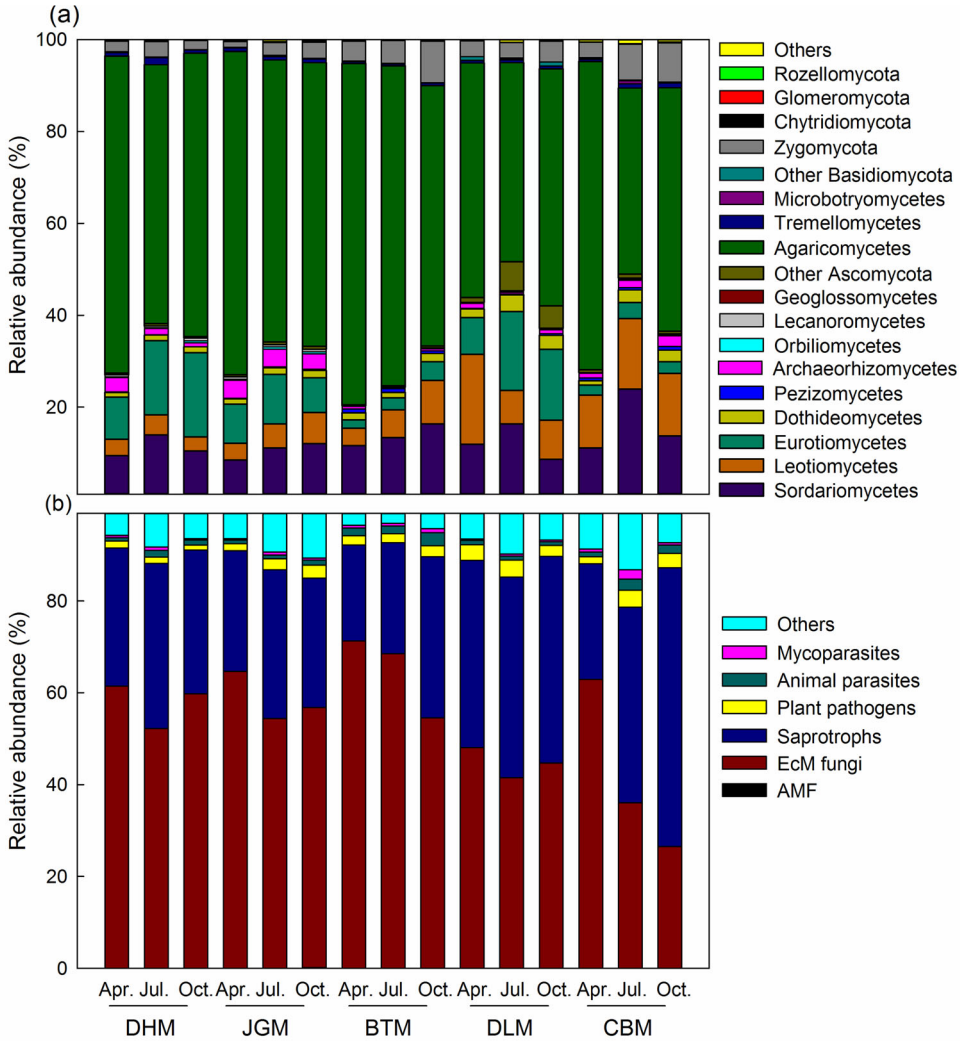

Figure 1. Relative abundance of the major taxonomic groups (**a**) and functional guilds (**b**) of soil fungal community in five latitudinal forests of eastern China at different seasons.

Table 1. ANOVA *F* and *P* values demonstrating the main and interactive effects of latitude and season on fungal diversity. These analyses were performed separately for the whole data set, the temperate deciduous forest (TDF) subset and the subtropical evergreen forest (SEF) subset. Bold typeface indicates statistical significance ($P < 0.05$).

			Overall community		EcM fungi		Saprotroph		Plant pathogen	
		df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Whole data set	Latitude (L)	4	4.75	**<0.001**	15.71	**<0.001**	4.51	**0.002**	26.48	**<0.001**
	Season (S)	2	5.77	**0.004**	0.85	0.43	9.74	**<0.001**	4.01	**0.021**
	L × S	8	3.09	**0.003**	6.04	**<0.001**	4.48	**<0.001**	4.95	**<0.001**
TDF	Latitude (L)	2	3.56	**0.03**	8.11	**<0.001**	0.20	0.82	0.42	0.66
	Season (S)	2	6.81	**<0.001**	1.56	0.22	10.59	**<0.001**	9.28	**<0.001**
	L × S	4	5.03	**<0.001**	8.53	**<0.001**	6.22	**<0.001**	3.48	**0.012**
SEF	Latitude (L)	1	0.55	0.46	0.12	0.73	18.14	**<0.001**	11.75	**0.002**
	Season (S)	2	0.58	0.56	0.04	0.96	0.63	0.5342	3.03	0.06
	L × S	2	1.29	0.28	5.41	**0.007**	1.44	0.25	3.87	**0.027**

data set or the data set of a given forest type, but latitude showed a much stronger effect (explaining more than 27%, 19% and 10% of the variation of FCC in the whole data set, the TDF data set and the SEF data set, respectively) than did season (explaining less than 7% of the variation of FCC for all three data sets) (Table 3). Similar patterns were found for the main functional guilds and taxonomic groups (Table 3; Supplementary Table S6).

By comparing the latitudinal and seasonal variation of FCC between the TDF and SEF subsets, we found that the latitudinal variation of FCC was larger in TDF ($R^2 = 0.20$) than in SEF ($R^2 = 0.11$) for the overall community as well as for the main

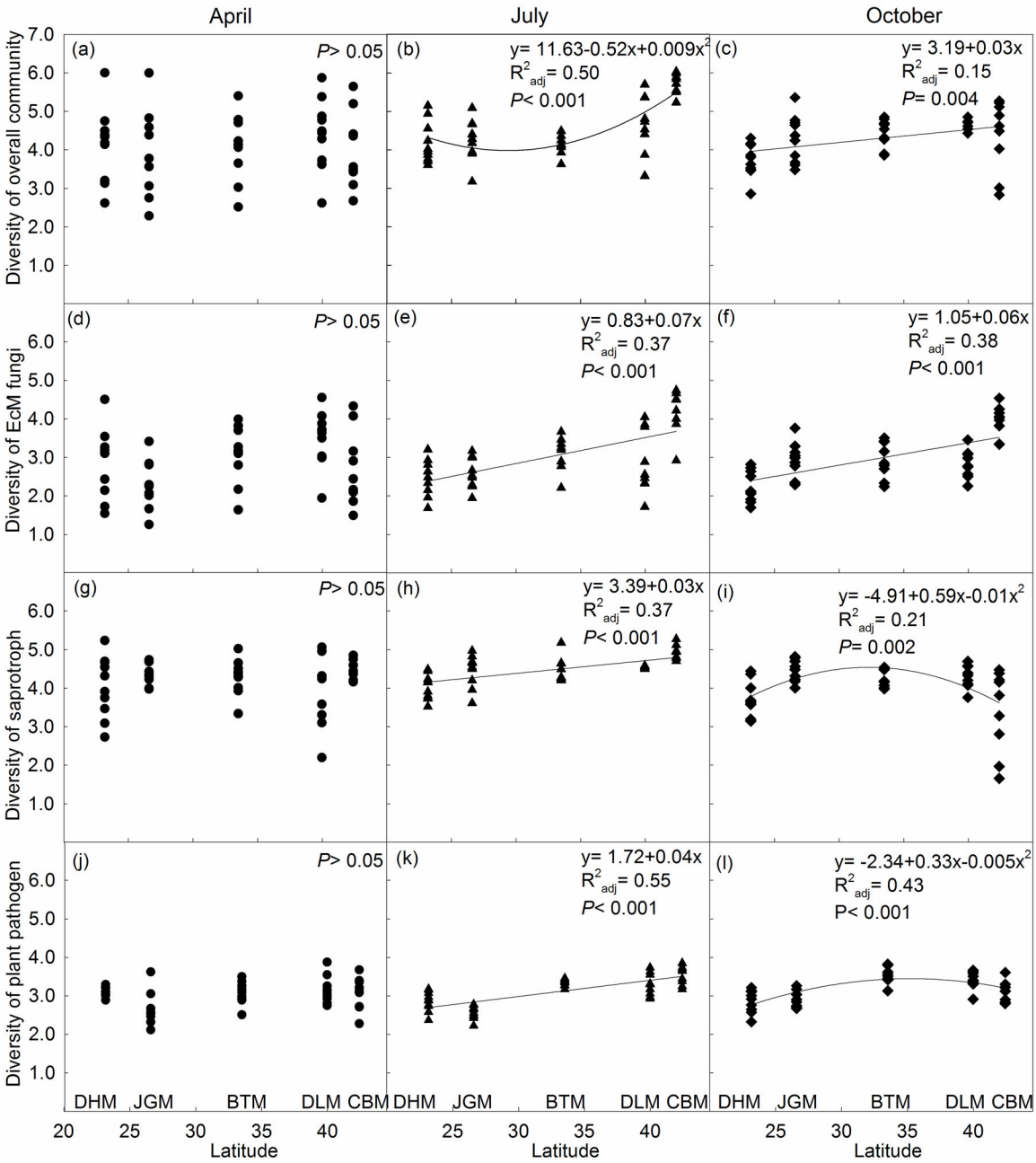

Figure 2. The latitudinal variations in Shannon diveristy of the overall fungal community (**a–c**) and the major functional guilds (**d–l**) in the three sampling seasons. Linear and polynomial regressions were used to derive the relationships between fungal diversity and latitude.

functional guilds and taxonomic groups. The seasonal variation of FCC was slightly smaller in TDF ($R^2 = 0.05$) than in SEF ($R^2 = 0.07$) (Table 3; Supplementary Table S6) for the overall fungal community, main functional guilds and most of the taxonomic groups. In April, the latitudinal variation of FCC was the highest in TDF but the lowest in SEF, while in July and October, the latitudinal variation tended to be less different between TDF and SEF. In each of the two sampling years, the seasonal variation of FCC at one specific site was slightly higher in SEF than in TDF (Supplementary Fig. S5).

We analyzed the relationships between FCC and environmental factors with the CCA method, showing that the overall FCC was strongly correlated with vegetation properties (plant diversity and forest age), soil pH and the contents of Mg^{2+} (pseudo-$F = 2.02$, $P < 0.001$; Fig. 5a). The plant diversity mainly influenced the FCC in SEF, while soil pH and the contents of Mg^{2+} mainly had an impact on the FCC in TDF. Similar patterns of influence of environmental properties on FCC were found for the main functional guilds (Fig. 5b–d). The relationships of environmental properties and FCC of the major functional guilds varied with

Table 2. Spearman correlations of fungal diversity (overall community and functional guilds) to climatic, soil and vegetation characteristics.These analyses were performed separately for the whole data set, the temperate deciduous forest (TDF) subset and the subtropical evergreen forest (SEF) subset. Bold typeface indicates statistical significance ($P < 0.01$). NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; TN, total nitrogen; TP, total phosphorous; N:P, the ratio of TN to TP; TOC, total organic carbon; C:N, the ratio of TOC to TN; SWC, matric soil water content; K^+, potassium ion; Ca^{2+}, calcium ion; Na^+, sodium ion; Mg^{2+}, magnesium ion; Fe^{3+}, iron ion; Al^{3+}, aluminum ion; Mn^{2+}, manganese ion; Temp, temperature; Prec, precipitation; FAG, forest age; PLD, plant diversity.

	Overall community			EcM fungi			Saprotroph			Plant pathogen		
	Whole	TDF	SEF	Whole	TDF	SEF	Whole	TDF	SEF	Whole	TDF	SEF
NH_4^+-N	0.17	0.10	0.31	**0.24**	0.18	0.28	**0.26**	0.16	**0.52**	0.19	0.17	−0.21
NO_3^--N	0.15	0.13	0.01	**0.23**	0.15	0.06	**0.26**	0.24	0.27	0.14	−0.09	−0.01
TN	**0.26**	0.15	0.16	**0.43**	**0.33**	0.12	**0.28**	0.20	**0.49**	**0.37**	0.07	**−0.38**
TP	**0.31**	0.13	0.24	**0.51**	**0.38**	**0.34**	**0.28**	0.14	**0.51**	**0.39**	−0.01	−0.13
N:P	−0.10	0.18	−0.07	**−0.22**	0.17	−0.28	−0.14	0.11	**−0.36**	−0.26	0.11	−0.14
TOC	**0.28**	0.15	0.23	**0.39**	**0.28**	0.20	**0.32**	0.25	**0.49**	**0.36**	0.10	**−0.38**
C:N	−0.07	0.07	0.10	**−0.27**	−0.17	0.11	−0.06	0.16	−0.19	−0.11	0.20	0.22
pH	**0.41**	**0.33**	**0.40**	**0.40**	0.04	0.17	**0.25**	0.18	**0.42**	**0.49**	0.11	**−0.48**
SWC	0.05	0.08	0.14	0.12	0.18	0.11	**0.33**	0.25	**0.53**	−0.07	0.04	**−0.43**
K^+	−0.17	−0.01	−0.31	−0.12	−0.04	−0.19	−0.07	−0.22	0.07	−0.05	−0.03	0.12
Ca^{2+}	**0.34**	0.17	0.26	**0.37**	−0.08	0.22	0.12	−0.10	0.20	**0.53**	0.01	0.16
Na^+	**0.25**	−0.0 3	0.10	**0.31**	−0.23	0.07	0.20	−0.06	**0.57**	**0.45**	−0.10	−0.31
Mg^{2+}	**0.42**	**0.35**	**0.39**	**0.40**	−0.04	0.31	**0.25**	0.25	0.26	**0.57**	0.19	−0.02
Fe^{3+}	−0.02	−0.18	−0.01	0.09	−0.19	0.08	−0.07	−0.27	0.08	**0.25**	0.04	0.13
Al^{3+}	0.10	−0.08	0.05	0.20	−0.13	0.23	−0.17	**−0.38**	−0.06	**0.24**	−0.24	0.32
Mn^{2+}	**0.25**	0.11	−0.01	**0.46**	**0.31**	0.09	0.19	0.02	**0.38**	**0.47**	0.03	−0.31
Temp	−0.05	0.08	0.20	−0.21	0.02	−0.03	0.10	**0.39**	−0.09	**−0.26**	0.17	−0.11
Prec	−0.01	0.18	0.01	−0.08	0.18	−0.03	0.11	**0.38**	−0.11	−0.11	0.15	0.06
FAG	0.02	**0.28**	−0.15	0.05	**0.33**	−0.09	−0.04	0.13	**−0.50**	-0.21	−0.09	**0.42**
PLD	−0.14	**0.28**	0.15	**−0.29**	**0.33**	0.09	−0.02	0.13	**0.50**	**−0.59**	−0.09	**−0.42**

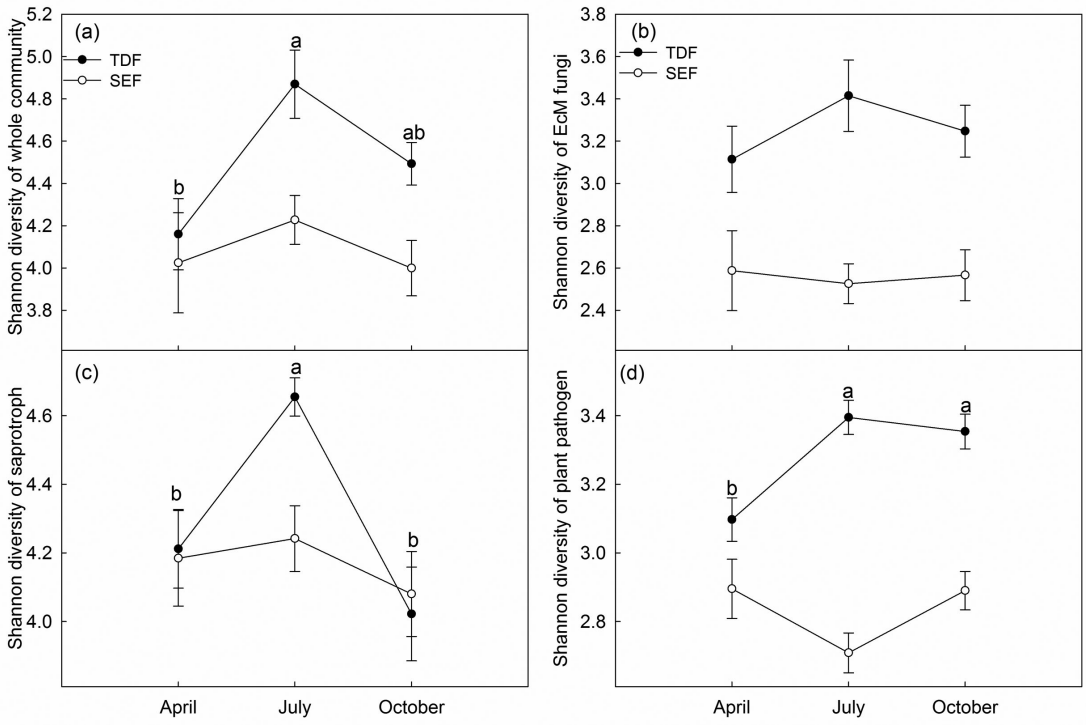

Figure 3. The seasonal variations in Shannon diversity of the overall fungal community (**a**) and the major functional guilds (**b–d**) in the temperate deciduous forests (TDF) and subtropical evergreen forests (SEF). Different lowercase letters indicate the diversity was significantly different among different seasons (ANOVA $P < 0.05$) for a given forest type.

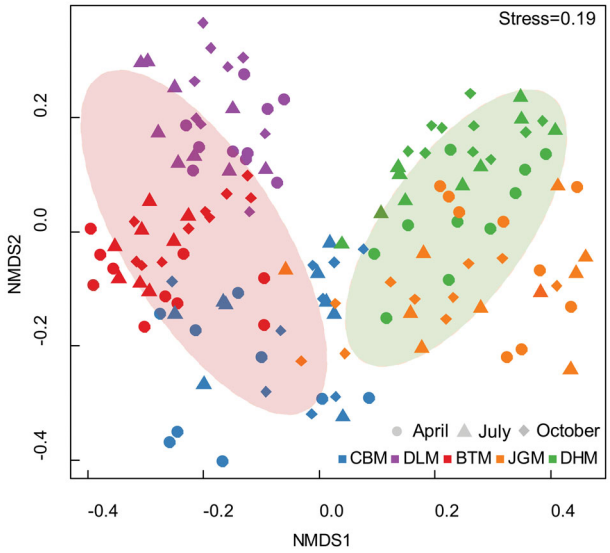

Figure 4. The temporal and spatial variation patterns of the overall fungal community composition revealed by non-metric multidimensional scaling (NMDS). Closer data points indicate more similar fungal community composition (FCC) between samples. The two forest types exhibit dissimilar FCC regardless of sampling season, with TDF being grouped in the red ellipse and SEF in the green one.

forest type. In TDF, soil pH, the concentration of soil cations, temperature and NH_4^+-N were the most important predictors ($P < 0.001$), while TOC, pH and soil cation concentrations drove FCC in SEF ($P < 0.001$; Supplementary Fig. S6).

DISCUSSION

Fungal diversity and influential factors

The latitudinal variation of soil fungal diversity has been found to differ among the land use/cover types involved and the spatial scale of analysis (Shi *et al.* 2014; Tedersoo *et al.* 2014; Liu *et al.* 2015). For example, soil fungal diversity showed a unimodal pattern with a peak at temperate forests (mid latitude), and this pattern was mainly driven by temperature in the forests of western China (Shi *et al.* 2014). Soil fungal diversity has also been found to decrease with increasing latitude at global geographic scales (Tedersoo *et al.* 2014) and in the arable black soils of northeastern China (Liu *et al.* 2015). Our results showed with the same land cover type (i.e. forests) that soil fungal diversity could exhibit linear, parabolic, or unclear latitudinal variation patterns among seasons. The season of soil sampling clearly imposed an effect on the latitudinal variations of fungal diversity due to the seasonal variations in climatic, edaphic and vegetational variables.

The community-level fungal diversity was significantly higher in TDF than in SEF and was positively correlated with soil pH and cation contents (e.g. Mg^{2+}, Ca^{2+} and Mn^{2+}; Table 2), indicating that soil cation concentrations could be important for fungal diversity. Base cations play important roles in many physicochemical processes such as plant photosynthesis. For instance, Mg^{2+} is a structural component of chlorophyll (St Clair and Lynch 2005). It can affect plant photosynthesis and therefore the amount of carbon available for soil bacteria and fungi (Shi *et al.* 2014). Mn^{2+} is a critical micronutrient that regulates microbial growth and reproduction and protects microbial communities against toxic metals and oxidative stress (Sujith and Bharathi 2011; Purahong *et al.* 2014). Ca^{2+} and pH can interact with each other to exert combined effects on fungal diversity (Tedersoo *et al.* 2014). In this study, the pH ranged from 3.8 to 6.2 (in TDF pH >5, in SEF pH <5; Supplementary Table S2). It has been discussed that the growth of fungal species would not be inhibited where the pH ranges across 5–9 (Wheeler, Hurdman and Pitt 1991; Nevarez *et al.* 2009). With the decreasing pH from TDF to SEF, some less endurable taxa may have become rare or even vanished in the presence of acidic stress, resulting in a decrease of soil fungal diversity (Beales 2004).

The differences in soil fungal diversity between TDF and SEF were not uniformly distributed among different growing seasons (Fig. 3; Supplementary Fig. S4), which indicated that seasonality imposed an impact on the latitudinal variation of fungal diversity. In July, fungal diversity exhibited a more drastic change than the other seasons in both TDF and SEF. The diversity of the overall fungal community was highest in July, which might be ascribed to the observation that in July the plants in both TDF and SEF show vigorous growth, producing and providing more substrates for fungal growth. What is more, the seasonal variations of fungal diversity were more distinct in TDF than in SEF (Fig. 3; Table 1). We argue that vegetation phenology may be responsible for the differentiated seasonal variations of fungal diversity between SEF and TDF. The vegetation growing season length declines with the increase of latitude (Yu *et al.* 2013). In the early and late growing seasons (April and October) in TDF, trees are in their sprouting and defoliation stages, respectively. Such distinct phenological phases, however, do not occur in SEF. So the plant-derived resources for fungal growth changed more drastically in TDF than in SEF, which could contribute to the more distinct seasonal variations of fungal

Table 3. Relative importance of latitude, season and their interaction on the fungal community composition at the overall community and functional guild levels, as revealed by the PERMANOVA. The whole data set and the subsets of temperate deciduous forests (TDF) and subtropical evergreen forests (SEF) were analyzed separately. Bold typeface indicates statistical significance ($P < 0.05$).

		Overall community		EcM fungi		Saprotroph		Plant pathogen	
		R^2	P	R^2	P	R^2	P	R^2	P
Whole data set	Latitude (L)	0.271	**0.001**	0.242	**0.001**	0.302	**0.001**	0.232	**0.001**
	Season (S)	0.027	**0.001**	0.024	**0.001**	0.027	**0.001**	0.030	**0.001**
	L × S	0.067	**0.001**	0.066	**0.001**	0.066	**0.001**	0.071	**0.001**
TDF	Latitude (L)	0.196	**0.001**	0.170	**0.001**	0.215	**0.001**	0.156	**0.001**
	Season (S)	0.054	**0.001**	0.047	**0.001**	0.056	**0.001**	0.063	**0.001**
	L × S	0.054	**0.003**	0.054	**0.003**	0.056	**0.002**	0.053	**0.002**
SEF	Latitude (L)	0.106	**0.001**	0.113	**0.001**	0.110	**0.001**	0.073	**0.001**
	Season (S)	0.066	**0.002**	0.069	**0.001**	0.062	**0.001**	0.079	**0.001**
	L × S	0.042	**0.026**	0.035	0.151	0.046	0.012	0.035	0.180

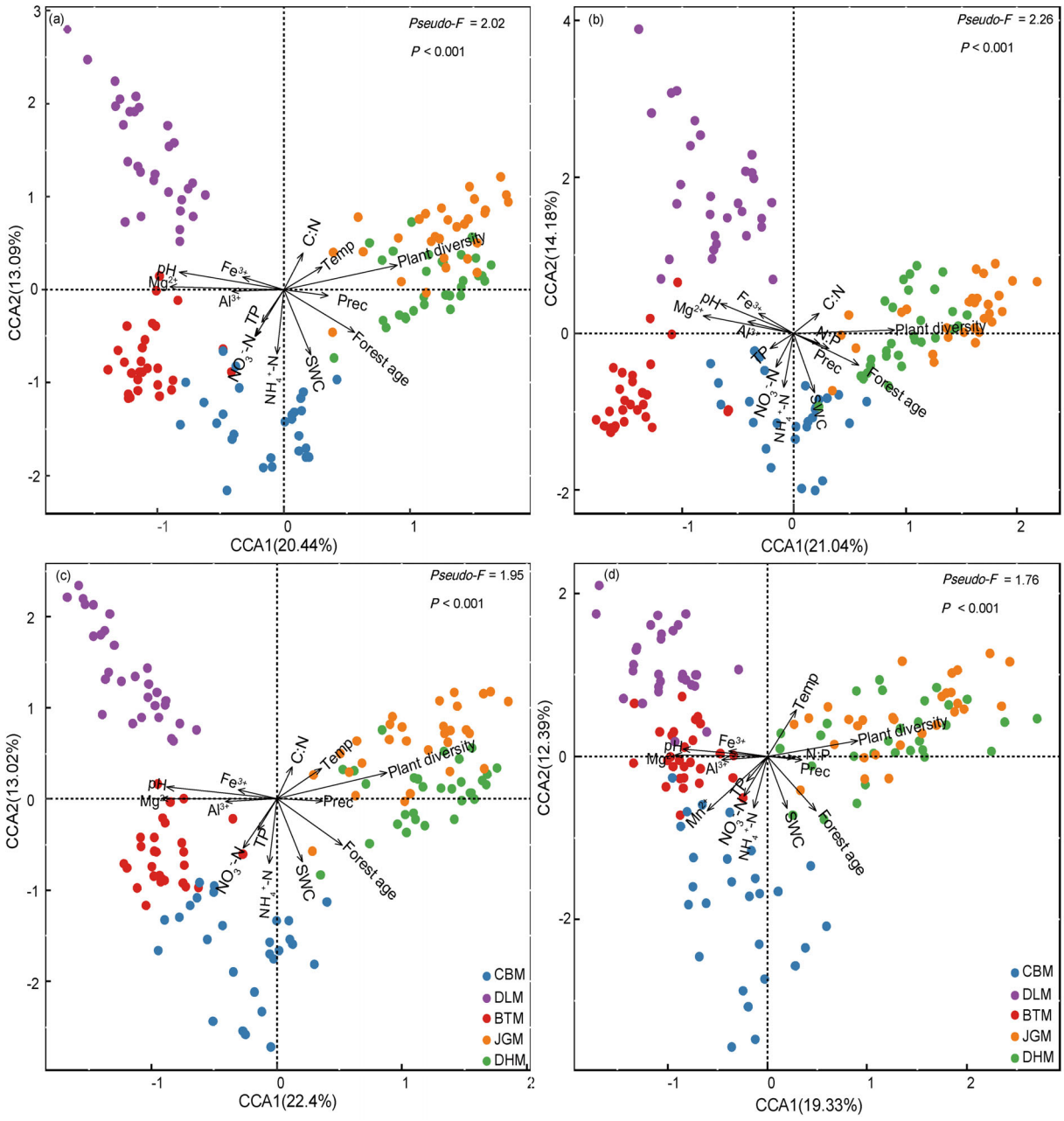

Figure 5. Correlations between the fungal community composition and the main predictors revealed by canonical correspondence analysis (CCA). (**a**) overall fungal community, (**b**) EcM fungi, (**c**) saprotrophs and (**d**) plant pathogens. NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; TP, total phosphorous; N:P, the ratio of TN to TP; C:N, the ratio of TOC to TN; SWC, matric soil water content; Mg^{2+}, magnesium ion; Fe^{3+}, iron ion; Al^{3+}, aluminum ion; Mn^{2+}, manganese ion; Temp, temperature; Prec, precipitation; CBM, Changbai Mountain; DLM, Dongling Mountain; BTM, Botianman Mountain; JGM, Jinggang Mountain; DHM, Dinghu Mountain.

diversity in TDF than in SEF. In addition, the soil properties such as the contents of soil Mg^{2+} and TP were higher in July than the other two months, and changed more drastically in TDF than in SEF (Supplementary Table S3), which can also be linked to the more distinct diversity changes in TDF than in SEF.

Spatial and seasonal variations of fungal community composition

The soil FCC was also significantly different between TDF and SEF (Fig. 4; Table 3). Plant diversity was an important factor affecting the soil FCC in forests of eastern China (Fig. 5). Similar results have been demonstrated for other forest soils (Peay, Baraloto and Fine 2013; Shi *et al.* 2014). In addition, the majority of soil nutrients we focused on were higher in TDF than in SEF (Supplementary Table S2), and it has been indicated that soil nutrient levels were the dominant predictors of FCC (Lauber *et al.* 2008). Specifically, high soil NH_4^+-N and NO_3^--N were characteristic of the samples from CBM, where the relatively high contents of soil dissolved inorganic nitrogen could cause a special fungal community fit to an N-saturation environment (Supplementary Table S2); soil pH and contents of metal ions also showed important roles in driving FCC differentiation (Fig. 5; Supplementary Fig. S6). Soil pH has been demonstrated

to be one of the most important parameters in shaping microbial community composition. Although in some studies pH was less important than soil fertility (such as TOC) on fungal FCC (Liu *et al.* 2015), our study still supported the essential role of pH in affecting soil FCC in forests of eastern China. The contents of soil metal ions could directly affect fungal growth by regulating the osmotic pressure of fungal cells (Jackson and Heath 1989), or indirectly by changing the growth state of aboveground plants and therefore the root exudates for fungal growth (Kozdrój and van Elsas 2000).

The influence of latitude on soil FCC was larger than that of season in both TDF and SEF, and season also imposed an effect on the latitudinal variation of soil FCC, which is confirmed by the significant interaction effects of latitude and season in most cases (Table 3). In April, the dissimilarity of FCC among samples was the highest in TDF but the lowest in SEF, contrasting with the situations in other seasons (Supplementary Fig. S5a). The difference might also be ascribed to the climatic and phenological differences between TDF and SEF, while the underlying mechanisms remain understudied. In general, the latitudinal variation of FCC within TDF were larger than those within SEF in a given season (Supplementary Fig. S5a). This may be explained by the latitudinal span within TDF and SEF. The latitudinal gradient was nearly 9° within TDF, which was larger than that within SEF (nearly 3°). Green *et al.* (2004) demonstrated that the similarity of fungal community composition declined with increasing distance at the continental scale. The increased distance therefore represents more spatial heterogeneity encountered by soil fungi, resulting in a higher latitudinal variability of soil FCC in TDF than SEF.

Spatial–temporal variations of functional guilds or taxonomic groups

The diversity of EcM fungi was significantly higher in TDF than in SEF (Fig. 3b), consistent with previous studies where the EcM fungi dominated in temperate forests (Shi *et al.* 2014; Tedersoo *et al.* 2014). One possible reason could be due to the greater dominance of their plant hosts (e.g. *Pinus* spp.) in TDF than in SEF (Supplementary Table S1), as pine trees are a common kind of plant host for EcM fungi (Hibbett, Gilbert and Donoghue 2000). Additionally, EcM fungi were found mainly in the neutral or sub-acidic soils (Tedersoo *et al.* 2014), which could explain the higher diversity of EcM fungi in TDF (with neutral/sub-acidic soils) compared with SEF (with strong acidic soils).

The diversity of saprotrophs was strongly correlated with temperature (Table 2). This has been evidenced by Burke *et al.* (2011) who noted that saprotrophic fungal diversity was positively correlated with soil enzymatic activities that were mainly affected by temperature (Wallenstein *et al.* 2010). Saprotrophs did not show an obvious latitudinal variation, which may be due to the different responses of saprotrophs to the soil pH and carbon contents. High diversity of saprotrophs was found in soils with low pH (Tedersoo *et al.* 2014) and high carbon content (Barnes *et al.* 2016). In our case, soil pH was lower in SEF but the carbon content was higher in TDF, which might have contributed to the similar saprotrophic diversity between the two types of forests.

Agaricomycetes (Basidiomycota) was the most abundant class in this study, consistent with other investigations of forest soils (Buée *et al.* 2009; Peay, Baraloto and Fine 2013; Shi *et al.* 2014; Purahong *et al.* 2016). Sordariomycetes was the dominant class in Ascomycota and its diversity did not display significant seasonal variation or spatial patterns (Supplementary Fig. S4f), indicating the high ecological plasticity of this group. The species of Sordariomycetes can play very versatile roles in ecosystems, such as plant pathogens, animal parasites, mycorrhizal symbionts and saprotrophs involved in decomposition and nutrient cycling (Zhang *et al.* 2006; Tedersoo and Smith 2013).

CONCLUSIONS

This study used high-throughput sequencing to investigate the diversity and composition of soil fungal communities and their latitudinal and seasonal variations in five forest ecosystems in eastern China. Soil fungal diversity was greater, with more distinguishable seasonal variation, in temperate deciduous forests than in subtropical evergreen forests, which was mainly affected by the spatiotemporal variation of soil pH, cation contents (mainly Mg^{2+}, Ca^{2+} and Mn^{2+}) and nutrient contents (TP, TOC and TN). Both diversity and composition variations were greater across latitude than among seasons, indicating the dominant roles of spatial heterogeneity in shaping soil fungal communities. At different organizational levels (e.g. overall community level or functional guild level), the latitudinal distribution patterns of fungal diversity differed in their responses to environmental variations, which points to a scale dependency of environmental selection of soil fungi in forests of eastern China.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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