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Host plant genus-level diversity is the best predictor of ectomycorrhizal fungal diversity in a Chinese subtropical forest

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Abstract

Microbial diversity is generally far higher than plant diversity, but the relationship between microbial diversity and plant diversity remains enigmatic. To shed light on this problem, we examined the diversity of a key guild of root-associated microbes, that is, ectomycorrhizal (EM) fungi along a plant diversity gradient in a Chinese subtropical forest. The results indicated that EM fungal diversity was positively correlated with host plant diversity. Furthermore, this relationship was best predicted by host genus-level diversity, rather than species-level diversity or family-level diversity. The generality of this finding was extended beyond our study system through the analyses of 100 additional studies of EM fungal communities from tropical and temperate forests. Here as well, EM fungal lineage composition was significantly affected by EM plant diversity levels, and some EM fungal lineages were co-associated with some host plant genera. These results suggest a general diversity maintenance mechanism for host-specific microbes based on higher order host plant phylogenetic diversity.

Keywords: ectomycorrhizal fungal diversity, EM fungal community, EM plant diversity, ITS barcoding, subtropical forest

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Introduction

Feedback between aboveground and belowground organisms plays a key role in biodiversity maintenance and ecosystem function (Wardle *et al.* 2004; De Deyn & Van der Putten 2005). Soil microbes, as an important component of belowground habitats, can influence plant diversity, productivity and community composition in natural ecosystems (van der Heijden *et al.* 1998, 2008; Torsvik & Ovreas 2002). However, as these organisms depend on the products of plant photosynthesis, microbial communities should be affected by plant community composition, diversity and productivity (Hooper *et al.* 2000; Waldrop *et al.* 2006; Wardle 2006; Bahram *et al.* 2012). Ectomycorrhizal (EM) fungi are a major component of soil microbial communities. These fungi form a symbiotic partnership with plants and can benefit their hosts by enhancing nutrient and water uptake from the soil and protecting EM plant roots from pathogens and toxic compounds (Smith & Read 2008). Despite the obvious link between plants and fungi, the role of aboveground EM plant communities in affecting EM fungal communities remains enigmatic (Dickie 2007; Ishida *et al.* 2007; Peay *et al.* 2010b; Bahram *et al.* 2012).

The *plant diversity hypothesis* is one of the prevailing theories to explain patterns of soil microbial diversity (Hooper *et al.* 2000; Waldrop *et al.* 2006). This hypothesis proposes that greater plant diversity increases the range of organic substrates entering the soil, thus creating more niche space that can accommodate a greater

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diversity of decomposer microbes (Hooper *et al.* 2000; Waldrop *et al.* 2006). While this hypothesis was originally focused on decomposers, the diversity of symbiotic microbes, such as mycorrhizal fungi, might also be controlled by similar mechanisms. For example, EM fungi have been shown to exhibit host preferences in most studies (e.g. Molina *et al.* 1992; Richard *et al.* 2005; Dickie 2007; Ishida *et al.* 2007; Tedersoo *et al.* 2008, 2010c; Ding *et al.* 2011). As a result, increasing the number of EM host plant species should lead to increasing EM fungal diversity through the addition of new niches. Such host specificity effects could be construed as an expansion of the original chemical resource-based mechanism of the *plant diversity hypothesis*. Interestingly, this suggests that symbiotic microbes and saprotrophic microbes might respond more strongly to different components of plant diversity, for example chemical vs. phylogenetic. A recent study by Sato *et al.* (2012) found that plant constraints on geographical distribution were stronger for EM fungi than for decomposer fungi. Nevertheless, studies examining this niche-based hypothesis for EM fungi have not yielded consistent results. For example, this hypothesis has been supported based on the studies of belowground EM fungi (Kernaghan *et al.* 2003; Dickie 2007) and a meta-analysis of EM fungal fruiting bodies (Schmit *et al.* 2005), showing positive linear correlations between EM plant species diversity and EM fungal species diversity in temperate forests. However, Kernaghan & Harper (2001) found that there was no relationship between EM plant species diversity and EM fungal species diversity across an alpine/subalpine ecotone. Furthermore, an increasing number of studies have found that EM fungal species diversity is relatively low in tropical forests that with a high species diversity of EM host plants (Peay *et al.* 2010b; Tedersoo *et al.* 2010c, 2012). To reconcile the contradiction between EM fungal species diversity in temperate and tropical forests, it has been proposed that EM fungal species diversity may be controlled by EM plant diversity at higher phylogenetic levels, such as genus or family diversity (Peay *et al.* 2010b; Tedersoo & Nara 2010). However, no study has quantitatively examined the effect of higher EM plant phylogenetic diversity on EM fungal species diversity.

Besides EM plant phylogeny, EM fungal community may be also controlled by other EM plant traits, such as EM plant density and identity (Ishida *et al.* 2007; Dickie *et al.* 2009; Peay *et al.* 2011), litter quality (Aponte *et al.* 2010) and leaf phenology traits (i.e. deciduous or evergreen plants) (Morris *et al.* 2008). Moreover, EM fungal community structure may also be influenced by non-EM plant and abiotic factors (Taniguchi *et al.* 2007; Smith & Read 2008). For example, EM fungal species diversity and community composition has been shown to be influenced by altitude (Bahram *et al.* 2012), forest productivity and soil organic matter content (Kernaghan 2005) and soil nitrogen and carbon contents (Twieg *et al.* 2009; Kjøller *et al.* 2012). Because abiotic and biotic factors often covary, it is important to disentangle the influences of EM plant community structure from the effects of non-EM plant, soil chemistry and topography.

To decouple these factors, in this study, we used molecular tools to examine EM fungal diversity along a gradient of EM plant diversity in a Chinese subtropical forest. Because this system is well characterized, we are able to explore the influences of both (i) EM plant phylogenetic diversity (species, genus and family levels) and abundance, and (ii) non-EM plant factors and abiotic factors on EM fungal species diversity. To generalize our main finding beyond a single system, we also compiled 83 temperate and 17 tropical forest data sets to test the effect of host plant phylogenetic diversity on EM fungal species diversity. Because of a number of studies have demonstrated host preferences of EM fungi, we predicted that there would be a strong correlation between host plant diversity and EM fungal diversity, in support of the *plant diversity hypothesis*. However, based on mixed results of plant diversity effects on EM fungi in the literature, we predicted that host plant diversity at higher phylogenetic levels might be a better predictor than species diversity.

Materials and methods

Study site

This study was conducted at a 24-ha permanent plot in a subtropical evergreen broad-leaved forest in the Gutianshan Nature Reserve (GNR) in Zhejiang province, southeast China (29°15′6″–29°15′21″ N, 118°07′01″–118°07′24″ E). The GNR occurs in a subtropical monsoon climatic zone, with an annual mean temperature of 15.38 °C and annual mean precipitation of 1964 mm (Zhu *et al.* 2008). The soil of the 24-ha plot is subtropical red soil and the topography is rugged, with the elevation ranging from 446.3 m to 714.9 m (Zhu *et al.* 2008). Currently, most of the forest is in middle and late successional stages (Legendre *et al.* 2009). The 24-ha permanent plot was divided into 600 quadrats (20 m × 20 m each), and all trees with diameter at breast height (DBH) $\geq$1 cm have been tagged, identified, measured and geo-referenced (Legendre *et al.* 2009). In total, 140 676 individual trees belonging to 159 species and 49 families were identified in the 24-ha plot. Observations of root morphology of suspected EM plants under a dissecting microscope confirmed a total of 11 EM plant species belonging to two families in the 24-ha plot, that is, *Pinus massoniana* Lamb. of the Pinaceae

and *Castanopsis eyrei* (Champion ex Bentham) Tutcher, *Castanopsis tibetana* Hance, *Castanopsis carlesii* (Hemsl.) Hayata, *Castanopsis fargesii* Franch, *Cyclobalanopsis glauca* (Thunb.) Oerst, *Cyclobalanopsis myrsinaefolia* (Blume) Oerst, *Cyclobalanopsis gracilis* (Blume) Oerst, *Quercus serrata* Murray, *Quercus phillyraeoides* A. Gray and *Lithocarpus glaber* (Thunb.) Nakai of the Fagaceae. Soil properties (total carbon (C), total nitrogen (N), total phosphorus (P), NH_4^+-N, NO_3^--N, bulk density, pH and moisture) and topographical characteristics (altitude, convexity, slope and aspect) of each quadrat have been determined and reported in previous studies (Legendre *et al*. 2009; Zhang *et al*. 2011).

Experimental design and sampling

To detect the influence of EM plant diversity on EM fungal diversity, four EM plant diversity levels were selected from the 24-ha plot, according to an increasing EM plant species richness gradient. EM plant species diversity level-I included two EM plant species, *C. eyrei* and *L. glaber* of the Fagaceae. Level-II included four EM plant species, *P. massoniana* of the Pinaceae and *C. eyrei*, *Cy. glauca* and *Q. serrata* of the Fagaceae. Level-III included five EM plant species, *C. eyrei*, *C. fargesii*, *L. glaber*, *Cy. myrsinaefolia* and *Cy. gracilis* of the Fagaceae. Level-IV included seven EM plant species, *P. massoniana* of the Pinaceae and *C. eyrei*, *C. fargesii*, *Cy. glauca*, *Cy. myrsinaefolia*, *Q. serrata* and *L. glaber* of the Fagaceae. For each EM plant diversity level, three non-neighbouring quadrats were selected as replicates (see Fig. S1, Supporting information). To account for other potential biotic and abiotic factors affecting EM fungal community structure, we also collected a range of additional explanatory variables for each quadrat, including species richness (the number of species per quadrat), genus richness (the number of genera per quadrat), family richness (the number of families per quadrat), abundance (the number of individuals per quadrat) of both EM and non-EM plants, EM plant basal area, dominance (Simpson dominance index) and Shannon diversity index, altitude, convexity, slope, aspect, total C, total N, total P, NO_3^--N, NH_4^+-N, pH, bulk density and moisture (Table S1, Supporting information). Simpson dominance index was calculated according to the following formula: $D = \sum_i^s p_i^2$, where p_i is the proportion of species i in the community and s is number of species (Simpson 1949). Shannon diversity index was calculated according to the following formula: $H\prime \sum_i^s (p_i)(\log_2 p_i)$, where p_i is the proportion of species i in the community and s is number of species (Shannon 1948). The abundance and range of the DBH of each EM plant species in each quadrat are presented in Table S2 (Supporting information).

To measure EM fungal diversity, we collected soil samples from each quadrat in October 2010. In each quadrat, we sampled a total of nine soil cores (10 cm in diameter × 10 cm deep), each placed about 0.5- to 1.0-m distance from an EM tree. The specific location of soil cores was determined systematically in each quadrat according to the following criteria: (i) all EM plant species that occurred in the quadrat were sampled at least once, (ii) sample cores were assigned to each EM plant species in proportion to their relative abundance and (iii) the soil cores were relatively evenly distributed in the quadrat. The spatial location of all soil cores and EM plants are presented in Fig. S2 (Supporting information). For each soil core, the roots within were washed free from soil over a 380-μm sieve in running tap water. All fine roots (<2 mm diam.) were trimmed into *ca*. 1-cm-long fragments from which 100 were randomly selected. EM root tips from the 100 root fragments were separated into different EM morphotypes based on morphological characteristics, such as shape, colour, size and texture under a stereomicroscope. We removed up to 20 root tips per EM morphotype in each sample. On average, this resulted in approximately 200 ± 20 EM root tips picked from each soil sample. While cores were selected from beneath one host, no plant root morphotyping was carried out so that samples likely contained roots from different co-occurring EM plant species. All EM root tips from a given soil sample were pooled and stored at −80 °C until DNA extraction.

Molecular analysis

The nine root samples from each quadrat were further consolidated into three pooled samples (consisting of three root samples each) for the purposes of DNA extraction and molecular characterization. Because all our statistical analyses are conducted at the quadrat level, pooling samples within quadrats should not have any effect on our results. Total DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method (Gardes & Bruns 1993). Briefly, root sample (including ~ 600 root tips each) was freeze-dried using liquid nitrogen and homogenized using mortar. The homogenized sample was then transferred into a 10-mL centrifuge tube with 2000 μL of 2× CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, pH 8.0) and incubated in a 65 °C water bath for 1.5 h with occasional gentle swirling. Equal volume of cold chloroform–isoamylol mixture (24:1 v/v) was added to each tube and mixed by brief vortexing. The mixture was spun at 12 000 g for 15 min at room temperature, and the aqueous phase was removed into a new centrifuge tube. The aqueous phase containing DNA was re-extracted with chloroform:isoamyl alcohol

(24:1) until no interface was visible. The aqueous phase was transferred into a new centrifuge tube, and DNA was precipitated with equal volume of cold isoproponal. The genomic DNA was precipitated at 12 000 g for 5 min at 4 °C. The DNA pellet was washed with 70% ethanol twice and dried using SpeedVac 2 (AES 1010; Savant, Holbrook, NY, USA) for 10 min or until dry. The DNA pellet was then resuspended in 150 μL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The internal transcribed spacer (ITS) regions of each DNA sample were amplified with the primers ITS5 and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) as recommended by Tedersoo *et al.* (2010b). Amplifications were performed in a Gene Amplification PCR System (East Win, Beijing, China) with initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 52 °C for 50 s and 72 °C for 1 min, and final extension at 72 °C for 10 min. Amplifications consisted of a 25-μL reaction mixture containing 1 U *Taq* (Takara, Dalian, China), 1 × PCR buffer, 2 mM $MgCl_2$, 0.2 μM dNTP, 0.4 μM primers and 1 μL of DNA template. Four replicate PCR products of each sample were pooled and purified using the Bioteke PCR Product Purification Kit (Bioteke, Beijing, China) and were cloned into the *p*GEM-T vector (Promega, USA). For each cloning library, ~80 positive *Escherichia coli* colonies were randomly selected and used as templates for PCR with primers ITS5/ITS4. Restriction fragment length polymorphism (RFLP) was carried within each clone library. Five microlitres of each PCR product was combined with 2.6 μL sterile deionized water, 2 μL buffer and 0.4 μL restriction endonuclease (either FastDigest *Hinf*I or FastDigest *Alu*I, Fermentas, USA). RFLP products were size fractionated on 2% agarose gels. Gels were stained with Goldview (Applied Biosystems, USA) under ultraviolet light. RFLP band sizes were estimated by comparison with a standard 100 base pair (bp) molecular weight ladder. RFLP matches were carried out in GERM (Dickie *et al.* 2003). RFLP types were only compared within each clone library, but not cross clone libraries (Smith *et al.* 2007). One representative clone of each unique RFLP type in each clone library was sequenced with a vector primer T7 using ABI Prism 3700 Genetic Analyzer (Applied Biosystems, USA).

Sequences obtained in this study were edited to obtain only the ITS regions using Mega v5.0 (Tamura *et al.* 2011) and defined into operational taxonomic units (OTUs) with a 97% threshold (Tedersoo *et al.* 2008) using Sequencher v4.2 (Gene Codes, Ann Arbor, MI, USA). Each OTU was assigned a mycorrhizal status based on the results from BLAST search against the UNITE (Abarenkov *et al.* 2010) and NCBI (National Center for Biotechnology Information) databases. OTUs that returned top BLAST results with high similarity to known EM fungi were assigned to phylogenetic lineages according to the nomenclature of Tedersoo *et al.* (2010a). Chimera check was carried out using the MAXCHI and CHIMAERA programs of Recombination Detection Program (RDP) (Martin & Rybicki 2000). Sequences of all EM fungal OTUs were submitted to European Molecular Biology Laboratory (EMBL) database (accession No. HE814066-HE814239).

Data source

We also explored the response of EM fungal species diversity to EM plant phylogenetic diversity (at the species, genus and family levels) across a range of previously published studies conducted in both tropical and temperate ecosystems. For statistical analysis, we systematically searched for published studies in ISI Web of Sciences using the key words 'ectomycorrh*' and 'diversit*'. The resulting studies were filtered by the following criteria: (i) the study sampled belowground root tip EM fungal communities, that is, studies focused on aboveground fruitbodies and belowground extramatrical mycelia in soil were excluded; (ii) sampling was carried out in a defined field area (plot or transect) within 10 km, and sites further than 10 km apart from each other were treated as different studies; (iii) species, genus and family information for EM plants was available; and (iv) EM fungal OTUs were identified based on DNA sequence or the database-terminal RFLP method. A total of 83 studies from temperate and 17 studies from tropical sites were included in our final analysis. Information on EM plant phylogenetic richness (species, genus and family levels) and EM fungal species richness is presented in Table S3 (Supporting information).

Data analysis

One-way analysis of variance (ANOVA) followed by least square difference (LSD) *post hoc* test was carried out to explore differences in EM fungal species richness among the four EM plant diversity levels included in our field study. Univariate regressions were carried out to explore the responses of EM fungal species richness to the species richness, genus richness, family richness and abundance of EM plants and non-EM plants, EM plant basal area and dominance and all abiotic variables (altitude, convexity, slope, aspect, total C, total N, total P, NH_4^+-N, NO_3^--N, bulk density, pH and soil moisture). Furthermore, a multiple regression model including all significant variables from univariate regressions was constructed and simplified using stepwise backward selection until $P < 0.05$ for all variables. In addition, the regression models using the Shannon

diversity index of EM fungi with Shannon diversity indices of EM plant species, genus and family were also carried out. For the 83 temperate and 17 tropical data sets obtained through our literature search, univariate and multiple regressions of EM fungal species richness with EM plant species, genus and family richness were carried out. However, because information on EM plant abundance, dominance and size could not be directly obtained from most of the compiled references, we were unable to test their influences on EM fungal species richness in the global analysis.

Global nonmetric multidimensional scaling (GNMDS) was carried out to visualize the Bray–Curtis dissimilarity among quadrats for both EM fungal species and lineage compositions (clone number data set, wisconsin —sqrt transformed). EM plant diversity levels were fitted as centroids onto the ordination plots using the '*envfit*' function in the *Vegan* package (Oksanen *et al.* 2007). Pearson correlations were carried out to explore the relationships between EM fungal lineages and EM plant species. Mantel tests were carried out to explore the relationships of EM fungal species and lineage compositions with species richness, genus richness, family richness and composition of EM plants and non-EM plants, soil and topography in the *Ecodist* package (Goslee & Urban 2007). All statistical analyses were carried out in R 2.14.1 (R Development Core Team 2010).

Results

Relationship between EM fungal diversity and EM plant diversity

Analysis of variance results showed that EM plant diversity levels had a significant effect on EM fungal species richness ($F_{3,\ 8}$ = 6.788, P = 0.014) in the subtropical forest. LSD *post hoc* test showed that EM fungal species richness was significantly lower at Level-I than at Level-II and IV, but there was no significant difference between Level-I and III and among Level-II, III and IV (Fig. 1). EM fungal species richness significantly increased with increasing EM plant species richness (R^2 = 0.491, P = 0.011, Fig. 2A), genus richness (R^2 = 0.684, P = 0.001, Fig. 2B), family richness (R^2 = 0.570, P = 0.005, Fig. 2C) and abundance (R^2 = 0.560, P = 0.005, Table 1), but not with EM plant dominance and basal area ($P > 0.05$, Table 1) and non-EM plant and abiotic variables ($P > 0.05$, Table 2). The Shannon diversity index of EM fungi was also significantly related with the Shannon diversity indices of EM plant species (R^2 = 0.353, P = 0.042) and genus (R^2 = 0.358, P = 0.040), but not with the Shannon diversity index of EM plant family (R^2 = 0.232, P = 0.113).

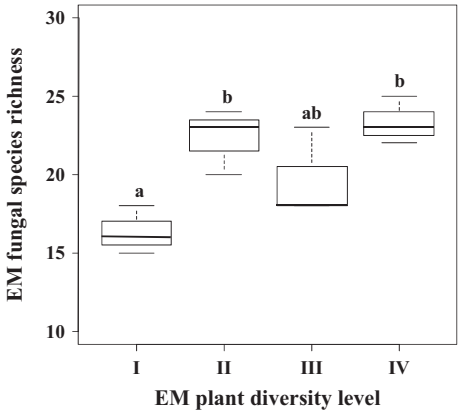


Fig. 1 Ectomycorrhizal (EM) fungal species richness in the four EM plant diversity levels ($F_{3,\ 8}$ = 6.788, P = 0.014). Different letters on bars denote significant difference at $P < 0.05$ level.

Results from the 83 temperate data sets indicated that EM fungal species richness significantly increased with increasing EM plant species richness (R^2 = 0.168, P = 0.001, Fig. 2D), genus richness (R^2 = 0.319, P = 0.001, Fig. 2E) and family richness (R^2 = 0.187, P = 0.001, Fig. 2F). Results from the 17 tropical data sets showed that EM fungal species richness also significantly increased with increasing EM plant species richness (R^2 = 0.343, P = 0.011, Fig. 2D) and genus richness (R^2 = 0.537, P = 0.001, Fig. 2E), but not significantly increased with EM plant family richness (R^2 = 0.102, P = 0.211, Fig. 2F).

A multivariate regression model including EM plant species richness, genus richness, family richness and abundance as independent variables was constructed for subtropical Gutianshan. Multivariate regression models including EM plant species richness, genus richness and family richness as independent variables were constructed for temperate and tropical data sets, respectively. Forward stepwise selections were carried out until all variables were significant. EM plant genus richness was the only variable retained in the final model for subtropical Gutianshan and also for both the temperate and tropical data sets, respectively (Table 1), indicating that EM plant genus richness was the best predictor of EM fungal species richness across temperate, subtropical and tropical forests.

EM fungal community structure

In total, we characterized EM fungi from approximately 21 600 EM root tips (200 ± 20 root tips per soil sample) from 108 samples in 12 quadrats. From this 36 clone libraries constructed (three clone libraries for each quadrat), we detected a total of 235 fungal OTUs at 97% sequence similarity. Based on BLAST results, 173 OTUs were assigned to EM fungal lineages and 62 to non-EM fungal

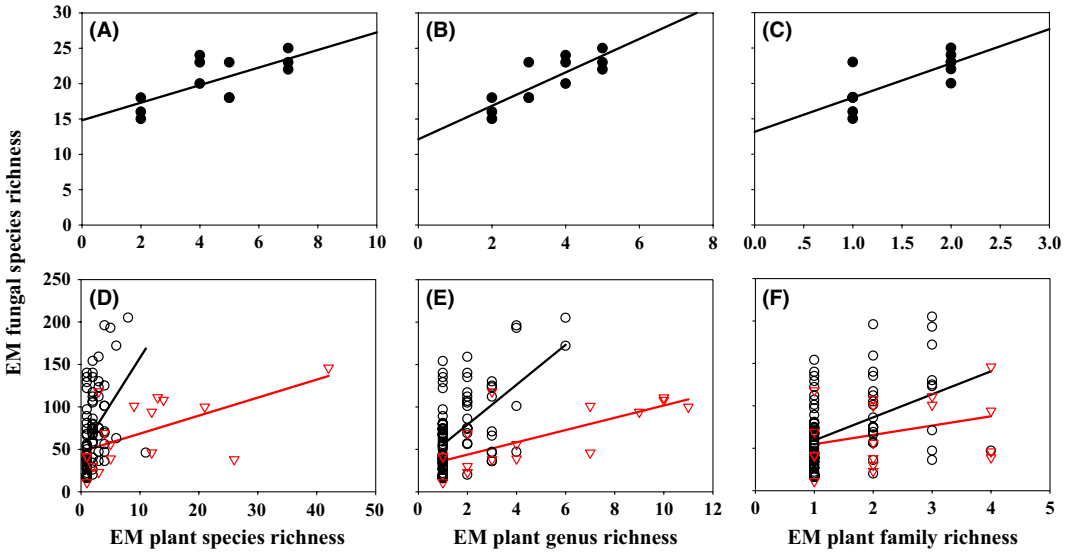


Fig. 2 Linear regressions of ectomycorrhizal (EM) fungal species richness with EM plant species richness, genus richness and family richness for (A–C) subtropical Gutianshan and (D–F) temperate (circles) and tropical (triangles) data.

Table 1 Univariate and multiple regressions of ectomycorrhizal (EM) fungal species richness with EM plant species richness, genus richness, family richness, abundance, basal area and dominance in the subtropical Gutianshan (n = 12); and univariate and multiple regressions of EM fungal species richness with EM plant species richness, genus richness and family richness for temperate (n = 83) and tropical (n = 17) data sets

	Univariate regression						Multiple regression†		
	Subtropical Gutianshan		Temperate data		Tropical data				
EM plant variable	Slope	R^2	Slope	R^2	Slope	R^2	Subtropical Gutianshan Parameter	Temperate data Parameter	Tropical data Parameter
Species richness	1.244	0.49*	11.12	0.17**	2.12	0.34*	$F_{1, 10}$ = 21.6	$F_{1, 81}$ = 42.07	$F_{1, 15}$ = 16.24
Genus richness	2.267	0.68**	23.53	0.32**	7.24	0.54**	R^2 = 0.68	R^2 = 0.32	R^2 = 0.54
Family richness	4.833	0.57*	27.15	0.19**	10.96	0.10	P = 0.001	P = 0.001	P = 0.001
Abundance	0.124	0.56*							
Basal area	0.0002	0.04							
Dominance	6.916	0.15							

†The original model for subtropical Gutianshan (R^2 = 0.685, P = 0.006) included EM plant species richness, genus richness, family richness and abundance as independent variables. The original models for temperate data (R^2 = 0.334, P < 0.001) and tropical data (R^2 = 0.682, P = 0.008) included EM plant species richness, genus richness and family richness as independent variables. The optimized models only retained EM plant genus richness for Gutianshan, temperate and tropical data, respectively. *0.01 < P < 0.05, **P < 0.01.

status. The 173 EM fungal OTUs accounted for 1858 clones (Fig. 3A). Of the 173 EM fungal OTUs, 48 belonged to lineage /tomentella-thelephora, 32 to /boletus, 25 to /russula-lactarius, 16 to /sebacina, 13 to /cortinarius, 10 to /helotiales, 6 to /sordariales, 4 to /clavulina, 3 to /amanita, 3 to /cenococcum, 2 to /cantharellus, 2 to /inocybe, 2 to /entoloma, 2 to /hygrophorus, 1 to /peziza, 1 to /byssocorticium, 1 to /ceratobasidium, 1 to /piloderma, and 1 to /tricholoma (Fig. 3B, Table S4, Supporting information). The results of GNMDS–*envfit* indicated that EM fungal lineage composition was significantly related to EM plant diversity levels (r^2 = 0.548, P = 0.02, Fig. 4A), but EM fungal species composition was not significantly related to EM plant diversity levels (r^2 = 0.381, P = 0.203, Fig. 4B). The EM fungal lineage/cortinarius was significantly related to *Q. serrata* (R = 0.813, P = 0.001), /piloderma to *P. massoniana* (R = 0.804, P = 0.002), /tricholoma to *Cy. glauca* (R = 0.813, P = 0.001) and *Cy. myrsinaefolia* (R = 0.845, P = 0.001), and /cenococcum to *L. glaber* (R = −0.703, P = 0.011,

Table 2 Regressions of ectomycorrhizal (EM) fungal species richness with species richness, genus richness, family richness and abundance of non-EM plants and abiotic variables (n = 12)

Variable	Slope	R^2	P
Non-EM plant species richness	0.289	0.228	0.117
Non-EM plant genus richness	0.324	0.227	0.117
Non-EM plant family richness	0.618	0.307	0.062
Non-EM plant abundance	−28.74	0.279	0.080
Altitude	0.044	0.304	0.063
Convexity	0.050	0.007	0.795
Slope	−0.084	0.011	0.741
Aspect	0.016	0.031	0.585
Total C	0.116	0.042	0.525
Total N	0.116	0.042	0.525
Total P	32.073	0.047	0.497
NO_3^--N	0.058	0.001	0.983
NH_4^+-N	0.252	0.145	0.222
Bulk density	−0.013	0.001	0.935
pH	3.663	0.016	0.694
Soil moisture	0.203	0.035	0.562

Fig. 4A). The results from Mantel tests showed that EM fungal lineage composition was significantly related to EM plant species composition (R = 0.353, P = 0.010) and non-EM plant species composition (R = 0.324, P = 0.016), and marginally significantly related to EM plant species richness (R = 0.195, P = 0.080) and genus richness (R = 0.201, P = 0.052), but EM fungal species composition was not significantly related to these factors (Table 3).

Discussion

Our finding of strong correlation between EM fungal diversity and EM plant diversity provides broad support for the *plant diversity hypothesis*. Furthermore, we found that this hypothesis was most strongly supported at the EM plant genus level, as EM plant genus diversity was the best predictor of EM fungal diversity in our study system (a Chinese subtropical forest), as well as in the analyses of temperate and tropical data sets. While the original *plant diversity hypothesis* was focused on resource diversity, host preference by EM fungi is the most plausible explanation for our results. Host preference has been demonstrated in temperate (Molina *et al.* 1992; Richard *et al.* 2005; Ishida *et al.* 2007; Tedersoo *et al.* 2008), subtropical (Ding *et al.* 2011) and tropical (Tedersoo *et al.* 2010c) studies and has been used to explain the increasing EM fungal species diversity with EM plant species diversity in temperate regions (Kernaghan *et al.* 2003; Dickie 2007). Furthermore, stronger host preference at higher EM plant phylogenetic levels has been demonstrated in a recent temperate study (Ishida *et al.* 2007), and EM plant species from different families have been shown to harbour more dissimilar EM fungal composition than that from the same genus (Morris *et al.* 2008; Smith *et al.* 2009). Thus, an increase of EM plant genus and family diversities would support a more diverse EM fungal community than if plant diversity was increased by adding closely related host species (e.g. within the same genus). This explanation fits well with the fact that EM plant genus diversity

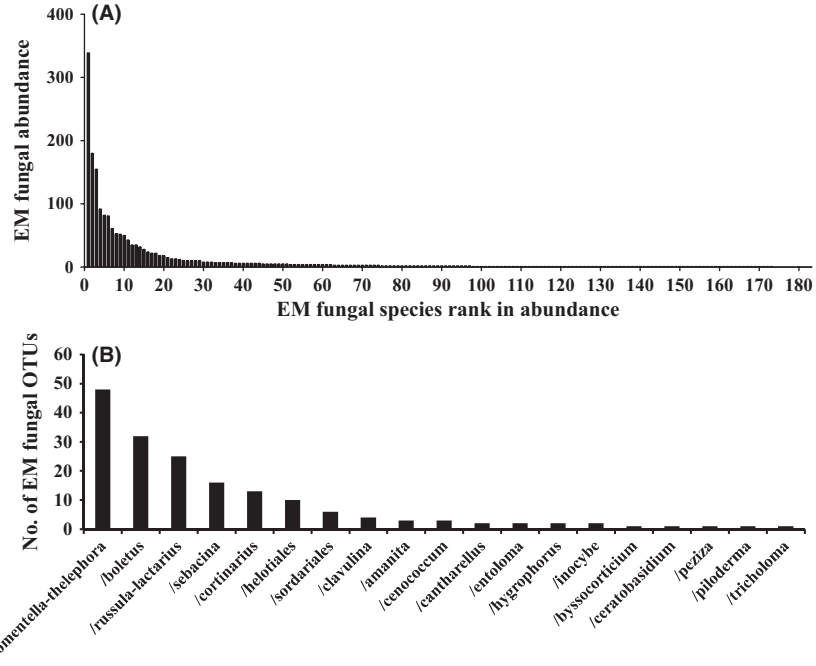


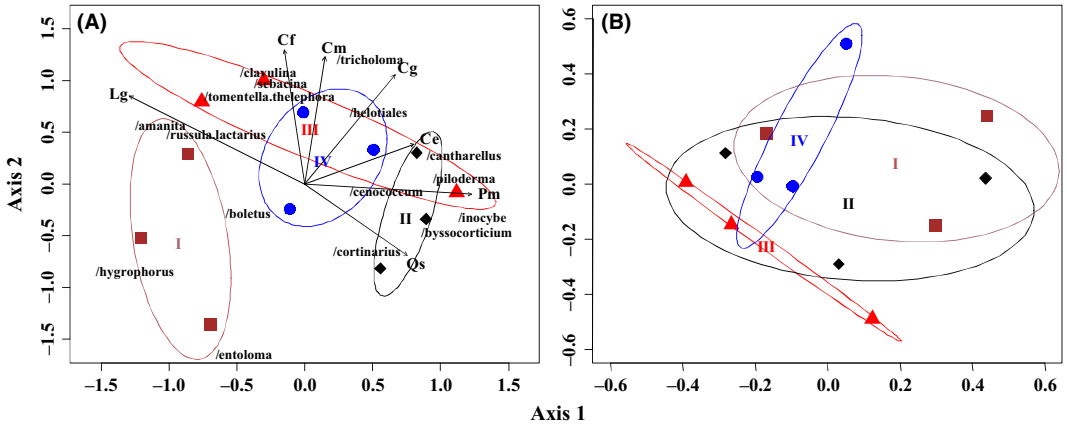
Fig. 3 The rank of (A) ectomycorrhizal (EM) fungal species in abundance (clone sequence numbers) and (B) EM fungal lineages in OTU numbers.

Table 3 Mantel tests of ectomycorrhizal (EM) fungal lineage and species compositions with plant species richness, genus richness, family richness and composition, soil and topography (n = 12)

	EM fungal lineage composition		EM fungal species composition	
Independent variable	*R*	*P*	*R*	*P*
EM plant species richness	0.195	0.080	−0.125	0.809
EM plant genus richness	0.201	0.052	−0.093	0.744
EM plant family richness	0.102	0.180	−0.066	0.753
EM plant species composition	0.353	0.010	−0.141	0.849
Non-EM plant species richness	−0.114	0.777	−0.060	0.621
Non-EM plant genus richness	−0.079	0.676	−0.119	0.755
Non-EM plant family richness	−0.109	0.751	−0.090	0.711
Non-EM plant species composition	0.324	0.016	0.081	0.285
Topography	−0.137	0.816	−0.002	0.451
Soil	−0.262	0.983	−0.024	0.586

was a better predictor of EM fungal diversity than EM plant species diversity. Because of the hierarchical nature of taxonomic data, increasing EM plant family diversity necessarily results in increasing diversity at the species and genus levels; therefore, the accumulating effects of host preference at EM plant species, genus and family levels should cause a compound response in EM fungal diversity. However, as the number of species and genera in different families varied strongly across the studies included in our global analysis, accounting for these effects in the analysis at the EM plant family level is not straightforward. This problem highlights the complexity of using EM plant family diversity in predicting EM fungal diversity. Variability in phylogenetic diversity across plant families is likely why EM plant family diversity was not the best predictor of EM fungal diversity in the subtropical study, as well as in the analyses of temperate and tropical studies. Overall, EM plant genus diversity was a robust predictor of EM fungal diversity in our study and across the analyses of temperate and tropical studies, indicating a general phylogenetic-based mechanism that structures EM fungal diversity in temperate, subtropical and tropical forest ecosystems.

The influence of host density on EM fungal community structure has been demonstrated in several previous studies (Dickie *et al.* 2009; Peay *et al.* 2011). While we observed a positive correlation between host abundance (number of individuals) and EM fungal diversity in the subtropical Gutianshan, this relationship did not hold when EM host genus diversity was also included in our multiple regression model. In addition, we found no significant effects of EM plant dominance and basal area on EM fungal species diversity in this study (Table 1). While this suggests EM host genus diversity was the main driver of EM fungal diversity in this system, it does raise interesting questions about the relationship between EM host abundance and genus diversity as well as the scale at which host density might affect EM fungal richness. One possibility for this result is that our sampling was not random (i.e. cores were intentionally sampled beneath EM hosts), which likely reduced the magnitude of host density on EM



Figs. 4 Global nonmetric multidimensional scaling (GNMDS) and envfit of EM plant diversity levels as centroids for (A) ectomycorrhizal (EM) fungal lineage composition (stress = 10.63, R^2 = 0.548, P = 0.019) and (B) EM fungal species composition (stress = 12.22, R^2 = 0.381, P = 0.203). Symbols: Squares, EM plant diversity level-I quadrats; Diamonds, EM plant diversity level-II quadrats; Triangles, EM plant diversity level-III quadrats; Circles, EM plant diversity level-IV quadrats. The EM fungal lineage/cortinarius was significantly related to *Quercus serrata* (Qs, R = 0.813, P = 0.001), /piloderma to *Pinus massoniana* (Pm, R = 0.804, P = 0.002), /tricholoma to *Cyclobalanopsis glauca* (Cg, R = 0.813, P = 0.001) and *Cy. myrsinaefolia* (Cm, R = 0.845, P = 0.001), and /cenococcum to *Lithocarpus glaber* (Lg, R = −0.703, P = 0.011).

fungal richness. In addition, it is possible that host density may not be as important in intact forest systems where EM hosts are generally abundant, as opposed to highly patchy landscapes (Peay *et al.* 2007; Dickie *et al.* 2009) or ecosystems where EM hosts are generally rare (Tedersoo *et al.* 2010c). It seems likely, however, that this effect of host abundance would be particularly important in explaining some of the global scale diversity differences that seem to be emerging between temperate and tropical systems (Peay *et al.* 2010a; Tedersoo & Nara 2010). Unfortunately, because information on host density, size and dominance was unavailable in most of the compiled temperate and tropical studies, we could not analyse their roles on EM fungal species diversity at this larger biogeographical scale. Therefore, incorporating host density into global EM fungal diversity models should be a priority for future research.

In addition, because each collected soil core likely contained mixed roots from co-occurring EM plant species, the effect of EM plant species identity on EM fungal species diversity was not possible to be directly tested in this study. However, some studies found that EM fungal species diversity did not significantly differ among three EM plant species *P. massoniana* (Pinaceae), *C. fargesii* and *Lithocarpus harlandii* (Fagaceae) in another Chinese subtropical forest (Ding *et al.* 2011), between two co-occurring plant species *Quercus douglasii* and *Quercus wislizeni* (Fagaceae) in USA (Morris *et al.* 2008) and *Quercus crassifolia* and *Quercus laurina* in Mexico (Morris *et al.* 2009), and among three co-occurring plant species *Dicymbe corymbosa*, *D. altsonii* and *Aldina insignis* (Fabaceae) in a neotropical rainforest (Smith *et al.* 2011). However, Ishida *et al.* (2007) did find differences in EM fungal species diversity across eight EM plant species from three families Betulaceae, Fagaceae and Pinaceae, suggesting that more studies on the role of individual host species on EM fungal diversity are necessary.

It should be noted that the slope of linear regression of EM fungal richness with EM plant genus richness was steeper in temperate than in tropical forests (Fig. 2E), indicating other factors may be responsible for the lower EM fungal diversity in tropical than in temperate regions, which is one of the most striking findings in EM fungal community ecology (Peay *et al.* 2010b; Tedersoo & Nara 2010; Tedersoo *et al.* 2012). Possible explanations have been proposed, including EM plant phylogenetic diversity (Peay *et al.* 2010b; Tedersoo & Nara 2010), resource availability and fragmentation (Tedersoo & Nara 2010; Tedersoo *et al.* 2010c), historical and biogeographical effects (Tedersoo & Nara 2010), and habitat conditions (Tedersoo *et al.* 2012). Regarding EM plant phylogenetic diversity, the present study indicates that differences in EM plant genus diversity cannot entirely reconcile the difference between tropical and temperate forest ecosystems. While Peay *et al.* (2007, 2010a, 2012) have demonstrated the effect of habitat fragmentation on landscape scale patterns of EM fungal diversity, no clear conclusion can yet be drawn relating these processes to the disparities between tropical and temperate biomes. Furthermore, there is not yet strong evidence to support historical and biogeographical effects due to the relatively poor knowledge of the tropical EM fungal community (Peay *et al.* 2010b; Tedersoo & Nara 2010). Tedersoo *et al.* (2012) recently analysed 77 published data sets and concluded that the global EM fungal diversity was mostly affected by temperature and precipitation, and proposed that weak soil stratification due to the rapid turnover of soil organic material may account for the low EM fungal diversity in tropical regions. Therefore, it is necessary to further explore EM fungal diversity globally, especially in tropical and subtropical regions (Kennedy *et al.* 2012; Tedersoo *et al.* 2012).

EM fungal lineage composition was significantly influenced by EM plant diversity levels. Moreover, some EM fungal lineages were significantly related to EM plants at genus or higher level, such as lineage/cortinarius to *Quercus*, /tricholoma to *Cyclobalanopsis* and /piloderma to *Pinus* (Fig. 4A). Similarly, EM fungal lineage composition has been demonstrated to be influenced by family-level EM plant composition globally (Tedersoo *et al.* 2012). Therefore, these results indicated that EM fungal lineages may be co-evolved with EM plants at genus or higher phylogenetic level. However, the influences of EM plant diversity level and EM plant composition on EM fungal species composition at the GNR study site were not significant. One potential explanation is that the variation of EM plant community structure was low (total inertia = 0.735 in correspondence analysis, CA) due to the nested nature of EM plant diversity inherence in the experimental design, but the variation of EM fungal communities was higher (total inertia = 6.146 in CA), thus the major proportion of variation in EM fungal community composition cannot be explained by EM plant species composition. Given the mixed results in our study and the general literature, more studies are clearly needed before generalizations on the plant controls over EM fungal community are possible.

In conclusion, EM plant genus diversity was a robust predictor of EM fungal diversity in a Chinese subtropical forest, as well as in temperate and tropical forests. Together, these results may indicate a general maintenance mechanism of EM fungal diversity in temperate, subtropical and tropical forest ecosystems. One important implication of this work is that many places that we would predict to be hotspots of EM fungal diversity remain largely unsampled (Dickie 2007). For example, areas such as the Southern USA, Central America,

subtropical Southeast Asia and the Mediterranean zone in Eurasia have high tree genus diversity, but diversity of EM fungi remains poorly sampled relative to the Western USA and Europe (Bahram *et al.* 2012). These regions are thus important targets for future studies aimed at describing both local and global patterns of EM fungal diversity and may deserve special attention for conservation efforts.

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C.G. and L.G. designed the experiment, C.G., N.S., Y.L. and Y.Z. collected samples and carried laboratory work, X.M. and K.M. provided soil data, C.G., Q.D. and L.G. analysed data, C.G., K.P., T.W., F.B. and L.G. wrote the manuscript.

Data accessibility

The representative sequences of the 173 ectomycorrhizal fungal OTUs have been submitted to the EMBL (accession No. HE814066-HE814239). Identification information of the 173 OTUs is available in the supplement Table S4 (Supporting information). Raw data of 83 temperate and 17 tropical studies are given in Table S3 (Supporting information).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 The distribution of 12 quadrats selected from 600 quadrats (20 m × 20 m each) in the 24-ha permanent plot in Gutianshan.

Figs. S2 The locations of collected soil cores (○, 10 cm in diameter × 10 cm deep) and EM plant individuals in each quadrat (20 m × 20 m).

Table S1 Ectomycorrhizal (EM) plant, non-EM plant, soil and topographical variables among the four EM plant diversity levels.

Table S2 Abundance and range of the diameters at beast height (DBH) of each ectomycorrhizal (EM) plant species in each quadrat.

Table S3 Data of temperate and tropical studies compiled.

Table S4 Molecular identification of ectomycorrhizal (EM) fungi in this study.