MOLECULAR ECOLOGY

Molecular Ecology (2011) **20**, 3071–3080 doi: 10.1111/j.1365-294X.2011.05145.x

Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of Continental Africa and Madagascar

LEHO TEDERSOO,*† MOHAMMAD BAHRAM,* TEELE JAIRUS,* ENEKE BECHEM,‡ STEPHEN CHINOYA,§ REBECCA MPUMBA,¶ MIGUEL LEAL,** EMILE RANDRIANJOHANY,†† SYLVAIN RAZAFIMANDIMBISON,‡‡ AVE SADAM,* TRIIN NAADEL* and URMAS KÕLJALG*†

*Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, 40 Lai St, 51005 Tartu, Estonia, †Natural History Museum of Tartu University, 46 Vanemuise Street 51005 Tartu, Estonia, ‡Department of Plant and Animal Sciences, University of Buea, PO Box 63, Buea, Cameroon, §Loloma Mission Hospital, PO Box 100 Manyinga, Kabompo, Zambia, ¶West Lunga Trust, PO Box 16 00 10 Mwinilunga, Zambia, **Missouri Botanical Garden, PO Box 299, St Louis, MO 63166, USA, ††Laboratoire de Microbiologie de l'Environnement, Centre National de Recherches sur l'Environnement, PO Box 1739, Antananarivo, Madagascar, ‡‡Bergius Foundation, the Royal Swedish Academy of Sciences and Botany Department, Stockholm University, SE-106 91 Stockholm, Sweden*

Abstract

Mycorrhizal fungi play a key role in mineral nutrition of terrestrial plants, but the factors affecting natural distribution, diversity and community composition of particularly tropical fungi remain poorly understood. This study addresses shifts in community structure and species frequency of ectomycorrhizal (EcM) fungi in relation to host taxa, soil depth and spatial structure in four contrasting African ecosystems. We used the rDNA and plastid trnL intron sequence analysis for identification of fungi and host plants, respectively. By partitioning out spatial autocorrelation in plant and fungal distribution, we suggest that African EcM fungal communities are little structured by soil horizon and host at the plant species and family levels. These findings contrast with patterns of vegetation in these forests and EcM fungal communities in other tropical and temperate ecosystems. The low level of host preference indirectly supports an earlier hypothesis that pioneer Phyllanthaceae may facilitate the establishment of late successional Fabaceae and potentially other EcM host trees by providing compatible fungal inoculum in deforested and naturally disturbed ecosystems of tropical Africa.

Keywords: ectomycorrhizal symbiosis, host specificity, Madagascar, miombo woodland, spatial analysis, *Uapaca* (Phyllanthaceae)

Received 20 December 2010; revision received 25 April 2011; accepted 2 May 2011

Introduction

The mycorrhizal symbiosis plays a key role in mineral nutrition of terrestrial plants. While arbuscular mycorrhizas dominate in tropical regions, the importance and distribution of ectomycorrhizas (EcM) has been only recently understood in these habitats (Alexander & Lee 2005; Ducousso *et al.* 2008). EcM-dominated wooded savannas and rain forests cover large areas on nutrient-poor soils in SE Asia and Africa (Burgess *et al.* 2004; Alexander 2006). In Sub-Saharan Africa, EcM vegetation includes many of the ecologically and economically important trees—Amhersteae (Caesalpinioideae, Fabaceae), Dipterocarpaceae, *Uapaca* (Phyllanthaceae) and Gnetaceae—that are heavily utilised for timber and charcoal production. In addition, these plants provide locally important forestry by-products such as fruits,

Correspondence: Leho Tedersoo, Tel/Fax: +372 7376222;
E-mail: leho.tedersoo@ut.ee

honey, edible caterpillars and edible mushrooms (Campbell *et al.* 2007). Similarly in Madagascar, the dominant trees of littoral forests and dry wooded savannas (Phyllanthaceae, Fabaceae, the endemic Asteropeiaceae and Sarcolaenaceae) form EcM (Ducousso *et al.* 2004, 2008). Many species of these trees are in danger of extinction due to habitat loss from annual burning and subsistence farming practices (Goodman & Benstead 2003).

Natural regeneration of EcM Fabaceae and Dipterocarpaceae is difficult in disturbed areas due to relatively slow growth and patchily distributed fungal inoculum (Onguene 2000). Early observations suggested that *Uapaca* spp. facilitate the recruitment and establishment of Fabaceae (Lawton 1978; Högberg & Piearce 1986). Further experimental studies confirmed that seedling establishment of Fabaceae strongly benefits from common mycelial networks that are sustained by the neighbouring adult trees (Onguene 2000; McGuire 2007). In most ecosystems, these belowground networks interconnect several host taxa as fungal communities on different hosts largely overlap (Molina *et al.* 1992; Ishida *et al.* 2007; Ryberg *et al.* 2009). Species of EcM fungi associate with several tree families in a West African rain forest (Diédhiou *et al.* 2010), but host-specific interactions prevail in the western Amazon (Tedersoo *et al.* 2010b). Besides strict host specificity that is relatively uncommon (Molina *et al.* 1992), EcM fungi may display host preference that probably reflects genetic and physiological compatibility (Morris *et al.* 2008; Tedersoo *et al.* 2008a). In addition, host trees modify soil properties by litter quality, stem flow, exudation of allelochemicals and depletion of soil water and nutrients (Dahlgren *et al.* 1997) that may differentially affect competitive abilities of EcM fungi (Morris *et al.* 2008). In addition to these host-derived effects, niche differentiation by soil profile and forest microsites contribute to the structural and functional properties of EcM fungal communities in temperate ecosystems (Buee *et al.* 2007; Lindahl *et al.* 2007; Tedersoo *et al.* 2008b). The relative importance of host and soil environments remains unknown in tropical habitats.

Tropical regions are regarded as hotspots for evolution and radiation of plants and animals as well as mutualistic interactions (Jablonski *et al.* 2006; Arita & Vazquez-Dominguez 2008; Schemske *et al.* 2009). EcM fungi probably evolved >60 times from non-mycorrhizal, mostly saprotrophical ancestors (Tedersoo *et al.* 2010a). All these lineages inhabit northern and/or southern temperate ecosystems, but very scattered information is available from tropical habitats due to paucity of studies on taxonomy and soil ecology of tropical fungi (Tedersoo & Nara 2010; Tedersoo *et al.* 2010a).

In this study, we hypothesised that African rain forests and woodlands harbour previously undescribed lineages of EcM fungi and that host and soil environments structure the communities of EcM fungi as suggested by earlier fruit-body observations and pure culture synthesis experiments (Thoen & Ba 1989; Ba & Thoen 1990; Sanon *et al.* 1997). We employed the rDNA ITS region and 28S gene for fungal identification, plastid trnL intron for plant identification and considered the confounding effects of spatial autocorrelation in statistical analyses to address these hypotheses.

Materials and methods

Study sites and sampling

Four study sites were established in different ecosystems of tropical Africa that support many EcM hosts from the Fabaceae and Phyllanthaceae families, supplemented by members of Dipterocarpaceae, Sarcolaenaceae or Asteropeiaceae in certain sites (Table S1, Supporting information). The Kashima site is located in an ecotone of miombo (woodland) and dambo (seasonally flooded wetland) ecosystems in Kabompo district of NW Zambia (13°19′S; 24°30′E), *c.* 1080 m a.s.l. The Tchimbele site is located in a primary lowland rain forest in Mbé National Park in Littoral province of Northwest Gabon (0°37′N; 10°24′E), *c.* 400 m above sea level (cf. plots 1 and 3; Sunderland *et al.* 2004). The Mandena site is located in a slightly disturbed littoral forest in Toliara province of SE Madagascar (24°58′S; 47°00′E), *c.* 40 m above sea level and is described in detail in Ganzhorn *et al.* (2007) as concession M15. The Korup site is located in a primary lowland rain forest in Korup National Park in Southwest Province of Cameroon (5°01′N; 8°48′E), *c.* 120 m above sea level. This site is described in Newbery *et al.* (1988). Specific information about the vegetation, soils, climate and sampling is given in Table S1 (Supporting information).

In all study sites, soil cores (15 × 15–10 cm depth) were collected from around each host tree (0.2–15 m distant) and at least 8 m distant from other samples to minimise the effect of spatial autocorrelation among samples. Core locations were mapped by averaging 200–500 replicate measurements recorded with a GPS Garmin 60CSx (Garmin International Inc., Olathe, KS, USA). To confirm dubious reports of EcM symbiosis in species of *Faurea* and *Pericopsis* (Högberg & Piearce 1986), we additionally sampled 20 plant individuals of *Faurea* spp. and two individuals of *Pericopsis angolensis* for mycorrhizal analyses in Kashima. Root samples were transported to the nearest villages and processed within four working days (except within 1.5 days in Tchimbele and Mandena) after collection. Samples were separated into mineral and organic horizon. EcM roots were separated from non-EcM roots and cleaned from

soil particles in water. More than 90% of root tips in all host trees (except *Faurea* spp. and *P. angolensis*) were EcM. EcM root tips were sorted into morphotypes based on their colour, mantle texture and structure of hyphae, cystidia and rhizomorphs. Morphotyping was performed on each root fragment separately to be able to relate fungal taxa and plant taxa. If available, 4–6 root fragments (uncut, typically 10–20 cm in length) were studied in each soil core. Clusters of EcM root tips from each morphotype per root fragment and soil core were stored in 0.2 ml-Eppendorf tubes containing 200 μl 1% CTAB DNA extraction buffer (1% cetyltrimethylammonium bromide, 100 mM Tris–HCl (pH 8.0), 1.4 M NaCl, and 20 mM EDTA) for transportation and further molecular analyses.

Molecular analyses

Between one and five individual root tips from each morphotype per soil core were subjected to DNA extraction with a Qiagen DNeasy 96 Plant kit (Qiagen, Crawley, UK), following the manufacturer's protocol. In total, 2120 root tips were analysed with molecular tools. The primer itsOF-T (5′-acttggtcatttagaggaagt-3′) combined with LB-W (5′-cttttcatctttccctcacgg-3′) was used for all fungi, except black ascomycetes, for which LA-W (5′-cttttcatctttcgatcactc-3′) was used instead. Unsuccessfully amplified DNA extracts were re-amplified using primers itsOF-T in combination with universal primers its4 (5′-tcctccgcttattgatatgc-3′) or its2 (5′-gctgcgttcttcatcgatgc-3′). DNA Extracts that resulted in low quality sequences assignable to known EcM taxa were re-amplified using itsOF-T in combination with any of the taxon-specific primers listed in Table 1. For most phylotypes, 28S rDNA was amplified with primers LR0R (5′-acccgctgaacttaagc-3′) paired with LB-Z (5′-aaaaatggccc-actagaaact-3′) or LR5 (5′-tcctgagggaaacttcg-3′). The PCR conditions follow Tedersoo *et al.* (2006). PCR products were checked on 1% agarose gels under UV-light and purified by use of Exo-Sap enzymes (Sigma, St. Louis, MO, USA). Sequencing was performed with the primer its5 (5′-ggaagtaaaagtcgtaacaagg-3′), LF340 (5′-ta cttgtkcgctatcgg-3′), and/or its4 for the ITS region and ctb6 (5′-gcatatcaataagcggagg-3′) for the 28S gene. Sequences were assembled into contigs and checked for quality by use of Sequencher 4.9 software (GeneCodes Corp., Ann Arbor, MI, USA). Species were delimited based on 97.0% ITS sequence (excluding the flanking rDNA genes) identity by use of single linkage algorithm as implemented in the online program Blastclust (http://toolkit.tuebingen.mpg.de/blastclust). Identification was performed by running bulk megablast queries against the international sequence database (INSD) and UNITE (Abarenkov *et al.* 2010a) as implemented in the PlutoF workbench (Abarenkov *et al.* 2010b). Fungal species were further assigned to EcM lineages following Tedersoo *et al.* (2010a). Host identification was performed based on a representative root tip of each root fragment per soil sample. From root tips and reference leaf samples, the plastid *trnL* region was amplified using primers trnC (5′-cgaaatcggtagacgctacg-3′) and trnD (5′-ggggatagagggacttgaac-3′), and sequenced with trnD. At the study sites, all EcM plant species differed from each other by at least one base substitution in the *trnL* region.

Statistical analyses

Host preference of fungal species was assessed at the species, genus and family levels of plants. Biases in frequency for both host and soil horizon (A-horizon vs. O-horizon) were studied by use of Fisher's Exact tests. False discovery rate was reduced by employing Benjamini–Hochberg correction (alternative to Bonferroni correction; Verhoeven *et al.* 2005) to each site and factor separately. Because distribution of host plants was spatially aggregated in Tchimbele, Moran's I was calculated for each common fungal species ($n > 3$) at this site

Table 1 List of primers used to amplify specific taxa of ectomycorrhizal fungi

Primer	Target taxon	Sequence (5′–3′)	Position in 28S gene (5′ end)	Reference
ITS4B	Agaricales, Boletales	caggagacttgtacacggtccag	150	Gardes & Bruns 1993
ITS4–Clav	/clavulina	ggtagtcccacctgattc	9	This study
ITS4–Russ	/russula–lactarius	agcgggtagtctcaccc	14	This study
ITS4–Seb	/sebacina	tcagcgggtartcctactc	14	This study
ITS4–Sord	Sordariomycetes	cccgttccagggaactc	100	Tedersoo *et al.* 2008a
ITS4–Tom	/tomentella–thelephora	aactcggacgaccagaggca	120	This study
LR21–Cer	Ceratobasidiaceae	cgactcgttgagagcacaa	250	This study
LR3–Pez	Pezizales	cmtcrggatcggtcgatgg	750	Tedersoo *et al.* 2008a
LR3–Tom	/tomentella–thelephora	ctaccgtagaaccgtctcc	700	Tedersoo *et al.* 2008a

to assess whether biases in fungal distribution can be ascribed to spatial factors. Specific attention was paid to the smallest distance class, <10 m that corresponds to a clump of conspecific saplings. Species- and genus-level host preference was not assessed in the Korup data set, because plant DNA was too degraded for amplification. Roots of Fabaceae and *Uapaca* were easily distinguished by differences in diameter and morphology. In Kashima, the effect of soil horizon was not addressed due to the lack of roots in shallow, recently burnt organic layer. Sample size in Mandena was relatively lower due to extreme floods resulting from a tornado during the time of sampling.

To address the relative importance of spatial effects and studied factors on the EcM fungal communities, we constructed vectors of principal coordinates of neighbour matrices (PCNM) according to Borcard & Legendre (2002). These eigenfunctions represent spatial relations among the samples over various geographical scales. PCNM vectors were calculated based on the geographical distance matrix and ordered by coefficients of determination based on forward selection and 1000 permutations as implemented in the Packfor package of R (R Core Development Team 2007). All significant ($P < 0.05$) PCNM vectors were included in the distance matrix for multivariate analysis. The relative effects of the PCNM vectors, host and soil horizon were addressed by use of Bray–Curtis distance measure (= Sørensen distance in binary data) as implemented in the Adonis permutational multivariate analysis of variance function of the Vegan package of R. Based on the same options, non-metric multidimensional scaling (NMS) plots were constructed to visualise the relative effects of these variables on EcM fungal communities. Vectors and centroids of variables were fitted into the NMS plots by use of the function envfit. Significance level of $\alpha < 0.05$ was used throughout the study.

To compare species richness among sites and host taxa, rarefied species accumulation curves and their 95% confidence intervals were calculated by use of EstimateS ver. 8 (Colwell 2006). Data from previous studies employing roughly similar sampling and identification techniques were plotted for comparative purposes.

Results

In total, 2120 root tips were subjected to molecular identification, of which 1063 (50.1%) were successfully identified. Successfully sequenced EcM root material was obtained from 77, 84, 38 and 106 root samples in Kashima, Tchimbele, Mandena and Korup, respectively. At these sites, respectively, 79.1%, 94.3%, 97.1% and 77.8% of morphotypes per soil sample were successfully identified. Lower success in Kashima and especially Korup are attributable to fluctuating electric power during sample preparation in the field laboratory. Sequencing revealed that, on average, morphotyping overestimated species richness per sample by 7.3%. In particular, different morphotypes were merged to the same species in 10.3% of cases, whereas 3.0% of morphotypes revealed more than one species in a sample. The sampling errors are estimated to be higher with greater sequencing effort and success rate. Plant identification was >90% successful in Kashima, Tchimbele and Mandena, but <50% in Korup. Plant species were always separated based on the sequence of the plastid trnL intron, although certain closely related host species differed by a single base substitution.

Clustering of the ITS sequences revealed 94, 101, 46 and 111 putative species of EcM fungi in Kashima, Tchimbele, Mandena and Korup, respectively. Of these taxa, 24 species occurred in two sites and two species in three sites. The rain forest sites Tchimbele and Korup shared 14 species. Two closely related species of Helotiales were considered as putatively EcM, because these fungi were associated with typical black EcM with ascomycetous morphology, although similar sequences were lacking from INSD. These species were recovered only once, permitting no unambiguous assignment of their lifestyle. A species of Atheliaceae, frequently found in both Tchimbele and Korup, did not match any known EcM lineage, but grouped with EcM isolates from SE Asia (not shown). All other species were confidently assigned to 18 of the 66 predefined EcM lineages (cf. Tedersoo *et al*. 2010a) or considered as non- EcM (e.g. *Leotia* sp, Atractiellales spp., *Penicillium* spp.). Only seven species (2.0%) were matched to previously identified fruit-bodies of African fungi, emphasising the paucity of molecular taxonomic work in this continent. In terms of species richness, the lineages of /russula-lactarius, /tomentella-thelephora and /boletus dominated in all sites. Other frequent EcM fungal taxa included the /sebacina, /clavulina, /sordariales, /pisolithus-scleroderma, /marcelleina-peziza gerardii and /elaphomyces lineages, but their relative contribution differed strongly among sites (Table S2, Supporting information).

With a few exceptions, host had no effect on the frequency of EcM fungal species at the study sites (Table S2, Supporting information). However, five out of 18 tested species displayed a significantly biased association towards host species in Tchimbele. In all but one of these cases, the observed results were ascribed to the confounding effects of spatial autocorrelation as revealed from statistically significant Moran's *I*-values in the <10 m distance class. Only a single species (in Korup) had a statistically significant preference for soil horizons. Reduction of the false discovery rate

shifted all *P*-values of host and soil effects below the significance threshold, except a single EcM fungal species at the Mandena site.

Inclusion of the PCNM vectors substantially reduced the effects of host taxon and soil on the EcM fungal community structure. The spatial component explained between 18.1% and 30.2% (except 7.6% at the host family level analysis in Tchimbele) of the community variance (Table 2). Host taxon and soil horizon explained 0.8–10.1% and 0.4–0.5% of the variance, respectively (Fig. 1; Table 2). The soil effect remained non-significant in all analyses.

The rarefied species accumulation curves were comparable among the study sites, but remained below the average values of three comprehensively sampled temperate forests (Fig. 2). Within sites, there were no significant differences in accumulating species richness among host taxa (not shown), suggesting that all hosts are equally receptive to the local pool of EcM mycobionts. In contrast to previous observations, *Faurea* spp. and *Pericopsis angolensis* lacked EcM colonization in Kashima.

Table 2 Relative importance of host, soil and PCNM eigenfunctions on the community composition of ectomycorrhizal fungi

	df	SS	*F*-value	R^2	*P*-value
Kashima, Zambia					
(a) Host species level					
PCNM vectors	12	10.45	2.06	0.205	**<0.001**
Host	4	1.67	0.98	0.033	0.518
Residuals	92	38.95		0.763	
(b) Host family level					
PCNM vectors	19	14.82	1.90	0.271	**<0.001**
Host	2	0.79	0.97	0.014	0.526
Residuals	95	39.06		0.714	
Tchimbele, Gabon					
(a) Host species level					
PCNM vectors	11	8.08	1.71	0.181	**<0.001**
Host	6	4.51	1.75	0.101	**<0.001**
Soil	1	0.17	0.40	0.004	0.999
Residuals	74	31.78		0.713	
(b) Host family level					
PCNM vectors	3	3.54	2.61	0.076	**<0.001**
Host	2	2.16	2.49	0.046	**<0.001**
Soil	1	0.20	0.44	0.004	0.999
Residuals	90	40.64		0.873	
Mandena, Madagascar					
Host species/family level					
PCNM vectors	10	7.64	2.12	0.302	**<0.001**
Host	3	2.07	1.92	0.082	**0.002**
Soil	1	0.13	0.37	0.005	0.969
Residuals	43	15.46		0.611	
Korup, Cameroon					
Host family level					
PCNM vectors	22	19.40	2.17	0.279	**<0.001**
Host	1	0.59	1.44	0.008	**0.044**
Soil	1	0.36	0.87	0.005	0.701
Residuals	121	49.21		0.708	

PCNM, principal coordinates of neighbour matrices.
Significant *P*-values are given in bold.

Discussion

Inclusion of spatial eigenfunctions enabled us to partition the variation explained by host and soil environments and spatial structure that results from non-random distribution patterns of both plants and fungi at different geographical scales. Both the host and soil horizon played a negligible role in niche differentiation of EcM fungal species in the four forest sites sampled in Continental Africa and Madagascar. Thus, our results extend the findings of a previous study in a 0.16 ha patch of a Guinean rain forest, where most EcM host trees associate with multiple mycobionts and the dominant fungal species colonise several host species (Diédhiou *et al.* 2010).

In contrast to these in situ below ground molecular identification studies, fruit-body collections and pure culture synthesis trials suggest that some African EcM fungi are host specific (Ba & Thoen 1990; Buyck *et al.* 1996; Sanon *et al.* 2009). The disparity of results can be ascribed to the lack of statistical testing in the latter studies, different phenology of host tree families (i.e. temporal partitioning of fruit-body production) and differential suitability of inoculation and growth conditions to host taxa. The observed lack of specificity in plant–fungal interactions provides indirect support to the hypothesis that the early successional, dual mycorrhizal *Uapaca* spp. may facilitate the establishment of the climax Fabaceae stands in heavily disturbed sites (Högberg & Piearce 1986). Such facilitation patterns are well known in other EcM-dominated ecosystems worldwide (Dickie *et al.* 2002; Nara 2006; McGuire 2007). In conservation and afforestation perspectives, low reciprocal preference between phytobionts and mycobionts may provide resistance to the detrimental effect of habitat fragmentation by improving access to mycorrhizal inoculum (Peay *et al.* 2010a; Tedersoo *et al.* 2010b).

In temperate ecosystems, host plant usually strongly affects EcM fungal community composition (Ishida *et al.* 2007; Morris *et al.* 2008; Tedersoo *et al.* 2008a), although only a few dominant fungal species display significant host preference. In addition to temperate ecosystems, most of the dominant EcM fungi in NW Amazon display host genus-level preference or specificity that is ascribed to inherent trends towards specificity in the Nyctaginaceae family independent from habitat (Haug *et al.* 2005; Suvi *et al.* 2010; Tedersoo *et al.* 2010b). *Alnus* spp. (Fagaceae) in the Northern Hemisphere (Molina *et al.* 1992; Tedersoo *et al.* 2009) and *Gnetum* (Gnetaceae) in the tropical regions (Bechem

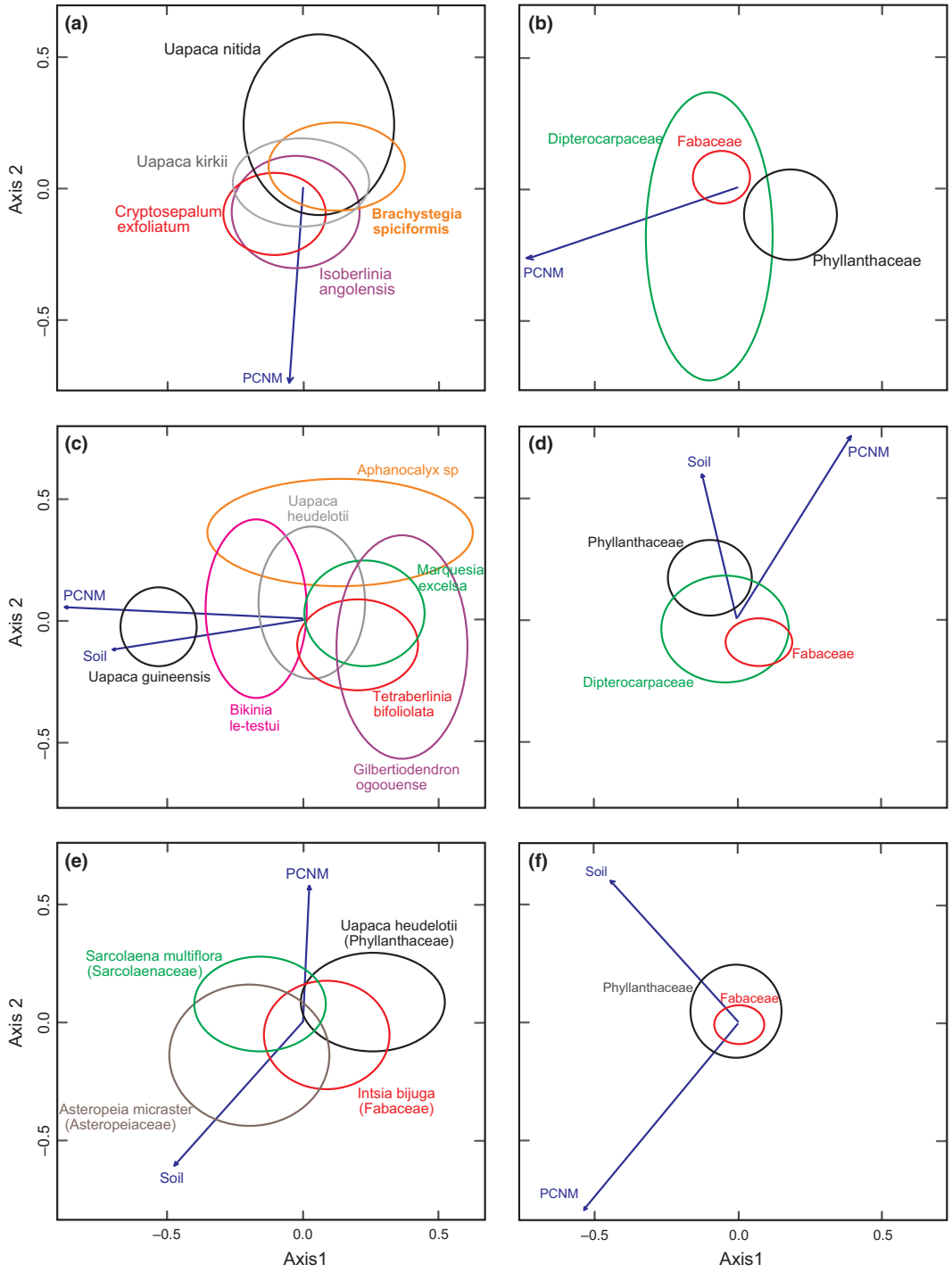

Fig. 1 The effects of host, soil and combined significant spatial (PCNM) vectors on community composition of ectomycorrhizal fungi in the four study sites as revealed by non-metric multidimensional scaling (NMS) ordination. (a) Kashima, Zambia: host species level; (b) Kashima: host family level; (c) Tchimbele, Gabon: host species level; (d) Tchimbele: host family level; (e) Mandena, Madagascar: host species/family level; (f) Korup, Cameroon: host family level. Arrows indicate the relative importance of variables (see also Table 2); ellipses indicate 95% confidence intervals around the average values for samples from each host taxon.

2004) are also highly selective for their EcM symbionts. In plants, specificity of EcM associations seems to be a function of phylogenetic distance (Ishida *et al.* 2007; Tedersoo *et al.* 2010b), extreme habitats (Tedersoo *et al.* 2009; Suvi *et al.* 2010) and the direction of carbon flow (Bruns *et al.* 2002; Hynson & Bruns 2010). In fungi, phy-

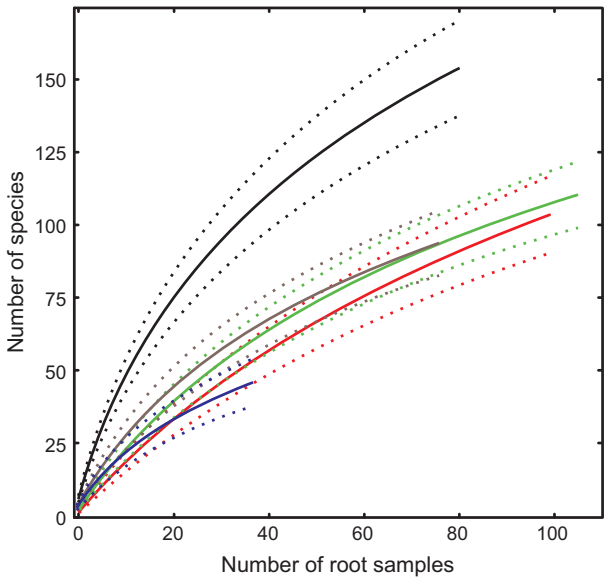

Fig. 2 Rarefied species accumulation curves and their 95% confidence intervals (pointed lines) of ectomycorrhizal fungi at the four study sites compared to the average rarefaction curve (and its 95% CI) of three comprehensively sampled temperate/subtropical sites (black; Tedersoo *et al.* 2006; Morris *et al.* 2009; Smith *et al.* 2009). Brown, Kashima, Zambia; red, Tchimbele, Gabon; blue, Mandena, Madagascar; green, Korup, Cameroon.

logenetic position (e.g. species of *Suillus*, *Leccinum*, *Lactarius* sect. *Deliciosi*) and co-evolution with the above-mentioned specific hosts seem to be the major factors determining specificity patterns. Whether specificity is beneficial to any partners or represents an evolutionary trap, remains a debate (Bruns *et al.* 2002).

Our failure to detect a soil effect on EcM fungal communities in African ecosystems contrasts with the niche differentiation of EcM fungi at the levels of soil horizons, nutrient availability, microsites and landscape in boreal forests (Toljander *et al.* 2006; Lindahl *et al.* 2007; Tedersoo *et al.* 2008b). These differences between ecosystems may stem from the poor structuring of soil that is characteristic of the vast majority of tropical forests. The humus layer is usually shallow or absent and mineral soil lacks stratification due to high decomposition and consumption rates (Tedersoo & Nara 2010). However, in Central Amazon's highly structured podzols, EcM morphotypes had a strongly biased distribution between the top mineral and organic horizons (L. Tedersoo unpublished). The lack of evidence for fungal belowground niche differentiation in Africa is particularly striking, because the African EcM vegetation is usually structured by soil humidity and fertility (Lawton 1978; Gartlan *et al.* 1986; Frost 1996). In particular, *Uapaca* and the EcM Fabaceae hosts have different ecophysiology and microhabitat preference in rain forests (Gartlan *et al.* 1986).

This study confirms the recent findings of EcM symbiosis in Asteropeiaceae and Sarcolaenaceae in Madagascar (Ducousso *et al.* 2004, 2008) and documents the mycota associated with roots of these endemic tree families for the first time. In contrast to previous reports (Högberg & Piearce 1986), we found no evidence for EcM formation in *Faurea* spp. and *Pericopsis angolensis* in Kashima. Although the dominance of mycorrhizal types may depend on soil and surrounding vegetation (Gehring *et al.* 2006), we believe that these trees are incapable of forming EcM, because the root systems of excavated saplings intermingled with EcM roots of Fabaceae that provided abundant fungal inoculum.

The diversity of species and phylogenetic lineages of EcM fungi was relatively low in African tropical forests. Across the four study sites, members of only 18 phylogenetic lineages were found. By contrast, individual sites commonly support >20 lineages in temperate ecosystems (Tedersoo & Nara 2010). Apart from a few species of Helotiales that were identified from small black mycorrhizal root tips and considered putatively EcM, no new EcM lineages were recovered. These findings corroborate the hypothesis of a lower phylogenetic richness of EcM fungi in tropical ecosystems that was raised based on fruit-body collections (Tedersoo *et al.* 2010a). The community composition of EcM fungi is highly similar across different African sites and resembles the structure of other tropical forests, where the /russula-lactarius, /tomentella-thelephora and /boletus lineages dominate (Diédhiou *et al.* 2010; Peay *et al.* 2010b; Jairus *et al.* 2011). Such a high similarity among the tropical EcM fungal communities may reflect the common climate or biogeographical history of hosts.

Conclusions

Compared to temperate ecosystems, African EcM fungal communities are less diverse in composition of both species and phylogenetic lineages. Host and soil profile play only a minor role in structuring EcM fungal communities that may respectively reflect the common biogeographical history of major host plants and poor differentiation of tropical soils. We advocate that variation partitioning of spatial autocorrelation and covariates along with targeted factors for multivariate analyses provides an efficient tool for understanding biological processes in spatially complex communities (Legendre *et al.* 2009; Dumbrell *et al.* 2010).

Acknowledgements

We thank M. Vincelette, L. Andriamahefarivo, G. Chuyong, Chiefs of Manyinga and Mundemba, Forestry Departments of

Cameroon, Gabon and Madagascar for providing research permits and/or facilities in Africa; D. M. Newbery and J. M. Norghauer for expertise in the field, M. Nõukas and K. Abarenkov for expertise in the lab, J.P.W. Young and four anonymous referees for constructive comments. Special thanks to M.E. Smith and M.H. Morris for providing raw data for rarefaction. This project was funded by Estonian Science Foundation grants 6939, 7434, 8235, JD-0092J; and FIBIR. TJ received a travel grant from Kaleva Travel.

References

Abarenkov K, Nilsson RH, Larsson K-H *et al.* (2010a) The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytologist*, **186**, 281–285.

Abarenkov K, Tedersoo L, Nilsson RH *et al.* (2010b) PlutoF—a web based workbench for ecological and taxonomic research with an online implementation for fungal ITS sequences. *Evolutionary Bioinformatics*, **6**, 189–196.

Alexander IJ (2006) Ectomycorrhizas—out of Africa? *New Phytologist*, **172**, 589–591.

Alexander IJ, Lee SS (2005) Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. In:*Biotic Interactions in the Tropics: Their Role in the Maintenance of Species Diversity* (eds Burslem DFRP, Pinard MA, Hartley SE). pp. 165–203, Cambridge University Press, London, UK.

Arita HT, Vazquez-Dominguez E (2008) The tropics: cradle, museum or casino? A dynamic null model for latitudinal gradients of species diversity. *Ecology Letters*, **11**, 653–663.

Ba AM, Thoen D (1990) First syntheses of ectomycorrhizas between *Afzelia africana* (Caesalpinioideae) and native fungi from West Africa. *New Phytologist*, **114**, 99–103.

Bechem EET (2004) *Mycorrhizal Status of* Gnetum *spp. in Cameroon: Evaluating Diversity with a View to Ameliorating Domestication Efforts*. PhD Thesis, University of Aberdeen, UK.

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

Bruns TD, Bidartondo MI, Taylor DL (2002) Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integrative and Comparative Biology*, **42**, 352–359.

Buee M, Courty PE, Mignot D, Garbaye J (2007) Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. *Soil Biology and Biochemistry*, **39**, 1947–1955.

Burgess N, D'Amico Hales J, Underwood E, Dinerstein E (2004) *Terrestrial Ecoregions of Africa and Madagascar*. Island Press, Washington DC, USA.

Buyck B, Thoen D, Watling R (1996) Ectomycorrhizal fungi in the Congo-Guinea region. *Proceedings of the Royal Society of London, series B*, **104**, 313–333.

Campbell BM, Angelsen A, Cunningham A, Katerere Y, Sitoe A, Wunder S (2007) Miombo woodlands—opportunities and barriers to sustainable forest management. CIFOR report. Available from http://www.cifor.cgiar.org/miombo/docs/Miombo2007.pdf.

Colwell RK (2006) *EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples. Version 8*. Persistent URL Available from purl.oclc.org/estimates.

Dahlgren RA, Singer MJ, Huang X (1997) Oak tree and grazing impacts on soil properties and nutrients in a California oak woodland. *Biogeochemistry*, **39**, 45–64.

Dickie IA, Koide RT, Steiner KC (2002) Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. *Ecological Monographs*, **72**, 505–521.

Diédhiou A, Selosse M-A, Galiana A *et al.* (2010) Multi-host ectomycorrhizal fungi are predominant in a Guinean tropical rainforest and shared between canopy trees and seedlings. *Environmental Microbiology*, **12**, 2219–2232.

Ducousso M, Bena G, Bourgeois C *et al.* (2004) The last common ancestor of Sarcolaenaceae and Asian dipterocarp trees was ectomycorrhizal before the India-Madagascar separation, about 88 million years ago. *Molecular Ecology*, **13**, 231–236.

Ducousso M, Ramanankierana H, Duponnois R *et al.* (2008) Mycorrhizal status of native trees and shrubs from eastern Madagascar littoral forests with specific emphasis on one new ectomycorrhizal family, the Asteropeiaceae. *New Phytologist*, **178**, 233–238.

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

Frost P (1996) The ecology of miombo woodlands. In:*The Miombo in Transition: Woodlands and Welfare in Africa* (ed. Campbell B). pp. 11–60, CIFOR, Bogor, Indonesia.

Ganzhorn JU, Goodman SM, Vincelette M (2007) *Biodiversity, Ecology and Conservation of Littoral Ecosystems in Southeastern Madagascar, Tolagnaro (Fort Dauphin)*. SI/MAB Series #11, Smithsonian Institution, Washington, DC.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizas and rusts. *Molecular Ecology*, **2**, 113–118.

Gartlan JS, Newbery DM, Thomas DW, Waterman PG (1986) The influence of topography and soil phosphorus on the vegetation of Korup forest reserve, Cameroun. *Vegetatio*, **65**, 131–148.

Gehring CA, Mueller RC, Whitham TG (2006) Environmental and genetic effects on the formation of ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. *Oecologia*, **149**, 158–164.

Goodman SM, Benstead JP (2003) *The Natural History of Madagascar*. The University of Chicago Press, Chicago, Illinois.

Haug I, Weiß M, Homeier J, Oberwinkler F, Kottke I (2005) Russulaceae and Thelephoraceae form ectomycorrhizas with members of the Nyctaginaceae (Caryophyllales) in the tropical mountain rain forest of southern Ecuador. *New Phytologist*, **165**, 923–936.

Högberg P, Piearce GD (1986) Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation type and successional patterns. *Journal of Ecology*, **74**, 775–785.

Hynson N, Bruns TD (2010) Fungal hosts for mycoheterotrophic plants: a non-exclusive, but highly selective club. *New Phytologist*, **185**, 598–601.

Ishida TA, Nara K, Hogetsu T (2007) Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. *New Phytologist*, **174**, 430–440.

Jablonski D, Roy K, Valentine AW (2006) Out of the tropics: evolutionary dynamics of the latitudinal diversity gradient. *Science*, **314**, 102–105.

Jairus T, Mpumba R, Chinoya S, Tedersoo L (2011) Invasion potential and host shifts of Australian and African ectomycorrhizal fungi in mixed eucalypt plantations. *New Phytologist*, doi: 10.1111/j.1469-8137.2011.03775.x.

Lawton RM (1978) A study of the dynamic ecology of Zambian vegetation. *Journal of Ecology*, **66**, 175–198.

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

Lindahl B, Ihrmark K, Boberg J *et al.* (2007) Spatial separation of litter decomposition and mycorrhizal nutrient uptake in a boreal forest. *New Phytologist*, **173**, 611–620.

McGuire KL (2007) Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. *Ecology*, **88**, 567–574.

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In:*Mycorrhizal Functioning. An Integrative Plant-Fungal Process* (ed. Allen M). pp. 357–423, Chapman and Hall, New York.

Morris MH, Smith ME, Rizzo DM, Rejmanek M, Bledsoe CS (2008) Contrasting ectomycorrhizal fungal communites on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland. *New Phytologist*, **178**, 167–176.

Morris MH, Perez-Perez MA, Smith ME, Bledsoe CS (2009) Influence of host species on ectomycorrhizal communities associated with two co-occurring oaks *(Quercus* spp.) in a tropical cloud forest. *FEMS Microbiology Ecology*, **69**, 274–287.

Nara K (2006) Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. *New Phytologist*, **171**, 187–198.

Newbery DM, Alexander IJ, Thomas DW, Gartlan JS (1988) Ectomycorrhizal rainforest legumes and soil phosphorus in Korup National Park, Cameroon. *New Phytologist*, **109**, 433–450.

Onguene NA (2000) *Diversity and dynamics of mycorrhizal associations in tropical rain forests with different disturbance regimes in South Cameroon. Tropenbos Cameroon Series* **3**. University of Wageningen, The Netherlands.

Peay KG, Garbelotto M, Bruns TD (2010a) Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. *Ecology*, **91**, 3631–3640.

Peay KG, Kennedy PG, Davies SJ, Tan S, Bruns TD (2010b) Potential link between plant and fungal distribuions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. *New Phytologist*, **185**, 529–542.

R Core Development Team (2007) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ryberg M, Larsson E, Molau U (2009) Ectomycorrhizal diversity in *Dryas octopetala* and *Salix reticulata* in an Alpine cliff ecosystem. *Arctic, Antarctic and Alpine Research*, **41**, 506–514.

Sanon KB, Ba AM, Dexheimer J (1997) Mycorrhizal status of some fungi fruiting beneath indigenous trees in Burkina Faso. *Forest Ecology and Management*, **98**, 61–68.

Sanon KB, Ba AM, Delaruelle C, Duponnois R, Martin F (2009) Morphological and molecular analyses in *Scleroderma* species associated with some Caesalpinioid legumes, Dipterocarpaceae and Phyllanthaceae trees in southern Burkina Faso. *Mycorrhiza*, **19**, 571–584.

Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K (2009) Is there a latitudinal gradient in the importance of biotic interactions? *Annual Reviews in Ecology, Evolution and Systematics*, **40**, 245–269.

Smith ME, Douhan GW, Fremier AK, Rizzo DM (2009) Are true multihost fungi the exception or the rule? Dominant ectomycorrhizal fungi on *Pinus sabiniana* differ from those on co-occurring *Quercus* species. *New Phytologist*, **182**, 295–299.

Sunderland T, Walters G, Issembe Y (2004) *A Preliminary Vegetation Assessment of the Mbé National Park, Monts de Cristal, Gabon*. CARPE report, Libreville, Gabon.

Suvi T, Tedersoo L, Abarenkov K, Gerlach J, Beaver K, Kõljalg U (2010) Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. *Mycologia*, **102**, 522–533.

Tedersoo L, Nara K (2010) General latitudinal gradient of biodiversity is reversed in ectomycorrhizal fungi. *New Phytologist*, **185**, 351–354.

Tedersoo L, Suvi T, Larsson E, Kõljalg U (2006) Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. *Mycological Research*, **110**, 734–748.

Tedersoo L, Jairus T, Horton BM *et al.* (2008a) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist*, **180**, 479–490.

Tedersoo L, Suvi T, Jairus T, Kõljalg U (2008b) Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. *Environmental Microbiology*, **10**, 1189–1201.

Tedersoo L, Suvi T, Jairus T, Ostonen I, Põlme S (2009) Revisiting ectomycorrhizal fungi of *Alnus*: differential host specificity, diversity and determinants of the fungal community. *New Phytologist*, **182**, 727–735.

Tedersoo L, May TW, Smith ME (2010a) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, **20**, 217–263.

Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M (2010b) Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. *The ISME Journal*, **4**, 465–471.

Thoen D, Ba AM (1989) Ectomycorrhizas and putative ectomycorrhizal fungi of *Afzelia africana* Sm. and *Uapaca guineensis* Müll. Arg. in southern Senegal. *New Phytologist*, **113**, 549–559.

Toljander JF, Eberhardt U, Toljander YK, Paul LR, Taylor AFS (2006) Species composition of an ectomycorrhizal fungal community along a local nutritional gradient. *New Phytologist*, **170**, 873–884.

Verhoeven KJF, Simonsen KL, McIntyre LM (2005) Implementing false discovery rate control: increasing your power. *Oikos*, **108**, 643–647.

L.T., M.B., T.J., E.B., E.R., T.N. and U.K. share the common interest on the factors underlying the diversity and community

composition of ectomycorrhizal fungi in the scales of individual seedlings to the globe. Research topics of S.C., M.L., S.R., E.B. and R.M. cover plant taxonomy, conservation and sustainable ecosystem management in African countries.

Data accessibility

The DNA sequences of fungal ITS and LSU regions are available in INSD (accession numbers FR731162–FR731964) and UNITE (see Table S2, Supporting information) databases. Raw species frequency data (arranged by sample, host and soil horizon) are given in supplementary material (Tables S3–S6, Supporting information).

Supporting information

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

Table S1 Characteristics of the study sites.

Table S2 Identification and frequency of ectomycorrhizal fungi at the study sites.

Table S3 Occurrence of ectomycorrhizal fungi in soil samples at Kashima, Zambia.

Table S4 Occurrence of ectomycorrhizal fungi in soil samples at Tchimbele, Gabon.

Table S5 Occurrence of ectomycorrhizal fungi in soil samples at Mandena, Madagascar.

Table S6 Occurrence of ectomycorrhizal fungi in soil samples at Korup, Cameroon.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.