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New Phytologist

Changes in richness and community composition of ectomycorrhizal fungi among altitudinal vegetation types on Mount Kinabalu in Borneo

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Received: *30 January 2017*
Accepted: *6 March 2017*

New Phytologist (2017) **215:** 454–468
doi: 10.1111/nph.14566

Key words: Borneo, biodiversity, ectomycorrhizal fungi, internal transcribed spacer (ITS), metabarcoding, mid-domain effect, tropical ecology.

Summary

- The distribution patterns of tropical ectomycorrhizal (ECM) fungi along altitudinal gradients remain largely unknown. Furthermore, despite being an iconic site for biodiversity research, virtually nothing is known about the diversity and spatial patterns of fungi on Mt Kinabalu and neighbouring mountain ranges.
- We carried out deep DNA sequencing of soil samples collected between 425 and 4000 m above sea level to compare richness and community composition of ECM fungi among altitudinal forest types in Borneo. In addition, we tested whether the observed patterns are driven by habitat or by geometric effect of overlapping ranges of species (mid-domain effect).
- Community composition of ECM fungi was strongly correlated with elevation. In most genera, richness peaked in the mid-elevation montane forest zone, with the exception of tomentelloid fungi, which showed monotonal decrease in richness with increasing altitude. Richness in lower-mid- and mid-elevations was significantly greater than predicted under the mid-domain effect model.
- We provide the first insight into the composition of ECM fungal communities and their strong altitudinal turnover in Borneo. The high richness and restricted distribution of many ECM fungi in the montane forests suggest that mid-elevation peak richness is primarily driven by environmental characteristics of this habitat and not by the mid-domain effect.

Introduction

Documenting spatial patterns in species richness and range sizes and understanding the underlying mechanisms have long been at the centre of ecological and biogeographic studies. In particular, changes in species richness along elevation gradients have been studied extensively in plants and animals and most taxa have been reported to display either a monotonal decline in richness with increasing elevation, a mid-elevation peak, or some combinations of the two, for example, low-elevation richness plateau followed by a mid-elevation peak or by a monotonal decline (Colwell *et al.*, 2004; Lomolino *et al.*, 2006; McCain, 2009). Patterns of monotonal decline have generally been attributed to the decrease in environmental energy (e.g. temperature) and the decrease in the suitable habitat area (Stevens, 1992; Rosenzweig, 1995). Possible explanations for mid-elevation peak in richness include increased rainfall and relative humidity and the mostly geometric effect of overlapping ranges of species with broad elevation range (Colwell & Lees, 2000; Colwell *et al.*, 2004). The latter is called the mid-domain effect (MDE) and it is supported by a substantial amount of empirical data from a range of organismal groups (Grytnes & Vetaas, 2002; Sanders, 2002; McCain, 2004; Cardelús *et al.*, 2006; Grytnes *et al.*, 2008).

The tropical high mountains in Sabah, Malaysian Borneo, provide a spectrum of moist forests from sea level to almost 4000 m. Because the precipitation varies relatively little among the elevation zones (ranging from 2000 to 3000 mm yr^{-1}) (Kitayama, 1992; Kitayama & Aiba, 2002), they are ideally suited to studying the effect of elevation on species richness and community composition. Mount Kinabalu, in particular, has one of the most species-rich biotas of the world, with > 5000 vascular plant species (Beaman & Anderson, 2004). Elevation patterns of species richness in various plant and animal groups on Mt Kinabalu have been studied by Kitayama (1996), Brühl *et al.* (1998), Nor (2001) and Liew *et al.* (2010). Kitayama (1996) and Brühl *et al.* (1998) found that richness decreased exponentially with elevation in trees and leaf litter ants, respectively, while Nor (2001) observed a mid-elevation peak in small mammal species richness at *c.* 1500 m above sea level (m asl), followed by a steep decline with increasing elevation. Finally, Liew *et al.* (2010) found that species density of land snails showed a decline with elevation, and weak MDE was found only on Mt Kinabalu, not on neighbouring Mt Tambuyukon.

On a global scale, the vast majority of studies on the effect of elevation on richness have focused on vascular plants and animals, while elevational patterns of richness in fungi, one of the largest groups of living organisms, remain scarcely studied. This is particularly true for Borneo, where there are numerous studies on the flora and fauna of Mt Kinabalu and the surrounding mountains, while virtually nothing is known about fungi inhabiting various elevation zones in this region. This gap in our knowledge seems particularly important if we consider the fact that fungi perform key roles in nutrient cycles (Van der Heijden *et al.*, 2015) and influence tropical rainforest plant diversity and composition (Bagchi *et al.*, 2014). Ectomycorrhizal (ECM) fungi, in particular, are among the most ecologically important groups of fungi in Borneo, as they provide water and nutrients to their host plants and enhance protection against various environmental stress factors (Brearley, 2012). Some of the dominant canopy trees from lowland to subalpine forests (e.g. members of Dipterocarpaceae, Fagaceae, Myrtaceae) are known ECM hosts (Aiba & Kitayama, 1999; Brundrett, 2009). Of these tree families, Dipterocarpaceae is perhaps the most notable for being the most characteristic component of low-elevation forests in Southeast Asia and for its economic importance (Brearley, 2012). Previous studies on ECM fungi in Borneo focused on fungi associated with dipterocarp forests in Sarawak (Sirikantaramas *et al.*, 2003; Peay *et al.*, 2010; Sato *et al.*, 2015), while other geographic areas and vegetation types in Borneo, including montane and subalpine forests, have remained unexplored. The aims of this work were to characterize ECM fungal communities in all major forest types in northern Borneo, including taxonomic composition and biogeographic affinities; to detect shifts in richness, function, and community composition of ECM fungi among altitudinal forest types; and to evaluate the possible roles of environmental variables and geometrical effects, such as MDE, in shaping the observed patterns.

Materials and Methods

Sampling locations

Soil samples were collected in September 2012 in Kinabalu and Crocker Range parks in Sabah, Borneo. The 25 sampling sites represent the entire elevation range, with five to eight sampling localities in each zone (Fig. 1). Forest type, elevation, geographic coordinates and localities are shown in Table 1.

The Crocker Range is the highest mountain range in Sabah with an average height of *c.* 1800 m asl. At 4095 m asl, Mt Kinabalu is the tallest mountain between the Himalayas and New Guinea. It is a relatively young and solitary ‘sky-island’, having emerged during the Pliocene and early Pleistocene as a granite pluton within the surrounding Tertiary sedimentary rocks of the Crocker Range (Jacobson, 1970). In addition, intrusions of ultramafic rock occur around the massif (Brunotte & Kitayama, 1987). The vascular flora mainly consists of Indo-Malaysian, East Asiatic, and Austral-Antarctic floristic elements that characterize the low, mid-, and high altitudes, respectively (Hotta, 1974; Kitayama, 1992). The vegetation of Mt Kinabalu can be divided into four discrete zones: lowland dipterocarp forests (< 1200 m asl), montane forests (1200–2700 m asl), ultramafic rock forests (2700–3000 m asl), granite boulder forests and summit zone (3000–4095 m asl) (Beaman & Beaman, 1990; Kitayama, 1992). In dipterocarp forests, ECM host tree genera include *Dipterocarpus*, *Hopea*, *Shorea*, *Vatica* (Dipterocarpaceae), *Castanopsis*, *Lithocarpus* (Fagaceae) and *Tristaniopsis* (Myrtaceae), while mainly *Castanopsis*, *Lithocarpus*, *Quercus* (Fagaceae) and *Tristaniopsis* (Myrtaceae) inhabit the montane forests. Above *c.* 2800 m asl, only *Leptospermum* and *Tristaniopsis* (Myrtaceae) are the primary ECM host trees that are further reduced to a single species, *Leptospermum recurvum*, at the high-elevation ultramafic sites (Kitayama, 1992; Aiba & Kitayama, 1999).

Sampling and molecular work

At each sampling site (*c.* 10 × 25 m), 40 soil cores, 2 cm in diameter and *c.* 20 cm deep, were taken *c.* 2 m from each other to minimize the probability of sampling the same genet repeatedly. The 40 cores were pooled, resulting in a composite soil sample per site. Approximately 20 g of each sample was dried immediately at 30–35°C. Soil chemical analyses were conducted following Sparks *et al.* (1996). Genomic DNA was extracted from 1 ml (*c.* 1 g) of dried soil from each of the 25 samples using a NucleoSpin soil kit (Macherey-Nagel Gmbh & Co., Düren, Germany). For each sample, two independent DNA extractions were carried out and the extracts were pooled. The ITS2 region (*c.* 250 bp) of the nuclear ribosomal DNA repeat was PCR-amplified using primers fITS7 (Ihrmark *et al.*, 2012) and ITS4 (White *et al.*, 1990). The ITS4 primer was labelled with sample-specific Multiplex Identification DNA-tags (MIDs). The amplicon library was sequenced at Naturalis Biodiversity Center (Naturalis) using an Ion 318™ Chip and an Ion Torrent Personal Genome Machine (Life Technologies, Guilford, CT, USA). Detailed protocols of the molecular work are described in Geml *et al.* (2014).

Bioinformatic work

During the initial clean-up of the raw data (7259 332 sequence reads) using GALAXY (https://main.g2.bx.psu.edu/root), sequences were sorted according to samples and MIDs were removed. Primers and poor-quality ends, based on 0.02 error probability limit, were trimmed in GENEIOUS PRO v.5.6.1 (Biomatters, Auckland, New Zealand). Sequences were filtered using MOTHUR v.1.32.1 (Schloss *et al.*, 2009) based on the following settings: no ambiguous bases (maxambig = 0), homopolymers no longer than 10 nucleotides (maxhomop = 10), and length range from 150 to 400 bp (minlength = 150; maxlength = 400), resulting in 4421 543 quality-filtered sequences with an average read length of 248.5 ± 44.6 (mean ± SD). Global singletons (1135 485) and putative chimeric sequences (32 358) were removed with USEARCH v.8.0 (Edgar, 2010). The remaining sequences were grouped into 11 409 operational taxonomic units (OTUs) at

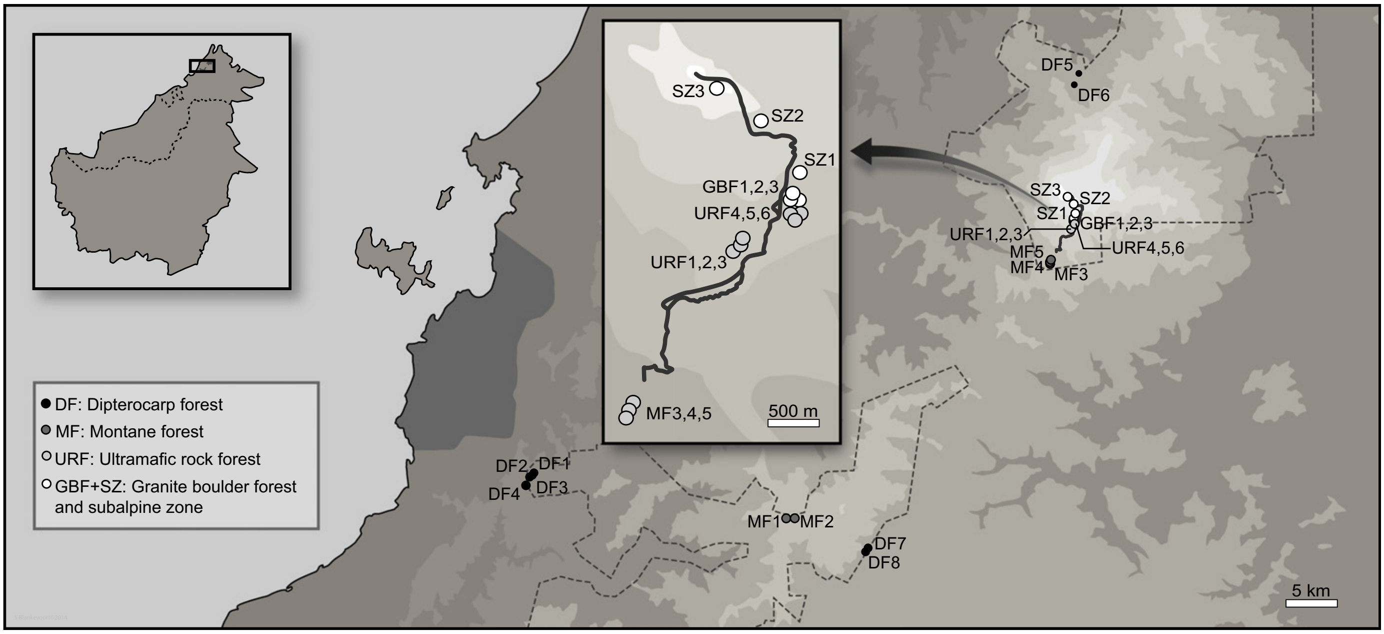

Fig. 1 A map of the sampling localities, with the location of the region of study in Borneo (inset left) and with the details of the summit trail in Kinabalu Park (inset middle). Dashed lines indicated the boundaries of Crocker Range and Kinabalu Parks. Full names, geographic coordinates and environmental variables corresponding to the sampling localities are listed in Table 1 and Supporting Information Table S1.

Table 1 Sampling sites in Sabah, Borneo, included in this study with code, forest type, locality, protected area, elevation, mean annual temperature and geographic coordinates. Locations are displayed in a map in Fig. 1

Site code	Forest type	Locality	National Park	Elevation (m asl)	Temperature (°C)	Latitude	Longitude
DF1	Dipterocarp	Inobong: Kibamabangan Trail	Crocker Range	425	24.4	5.856 925	116.140 05
DF2	Dipterocarp	Inobong: Kibamabangan Trail	Crocker Range	478	24.4	5.855 532	116.1392
DF3	Dipterocarp	Inobong: Salt Trail	Crocker Range	551	24.4	5.853 614	116.136 97
DF4	Dipterocarp	Inobong: Salt Trail	Crocker Range	624	24	5.847 208	116.134 49
DF5	Dipterocarp	Sayap: Kemantis Trail	Kinabalu	1000	21.4	6.168 402	116.568 18
DF6	Dipterocarp	Sayap: Kemantis Trail	Kinabalu	1020	20.9	6.159 855	116.565 18
DF7	Dipterocarp	Mahua waterfall valley	Crocker Range	1067	20.9	5.798 519	116.404 07
DF8	Dipterocarp	Mahua waterfall ridge	Crocker Range	1120	20.9	5.794 901	116.401 44
MF1	Montane	Mt Alab: Waken Trail	Crocker Range	1900	17.9	5.821 002	116.339 58
MF2	Montane	Mt Alab: Minduk Sirunk Trail	Crocker Range	1900	17.4	5.821 667	116.343 36
MF3	Montane	Kinabalu Trail near Pondok Ubah	Kinabalu	2039	18	6.023 542	116.546 34
MF4	Montane	Kinabalu Trail near Pondok Ubah	Kinabalu	2080	18	6.0245	116.546 52
MF5	Montane	Kinabalu Trail near Pondok Ubah	Kinabalu	2100	17.5	6.025 778	116.547 53
URF1	Ultramafic	Kinabalu Trail below Pondok Villosa	Kinabalu	2850	12.6	6.047 807	116.562 45
URF2	Ultramafic	Kinabalu Trail below Pondok Villosa	Kinabalu	2896	12.6	6.048 198	116.5629
URF3	Ultramafic	Kinabalu Trail below Pondok Villosa	Kinabalu	2908	12.6	6.048 888	116.563 32
URF4	Ultramafic	Kinabalu Trail above Pondok Villosa	Kinabalu	3023	11	6.0525	116.564 79
URF5	Ultramafic	Kinabalu Trail above Pondok Villosa	Kinabalu	3050	11	6.052 932	116.564 83
URF6	Ultramafic	Kinabalu Trail above Pondok Villosa	Kinabalu	3070	11	6.053 699	116.565 24
GBF1	Granite/subalpine	Kinabalu Trail near Pondok Paka	Kinabalu	3080	11	6.054 066	116.565 25
GBF2	Granite/subalpine	Kinabalu Trail near Pondok Paka	Kinabalu	3100	11	6.054 316	116.5654
GBF3	Granite/subalpine	Kinabalu Trail near Pondok Paka	Kinabalu	3120	11	6.054 728	116.565 44
SZ1	Granite/subalpine	Kinabalu Trail near Laban Rata	Kinabalu	3280	9.1	6.058 88	116.566 69
SZ2	Granite/subalpine	Mt Kinabalu summit	Kinabalu	3600	6.4	6.067 035	116.565 41
SZ3	Granite/subalpine	Mt Kinabalu summit	Kinabalu	4000	6.4	6.072 942	116.561 25

asl, above sea level.

97% sequence similarity in USEARCH, and representative sequences were assigned to taxonomic groups based on pairwise similarity searches. For both chimera checking and taxonomic assignments, we used the curated UNITE + INSD fungal internal transcribed spacer (ITS) sequence database (version released on 22 August 2016), containing identified fungal sequences with assignments to species hypothesis groups delimited based on dynamic similarity thresholds (Kõljalg *et al.*, 2013). After excluding OTUs with $< 80\%$ similarity or < 150 bp pairwise alignment length to a fungal sequence, 7535 fungal OTUs were retained. These fungal OTUs contained a total of 1 556 155 high-quality sequences with an average of $62\,246.2 \pm 37\,814.3$ reads per sample, ranging from 26 782 to 167 006. The number of sequences did not correlate with the detected total fungal and ECM fungal OTUs ($R^2 = 0.029$, $P = 0.421$; $R^2 = 0.024$, $P = 0.456$, respectively). Although next-generation sequencing libraries often are normalized by random subsampling to the size of the smallest library, such rarefied counts often represent only a small fraction of the data. This practice has been shown to increase type I error (decreased specificity) and type II error (decreased sensitivity), and is strongly discouraged by McMurdie & Holmes (2014). Because the sampled ecosystems were presumed to be highly diverse and because no previous data from the sampled region were available, we estimated the richness of ECM fungi in two ways: by including all high-quality sequences in the analyses with the goal of assessing all ECM fungal diversity in the samples; and by rarefying the number of high-quality fungal sequences to the smallest library size (26 782 reads).

For the in-depth analyses presented in this paper, we selected all OTUs that showed $\geq 90\%$ ITS2 sequence similarity, suggesting congeneric relationships (Nilsson *et al.*, 2011), with reference sequences of phylogenetic lineages of ECM fungi as reviewed by Tedersoo & Smith (2013). We did not include representatives of the genus *Entoloma*, because our preliminary phylogenetic analyses showed that almost all OTUs fell outside the only ECM clade within the genus (subgenus *Rhodopolia*) and hence were considered nonECM (Co-David *et al.*, 2009). In the order Sebacinales, owing to the wide variety of ecological functions (e.g., saptrotrophic, endophytic, ericoid mycorrhizal, and ECM) and the high number of reference sequences with incomplete taxonomic classification, ECM OTUs were selected based on their supported phylogenetic placement (with $\geq 70\%$ bootstrap and/or ≥ 0.95 posterior probability) among sequences of known ECM taxa published by Urban *et al.* (2003), Ryberg *et al.* (2009) and Tedersoo & Smith (2013). Data on known geographic locations and host identities of the matching species hypotheses were gathered to reveal patterns of biogeographic and host affinities of ECM fungi in Borneo. OTU sequences of ECM fungi analyzed for this paper have been submitted to GenBank (KT273628–KT273899).

We classified the OTUs according to extramatrical mycelial exploration type (ET) characteristics following Agerer (2006), Tedersoo & Smith (2013) and an online information system for the determination and characterization of ECM fungi the DEEMY database (http://deemy.de). We classified the OTUs into two aggregate categories corresponding to hydrophobicity: contact/short-distance/medium-distance smooth with hydrophilic hyphae (C/SD/MDS) and medium-distance fringe/long-distance with hydrophobic hyphae (MDF/LD). Among the genera encountered in our samples, only *Byssocorticium* and *Hydropus* contain species from both ET categories and, therefore, these genera (eight OTUs) were excluded from the ET analyses. Because hyphal characteristics of ECM fungi in the tropics are not well known, the assignment of ECM taxa to ETs in this manuscript is based on data accumulated primarily from temperate and boreal studies, which will need to be re-evaluated, when more data on the hyphal characteristics of tropical ECM fungi become available.

Statistical analyses

Values of observed richness of all ECM fungal OTUs (S), Simpson's diversity ($D = 1 - \text{sum}\ (p_i^2)$, where p_i is the importance probability in element i), and OTU richness for ECM lineages were computed using PC-ORD v.6.0 (McCune & Grace, 2002). In addition, we calculated Fisher's alpha, which is a parameter of the log series model calculated from richness and the number of individuals (Magurran, 2004). We compared species turnover within and between forest types calculated by pairwise comparisons of communities in the corresponding sampling sites. These beta diversity measures included Sørensen and Bray–Curtis similarity indices that were calculated from presence–absence and abundance data, respectively. In addition, we used the Chao–Sørensen index to assess the probability that individuals belong to shared vs unshared species following Chao *et al.* (2005). All beta diversity measures were calculated using ESTIMATES v.9.1.0 (Colwell *et al.*, 2012). These indices as well as richness of various phylogenetic lineages and ETs were compared across forest types using analysis of variance (ANOVA) in R, with means compared with Tukey's honestly significant difference test (Faraway, 2002).

We assessed to what extent the MDE could contribute to the observed richness patterns across the forest types using RANGEMODEL v.5 (Colwell, 2005). We followed the common practice in studies on highly diverse tropical communities (Rahbek, 2005; Cardelús *et al.*, 2006) and calculated empirical richness as interpolated richness, based on the assumption that each OTU inhabited all sampling sites between its lowest and highest recorded occurrences regardless of whether or not it was recorded at all intermediate sites. Expected richness under the influence of MDE was generated by randomizing the placement of the complete set of empirical ranges along a continuous gradient, as suggested by McCain (2003) and Colwell *et al.* (2004). In general, incomplete sampling tends to underestimate range size (Colwell & Hurtt, 1994) and OTUs recorded at a single site (with an observed elevation range of zero) would have an interpolated range of zero and would fail to properly contribute to the predicted richness (Cardelús *et al.*, 2006). Therefore, we assumed that all OTUs, regardless of their observed ranges, have actual elevation ranges 250 m greater than what we observed, while maintaining the observed midpoints. Because environmental conditions are similar within 250 m of elevation difference and because fungi generally are good dispersers, we think that our assumption of any given species occurring within an elevation range extended by 250 m beyond the observed end points is reasonable and would, to some extent, compensate for the incomplete occurrence records inherent in sampling hyperdiverse communities. Randomizations (1000) were carried out for the full dataset as well as separate datasets containing large-range and small-range OTUs, with empirical ranges of > 500 m and

$<$ 500 m, respectively. The mid-domain theory predicts that the MDE, that is, the geometric overlaps of ranges, will be stronger for large-range species than for small-range ones (Colwell *et al.*, 2004). Quadratic regression analyses were used to examine relationships between environmental variables and ECM fungal richness in two separate analyses: one based on observed ECM fungal richness and another based on residuals of observed from predicted richness under the MDE model. Environmental variables included mean annual temperature (MAT), mean annual precipitation (MAP), soil pH, carbon (C), nitrogen (N), and C : N (Supporting Information Table S1).

To compare community composition across the forest types, we used PC-ORD to run detrended correspondence analysis (DCA) on the rarefied OTU table and a secondary matrix containing environmental variables, that is, elevation, forest type, MAT, MAP, and edaphic characteristics. Climatic data for the sampling locations were downloaded from the WorldClim database (www.worldclim.org) (Table S1). Despite the theoretical limitations of raster data in accurately capturing steep topography-driven gradients, the obtained temperature and precipitation values were in agreement with field measurements of Kitayama (1992) and Nor (2001) on Mt Kinabalu. The ordination was carried out based on presence/absence, where 'presence' was defined as more than or equal to two sequences on a per-sample basis to minimize false positives. The sample *SZ3* (4000 m asl) contained a single ECM fungal OTU (/sordariales1) and was therefore excluded from the ordination analyses. Correlation between the DCA axis 1 scores and elevation was assessed using linear regression. Also, we performed empirical semivariance analyses to describe the spatial structure of the ordination axes following Mundra *et al.* (2015), where standardized isotrophic semivariance was calculated for each of the 10 lag classes into which the range of distances between the sampling sites was divided. In addition, we used partial Mantel tests in PC-ORD to differentiate the effects of spatial distance and elevation on community structure. We tested whether fungal communities were statistically different across forest types using the multiresponse permutation procedure (MRPP). Finally, we determined any preferences of individual OTUs for specific forest types using indicator species analyses (Dufrêne & Legendre, 1997), also in PC-ORD.

Tree community analyses

Because we did not collect vegetation data during the sampling because of logistical constraints, we analysed tree community data gathered by Kitayama (1992) and Aiba & Kitayama (1999) to obtain information on the distribution of ECM host tree genera on Mt Kinabalu. The cited studies sampled tree communities in elevations from 600 to 3400 m asl and provide virtually the only tree community dataset spanning altitudinal forest types in Borneo. In addition, these authors focused on the same areas where the majority of our sampling sites were located, although the precise locations probably differed in many cases, precluding combined statistical analyses of host and fungal datasets. Therefore, we analysed the tree community independently and used linear regression to examine relationships between elevation and generic richness and relative basal area of ECM host trees.

Data accessibility

The representative sequences of ECM fungal OTUs have been submitted to GenBank (KT273628–KT273899). The list of OTUs with identities of the most similar reference sequences and their distribution matrix with read abundance for the altitudinal forest types are shown in the Supporting Information.

Results

Patterns of richness

In total, we found 340 ECM fungal OTUs in our samples, of which 313 belong to Basidiomycota and 27 to Ascomycota, representing at least 34 ECM genera of 25 phylogenetic lineages (Table S2). Of these, *Tomentella* (including *Thelephora*) was the most OTU-rich genus with 75 OTUs, followed by *Cortinarius* (57 OTUs) and *Russula* (47 OTUs). Although the rarefied dataset contained less than half of all high-quality sequences, $>$ 95% of the ECM fungal OTUs were still recovered. Diversity measures differed significantly among the forest types and showed the highest values in the mid-elevation montane forest zone: observed ECM fungal richness ($F = 5.76$, $P = 0.0049$), rarefied ECM fungal richness ($F = 6.26$, $P = 0.0033$), and Fisher's alpha ($F = 5.72$, $P = 0.0054$) (Fig. 2). On the other hand, Simpson's diversity ($F = 2.45$, $P = 0.0918$) did not differ among the vegetation types, possibly because the distribution of relative abundances, which heavily influences D, was similar in all samples: a few abundant and many rare OTUs. Fourteen phylogenetic lineages were represented by at least three OTUs and their observed richness values were compared among the forest types. ECM ascomycetes and eight ECM basidiomycete lineages with a low number of OTUs showed no significant difference in richness across the forest types, while six basidiomycete lineages and both ET categories showed significant differences in the observed number of OTUs in different elevation zones: /ceratobasidium ($F = 7.01$, $P = 0.0019$), /cortinarius ($F = 8.59$, $P = 0.0007$), /hebeloma-alnicola ($F = 4.03$, $P = 0.0207$), /russula-lactarius ($F = 7.3$, $P = 0.0016$), /tomentella-thelephora ($F = 4.94$, $P = 0.0095$), and /xenasmatella ($F = 4.99$, $P = 0.0091$). Most of these exhibited a distinct mid-elevation peak in richness, with the exception of /tomentella-thelephora and /ceratobasidium, which had the highest number of OTUs in the dipterocarp forest and showed a monotonal decline in richness with increasing elevation (Fig. 3a). Both ET categories showed a mid-elevation peak in OTU richness corresponding to the montane forest zone, although this pattern was more pronounced in the MDF/LD: C/SD/MDS ($F = 4.7$, $P = 0.0116$), MDF/LD ($F = 4.73$, $P = 0.0112$) (Fig. 3b). All three beta diversity measures indicated that sampled fungal communities within the altitudinal zones were most similar to one another in the montane forest, while species turnover was greatest among dipterocarp forest sites, although significant differences were only observed in presence–

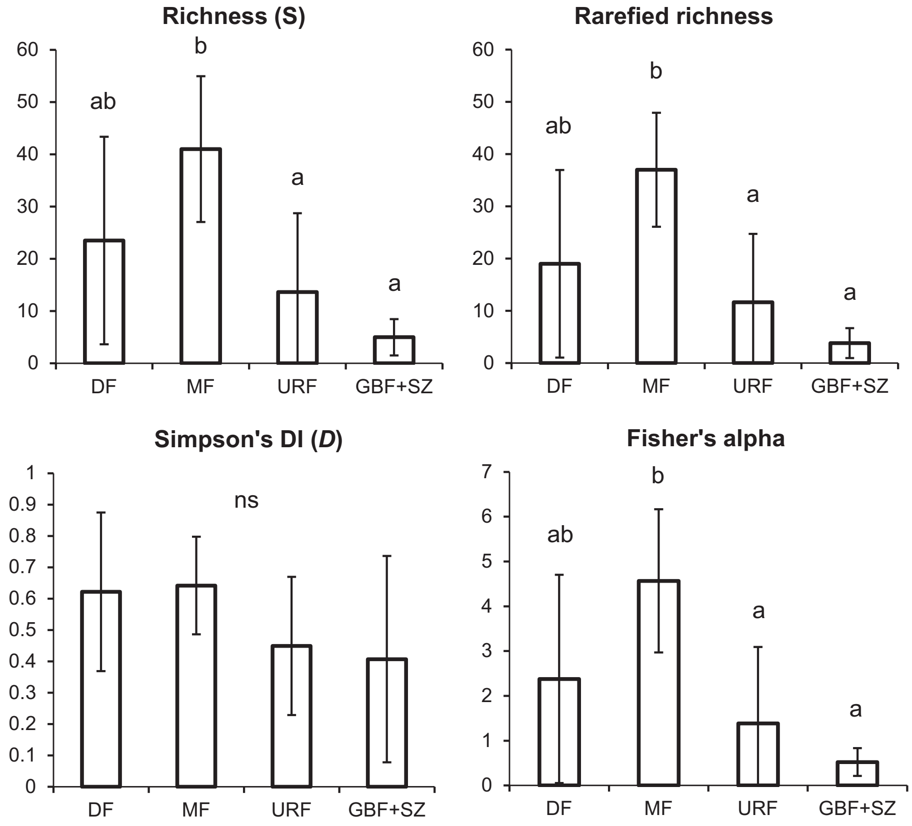

Fig. 2 Operational taxonomic unit (OTU) richness, rarefied richness, Simpson's diversity index (DI), *D*, and Fisher's alpha of ectomycorrhizal fungi per sampling site, across elevational forest types in Borneo. Rarefied richness of ectomycorrhizal fungi was calculated after rarefying the number of high-quality fungal sequences for each sample to the smallest library size (26 782 reads). Error bars represent ± SD. Letters above bars represent pairwise comparisons between forest types. ns, not significant. URF, ultramafic rock forest; MF, montane forest; DF, dipterocarp forest; GBF+SZ, granite boulder forest and subalpine zone.

absence-based comparisons (Sørensen, $F = 5.35$, $P = 0.0023$; Bray–Curtis, $F = 1.14$, $P = 0.3397$; Chao–Sørensen, $F = 1.75$, $P = 0.1657$) (Table 2). Pairwise similarity indices among the forest types were highest between adjacent vegetation zones in mid- to high elevations, while the dipterocarp forest sites shared no ECM fungal OTUs with any sites above 2500 m (Sørensen, $F = 10.49$, $P < 0.0001$; Bray–Curtis, $F = 5.2$, $P = 0.0006$; Chao–Sørensen, $F = 1.14$, $P = 0.3393$) (Table 2).

Patterns of OTU richness for interpolated empirical distributions as a function of elevation are shown in Fig. 4. Both observed (grey triangles) and interpolated empirical richness (closed circles) of ECM fungi peak at lower mid-elevations, between *c.* 1000 and 2000 m asl. Computed OTU richness (open circles) at the sampled elevations, as predicted by the MDE randomization model, are shown with 95% confidence intervals (fine lines). The data presented here clearly show that empirical richness values of large-range OTUs yield a better fit to the MDE model ($R^2 = 0.552$) than when only small-range ($R^2 = 0.175$) or all OTUs are considered ($R^2 = 0.331$), as predicted by the theory. However, we found significant deviations from predicted richness in all three analyses (Fig. 4). These deviations were most pronounced at lower-mid- and mid-elevations, where empirical ECM fungal richness values were substantially higher than predicted by the MDE model. In addition, sampling sites above 3000 m asl showed lower-than-predicted richness values. Although these deviations were most pronounced in OTUs with small ranges, even large-range OTUs showed significantly higher empirical richness values at low- to mid-elevations than predicted under the MDE. Neither observed ECM fungal richness nor residuals of observed ECM fungal richness from the MDE model were correlated with environmental variables in quadratic regressions, with the exception of MAT, which showed strong correlation in both regression analyses (Table S3).

Comparing ECM fungal community composition among the sampling sites

The first axis of the DCA (eigenvalue 0.9503) identified elevation (or MAT) as the major determinant of fungal community composition, while the second axis (eigenvalue 0.74934) was correlated with MAP. Consequently, ECM fungal communities clearly clustered according to elevational forest types (Fig. 5). Standardized semivariance for DCA axis 1 was below the lower bound of the confidence envelope in lag classes 1–3, suggesting spatial clustering at local scale (< 3.2 km), but not at greater distances (Fig. S1). As expected, partial Mantel tests indicated correlation between elevation and spatial distance ($R = 0.620\,24$), probably caused by the fact that high-elevation (> 2500 m asl) vegetation types are only found on Mt Kinabalu. Nevertheless, elevation in itself had a strongly significant effect on community structure ($R = 0.421\,27$, $P = 0.000\,06$) when spatial distance was accounted for (control matrix), while the effect of spatial distance was not significant ($R = 0.000\,01$, $P = 0.400\,84$), when elevation was controlled. This was also confirmed by the strong correlation ($R^2 = 0.9072$, $P < 0.000\,01$) between elevation and the DCA axis

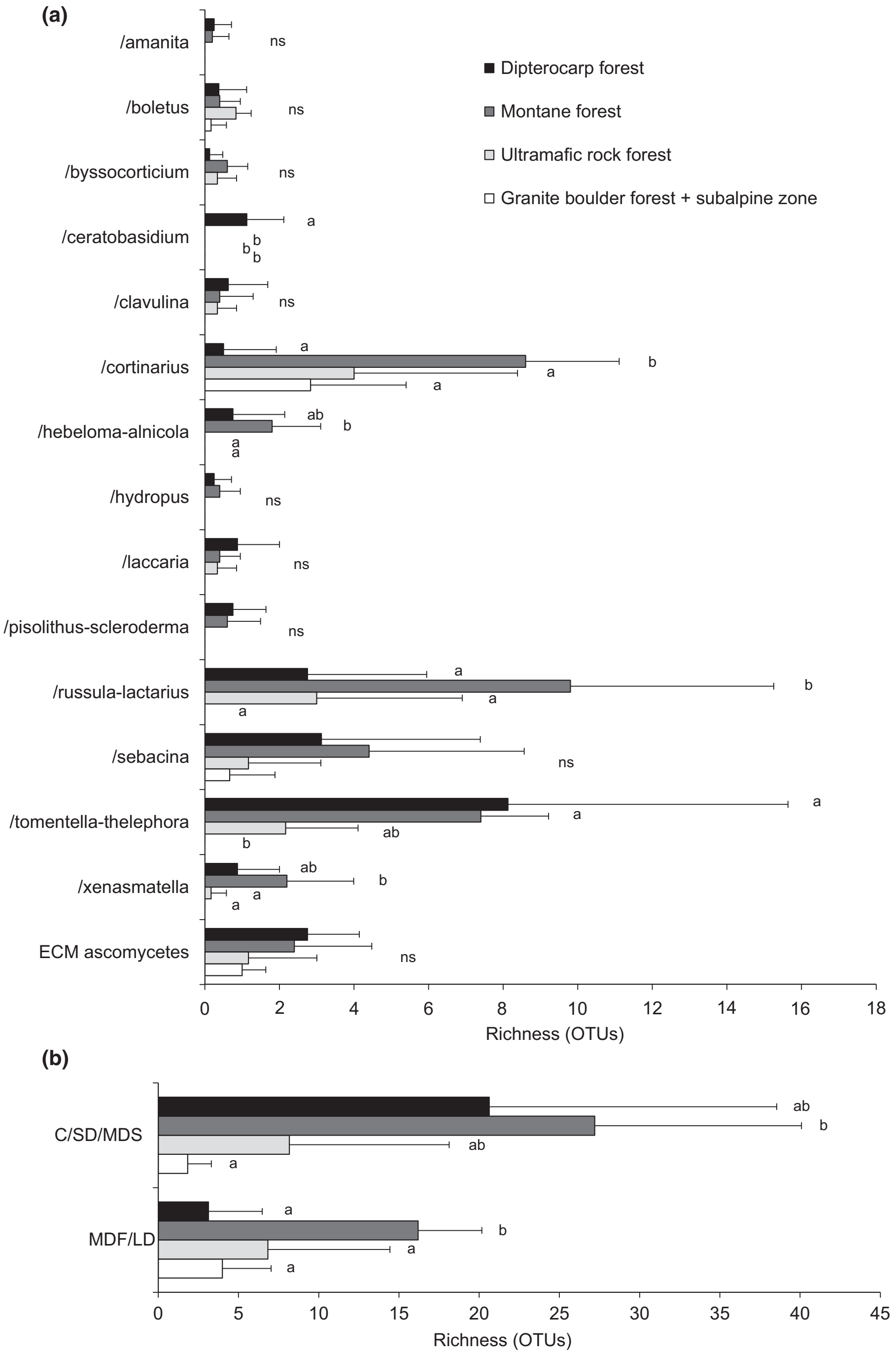

Fig. 3 Operational taxonomic unit (OTU) richness of: (a) ectomycorrhizal fungal phylogenetic lineages *sensu* Tedersoo & Smith (2013) represented by at least three OTUs; and (b) mycelial exploration type categories with SDs. C/SD/MDS, contact/short-distance/medium-distance smooth with hydrophilic hyphae; MDF/LD, medium-distance fringe/long-distance with hydrophobic hyphae. Error bars represent ± SD. Letters above bars represent pairwise comparisons between forest types for each genus. ns, not significant.

1 scores for the sampling sites, where sites representing different mountains aligned primarily by elevation and not by spatial distance (Fig. S2). MRPP confirmed the importance of elevational forest types in shaping fungal community composition (effect size $A = 0.13673$, probability $P < 0.00001$). As expected, MAT was strongly negatively correlated with elevation ($R = -0.9902$), while other environmental variables showed weak correlation with elevation (all $|R| < 0.5$). There were 23 significant ($P < 0.05$) indicator ECM fungal OTUs of a certain forest type, 16 of which represented taxa in the montane forests (Table 3).

Biogeographic affinities

Analysing the geographic data obtained from species hypotheses matching the ECM fungal OTUs in our dataset revealed clear differences among the altitudinal forest types. In the dipterocarp forests, matching species hypotheses represented taxa mainly from tropical Asia (42%) and temperate Asia (27%), with smaller influence from Australasia (9%), Europe (8%) and the other regions. In forest types inhabiting successively higher elevation, the influence of tropical Asian taxa declined markedly, being

Table 2 Beta diversity measures of ectomycorrhizal fungal communities within and between elevational forest types, that is, dipterocarp forest (DF), montane forest (MF), ultramafic rock forest (URF), and granite boulder forest and subalpine zone (GBF + SZ), calculated by pairwise comparisons of communities in the corresponding sampling sites

Forest types	Sørensen	Bray–Curtis	Chao–Sørensen
DF	0.0727 ± 0.0845 a	0.0294 ± 0.1128	0.0571 ± 0.1543
MF	0.2852 ± 0.1337 b	0.0882 ± 0.1626	0.1658 ± 0.2736
URF	0.1379 ± 0.1833 ab	0.0189 ± 0.0316	0.0989 ± 0.2429
GBF + SZ	0.2212 ± 0.2511 ab	0.0548 ± 0.0761	0.2269 ± 0.3418
DF vs MF	0.0134 ± 0.0300 AB	0.0013 ± 0.0029 A	0.0173 ± 0.0916
DF vs URF	0 ± 0 A	0 ± 0 B	0 ± 0
DF vs GBF + SZ	0 ± 0 A	0 ± 0 B	0 ± 0
MF vs URF	0.0422 ± 0.0488 BC	0.0055 ± 0.0072 C	0.0156 ± 0.0281
MF vs GBF	0.0065 ± 0.0186 A	0.0018 ± 0.0054 D	0.0024 ± 0.0074
URF vs GBF	0.0526 ± 0.0798 C	0.0057 ± 0.0131 E	0.0116 ± 0.0242

Numbers represent the mean of vegetation type replicates ± SD. The Sørensen and Bray–Curtis similarity indices are calculated from presence–absence and abundance data, respectively, while the Chao–Sørensen is an abundance-based probabilistic index (Chao *et al.*, 2005). Different letters beside the numbers represent significant differences ($P \leq 0.05$) within (lowercase) and between (uppercase) forest types in pairwise comparisons.

23% in the montane forest, 14% in ultramafic forests, and 0% in the granite boulder forest and subalpine zone. By contrast, Australasian elements increased in prominence in mid- and high-elevation forest types: 23% in montane forests, 31% in ultramafic forests, and reaching 50% in granite boulder forests and the subalpine zone. On the other hand, the proportion of temperate Asian taxa remained comparable throughout the gradient: 28% in montane forests, 26% in ultramafic forests, and 19% in the granite boulder forest and subalpine zone. Holarctic taxa with wide distributions in Europe and North America had highest representation in mid-elevation montane and ultramafic forests (Fig. 6).

Tree community analyses

Generic richness of ECM host trees was highest in low-elevation dipterocarp forests, where four to five genera, belonging to Dipterocarpaceae, Fagaceae, and Myrtaceae, were found in each 1 ha plot. Host richness showed strong negative correlation with elevation ($R^2 = 0.772$; $P < 0.000\,01$), resulting in only one ECM host tree genus above 3000 m asl (Fig. S3). On the other hand, relative basal area of ECM host trees was comparable throughout the gradient, showing nonsignificant decline with increasing elevation ($R^2 = 0.027$; $P = 0.2329$) (Fig. S3).

Discussion

Altitudinal patterns and possible underlying factors

The deep sequence data presented here clearly show that ECM fungal community composition in northern Borneo is strongly structured according to altitudinal forest types. We found that the majority of ECM fungal OTUs are restricted to certain vegetation types, including several indicators for the different elevation zones. In addition, total ECM fungal richness peaks at lower-mid- and mid-elevations (*c.* 1000–2000 m asl). This richness pattern is similar to those observed in the Neotropics (Gómez-Hernández *et al.*, 2012; Geml *et al.*, 2014) and it differs from the pattern of monotonal decrease with increasing elevation in temperate mountains (Bahram *et al.*, 2012; Nouhra *et al.*, 2012; but see Miyamoto *et al.*, 2014).

On a global scale, ECM fungal richness are primarily influenced by the relative proportion and richness of host plants, soil pH, MAT, and MAP (Tedersoo *et al.*, 2012, 2014). In addition, habitat area is often positively correlated with species richness in many taxonomic groups (Gotelli, 1998), including ECM fungi (Peay *et al.*, 2007). Therefore, it is possible that, in addition to other factors discussed later, the smaller habitat area may contribute to the relatively low richness of ECM fungi at higher elevations. However, the species–area theory does not seem to offer a satisfying explanation for the mid-elevation peak in richness for most ECM lineages in Borneo. In terms of soil pH, ECM fungi are known to prefer slightly acidic to neutral pH and there is little difference among the sampled forest types in this regard, except for the somewhat higher pH values in ultramafic sites.

Richness and density of host plants have been observed to correlate positively with ECM fungal richness on both global and more regional scales (Tedersoo *et al.*, 2014). The majority of ECM fungi have broad host range and associate with hosts representing a wide range of taxonomic groups, while several others are more specific to plant families or even genera (Molina *et al.*, 1992). The importance of hosts on ECM fungal richness tends to vary by taxonomic groups of hosts and by geographic regions and scales (Tedersoo *et al.*, 2011; Murata *et al.*, 2013). Furthermore, the host effect is generally less easy to detect when the distribution of ECM hosts is determined by environmental conditions and/or is geographically limited (Miyamoto *et al.*, 2014). Our results suggest that neither richness nor relative basal area of ECM hosts can explain the mid-elevation bulge in ECM fungal richness on Mt Kinabalu. Host tree generic richness shows a strong decline with increasing elevation, while relative basal area remains comparable throughout the gradient (Fig. S2). Somewhat surprisingly, despite the substantial differences in richness of ECM hosts and other plants (Aiba & Kitayama, 1999; Grytnes *et al.*, 2008), the sampled low-elevation dipterocarp forest and high-elevation ultramafic cloud forest sites apparently harbour comparably rich ECM communities. Furthermore, in several lineages (e.g. /cortinarius, /russula-lactarius), richness appeared to

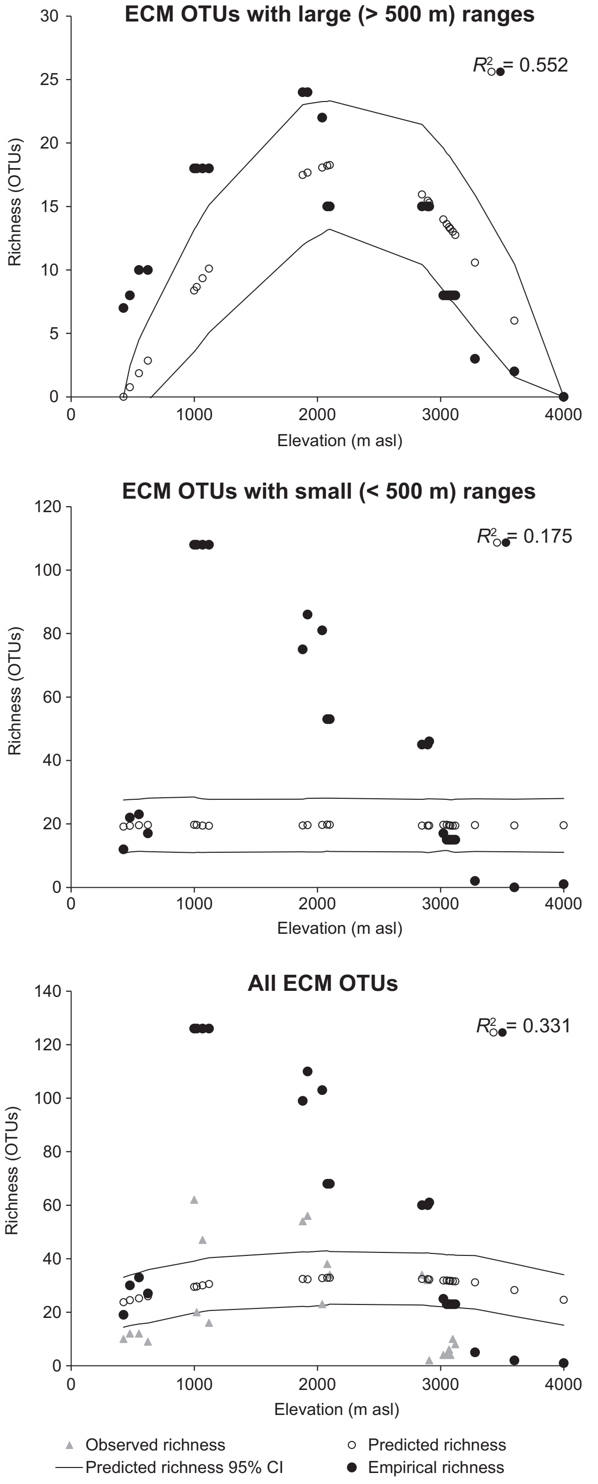

Fig. 4 Observed, empirical and predicted mean operational taxonomic unit (OTU) richness as a function of elevation for ectomycorrhizal (ECM) fungi. The bottom graph displays results for all OTUs, whereas the middle and the uppermost graphs show results for OTUs with empirical elevation ranges smaller and greater than 500 m, respectively. Grey triangles and closed circles indicate observed and interpolated empirical richness, respectively, for each field sampling elevation. Open circles show mean richness with 95% confidence intervals (CIs; fine lines) predicted by the mid-domain effect randomization model. The coefficient of determination for the regression of empirical on expected richness appears on each graph. m asl, m above sea level.

be somewhat higher in ultramafic forests than in dipterocarp forests, although the differences were not statistically significant as a result of the substantial variation among the replicates. The availability of colonizable roots may counterbalance the low host richness in ultramafic forests, as *Leptospermum recurvum* can be dominant with up to 87% relative basal area in ultramafic forests *c.* 3000 m asl (Aiba & Kitayama, 1999).

The most notable exception to the general pattern of mid-elevation peak is the /tomentella-thelephora lineage which had very high OTU richness in the dipterocarp forests and exhibited a monotonal decrease in richness with increasing altitude. The low richness of this lineage at high-elevation sites is surprising, because *Tomentella* is the most diverse ECM-forming genus in arctic and alpine habitats (Bjorbækmo *et al.*, 2010; Geml *et al.*, 2015; Morgado *et al.*, 2016). Because available host data from matching species hypotheses included mostly Fagaceae, Pinaceae, and Dipterocarpaceae, and only one with Myrtaceae (Table S2), it is possible that the gradual disappearance of Dipterocarpaceae and Fagaceae contributes to the decreasing richness of /tomentella-thelephora with increasing elevation. The /ceratobasidium lineage showed similar decrease in richness, although the number of OTUs was much lower. Similar trends were seen in /clavulina, /laccaria and /pisolithus-scleroderma, and in ECM ascomycetes, although the differences were not statistically significant, partly as a result of the low number of OTUs and the substantial spatial variation.

Patterns of a mid-elevation peak in richness have also been observed in many other groups of organisms on Mt Kinabalu, for example, in orchids (Wood *et al.*, 1993), ferns (Parris *et al.*, 1992), gymnosperms (Beaman & Beaman, 1993), small mammals (Nor, 2001) and snails (Liew *et al.*, 2010). This suggests that the underlying factors may be more general across many organismal groups instead of being specific to ECM fungi. These include environmental factors as well as the geometric effects (e.g. MDE) of overlapping ranges of species with relatively broad elevation range. It is well known that environmental factors change along elevation gradients and that species found at different elevations are differentially adapted to these varying conditions. However, differentiating between the effects of environmental factors and geometric constraints is important to better understand their contributions to present distributional patterns. Species richness patterns in various vascular plant groups have been shown to exhibit varying degrees of MDE effect on Mt Kinabalu (Grytnes *et al.*, 2008) and on Neotropical mountains (Cardelús *et al.*, 2006), although MDE alone cannot fully explain the observed patterns. The results presented here suggest that MDE probably has a relatively small effect on ECM fungal richness on Mt Kinabalu. Namely, in addition to the significant deviations of empirical from predicted richness under the MDE model, ECM fungal richness peaks at low- to mid-elevations rather than at the symmetrical midpoint as predicted by the MDE. This finding is markedly different from patterns found in ECM fungal communities on Mt Fuji in Japan, where MDE was found to explain most of the richness patterns (Miyamoto *et al.*, 2014). The elevation gradient sampled in our study (3575 m) is more than three times larger than

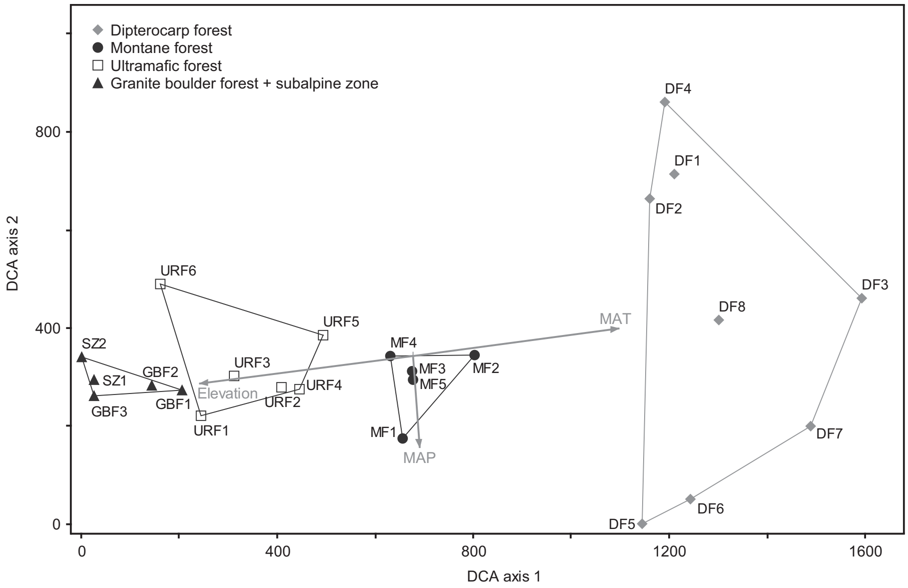

Fig. 5 Detrended correspondence analysis (DCA) ordination plot for ectomycorrhizal fungal communities from various forest types. Labels, localities and descriptions of the sampling sites are given in Table 1. Vectors of environmental variables correlated with ordination axes are displayed. MAT, mean annual temperature; MAP, mean annual precipitation.

Table 3 Ectomycorrhizal fungal operational taxonomic units (OTUs) considered as significant indicators of the altitudinal forest types with corresponding *P*-values, and with accession numbers, sequence similarity, pairwise alignment length, name, phylogenetic lineage, species hypothesis numbers (SH), and taxonomic classification of the most similar sequence in the UNITE + INSD database

OTU	Forest type	*P*	Accession	Percentage (%)	bp	Name	Phylogenetic lineage	SH	Order
OTU 7623	DF	0.009	GQ240903	91.4	314	*Tomentella* sp.	/tomentella-thelephora	SH007323.07FU	Thelephorales
OTU 4484	DF	0.047	UDB013022	95.4	259	Sordariales sp.	/sordariales1	SH031934.07FU	Sordariales
OTU 1909	MF	0.009	FJ039619	95.2	274	*Cortinarius scaurus*	/cortinarius	SH222368.07FU	Agaricales
OTU 4332	MF	0.0014	AY669583	92.5	173	*Cortinarius malicorius*	/cortinarius	SH222518.07FU	Agaricales
OTU 2174	MF	0.011	JQ991694	91.8	306	*Cortinarius* sp.	/cortinarius	SH494172.07FU	Agaricales
OTU 3608	MF	0.0014	JX316452	91.4	303	*Cortinarius* sp.	/cortinarius	SH222701.07FU	Agaricales
OTU 1976	MF	0.0014	GU591646	97.4	311	*Hebeloma aminophilum*	/hebeloma-alnicola	SH218848.07FU	Agaricales
OTU 2816	MF	0.0094	JQ991775	91.7	241	*Ramaria* sp.	/ramaria-gautieria	SH479120.07FU	Gomphales
OTU 5568	MF	0.0094	KF225491	92.1	239	*Russula chiui*	/russula-lactarius	SH210581.07FU	Russulales
OTU 5594	MF	0.0096	DQ384580	95.8	306	*Russula crassotunicata*	/russula-lactarius	SH189293.07FU	Russulales
OTU 3032	MF	0.0002	UDB013176	96.9	159	*Russula* sp.	/russula-lactarius	SH218439.07FU	Russulales
OTU 1187	MF	0.0002	AB807952	90.3	269	Russulaceae sp.	/russula-lactarius	SH001515.07FU	Russulales
OTU 6849	MF	0.0002	GQ268673	91.5	293	Thelephoraceae sp.	/tomentella-thelephora	SH010150.07FU	Thelephorales
OTU 434	MF	0.0262	GQ268673	92.4	275	Thelephoraceae sp.	/tomentella-thelephora	SH010150.07FU	Thelephorales
OTU 4077	MF	0.0094	KC952675	95.3	316	*Tomentella* sp.	/tomentella-thelephora	SH177859.07FU	Thelephorales
OTU 2169	MF	0.0014	UDB013069	98.8	324	*Tomentella* sp.	/tomentella-thelephora	SH010044.07FU	Thelephorales
OTU 1404	MF	0.0026	EF655700	90.3	207	*Tomentella* sp.	/tomentella-thelephora	SH493506.07FU	Thelephorales
OTU 440	MF	0.0096	GQ221633	90.9	320	*Tomentella* sp.	/tomentella-thelephora	SH177816.07FU	Thelephorales
OTU 1764	URF	0.0054	UDB013183	94.4	251	Boletales sp.	/boletus	SH016689.07FU	Boletales
OTU 3474	URF	0.0186	UDB000824	97.2	355	*Lactarius hysginus*	/russula-lactarius	SH176435.07FU	Russulales
OTU 1674	GBF+SZ	0.0054	KP087990	98.3	297	*Cortinarius croceus*	/cortinarius	SH135194.07FU	Agaricales
OTU 395	GBF+SZ	0.0124	AY669651	94.8	193	*Cortinarius* sp.	/cortinarius	SH222441.07FU	Agaricales
OTU 539	GBF+SZ	0.0042	JX679100	97.3	258	*Cortinarius olivaceopictus*	/cortinarius	SH222615.07FU	Agaricales

DF, dipterocarp forest; MF, montane forest; URF, ultramafic rock forest; GBF + SZ, granite boulder forest and subalpine zone.

that sampled by Miyamoto *et al.* (2014), which implies greater variation in environmental conditions and vegetation. Furthermore, temperate species often have greater elevation ranges than tropical species (Janzen, 1967; Ghalambor *et al.*, 2006). In agreement with Janzen (1967) and the Rapoport's rule (Stevens, 1992), our data suggest that most tropical ECM fungi have small elevation ranges in the mountains of northern Borneo, while Miyamoto *et al.* (2014) found that the vast majority of temperate ECM species on Mt Fuji had continuous distribution over most of the elevation gradient, similar to their hosts.

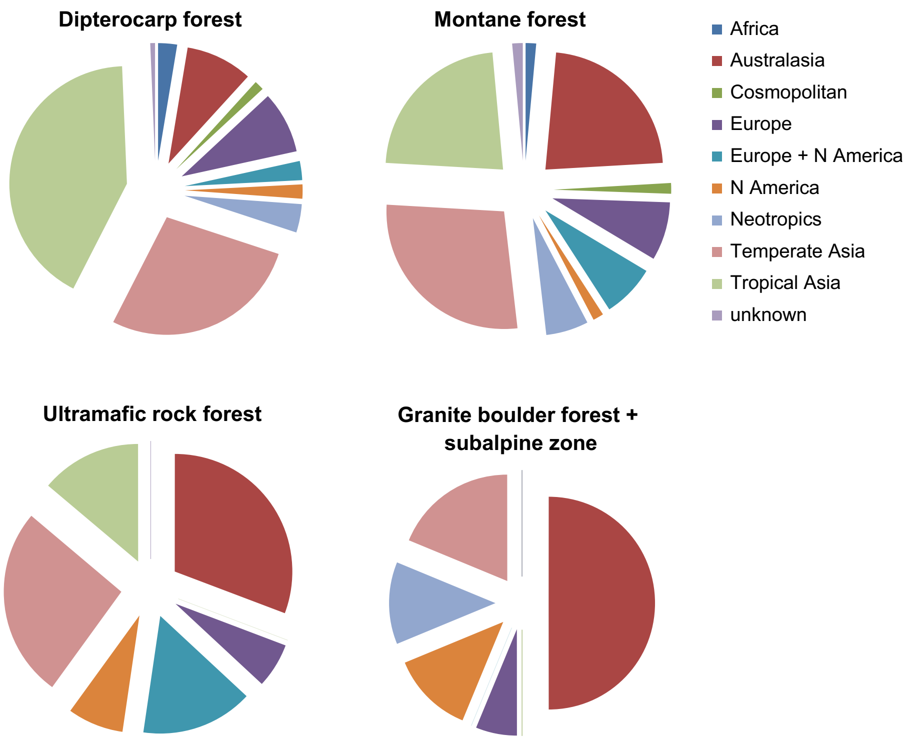

Fig. 6 Biogeographic affinities of ectomycorrhizal fungi inhabiting the altitudinal forest types in Borneo, based on the relative representation of geographic areas among the reference sequences matching the ectomycorrhizal fungal operational taxonomic units (OTUs) generated in this study. Geographic distribution data were obtained from the UNITE + INSD database for the corresponding species hypotheses.

In most organismal groups, the environmental variables most commonly related to species richness are generally measures of energy (temperature) and water availability (Rahbek, 2005; Grytnes & Beaman, 2006). Specifically, on a global scale, richness of ECM fungi has repeatedly been shown to peak at intermediate annual temperatures, which correspond to mid-latitudes (Tedersoo *et al.*, 2014), and the mid-elevation richness peak of ECM fungi reported here is somewhat similar to this pattern. Furthermore, as a result of decreasing temperature with increasing elevation, tropical mountain environments are characterized by mid-elevation condensation zones with high relative humidity (Whitmore, 1984; Rahbek, 1995), which may affect ECM fungal richness directly or indirectly. Even though precipitation varies less among the elevation zones in Kinabalu than in most other tropical mountain regions, mid-elevation montane forests have been shown to have the highest relative humidity (Kitayama, 1992; Nor, 2001; Kitayama & Aiba, 2002) as well as the highest soil moisture (Kitayama *et al.*, 1998). The data presented here suggest that ECM fungal richness is highest in the elevation zone with the most available water.

Adaptation of ECM fungi to elevation and forest types

The differences in community composition among the forest types are also apparent in the observed patterns of extramatrical mycelial ETs. In temperate forests, ETs have been shown to correlate with functional roles of ECM fungi, such as foraging for and translocation of soil nutrients and water (Anderson & Cairney, 2007). Consequently, elevation patterns of ET categories may reflect functional differences in ECM fungi among the forest types. Taxa with medium MDF/LD ETs with hydrophobic hyphae are better adapted to foraging the soil for recalcitrant N (Lilleskov *et al.*, 2002; Bödeker *et al.*, 2014). On the other hand, species with C/SD/MDS ETs with hydrophilic hyphae are more suited to environments with labile N, because most of them showed reduced proteolytic capabilities in laboratory experiments (Lilleskov *et al.*, 2002).

The indicator species analyses suggest that mid-elevation ECM fungal communities in montane forests of Borneo are particularly rich in taxa with high specificity and fidelity to their habitat, as also suggested by the highest measured values in community similarity indices within forest types. Similarly, a large proportion of ECM fungi are known to be restricted to montane cloud forests in the Neotropics (Mueller *et al.*, 2006; Wicaksono *et al.*, 2017). Our work provides the first evidence for such altitudinal patterns in the Palaeotropics, indicating a high degree of specialization to the tropical montane forest habitat in many ECM fungi. In the montane zone, the higher precipitation and lower temperatures result in greater accumulation of litter and, even though the total N and organic C contents in the montane forest soils on Mt Kinabalu are greater than in the lowland and in the high-altitude zones, a considerable fraction of N may not be available for plants, as indicated by the higher C : N ratios (Table S3; Kitayama, 1992). Therefore, ECM fungi with MDF/LD ETs may be particularly well suited to the montane forest habitat, as they are capable of breaking down complex organic compounds and allocate otherwise unavailable N to their host trees (Smith & Read, 2008). In addition, the predominantly hydrophobic hyphae of the MDF/LD ETs can prevent leakage of nutrients during transport in the often water-saturated montane forest zone. By contrast, the marked dominance of ECM fungi with C/SD/MDS ETs at low elevations may be explained in part by the faster turnover of decomposing organic matter that is likely to result in greater labile N pools (Rastin *et al.*, 1990; Osono, 2007; Liu *et al.*, 2013).

Even though dipterocarp forests are markedly different in composition from other forest types, the number of indicator species appears very low. There is probably a greater variability in local climatic and edaphic factors and stochastic or historical differences in plant species composition in dipterocarp forests than in other sampled elevation zones. Many ECM fungi may prefer specific habitats within dipterocarp forests which may result in low fidelity measures for the elevation zone as a whole. For example, Peay *et al.* (2010) found substantial compositional differences in ECM fungal communities corresponding to ecotones in dipterocarp forests of Sarawak. The low number of indicator species in the serpentine soils of the ultramafic forests is in agreement with Branco (2010) in that serpentine soils in general do not represent a strong physiological barrier for ECM fungi and that ECM fungal communities found in serpentine soils are not depauperate or specialized. The genus *Cortinarius* was by far the most OTU-rich ECM genus in the high-elevation zones above 3000 m asl, particularly in the granite boulder forest and the subalpine zone, which is in agreement with their high diversity in cold-dominated ecosystems (Geml *et al.*, 2012; Timling *et al.*, 2012; Morgado *et al.*, 2015).

Biogeographic affinities

Overall, the observed pattern of biogeographic affinities of ECM fungi is similar to that observed in vascular plants, where Indo-Malaysian, East Asiatic and Austral-Antarctic floristic elements characterize low, mid- and high elevations, respectively (Hotta, 1974; Kitayama, 1992). Dipterocarp forests harbour the largest proportion of ECM fungi characteristic of tropical Asia. Many ECM fungi in the montane forest zone in Borneo are closely related to, and are often even conspecific with, species that occur in mountains of New Guinea and subtropical and temperate forests of Australia, New Zealand, temperate Asia, and other parts of the Holarctic (Fig. 6; Table S2). Similarly, recent molecular data suggest that many plant mountain endemics in Borneo are representatives of immigrant lineages preadapted to cool and moist conditions, particularly in lineages with effective dispersal strategies, such as bryophytes and ferns (Merckx *et al.*, 2015). Finally, at high elevations, the high proportion of Australasian elements may be explained by the dominance of myrtaceous hosts.

Conclusions

This is the first study investigating community structure and richness of ECM fungi along elevation gradients from lowland to subalpine forests in the Paleotropics. Even though the true richness of ECM fungi in Borneo almost certainly exceeds our estimates based on a limited number of samples, the data presented here clearly indicate strong correlations of richness and community structure with elevation in northern Borneo. Climate appears to be important in shaping the distribution of ECM fungi along altitudinal gradients in a variety of ways, for example, by affecting microbial processes (e.g. decomposition) and edaphic factors, and by altering species interaction dynamics among fungi and other members of the soil biota. The habitat specificity exhibited by many fungi offers possibilities for monitoring and habitat characterization and we strongly advocate incorporating fungi in biodiversity assessments and conservation efforts.

Acknowledgements

Financial support was provided by the Crocker Range – Kinabalu Expedition sponsored by Naturalis and Sabah Parks, the Naturalis personal research budget of J.G., the Alberta Mennega Stichting travel grant to L.N.M. (fieldwork), and a Naturalis Research Initiative grant to J.G. (molecular work). The authors thank Sabah Parks for providing permits for the scientific work, Marcel Eurlings and Elza Duijm (Naturalis) for conducting the Ion Torrent sequencing, Eszter Draskovits (Institute for Soil Sciences and Agricultural Chemistry, Budapest, Hungary) for the chemical analyses, Hans ter Steege (Naturalis) for his guidance in the multivariate analyses, and Bas Blankevoort (Naturalis) for preparing the map for Fig. 1. The authors declare no conflict of interest.

Author contributions

J.G. and L.N.M. planned and designed the research. J.G., L.N.M. and M.S. conducted the fieldwork. T.A.S-N. performed the laboratory work. J.G. and L.N.M. analysed the data. All authors wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Standardized semivariograms for detrended correspondence analysis (DCA) ordination axis 1 for ectomycorrhizal fungal communities.

Fig. S2 Detrended correspondence analysis (DCA) axis 1 score as function of elevation with correlation coefficient and *P*-value inferred using linear regression.

Fig. S3 Generic richness and relative basal area of ectomycorrhizal host trees on Mt Kinabalu as functions of elevation.

Table S1 The list of environmental factors included in regression analyses

Table S2 The distribution matrix of all ECM basidiomycete OTUs among the sampled sites

Table S3 Results of quadratic regression analyses between environmental variables and ectomycorrhizal fungal richness

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