

Comparative sampling of Neotropical and Paleotropical elevation gradients reveals the role of climate in shaping the functional and taxonomic composition of soil-borne fungal communities in tropical forests

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Abstract

Because of their steep gradients in abiotic and biotic factors, mountains offer an ideal setting to enhance our understanding of mechanisms that underlie species distributions and community assemblies. We compared the structure of taxonomically and functionally diverse soil fungal communities in soils along elevational gradients in the Neo- and Paleotropics (northern Argentina, Central America, and Borneo). We found that soil fungal community composition reflects environmental factors at both regional and pantropical scales, particularly temperature and soil pH. Elevational turnover is driven by contrasting environmental preferences among functional groups and replacement of species within functional guilds. In addition, we found that habitat preference can already be observed at the level of taxonomic orders, often irrespective of functional guild, which suggests shared physiological constraints and environmental optimum for relatively closely related taxa. Strong biogeographic structure likely reflects dispersal limitation and resulting differences in local species pools of fungi, as well as their hosts or substrates. Although the number of species shared among regions is low, remarkable similarity of functional profiles across regions suggests functional niche proportions may be driven by similar mechanisms across moist tropical forests, resulting in relatively predictable proportions of functional guilds. The pronounced compositional and functional turnover along elevation gradients driven mainly by temperature and correlated environmental factors implies that tropical montane forest fungi will likely be sensitive to climate change, resulting in variation in composition and functionality over time.

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Running title: Tropical montane fungi

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Abstract

Because of their steep gradients in abiotic and biotic factors, mountains offer an ideal setting to enhance our understanding of mechanisms that underlie species distributions and community assemblies. We compared the structure of taxonomically and functionally diverse soil fungal communities in soils along elevational gradients in the Neo- and Paleotropics (northern Argentina, Central America, and Borneo). We found that soil fungal community composition reflects environmental factors at both regional and pantropical scales, particularly temperature and soil pH. Elevational turnover is driven by contrasting environmental preferences among functional groups and replacement of species within functional guilds. In addition, we found that habitat preference can already be observed at the level of taxonomic orders, often irrespective of functional guild, which suggests shared physiological constraints and environmental optimum for relatively closely related taxa. Strong biogeographic structure likely reflects dispersal limitation and resulting differences in local species pools of fungi, as well as their hosts or substrates. Although the number of species shared among regions is low, remarkable similarity of functional profiles across regions suggests functional niche proportions may be driven by similar mechanisms across moist tropical forests, resulting in relatively predictable proportions of functional guilds. The pronounced compositional and functional turnover along elevation gradients driven mainly by temperature and correlated environmental factors implies that tropical montane forest fungi will likely be sensitive to climate change, resulting in variation in composition and functionality over time.

Key Words: Argentina, Borneo, ITS, DNA metabarcoding, Panama, soil fungi

INTRODUCTION

Montane ecosystems generally are recognized as biodiversity hotspots as well as areas of high endemism (Lomolino 2001). Despite representing about one-eighth of the world's land area outside Antarctica, mountains harbor about one-third of all terrestrial species (Spehn *et al.* 2012; Antonelli 2015). Since the early scientific studies of Darwin, Wallace and von Humboldt on mountain biota, documentation of changes in species richness and community composition have been central to ecological and biogeographic studies (Lomolino 2001; McCain and Grytnes 2010). Mountains provide unique opportunities to test various ecological hypotheses,

such as those relevant to climate change, as they are characterized by gradients of abiotic factors such as temperature and available moisture (Guo *et al.* 2013). However, in most organismal groups we still lack answers to fundamental questions regarding diversity, distributional patterns, and community composition in montane systems (Lomolino 2001; Guo *et al.* 2013; Perrigo *et al.* 2020).

Numerous abiotic factors that shape biological communities change more or less predictably with increasing elevation. Among these, temperature is the most predictable, with an average decrease of ca. 0.6 °C per 100 m increase in elevation (Barry 2008). Despite the crucial importance of water for living organisms, changes in precipitation along elevation gradients are much less predictable in general due to complex relationships of regional climate and topography (Barry 2008). In mid- and high latitudes, precipitation tends to increase with elevation, whereas tropical mountains typically show little variation in rainfall along an elevation gradient or exhibit a moderate mid-elevation peak (McCain and Grytnes 2010). Related environmental factors interplay with temperature and precipitation to determine biological productivity, including solar radiation, cloud cover, soil type and nutrient content, as well as habitat surface area due to geometric constraints (Stevens 1992; Rosenzweig 1995). For example, cloud forests, perhaps the most characteristic vegetation of tropical montane habitats, are characterized by largely persistent clouds at mid- to high elevations. Organisms occupy different habitats along elevation gradients according to their physiological requirements for abiotic factors and their interaction dynamics with other species. Resulting changes in community structure with increasing elevation have been a focal point for ecological and evolutionary research and have contributed to the understanding of spatial patterns of biodiversity and their underlying mechanisms.

On a global scale, most studies of species richness along elevation gradients have focused on vascular plants and animals (e.g., Parris *et al.* 1992; Wood *et al.* 1993; Nor 2001; Cardelús *et al.* 2006; Ghalambor *et al.* 2006; Grau *et al.* 2007; Grytnes *et al.* 2008; Liew *et al.* 2010). Fungi represent one of the largest groups of living organisms with key roles in the functioning of ecosystems, and their importance is increasingly recognized with respect to food safety and human health. Several studies have been conducted in mountains in temperate regions on the distribution of specific fungal functional groups: phyllosphere fungi (Cordier *et al.* 2012; Coince *et al.* 2014), bryophyte-associated fungi (Davey *et al.* 2013), wood-inhabiting fungi (Meier *et al.* 2010), arbuscular mycorrhizal (AM) fungi (Gai *et al.* 2012), and ectomycorrhizal (ECM) and other root-associated fungi (Bahram *et al.* 2012; Nouhra *et al.* 2012; Coince *et al.* 2014; Miyamoto *et al.* 2014; Javis *et al.* 2015; Rincón *et al.* 2015; Bowman and Arnold 2018; Schön *et al.* 2018). However, species richness and composition of fungal communities in tropical mountains remain virtually unknown. This gap in our knowledge seems particularly concerning because fungi have been reported as major drivers of the diversity and composition of plant communities in tropical forests (Bagchi *et al.* 2014) and because fungi, through their interactions with plants, contribute to ecosystem services such as the provision of clean water, food and air (Bakker *et al.* 2019). The few studies that have examined fungi in tropical mountains were reviewed recently by Geml (2017). These include morphological studies of macrofungi in eastern Mexico (Gómez-Hernández *et al.* 2012) and freshwater ascomycetes in the Peruvian Andes (Shearer *et al.* 2015), environmental DNA studies of total fungal communities (Geml *et al.* 2014) and AM fungi (Geml 2017) in the Tucuman-Bolivian Yungas, and AM fungi (Merckx *et al.* 2015) and ECM fungi (Geml *et al.* 2017) in Malaysian Borneo. Consequently, alpha and beta diversity of a wide range of fungal groups in tropical mountains remain mostly unexplored.

In this study, we compared community composition and richness of diverse functional groups of fungi in forest soils along elevation gradients in three tropical mountain areas: Mt. Kinabalu and Crocker Range in Sabah, Malaysian Borneo; the rainforests of western Panama; and the Andean Yungas in northwestern Argentina. The aims of this work were to 1) characterize soil fungal communities in major elevational forest types; 2) compare elevation patterns of richness in taxonomic and functional groups of fungi among Neotropical and Paleotropical mountains; 3) evaluate the possible influence of climatic and edaphic factors on fungal community composition in tropical mountains; and 4) provide the first characterization of Pantropical core mycobiomes in soils of lowland and montane forests.

We hypothesized that elevation gradients would structure fungal communities through changes in temperature and precipitation, reflecting effects on soil edaphic variables and plant community composition. Fungal

richness and distribution on a global scale is strongly influenced by mean annual temperature (MAT), mean annual precipitation (MAP), soil pH, and, in the case of ECM fungi, by diversity and abundance of host plants (Tedersoo *et al.* 2014; Větrovský *et al.* 2019). Therefore, we expected MAT, MAP, and soil pH to be the strongest drivers of fungal community structure along the sampled elevation gradients irrespective of geographic region (Hypothesis 1). We also hypothesized that elevational patterns of community structure would differ among functional groups, reflecting different environmental optima corresponding to distinct life strategies and resulting in differences in inferred functionality among elevation zones (Hypothesis 2). For example, we expected fungi not associated with plants to be less affected by vegetation type than those intimately associated with plants, such as ECM, plant pathogens, root-associated fungi, and to some extent wood decomposers, resulting in greater differences in richness and community composition for plant-associated fungi among elevational forest types (Hypothesis 2a). In addition, we expected higher species richness of plant pathogens, wood decomposers and generalist saprotrophs at lower elevations due to higher host- and substrate richness at low to mid-elevations (Rahbek 2005, McCain and Grytnes 2010, Nouhra *et al.* 2018) and more energy available for decomposition (Hypothesis 2b). Finally, we expected fungal species pools to be different among the three biogeographic regions due to distinct biogeographic histories, with substantially more overlap between the two Neotropical regions due to land connection and consequent similarities in flora and fauna (Hypothesis 3).

Materials and Methods

We analyzed data from three datasets. Two of these are novel: Borneo and Panama, except for a small subset of the data representing ECM fungi from Borneo published by Geml *et al.* (2017). These were combined with the Yungas dataset from northwestern Argentina originally published by Geml *et al.* (2014), which is re-analyzed here.

In Argentina, the Yungas comprise tropical and subtropical humid montane forests developed on the eastern slopes of the Andes as a result of orographic rains. The flora and fauna of the Yungas have been relatively well studied and are very diverse and rich in endemics (e.g., Ojeda and Mares 1989; Lavilla and Manzano 1995; Blake and Rougés 1997; Brown *et al.* 2001). Together with adjacent, seasonally dry piedmont forests, the Yungas constitute the southern limit of the Amazonian biogeographic domain (Cabrera 1976; Prado 2000). The forests in this region are classified into three major elevational types: piedmont forest (400–700 masl), montane forest (700–1500 masl), and montane cloud forest (1500–3000 masl) (Brown *et al.* 2001; Brown *et al.* 2005), here considered as lowland, low montane and upper montane forests, respectively, for comparative purposes.

In Borneo, the Crocker Range is the highest mountain range in Sabah (Malaysia) with an average height of ca. 1800 masl. With an elevation of 4095 masl, Mt. Kinabalu is the tallest mountain between the Himalayas and New Guinea. Mount Kinabalu has one of the most species-rich biotas of the world, and includes more than 5000 vascular plant species (Beaman and Anderson 2004). The vegetation of Mt. Kinabalu can be divided into four discrete zones: lowland dipterocarp forests (< 1200 masl), montane forests (1200–2700 masl), ultramafic rock forests (2700–3000 masl), and granite boulder forests of the summit zone (3000–4095 masl) (Beaman and Beaman 1990; Kitayama 1992). Of these, only dipterocarp forests and montane forests occur in other parts of the Crocker Range. For this paper, ultramafic rock forest samples were excluded because the vegetation is not typical for the elevation zone: it is made up of a relatively small number of plant species capable of surviving serpentine soils that are poor in nutrients and rich in heavy metals. For comparisons with the other regions, the dipterocarp, montane, and granite boulder forests were labelled as lowland, lower montane and upper montane forests, respectively.

Panama and neighboring countries in Central and South America represent a biodiversity hotspot that is one of the richest in the world. With respect to trees alone, at least 2300 species are known to occur in Panama (Condit *et al.* 2011). The Caribbean side of the isthmus and the central mountains receive more than 3000 mm precipitation per year. The Pacific side tends to be somewhat drier, but there is greater regional variation in rainfall, resulting in a mosaic of wet (>3000 mm per year), moist (1500–3000 mm per year), and dry (<1500 mm per year) forests. The major forest types sampled in this study were classified

as lowland wet and moist forests (0–800 masl), lower montane forest (800–1500 masl), and upper montane forests (1500–3000 masl) (Condit *et al.* 2011).

Sampling and molecular work

Soil samples were collected in September, 2012, in Kinabalu and Crocker Range Parks of Sabah, Borneo; in May, 2011 and 2013, in Jujuy, Salta and Tucumán provinces of Argentina; and in June, 2016, in Bocas del Toro, Chiriquí, Colón and Panamá provinces of Panama. The sampling sites represent the entire elevation range of various types of forests in the respective regions. Forest type, elevation, geographic coordinates, and localities are shown in Table S1 and on maps corresponding to the three regions (Figs. S1-3).

In Argentina and Borneo, 40 soil cores, 2 cm in diameter and ca. 20 cm deep, were taken at each sampling site (ca. 10 × 25 m). Cores were collected ca. 2 m from each other to minimize the probability of sampling the same genet repeatedly. In Panama, 10 soil cores of the above dimensions were collected in each site (ca. 4 × 5 m). Soil cores taken at a given site were pooled, resulting in a composite soil sample for each site. With respect to the samples collected in Argentina and Panama, ca. 20 g of each sample was kept frozen until lyophilization ca. 2 weeks later. In Borneo, because of the more remote location, soil samples were dried immediately at 30–35 °C. Because of these differences, we handled the data separately for each region for the analyses that are the focus of this paper. We did use the combined dataset to provide the first characterization of the putative ‘core’ pantropical mycobiome of montane and lowland forests, i.e., fungi shared among the Neotropical and Paleotropical regions and characteristic of the different elevation zones.

Genomic DNA was extracted from 0.5 ml of dry soil from each sample with the NucleoSpin® soil kit (Macherey-Nagel GmbH & Co., Düren, Germany) according to manufacturer’s protocol. For each sample, two independent DNA extractions were carried out and the extracts were pooled. The ITS2 region (ca. 250 bp) of the nuclear ribosomal DNA repeat was amplified via PCR with 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) with an Ion 318™ Chip and an Ion Torrent Personal Genome Machine (Life Technologies, Guilford, CT, U.S.A.). Chemical analyses of soil samples from Argentina and Borneo were carried out as described in Geml *et al.* (2014) and Geml *et al.* (2017), respectively. The samples from Panama were analysed by the Instituto de Investigación Agropecuaria de Panamá (IDIAP). Climate data were obtained from the WorldClim database (www.worldclim.org) based on the geographic coordinates of the sampling sites.

Bioinformatics

Sequences were sorted according to samples and adapters (identification tags) were removed in Galaxy (<https://main.g2.bx.psu.edu/root>). Primer sequences were removed and poor-quality ends were trimmed based on a 0.02 error probability limit in Geneious Pro 5.6.1 (BioMatters, New Zealand). Sequences were filtered with USEARCH v.8.0 (Edgar 2010) with the following settings: all sequences were truncated to 200 bp and sequences with expected error > 1 were discarded. For each sample, identical sequences were collapsed into unique sequence types while preserving their counts. The quality-filtered sequences from all samples were grouped into operational taxonomic units (OTUs) at 97% sequence similarity in USEARCH, and global singletons and putative chimeric sequences were removed. We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE+INSD fungal ITS sequence database (released August 22, 2016), containing identified fungal sequences with assignments to Species Hypothesis groups based on dynamic similarity thresholds (Kõljalg *et al.* 2013). We excluded OTUs with < 80% similarity or < 150 bp pairwise alignment length to a fungal reference sequence. To minimize artifactual OTUs that may have been generated during the molecular work, in subsequent analyses we only included OTUs that occurred in at least two samples. The final dataset contained 20 620 fungal OTUs, representing a total of 3 962 204 high-quality sequences.

We assigned fungal OTUs to functional groups in two steps. First, we assigned putative functional guilds using FunGuild (Nguyen *et al.* 2015). We recognize the limitations of functional inference based on partial ITS sequences, and here use these guilds as hypothetical functional groups. Secondly, for genera that

comprise species from multiple functional guilds (e.g., *Amanita*, *Entoloma*, *Ramaria*, *Sebacina* and related genera in the Sebacinales), we assigned ecological function to each OTU on a case by case basis with available ecological information for the matching Species Hypothesis in the UNITE database. Fungi that can grow as saprotrophs as well as animal or plant pathogens were designated as animal or plant pathogens, respectively, to acknowledge the ecological distinction from purely saprotrophic fungi. Similarly, we separated fungi capable of degrading complex carbohydrates of wood from generalist saprotrophs that generally can utilize only simple sugars. In total, functional assignments were made for 13 356 OTUs (ca. 65%). The quality-filtered and rarefied dataset contained 8720, 7829, and 4498 fungal OTUs in the samples from Argentina, Borneo, and Panama, respectively, of which 5917, 5078, and 2896 OTUs were assigned to functional groups, respectively. Targeted Locus Study projects corresponding to the three study regions have been deposited at DDBJ/EMBL/GenBank under accessions KDPX00000000 (Argentinian Yungas), KDPY00000000 (Malaysian Borneo), and KDPZ00000000 (Panama). The versions described in this paper are the first versions, i.e. KDPX01000000, KDPY01000000, and KDPZ01000000, respectively.

Statistical analyses

For each biogeographic region, we normalized the OTU table for subsequent statistical analyses by rarefying the number of high-quality fungal sequences to the smallest library size in that region (14 241 reads for the Argentinian Yungas, 24 812 for Borneo, and 2000 for Panama). For the characterization of putative core communities of pantropical montane and lowland fungi, we used a dataset rarefied to 2000 reads to estimate species overlap among geographic regions and to evaluate and distinguish the possible effects of biogeography and environmental variables on community composition.

In each geographic region, total fungal richness as well as OTU richness of taxonomic classes and functional groups were compared among the three major elevational forest types via ANOVA with Tukey's HSD test, implemented in R (R Development Core Team 2013). We calculated values of proportional richness and proportional abundance of functional groups on a per-sample basis and compared them among forest types as above. We used quadratic regression analyses, also in R, to examine relationships between elevation and environmental variables such as MAT, MAP, soil pH, organic matter, total nitrogen content, and carbon/nitrogen (C/N) ratio, as well as total P content in Argentina and Panama.

To compare community composition across elevational forest types, we used the *vegan* R package (Oksanen *et al.* 2015) to run Generalized Nonmetric Multidimensional Scaling (GNMDS) ordinations on the Hellinger-transformed OTU table and a secondary matrix containing environmental variables mentioned above. Ordinations were run separately for functional groups as well as for all fungi in each geographic region with the *metaMDS* function, which uses several random starts to find a stable solution. Data were subjected to 999 iterations per run with Bray-Curtis distance measure. Pearson correlation coefficient (r) values and statistical significance between environmental variables and fungal community composition were calculated with the *envfit* function, and vectors of variables with statistically significant correlations were plotted in ordinations. We plotted isolines of elevation on the GNMDS ordinations with the *ordisurf* function.

Statistical tests of the equality variances via the *betadis* function indicated no significant difference in multivariate homogeneity of group dispersions across elevational forest types in any region. To estimate the relative importance of forest type (categorical) and environmental (continuous) variables as sources of variation in fungal community composition, permutational multivariate analysis of variance (PerMANOVA) was carried out for all fungi and each functional group with the *adonis* function in *vegan*. To account for correlations among environmental variables, we performed a forward selection of parameters, including only significant environmental variables in the final model. For each geographic region as well as for the cross-regional comparison, we used partial Mantel test in *vegan* to differentiate the effects of spatial distance and abiotic environmental variables, standardized with the *scale* function in R, on community structure. Finally, we carried out indicator species analysis (Dufrêne and Legendre 1997) to quantify associations of individual OTUs with specific elevational forest types at a pantropical scale with the *multipatt* function in the *indicspecies* package in R (De Cáceres *et al.* 2012).

Results

Environmental drivers

Abiotic environmental variables correlated with elevation to a varying degree in the three sampled regions. As expected, MAT showed strong negative correlation with elevation in all three regions. MAP generally was not correlated with elevation except in Argentina, where it was highest in the lower montane forest zone (Fig. S4). Soil pH tended to decrease with elevation in all regions, although in Panama the trend was not significant. In Borneo, pH increased again above 3500 masl, representing the sparsely vegetated summit zone of Mt. Kinabalu. In a related manner, soil organic material (OM), and to a lesser extent N, were correlated positively with elevation in general, again with change of trend in the summit zone of Mt. Kinabalu (Fig. S4).

GNMDS ordinations revealed strong structuring of fungal communities according to elevation in all three regions (Pearson's correlations values: $r^2 = 0.8633$, $r^2 = 0.9406$, and $r^2 = 0.9661$; all $p = 0.001$) in Argentina, Borneo, and Panama, respectively (Fig. 1). With respect to abiotic variables, MAT and soil pH were correlated strongly with fungal community composition in all three regions (all $p = 0.001$), whereas other climatic and edaphic variables differed in their importance among the regions. Partial Mantel tests indicated that abiotic environmental variables were linked robustly to community structure in all sampling areas (Argentina: $r = 0.636$, $p < 0.001$; Borneo: $r = 0.444$, $p < 0.001$; Panama $r = 0.576$, $p < 0.001$), when spatial proximity was accounted for (control matrix), whereas spatial proximity was not significant (all $p > 0.1$) when abiotic variables were controlled.

In the PerMANOVA analyses, MAT and pH tended to explain the greatest variation in the total fungal community composition and remained significant contributors in the combined model in all three regions when correlation among parameters was accounted for. The contribution of other abiotic variables varied among functional groups and geographic regions, with P and OM explaining significant proportions of variation in fungal community structure in at least two regions and MAP being especially important in Argentina (Table 1). These results are consistent with Hypothesis 1, particularly with respect to MAT, pH, and to a lesser extent MAP, as drivers of soil fungal community composition.

Elevational patterns of fungal functional groups

All functional groups exhibited significant ($p < 0.05$) differences in community composition among the three elevational forest types in all geographic regions, consistent with our general Hypothesis 2. GNMDS plots of the datasets corresponding to functional groups showed similar correlations with elevation and edaphic variables as detailed above. The GNMDS ordinations of the four largest functional groups in terms of OTU richness are shown in Fig. 2, with richness of taxonomic orders displayed as vectors. In all regions, forest type was a significant source of variation in community composition in almost all functional groups, explaining ca. 20% of the variation in all fungi and between 11.5% and 26.5% in the functional groups in Argentina, between 15.3% and 32.6% in Borneo, and between 13.0% and 31.5% in Panama (Table 1). We did not find support for greater community turnover among elevational forest types in plant-associated than in non-plant-associated functional groups, in contrast to Hypothesis 2a.

Differences in richness among elevational forest types also were observed in all functional groups in at least one region. Total fungal richness was generally similar in all three elevational forest types, except in Panama, where lower montane forests harbored significantly more fungal OTUs than the other forest types (Fig. 3). We found three functional groups, all associated with plants (ECM fungi, plant pathogens, and wood decomposers), for which observed OTU richness as well as proportional richness differed significantly among elevational forest types in all three regions (Fig. 3, Fig. S5). Root-associated fungi, the only remaining plant-associated guild, and animal pathogens, differed significantly in richness in two regions, while richness of mycoparasites and generalist saprotrophs generally did not differ meaningfully among the elevational forest types. Greater differences among vegetation types in plant-associated fungi compared to non-plant-associated guilds are consistent with Hypothesis 2a.

Observed richness and proportional richness of plant pathogens and wood decomposers, and, to a lesser extent, animal pathogens and generalist saprotrophs, tended to be higher in lowland and/or lower montane forests than in upper montane forests, largely consistent with Hypothesis 2b. In contrast, ECM fungi, mycoparasites, and non-ECM root-associated fungi showed mixed patterns (Fig. 3, Fig. S5). For example, in Neotropical forests, ECM fungi peaked in richness in the upper montane forests, but they were most diverse in the lower montane forests in Borneo. Similar patterns also were observed with respect to proportional richness and to some extent in proportional abundance, with the notable exceptions of a pronounced abundance peak of mycoparasitic fungi and the abundance minimum of saprotrophs in the lower montane forest in Borneo, and the lack of significant differences in the proportional abundance of wood decomposers among elevation zones in all three regions (Fig. S6).

Elevational patterns of taxonomic groups

All taxonomic orders of ECM and root-associated fungi in the Neotropical sites that correlated significantly with the ordination axes exhibited highest richness in upper montane forests (e.g., Agaricales, Archaeorhizomycetales, Eurotiales, Helotiales, Hysteriales, Russulales, Sebaciniales, and Thelephorales). In Borneo, vectors of richness for most taxonomic orders of ECM and root-associated fungi were directed towards the lower montane forest sites, with the exception of Sordariales and Thelephorales with highest richness in lowland forests, and Helotiales in upper montane forests (Fig. 2).

Vectors representing the richness of most taxonomic groups of plant pathogens with significant correlations (e.g., in Botryosphaeriales, Cantharellales, Diaporthales, Hypocreales, Ophiostomatales, Polyporales, Rhizophydiales, and Xylariales) were directed mostly towards lowland forests, whereas putatively pathogenic Helotiales were more rich in lower montane forests (Borneo) or upper montane forests (Panama).

Orders of saprotrophic fungi that were richer in forests at lower elevations included Agaricales, Eurotiales, Geastrales, Hypocreales, Kickxellales, Saccharomycetales, Sporidiobolales, and Sordariales, each with significant Pearson's correlation values in at least two regions. In contrast, vectors for saprotrophic Chaetothyriales, Helotiales, and Sebaciniales indicated higher richness in montane forests. Among wood decomposers, Helotiales consistently showed highest richness in forests at higher elevations, and Agaricales and Xylariales at lower elevations (Fig. 2).

Overall, Archaeorhizomycetes, Leotiomycetes, Saccharomycetes, and Sordariomycetes differed significantly in richness among the elevational forest types in all three regions (Fig. S7). In particular, richness of Leotiomycetes and Sordariomycetes consistently was higher and lower, respectively, in upper montane than in lowland forests. Richness of Archaeorhizomycetes and Saccharomycetes peaked in lower montane forests in Borneo and Panama, but they showed contrasting patterns in Argentina (i.e., their richness increased and decreased with elevation, respectively). The Mortierellomycotina subphylum was always more rich in montane forests than in the lowlands, though the difference was not significant in Argentina. Similarly, significant differences in richness of Agaricomycetes among the elevational forest types were only detected in Borneo and Panama, where contrasting patterns were observed (i.e., higher and lower richness in lowland than in upper montane forests, respectively) (Fig. S7).

Pantropical core mycobiome of lowland and montane forests

The rarefied, combined dataset included 7612 OTUs. Of these, only 211 OTUs were shared among all three regions. The majority of OTUs were restricted to one geographic region, but 376 were shared between the two Neotropical regions, 188 were shared between the Yungas and Borneo, and 192 were shared between Borneo and Panama (Fig. 4). These findings are consistent with Hypothesis 3, i.e., little overlap among species pools of the three biogeographic regions, with more shared species between the two Neotropical regions.

Both abiotic environmental variables and geographic location were strongly correlated with fungal community structure in separate Mantel tests ($r = 0.658$ and $r = 0.497$, respectively; all $p < 0.001$), and when they were combined in partial Mantel tests, resulting in significant correlation of environmental factors with fungal

community structure when location was accounted for ($r = 0.553$, $p < 0.001$), and *vice versa* ($r = 0.279$, $p < 0.001$). PerMANOVA indicated that geographic region and forest type explained 7.3% and 5.8% of the variation in community composition, respectively, while among abiotic variables, MAP explained 7.4%, pH 6.9%, and MAT 5.7% of the variation, all with significant contributions in the combined model, consistent with Hypothesis 1.

Of 360 fungal OTUs with significant ($p < 0.05$) indicator value for a forest type, 136 occurred both in the Neo- and Paleotropics and were included in the pantropical comparison. Of these, 40, 24, and 72 were indicators for the lowland, lower montane, and upper montane forests, respectively (Table S2).

Discussion

Fungal biodiversity in tropical forests remains little known, and opportunities to compare data from similar guilds across diverse tropical forest types at local and global scales are rare. The deep sequence data presented here show that composition of the total fungal community, as well as that of all functional groups, is strongly structured according to elevational forest types in both the Neo- and the Paleotropics. Contrary to vascular plants, where the lowland and the lower montane forests typically harbor more species than upper montane forests (Aiba and Kitayama 1999; Brown *et al.* 2001; McCain and Grytnes 2010), we did not find substantial differences in soil fungal richness among the three elevational zones in Argentina and Borneo. The lack of a strong elevational pattern in fungal richness is similar to the lack of latitudinal differences in fungal richness on a global scale (Větrovský *et al.* 2019). Panama was an exception, where the mid-elevation peak in fungal richness is concordant with vascular plant richness in Central American mountains (Cardelús *et al.* 2006, Prada *et al.* 2017). However, in all functional groups and in all regions, compositional structure appears to be driven by elevation and the resulting environmental filtering according to contrasting climatic and edaphic conditions, and to a smaller extent, differences in vegetation. Furthermore, composition of fungal communities in the lower montane forests may be regarded as intermediate between communities of the lowland and upper montane forests, although, as the other two forest types, the lower montane forest also possesses several characteristic taxa.

Environmental drivers

The effect of elevation on fungal community composition and richness is mediated through abiotic and biotic factors driven either directly or indirectly by differences in temperature, which strongly influences relative humidity, soil moisture, and soil chemical processes. Consequently, the forest types found in different elevation zones have distinct mesoclimatic and edaphic conditions. Moreover, higher elevation habitats generally cover smaller areas than lower elevation ones and habitat area often is correlated positively with species richness in many taxonomic groups (Gotelli 1998). However, for fungi, the decreasing habitat area with increasing elevation does not seem to offer a satisfying explanation for the observed richness patterns: there were no statistical difference in total fungal richness among lowland and upper montane forests in any of the three regions, even though lowland forests cover much larger areas, particularly in Borneo and Panama (Figs. S1-3). Although some functional groups decreased in richness with increasing elevation, other groups showed the opposite trend. It is likely that climatic and edaphic factors as well as composition of biological communities are far more influential drivers of fungal richness in mountains than habitat area alone.

Our results indicate that MAT is the most influential driver of fungal community composition (Figs. 1-2, Table 1), while the role of MAP seems to be more region-dependent. For example, in terms of community composition, MAP was the strongest contributor of explained variation in the majority of the functional groups in Argentina, while it remained marginal or insignificant in Borneo and Panama. The mainly subtropical Yungas has pronounced seasonality, particularly at lower elevations (Brown *et al.* 2001). Local differences in moisture, and the resulting temporary drought stress, may represent a stronger environmental filter for community assembly than the limited variability and greater amounts of available moisture in the wet tropical forests of Borneo and Panama, which have far less pronounced seasonality.

Soil pH also plays an important role in shaping belowground fungal communities (Porter *et al.* 1987; Coughlan *et al.* 2000; Lauber *et al.* 2008; Rousk *et al.* 2010; Geml *et al.* 2014; Tedersoo *et al.* 2014; Glassman *et*

al. 2017, Geml 2019). Because many fungal species have a relatively wide pH optimum (e.g., Wheeler *et al.* 1991; Nevarez *et al.* 2009), it is likely that the observed correlation of pH with community composition is mainly indirect, e.g., by altering nutrient availability and competitive interactions between soil fungi and bacteria (Rousk *et al.* 2010) and other soil biota. In all regions, we observed significant decrease in pH and increase in OM with elevation, and it is difficult to disentangle their effects from that of MAT. However, the observed increase in pH and decrease in OM in the sparsely vegetated granitic summit zone of Mt. Kinabalu suggest that within the forest habitat, lower temperatures are associated with greater OM accumulation and a simultaneous decrease in pH in the montane forests, whereas OM accumulation drops sharply in sparsely vegetated habitats near the summit and does not result in low soil pH.

Elevational dynamics in soil fungal communities

The results presented here show that fungi with different life strategies seem to be favored by different environmental conditions, as indicated by functional differences among the elevational zones. Even in functional groups with no significant differences in richness, we observed strong differences in composition as a function of elevation. Similar elevational differences in richness and composition also have been observed in various functional groups of plants and animals (Cardelús *et al.* 2006; McCain 2009; McCain and Grytnes 2010; Guo *et al.* 2013).

Our results suggest the importance of vegetation type for species richness and community composition of functional groups of fungi associated with plants. For ECM fungi, richness correlates positively with taxonomic richness and density of ECM host plants both on global and regional scales (Tedersoo *et al.* 2014). Similarly, the data presented here suggest that ECM fungal richness mirrors host richness and density along elevation gradients in both the Neo- and Palaeotropics, as inferred from previous studies on tree community richness and composition of the sampled forest types (Aiba and Kitayama 1999; Malizia *et al.* 2012). This also results in important differences in the elevational patterns of ECM fungi between the Neo- and Palaeotropics. In the Neotropics, upper montane forests tend to harbor the highest density of ECM trees, i.e., with *Alnus acuminata* as the principal ECM host in the Yungas in Argentina, and *Alnus acuminata* and several *Quercus* spp. in the mountain range spanning southern Costa Rica and northern Panama (Malizia *et al.* 2012; Kappelle 2016; Wicaksono *et al.* 2017; Nouhra *et al.* 2018). These findings are in agreement with sporocarp-based studies in other areas of the wet Neotropics that reported highest richness of ECM fungi in upper montane forests (Mueller *et al.* 2006; Gómez-Hernández *et al.* 2012). In Borneo, in non-ultramafic soils, richness and density of ECM trees are high in lowland and lower montane forests and decline in upper montane forests, which is reflected here and is discussed in detail by Geml *et al.* (2017). At high elevations, ECM hosts such as *Leptospermum recurvum* tend to be dominant only in ultramafic soils (Aiba and Kitayama 1999), which were not included in this study. The above patterns in tropical mountains differ from those observed in temperate mountains, where ECM fungal richness tends to decrease monotonically with increasing elevation (Bahram *et al.* 2012; Nouhra *et al.* 2012; but see Bowman and Arnold 2018). The greater diversity and abundance of ECM fungi in montane forests likely contribute to the accumulation of soil OM through the continuous addition of fixed C to the rhizosphere, as in ECM-dominated boreal forests (Clemmensen *et al.* 2013), and as a result of negative interactions with saprotrophic fungi that inhibit decomposition (Gadgil and Gadgil 1971; Fernandez and Kennedy 2016).

Richness of plant pathogenic and saprotrophic fungi generally correlates positively with temperature as reported by a wide range of studies from landscape-level to global scales (Gómez-Hernández *et al.* 2012, Tedersoo *et al.* 2014, Geml 2019). In our study, richness (both proportional and absolute) of wood decomposers, and to some extent plant pathogenic fungi, was predictably higher at lower elevations, but richness patterns of saprotrophs were less predictable. In all three regions, the lowest proportional richness of saprotrophs always coincided with the highest proportional richness of ECM fungi, possibly as a result of competitive interactions mentioned above, i.e. “the Gadgil effect”. Regardless of differences in richness, strong community structure as a function of elevation, observed in all functional groups, suggests habitat specificity and elevational turnover of species within functional guilds as well. We note that some of the fungi identified under the functional guilds of plant pathogens and saprotrophs may prove to be endophytes, raising an

interesting direction for future study. Our work suggests that given the ecological and phylogenetic overlap of endophytic, saprotrophic, and pathogenic taxa in terms of ITS sequences, endophytes will broadly follow the patterns described here.

We observed several consistent patterns regarding the distribution of taxonomic groups across functional guilds, suggesting a certain level of phylogenetic conservatism with respect to environmental niches. For example, the consistently higher richness of Sordariomycetes, particularly Hypocreales, Sordariales and Xylariales, at lower elevations is apparent in various functional groups and is in agreement with higher host and substrate diversity for these fungi, which include plant pathogens as well as saprotrophs. Unlike in boreal and temperate forests, Sordariomycetes are the dominant class of plant endophytic fungi in tropical lowland forests (Arnold and Lutzoni 2007), in agreement with their prevalence in soil at low elevations. In a similar manner, the higher richness of Leotiomycetes, particularly Helotiales, in montane cloud forests is apparent in several functional groups, such as root-associated fungi, plant pathogens, saprotrophs, and wood decomposers. Helotiales appear to be the most diverse order of ascomycetes in arctic tundra ecosystems (Semenova *et al.* 2015), and the above trend confirms that many Helotiales taxa thrive in relatively colder climates. The fact that habitat preference can already be observed at the level of taxonomic orders, often irrespective of functional guild, suggests shared physiological constraints and environmental optimum for relatively closely related taxa.

Emerging pantropical patterns

The observed compositional differences among geographic regions indicate a relatively high level of endemism even within different parts of the Neotropics. Because our sampling of the hyperdiverse mycota in these regions is incomplete, it is possible that more shared taxa will be identified through the accumulation of more field data in the future. Nonetheless, regional endemism is widespread in fungi, as many species in low- to mid-latitudes show dispersal limitation and pronounced phylogeographic patterns (e.g., Geml 2011; Peay *et al.* 2012; Branco *et al.* 2015), resulting in differences in the composition of regional species pools.

Despite the low number of shared species, environmental variables are still at least as important drivers of community composition of tropical fungi as geography (i.e. biogeographic history). Almost two-thirds of the putatively pantropical OTUs were indicators for an elevational zone. This suggests that even among fungi with widespread geographic distributions, many are habitat specialists for a certain elevational forest type. This observation is in agreement with the importance of abiotic factors in shaping the distributions of the most common fungi on a global scale (Větrovský *et al.* 2019).

Fungal communities in upper montane forests are particularly rich in taxa with high specificity and fidelity to their habitat, as shown by indicator species analyses. The upper montane forest likely represents a unique habitat with a higher proportion of species specialized to the characteristic environmental conditions there (i.e. lower temperature and pH, increased OM accumulation, higher relative humidity and cloud formation).

Likewise, the similarity of proportional richness of the functional groups among the three regions is remarkable (Fig. S5). We hypothesize that functional niche proportions may be driven by similar mechanisms of environmental filtering along elevation gradients throughout Neo- and Paleotropical forests, resulting in relatively predictable representations of the functional guilds in lowland forests: saprotrophs representing ca. 35-40%, plant pathogens 11-17%, wood decomposers 6-15%, animal pathogens 2-4%, and mycoparasites 1-2% of the OTUs (as calculated for the whole community including fungi with currently unknown function). In addition, ECM and non-ECM root-associated fungi represent 1-2% and 1-5% of OTUs in Neotropical and 4-8% and 1-2% in Paleotropical lowland rainforests, and reach 4-12% and 2-4% of OTUs in Neotropical upper montane forests, respectively.

Conclusions

This is the first study comparing community structure of fungi along elevation gradients in the Neo- and Paleotropics. Climate, particularly temperature, appears to be the driving factor shaping the distribution of fungi along elevational gradients in a variety of ways, e.g., by affecting microbial processes (e.g., decompo-

sition), vegetation, and edaphic factors, and by altering species interaction dynamics. Montane forests are among the most vulnerable terrestrial ecosystems to climate change and warming will undoubtedly affect fungal communities in these ecosystems. Given the contrasting habitat preferences of several taxonomic groups and the possible functional differences among them within the broad functional guilds, future communities at a given site may differ considerably from current ones not only in composition, but also in functionality. Habitat specificity exhibited by many fungi offers possibilities for monitoring and habitat characterization and we advocate incorporating fungi in biodiversity assessments and conservation efforts. With the accumulating spatial data points for fungal taxa from metabarcoding studies, it will be possible in the near future to determine the climatic niches and model the suitable habitats for many fungi.

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[dataset] Geml J; 2020; Soil metagenome; DDBJ/EMBL/GenBank; KDPX01000000, KDPY01000000, and KDPZ01000000

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Wood JJ, Beaman RS, Beaman JH (1993) *The plants of Mount Kinabalu. 2. Orchids*. Royal Botanic Gardens, Kew, London, U.K.

Data Accessibility:

Targeted Locus Study projects corresponding to the three study regions have been deposited at DDBJ/EMBL/GenBank under accessions KDPX00000000 (Argentinian Yungas), KDPY00000000 (Malaysian Borneo), and KDPZ00000000 (Panama). The versions described in this paper are the first versions, i.e. KDPX01000000, KDPY01000000, and KDPZ01000000, respectively.

Data Availability:

The data that support the findings of this study are openly available in DDBJ/EMBL/GenBank at <https://www.ncbi.nlm.nih.gov/genbank/>, reference numbers KDPX01000000, KDPY01000000, and KDPZ01000000.

Author contributions:

J. Geml, E.R. Nouhra, F. Lutzoni, and A.E. Arnold designed the research and selected sampling sites. J. Geml, E.R. Nouhra, F. Lutzoni, A.E. Arnold, A. Ibáñez and L.N. Morgado performed the fieldwork. J. Geml and T.A. Semenova-Nelsen performed the labwork. J. Geml completed the bioinformatics and the statistical analyses, to which O. Grau and L.N. Morgado contributed R scripts. B. Hegyi prepared the maps for Figures S1-3. J. Geml wrote the first draft of the paper and all authors contributed to the revisions of the manuscript that resulted in the first submitted version.

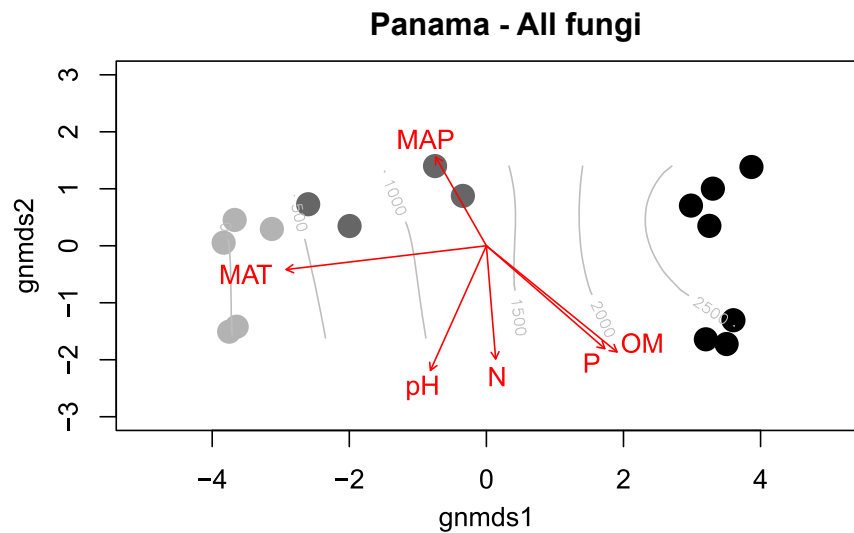
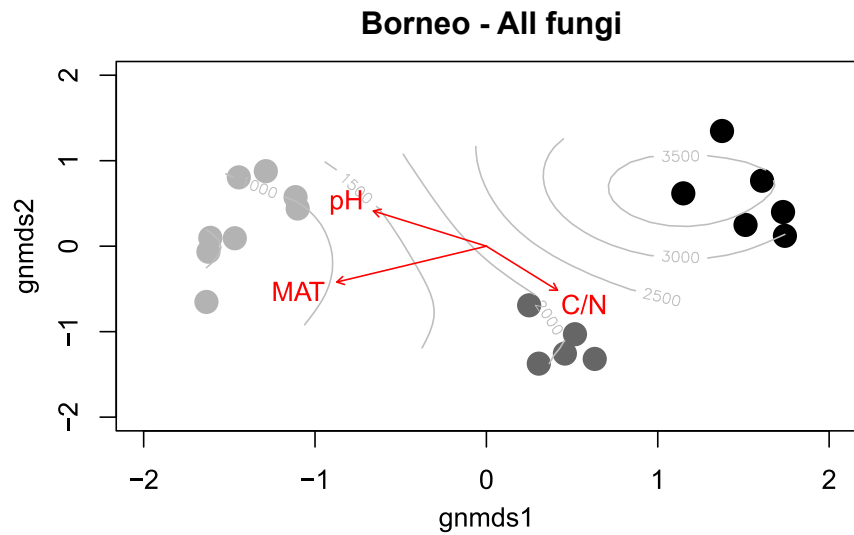
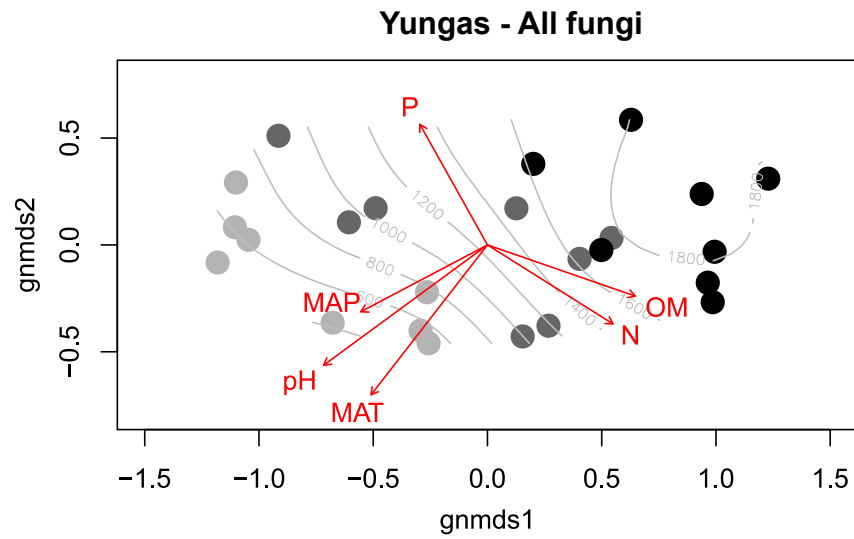
Table 1. Proportion of variation (%) in fungal community composition explained by elevational forest type (categorical) and continuous environmental variables calculated independently with permutational multivariate analysis of variance, based on the fungal community matrix. Significant results are in bold. Climatic and edaphic variables that remained significant in the final composite model (without elevation) for each fungal group are indicated by asterisk (*).

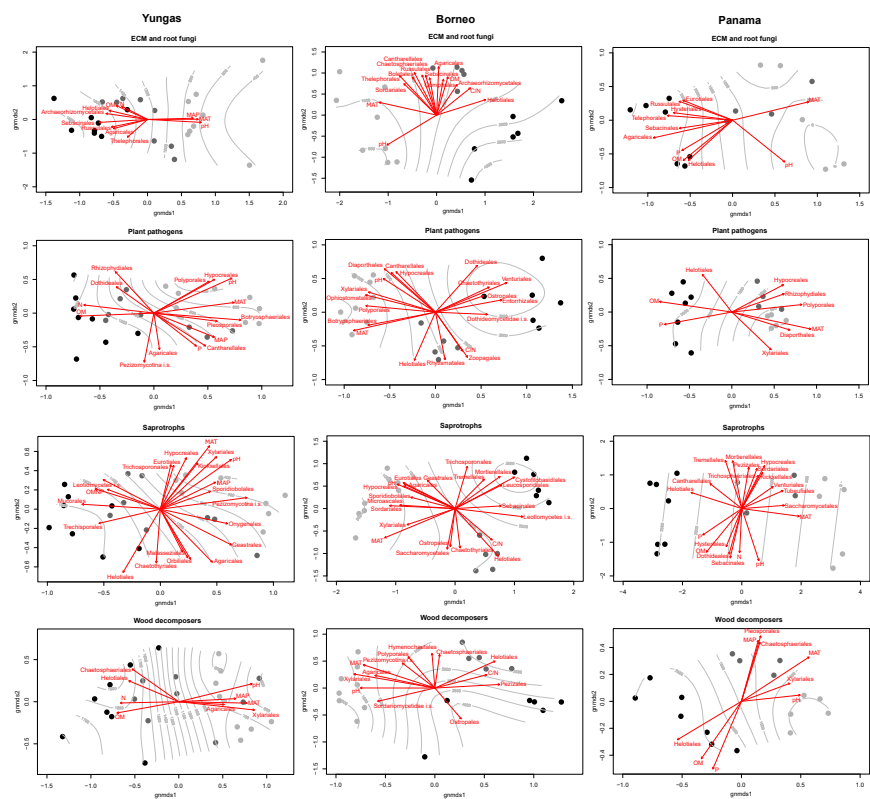
Fig. 1. Generalized non-metric multidimensional scaling (GNMDS) ordination plots of fungal communities in the sampled elevational forest types of the three regions based on Hellinger-transformed data, with elevation displayed as isolines. Localities and descriptions of the sampling sites are given in Table S1. Vectors of environmental variables with significant correlation with ordination axes are displayed. Lowland, lower montane and upper montane forest sites are indicated with light grey, dark grey, and black symbols, respectively. Abbreviations: MAT = mean annual temperature, MAP = mean annual precipitation, OM = soil organic matter content, N = soil nitrogen content, P = soil phosphorus content.

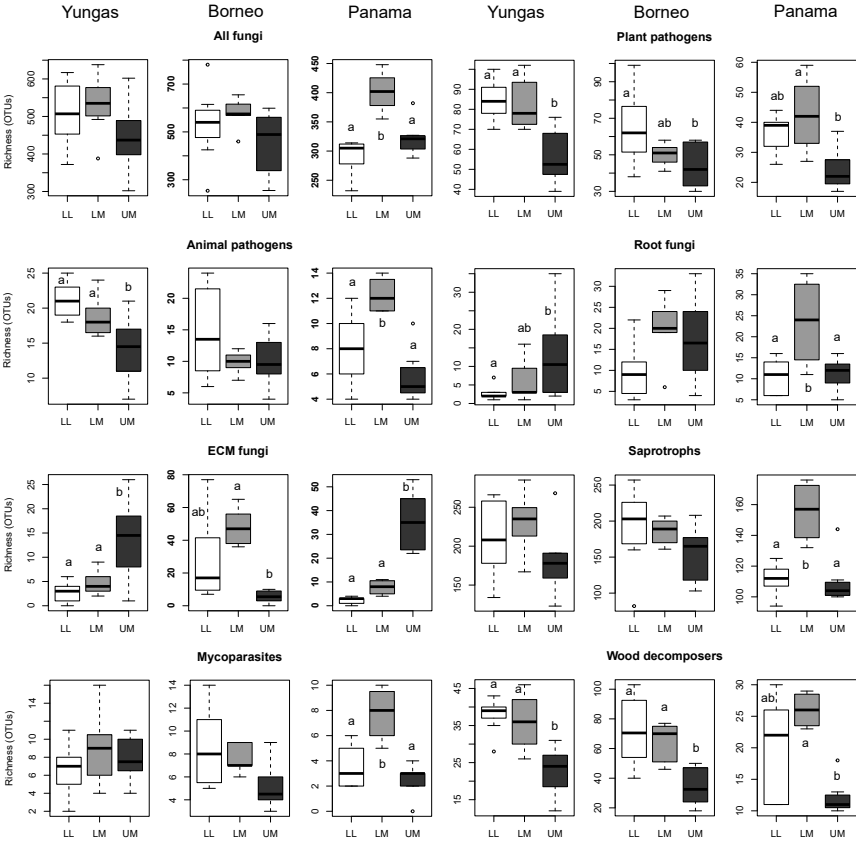
Fig. 2. Generalized non-metric multidimensional scaling (GNMDS) ordination plots of fungal functional groups in the sampled elevational forest types based on Hellinger-transformed data, with elevation displayed as isolines. Vectors of environmental variables and taxonomic orders with significant correlation with ordination axes are displayed. Lowland, lower montane and upper montane forest sites are indicated with light grey, dark grey, and black symbols, respectively. Abbreviations: MAT = mean annual temperature, MAP = mean annual precipitation, OM = soil organic matter content, N = soil nitrogen content, P = soil phosphorus content.

Fig. 3. Comparison of total fungal richness and richness of functional groups across the elevational forest types in the three sampled geographic regions. Means were compared using ANOVA and Tukey's HSD tests, with letters denoting significant differences ($p < 0.05$). Abbreviations: LL = lowland forest, LM = lower montane forest, UM = upper montane forest. Forest types are described in detail in the text.

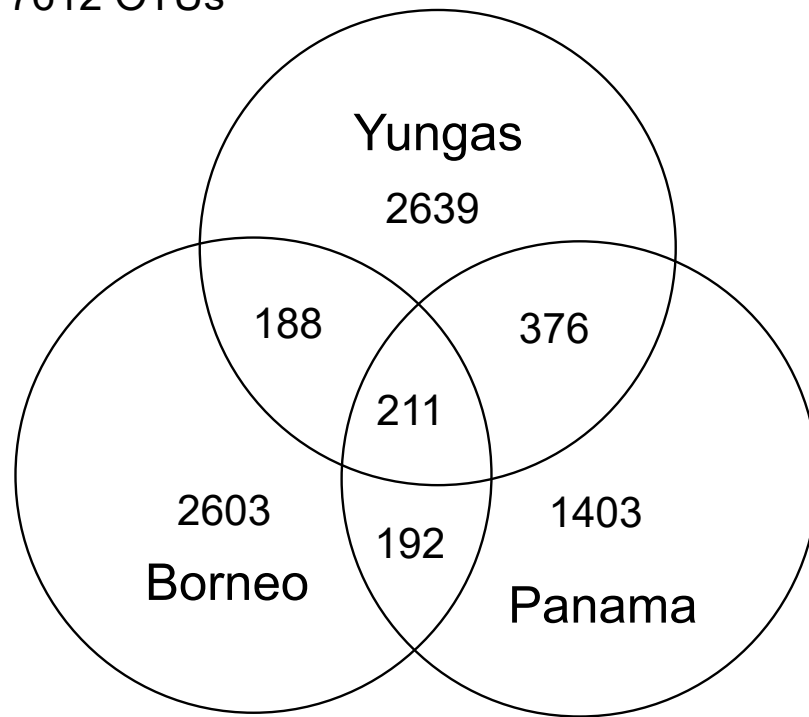
Fig. 4. Shared and exclusive OTUs across the three sampled geographic regions based on the rarefied Pan-tropical dataset.







7612 OTUs



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Table1.doc available at <https://authorea.com/users/301763/articles/431622-comparative-sampling-of-neotropical-and-paleotropical-elevation-gradients-reveals-the-role-of-climate-in-shaping-the-functional-and-taxonomic-composition-of-soil-borne-fungal-communities-in-tropical-forests>