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Fungal Communities across an Edaphic Gradient in Central Borneo †

Francis Q. Brearley

Department of Natural Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK; f.q.brearley@mmu.ac.uk; Tel.: +44-161-2471717

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Abstract: To examine the role of soil properties in influencing tropical fungal communities, soils were collected from Barito Ulu, in central Borneo, across an edaphic gradient from clay-rich ultisols to sandy spodosols and from upper and lower horizons, and subjected to high-throughput Illumina sequencing of the ITS1 region. The fungal community was clearly distinct between contrasting soils and depths, but diversity metrics did not show significant differences. Differentiation by depth was more marked as soils became less fertile. There were few marked impacts on phyla or functional guilds at a broad level, but Ascomycota were more abundant in less acidic soils with a narrower C:N ratio. Forests of South-east Asia remain an underexplored frontier for fungal diversity.

Keywords: acrisol; carbon; ectomycorrhizas; eDNA; fungi; *kerangas*; podzol

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1. Introduction

Whilst our understanding of patterns and drivers of fungal community composition is increasing, we have a paucity of data from tropical regions which needs addressing due to the key role fungi play in ecosystem processes [1] and their importance in contributing to high overall tropical biodiversity [2]. Molecular surveys of tropical soil fungi are increasing but much of the data is from Amazonia [3–7] with South-east Asia being less explored [8,9]. Soil chemical properties are known to play an important role in structuring fungal communities with pH and soil carbon noted as particularly important [7] along with soil phosphorus [5] and calcium [10]. Furthermore, we know that soil depth can influence the fungal community, at least partly due to separation of the soil depth niche by saprotrophic and ectomycorrhizal fungi with saprotrophs being stronger competitors in recently shed leaf litter [3,11]. The island of Borneo is a valuable study system in this regard as it contains high pedodiversity at local scales which is known to influence tree communities [12,13]. However, how this pedodiversity influences soil microbes and especially fungi is poorly explored [14]. In this study, we examined soil fungal community composition across edaphic gradients using Illumina sequencing, presenting here a detailed study of soil fungi in tropical forests of Borneo.

2. Methods

Soils were collected from the Project Barito Ulu research area in central Borneo (114°0′ E, 0°6′ S) which has a mean annual precipitation of 3800 mm [15] between an elevation range of about 150 to 200 m a.s.l. Whilst the geology is based on a Tertiary sedimentary formation, the research area contains a range of forest types developed on contrasting soils from more clay-rich udult ultisols (hosting a tall lowland evergreen rain forest (LERF2; *sensu* Proctor [16]) grading through sandy humult ultisols (hosting a shorter

lowland evergreen rain forest: LERF1) to spodosols hosting heath forests (HF1 & HF2) (*kerangas*). From each location, a soil sample was collected from the surface 0–5 cm or deeper at 15–20 cm depth after removing the superficial litter layer.

The number of fungal colony forming units (CFUs) was determined adding soil in a 1:10 ratio to sterile water and then serially diluted and added to potato dextrose agar (with 0.05% (*w*/*v*) chloramphenicol) in petri dishes before incubation for 96 h at 25 °C [17].

DNA was extracted from soil using a MoBio PowerSoil DNA Extraction Kit with one minute in a FastPrep 120 at the first stage. To amplify the fungal ITS1 region, the primers ITS5 and 5.8S_fungi [18] were used for PCR and sequencing on Illumina HiSeq 2500 at 2 × 300 bp paired-end sequencing following the protocol in Brearley [19]. Basecalling and de-multiplexing was performed by CASAVA v.1.8.2 with trimming of primers and adapter sequences using Cutadapt v.1.2.1 [20] and removal of low-quality bases using Sickle v. 1.200 [21]. FLASH v.1.2.8 [22] was used to assemble each pair of reads into a single sequence. Sequences shorter than 200 bp or longer than 600 bp were removed using a custom script, as were those matching PhiX. Clustering of sequences (at 99%), removal of chimeras, and defining OTU abundances was done in USEARCH7 [23]. After OTU-picking, taxonomic assignment of each OTU was carried out using the QIIME [24] script *assign_taxonomy.py*, using the RDP classifier [25] to match a representative sequence from each OTU to a sequence from the UNITE ITS database [26] at 97% similarity. Sequences not matching the Kingdom Fungi were removed from further analysis.

Soil pH was measured in a 1:2.5 soil:water ratio after 1 hr equilibration using a Sartorius PB-11 pH meter, and total carbon and nitrogen were analysed using a Vario ELCube elemental analyser.

Functional guilds based on trophic mode were assigned using FUNGuild [27]: only guild assignments where confidence ranking was "highly probable", "probable" or "possible" were used. Krona [28] was used to visualize fungal taxa, an NMDS ordination was conducted using Vegan [29] in R, and general linear models (GLMs) and correlations were conducted in Minitab v.19.

3. Results

Fungal CFUs were significantly more abundant in surface (0–5 cm) soil layers than deeper (15–20 cm) layers (GLM: $F = 9.1$, $p = 0.013$) with this difference being more marked for the spodosol and humult soils than the udult soil (GLM: $F = 6.6$, $p = 0.015$) (Figure 1).

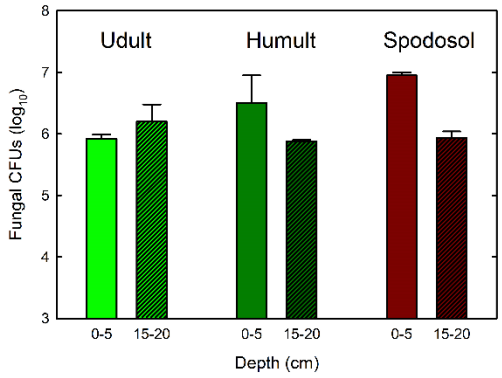

Figure 1. Abundance of fungal colony forming units (CFUs) (mean ± standard error) across different soil types and depths at Barito Ulu, central Borneo.

A total of 8536 fungal OTUs were found, of which 4197 could not be assigned to a phylum. Of those that could be assigned to a phylum, Ascomycota dominated the community with 76% of the assigned sequences followed by the Basidiomycota (23%) and the Glomeromycota (<1%). Other phyla, Chytridiomycota, Neocallimastigomycota and Zygomycota totaled less than 1% of all the sequences (Figure 2). The most abundant assigned

Ascomycota classes were the Sordariomycetes (38%), Eurotiomycetes (21%) and Archaeorhizomycetes (4.7%). In the Basidiomycota, the most abundant orders were Agaricales, Russulales and Catharellales (which contain a large number of ectomycorrhizal families between them) (Figure 2). The majority of sequences (70%) could not be confidently assigned to a functional guild, but of those that could, Symbiotroph was the most abundant guild (14%) followed by Pathotroph-Saprotroph-Symbiotroph (6.4%) and then Saprotoph (3.5%). There was no significant influence of either soil type or depth (or their interaction) on the relative abundance of any of the phyla or functional guilds (GLM: $p > 0.10$ in all cases).

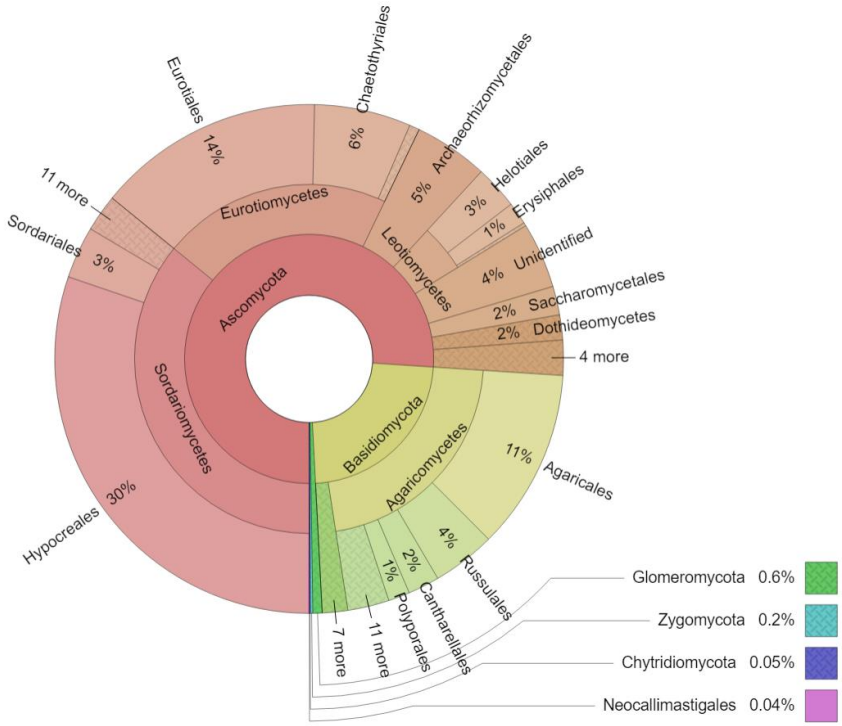

Figure 2. Abundance of fungal OTU sequences by dominant phyla, orders and classes across all samples from different soil types and depths at Barito Ulu, central Borneo.

There was a mean of 784 (±s.d. 269) OTUs per sample and a mean Chao1 estimator of 1536 (±s.d. 451) OTUs per sample. There was no indication of either of these diversity metrics differing by soil type or depth (GLM: $p > 0.10$). However, there was a clear separation of the fungal communities in the NMDS ordination plot (Figure 3) with the x-axis representing a soil type gradient and the y-axis representing and soil depth differentiation that was more marked in the spodosol than the ultisols.

Surface soils were significantly richer in carbon (C) and nitrogen (N) but more acidic than the deeper soils with increasing C and N and decreasing acidity through the udult ultisol to spodosol sequence (Table 1); the differences between surface and deeper soils were more marked for the humult and spodosol soils then the udult soil (Table 1). There were few correlations between soil properties and fungal richness/diversity or guild and phylum proportional abundance with the exception being a declining abundance of Ascomycota in more acidic soils ($\varrho = 0.47$, $p = 0.072$) with greater C ($\varrho = -0.46$, $p = 0.07$) and wider C:N ratios ($\varrho = -0.53$, $p = 0.035$), and a greater number of fungal CFUs in acidic, C- and N-rich soils ($|\varrho| = 0.55$–0.63, $p < 0.028$).

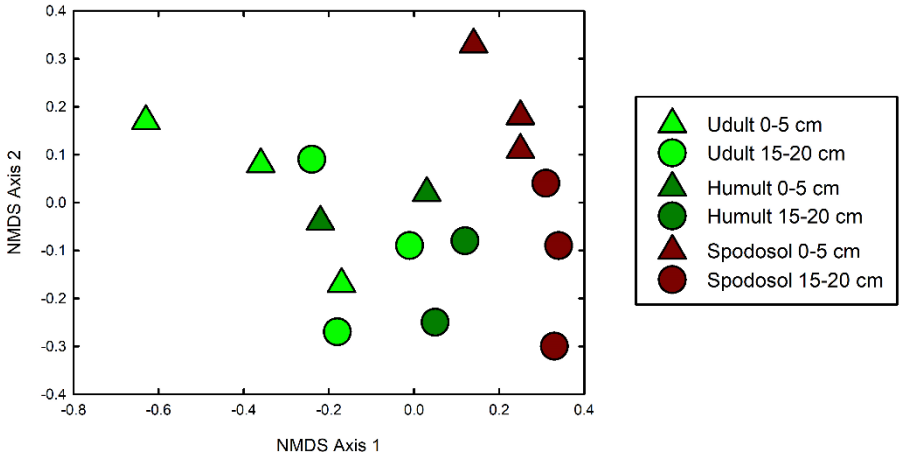

Figure 3. Ordination (NMDS) of fungal OTU sequences from different soil types and depths at Barito Ulu, central Borneo.

Table 1. Soil parameters (mean ± standard error) from different soil types and depths at Barito Ulu, central Borneo. Under each parameter, significance codes from a GLM are presented for soil type (S), depth (D) and their interaction (SxD) as *** $p < 0.001$, **, $p < 0.01$, n.s. = not significant.

Parameter	Soil Type	Depth (cm)	
		0–5	15–20
pH S ***, D ***, SxD **	Udult	3.36 ± 0.08	3.55 ± 0.09
	Humult	2.81 ± 0.04	3.63 ± 0.05
	Spodosol	2.76 ± 0.07	3.14 ± 0.07
N (%) S ***, D ***, SxD n.s.	Udult	0.27 ± 0.10	0.09 ± 0.02
	Humult	1.11 ± 0.08	0.15 ± 0.02
	Spodosol	1.25 ± 0.09	0.17 ± 0.04
C (%) S ***, D ***, SxD **	Udult	4.09 ± 0.69	1.07 ± 0.10
	Humult	39.8 ± 4.95	2.31 ± 0.29
	Spodosol	48.9 ± 1.05	4.71 ± 1.36

4. Discussion

I show here that soils can influence the fungal communities inhabiting them through differences in their chemical and textural properties, and some aspects of the fungal community correlate with soil chemical parameters at the individual sample level. These patterns are all found over a small spatial scale (less than 1 km^2) within a mosaic of soil and forest typologies (F.Q. Brearley & J. Proctor, unpublished) contrasting with other similar studies that have examined forests and soils over a broader geographical extent [4,7,14]. In common with other studies [7,14], forests over spodosols (a.k.a. *kerangas* or white sand forests) showed a distinctive fungal community composition from those in more clay-rich udult ultisols, but, in this study, humult ultisols were intermediate between these two showing a continuum of responses to the edaphic environment.

Whilst there were clear influences of both soil type and soil depth on the fungal community overall, it was difficult to elucidate a clear influence on individual fungal phyla or functional guilds. Additionally, soil type did not influence fungal alpha diversity, corroborating results of Tripathi et al. [14] in other Bornean heath forests although contrasting with Vasco-Palacios et al. [7] who found lower fungal diversity in Amazonian white sand soils. However, Tripathi et al. [14] did find greater beta-diversity in ultisols that they suggested might be due to more diverse plant communities in lowland evergreen rain forests

on these soils compared to white sand forests on spodosols. This is supported by the results of Peay et al. [4] who found plant and fungal beta diversity to be correlated across forest of western Amazonia.

At the phylum level, Tripathi et al. [14] found Ascomycota to be least common in dipterocarp forest (analogous to our udult ultisol) in contrast with this study where they were most common in this soil type (albeit not significantly) and became less abundant in more acidic soils with wider C:N ratios. Although dominant Ascomycota have a range of stress-tolerance genes [30], the greater recalcitrance of soil carbon in the spodosols may be a factor leading to their reduced abundance due to competition with Basidiomycota that have a greater capacity to degrade more complex organic substrates [31,32]. The relatively high abundance of Archaeorhizomycetes found here agrees with other work from Amazonia [7]. The large proportion of OTUs that could not be assigned even to phylum was surprising and could represent either bioinformatic issues or a number of unknown fungal lineages remaining to be discovered [19] in this underexplored biodiversity hotspot.

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