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# Title: Conversion of rainforest to rubber plantations impacts rhizosphere soil mycobiome and alters soil biological activity

**Running title: Expansion of rubber plantations threaten soil microbes**

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**Abstract**

In Asia, large swathes of rainforest have been converted to rubber plantations, with major consequences for biodiversity and ecosystem services. However, the impact of this land use conversion on rhizosphere soil mycobiome has not yet been addressed. This study aims to investigate how rhizosphere soil fungal communities and their associated biological activity (soil respiration, soil methane ($CH_4$) and potential soil enzyme production) are impacted by the conversion of rainforest to rubber plantations. Fungal richness and community composition in rhizosphere soils collected from natural rainforests, immature rubber, and mature rubber plantations were analyzed using paired-end Illumina sequencing. The conversion of natural rainforest to rubber plantations significantly altered fungal community composition of specific functional groups (saprotrophs, pathogens and mycorrhiza). We observed significant loss of saprotrophic and ectomycorrhizal fungi in natural rainforests, but enrichment of plant pathogenic fungi in immature rubber plantations. The mechanism underlying the effects of forest conversion on changes of fungal communities is related to reductions in soil pH, total nitrogen (N) and ammonium ($NH_4$) in rubber plantations. Conversion to rubber plantation also resulted in decline of soil respiration rates and less potential for cellulase and chitinase productions. The significant negative correlations between fungal richness and soil respiration in mature rubber plantations indicated high competition among fungi and low nutrient availability in this system. We demonstrate the negative consequences of the conversion of rainforest to rubber plantations on soil biological activity and significant changes in fungal community composition that could threaten long-term ecosystem functions.

**Keywords:** Forest conversion, Fungal diversity, *Hevea brasiliensis*, Illumina sequencing, Rhizosphere soil

## 1. Introduction

The expansion of rubber plantations is an important driver of land use change in Southeast Asia, where 97% of global natural rubber production occurs (FAO, 2013). Prior to the 1990s, monoculture rubber plantations were mainly restricted to southern Thailand, Malaysia and Indonesia (Chen *et al.*, 2016). However, due to the increased demand for rubber from China's emergent car industry, millions of hectares of rubber plantations have been established, with plantations encroaching northwards in Thailand, Cambodia, Laos, Vietnam, Myanmar and South China (Li & Fox, 2012). Up to 70% of rubber plantations have been planted at high elevation and on steeply sloping areas of upland Southeast Asia and South China, which has reduced sustainability and become a major threat to biodiversity (Ahrends *et al.*, 2015; Chen *et al.*, 2016). Increased rates of habitat loss are now of conservation concern in Southeast Asia and South China, both of which are considered biodiversity hotspots, as the area harbors high numbers of endemic and threatened species (Myers *et al.*, 2000; Sodhi *et al.*, 2010). Numerous studies have reported that habitat conversion to rubber plantations adversely influences biodiversity and ecosystem functions (e.g., reduces above- and below-ground carbon stocks, decreases soil fertility, and increases soil erosion) (de Blécourt *et al.*, 2013; Sarathchandra *et al.*, 2018; Sreekar *et al.*, 2016; Warren-Thomas *et al.*, 2015; Zhang *et al.*, 2007). Most studies have focused on the impact of rubber tree expansion on plant and animal diversity and found high losses of bird, mammal and invertebrate diversity in converted rubber plantations (Beng *et al.*, 2016; Liu *et al.*, 2019; Phommexay *et al.*, 2011; Sreekar *et al.*, 2016; Warren-Thomas *et al.*, 2015). However, the impact of forest conversion to rubber plantation on microbial diversity has been seldom examined.

The rhizosphere is a hotspot for complex plant-microbe interactions, which are involved in nutrient cycling, carbon sequestration, regulating plant diversity and productivity, and overall ecosystem functioning (Berg & Smalla, 2009; Mendes *et al.*, 2013; Singh *et al.*, 2004). In the rhizosphere, microbial populations and activities are higher than in bulk soil due to root exudates and high levels of organic matter, which help determine the presence of individual microbial taxa (Berendsen *et al.*, 2012; Broeckling *et al.*, 2008). For this reason, the rhizosphere is an important habitat for studying shifts in the diversity and composition of soil microbial communities. Fungi that are associated with rhizosphere soil play a major role in the mineralization of nutrients; degradation of recalcitrant organic matter; enhancement of plant growth and nutrition; the production of antibiotics; and the suppression of plant disease (Berendsen *et al.*, 2012; Buée *et al.*, 2009; Mendes *et al.*, 2013).

Conversely, pathogenic fungi in the rhizosphere have detrimental effects on plant growth and health (Mendes *et al.*, 2013). Rhizosphere-associated fungi are therefore critical components of terrestrial ecosystems (Berg & Smalla, 2009; Buée *et al.*, 2009). Environmental factors (soil moisture and soil temperature), site microclimates, root density and soil physicochemical properties such as soil pH, soil organic C, and soil nutrients directly and indirectly impact rhizosphere fungal communities (Berg & Smalla, 2009; Broeckling *et al.*; 2008; Buée *et al.*, 2009). Soil respiration is the sum of the processes that release $CO_2$ from soil to atmosphere (Schlesinger & Andrews, 2015), which is highly related important soil ecosystem functions, including decomposition and nutrient cycling (especially for C) (Ohlinger *et al.*, 1996). Both, $CO_2$ and $CH_4$, are important greenhouse gases, and tropical forest soils have been identified as both significant $CO_2$ sources and $CH_4$ sinks (Dalal & Allen, 2008; Raich & Potter, 1995; Veldkamp *et al.*, 2013; Werner *et al.*, 2006). Soil enzymes are a key determinant of metabolic processes in soil (Das & Varma, 2010), which are an important indicator for soil biological activity and fertility (Haney *et al.*, 2018; Ohlinger *et al.*, 1996). Therefore, it is important to investigate key biological indicators, including soil respiration, soil $CH_4$ and potential soil enzyme activities, and possible links between these indicators and abundance and composition of rhizosphere fungal communities.

Previous studies reported that conversion of rainforests to rubber plantations has pronounced effects on the composition of soil fungal communities (Brinkman *et al.*, 2019; Kerfahi *et al.*, 2016; Lan *et al.*, 2017, 2020; Song *et al.*, 2019). Brinkman *et al.* (2019) and Kerfahi *et al.* (2016) found that the diversity of soil fungi was not significantly lower in rubber plantations after rainforest conversion; however, there was a strong shift in fungal community composition. In contrast, conversion of tropical rainforests to rubber plantations resulted in a substantial loss of β-diversity (Lan *et al.*, 2020; Song *et al.*, 2019). Song *et al.* (2019) revealed that network complexity of fungi decreased due to the forest conversion to rubber plantation. Brinkman *et al.* (2019) showed that the major difference of fungal trophic groups in rubber plantation was caused by the reduction of symbiotrophic fungi and the increase of saprotrophic and pathotrophic fungi. These studies investigated fungal communities from bulk soil; however, within the rhizosphere, the effect of rainforest conversion on rubber plantations remains poorly understood. Moreover, studies investigating the effects of rainforest conversion on rubber plantations regarding the composition of microbial communities usually neglect the consequences of such effects on soil biological activity and ecosystem function (de Blécourt *et al.*, 2013; Goldberg *et al.*, 2017). The conversion of forests to tree plantations, including rubber, has been shown to reduce both soil respiration and $CH_4$ uptake by soils (e.g., Hassler *et al.*, 2015; Lang *et al.*, 2017; Veldkamp *et al.*, 2008). This was also true for

our study sites, where soil respiration and $CH_4$ uptake were found to be significantly reduced in rubber plantations compared to neighboring natural forests (Goldberg *et al*., 2017; Lang *et al*., 2019). To better understand the mechanism underlying changes in soil fungal communities, it is essential to determine soil respiration and metabolic functions and explore how these factors control changes in soil fungal communities following the forest conversion to rubber plantation.

Therefore, the aims of this study were to evaluate the impact of converting rainforests to rubber plantations on rhizosphere soil mycobiome (fungal community composition and richness) and their associated biological activity (soil respiration, soil $CH_4$ and potential soil enzyme production), and also to identify the key drivers that are responsible for any observed changes. We employed unique criteria to select the two main types of converted plantations (immature and mature rubber plantations) for observing changes in soil mycobiome after forest conversion over time. Three forest conversion types in Xishuangbanna, China were selected: natural rainforest (no conversion); immature rubber plantations (6-8 years old); and mature rubber plantations (15-20 years old). The richness and community composition of rhizosphere soil fungi in the studied forest conversion types were analyzed by paired-end Illumina MiSeq sequencing of the fungal Internal Transcribed Spacer (ITS2) fragment. We hypothesized that (i) the conversion of rainforest to rubber plantations would alter the community composition of specific fungal functional groups, especially the groups known to have biotrophic strategies, and strongly interact with plant hosts such as pathogens and mycorrhizae (Peay *et al*., 2013); and (ii) rubber plantations would introduce new fungal pathogens and mycorrhizae due to rubber trees containing many plant pathogenic fungi and are associated with arbuscular mycorrhizal fungi, but rarely associated with ectomycorrhizal fungi, whereas dominant trees in natural rainforests are associated with both arbuscular mycorrhizal and ectomycorrhizal fungi. (iii) We expected that for dominant plant species, pH, nitrogen (N) and phosphorus (P) are the main drivers causing changes in fungal community composition. Fertilization with urea has been found to reduce soil pH (Fageria *et al*., 2010). Even with intensive fertilization, due to high demand of nutrients in rubber plantation, we expected the depletion of macronutrients (N and P) in the soil of rubber plantations. Reduction of soil pH and macronutrients are known to cause significant changes in the rhizosphere fungal community composition (Deng *et al*., 2021).

## 2. Materials and methods

### 2.1 Site description and soil sampling

The research was conducted at the Naban River Watershed National Nature Reserve (NRWNNR), Xishuangbanna, Yunnan Province, China (22° 04'- 22° 17'N, 100° 32'- 100° 44'E). The reserve has a total area of 21,100 ha and is surrounded by mountains and covered by a tropical monsoon forest (YEPB, 2006). The region is characterized by a tropical monsoon climate, with a distinct dry season from November to April and a rainy season from May to October. Mean annual air temperature was 21.3°C and cumulative precipitation was 1106 mm from September 2014 to August 2015 (data from a local meteorological station). Soil in the study sites is classified as Latosol (Ferrosol) based on FAO classification (YEPB, 2006). Beginning in 1990, rubber plantations (*Hevea brasiliensis*) were established in NRWNNR after clearance of rainforest (Sarathchandra *et al.*, 2018).

In this study, we selected a study area in Xishuangbanna, Yunnan, Southwest China. Since Xishuangbanna is a biodiversity hotspot with expansive rubber plantation, it was selected as a model site to represent the forest conversion dominating South China. Using a space-for-time substitution approach, we selected three forest conversion types, including natural rainforests (NF), immature rubber (IR) and mature rubber (MR) plantations, which are clustered within each site. To minimize other effects (land use history, geographic and anthropogenic) other than the effects of land use conversion, we selected three sites within the national nature reserve, each with homogeneous management practices and land use histories. The three study sites (Mandian, Manlu, and Manfei) were located approximately 10 km apart from each other. At each site, three replicate plots were selected per conversion type; thus, the total study comprised nine replicate plots per conversion type for a total of 27 plots (Fig. S1 a).

All study sites have elevations ranging from 600-800 m above sea level and slope gradients between 22-28°. Rubber trees were planted on terraces with a mean tree spacing of 2.5 m in row and 6 m between rows. Immature rubber plantations were 6-8 years old, characterized by pre-canopy closure and were not yet being tapped for latex. Fertilizers are applied in immature rubber plantations. Farmers apply 46% urea at a rate of 0.5 kg per tree and 45% compound fertilizer (N-P-K=15-15-15) at 0.75 kg per tree once a year in July, but herbicides and fungicides are not applied. Mature rubber plantations were 15-20 years old with post-canopy closure, and latex

was being tapped. In mature plantations, farmers apply 45% compound fertilizer (N-P-K=15-15-15) at a rate of 1.5 kg per tree once a year in July. Farmers also use herbicides and fungicides at this stage, applying 30% glyphosate at 6 kg $ha^{-1}$ twice a year in July and December and 99% sulfur powder at 10 kg $ha^{-1}$ once a year during January to March to control powdery mildew disease. The farmers did not apply lime to the soils but applied herbicide and fungicide when the rubber trees reached their maturity at 6-8 years after plantation and had already been tapped for latex. Dominant plant species were selected from the rainforest, including *Toona ciliata*, *Microcos paniculata* and *Baccaurea ramiflora* in the Mandian, Manlu, and Manfei study sites, respectively.

Roots with the rhizosphere soil still attached were collected from three randomly selected trees at a depth of 0–20 cm for each plot. Rhizosphere soil was later removed by shaking soil from the root using sterilized gloves. The soil was then sieved through a 2 mm mesh sieve to remove root and plant materials. Two aliquots of the rhizosphere soil, 10g for molecular study and 100g for soil physico-chemical analysis, were packed in plastic bags, placed in an ice-box and cold-transported to the lab. Samples were immediately transferred and kept in a freezer at -20 °C prior to analysis. Rhizosphere soil collections were carried out in the wet (September 2014) and dry (March 2015) seasons.

**2.2 Soil physicochemical analyses**

Soil samples were sent for analysis of physical and chemical properties to the Biogeochemical Laboratory, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. All methods described below were adapted from the National Forest Service of China standards (National Forest Service of China, 1999). Soil particle size was examined using the pipette method, which fractionated the soil into clay (0–2 mm), silt (2–50 mm) and sand (50–2000 mm) according to the USDA classification system (FAO, 2006). For the sake of consistency, we analyzed soil texture in both the wet and dry season, even though soil texture is not affected by seasonality. Soil pH was measured potentiometrically in $H_2O$ at a soil: water ratio of 1:2.5. Organic matter (OM) was quantified by oxidation with a potassium dichromate solution in sulfuric acid ($H_2SO_4$-$K_2Cr_2O_7$). Organic carbon (OC) was calculated from the levels of organic matter divided by 1.724. Total nitrogen (TN) was measured using a CN Analyzer (Vario MAX CN, ElementarAnalysensysteme GmbH, Germany). Total phosphorus (TP) was digested with perchloric acid and hydrofluoric acid ($HClO_4$-HF) solutions and determined using an inductively coupled plasma atomic emission spectrometer (iCAP6300, Thermo Fisher Scientific, U.S.A). Cation exchange capacity (CEC) was assessed with 1 M ammonium acetate ($CH_3COONH_4$) (pH=7.0)

and tested using an auto Kjeldahl unit (K370, BUCHI Labortechnik AG, Schweiz). Available nitrogen was measured as ammonium nitrogen ($NH_4^+$-N) and nitrate nitrogen ($NO_3^-$-N) concentrations in 2 M KCl extracts, and concentrations were determined using a continuous flow analyzer (Auto Analyzer 3, SEAL Analytical GmbH, Germany).

**2.3 Soil respiration and $CH_4$ fluxes**

Soil respiration was measured using a closed dynamic system (Goldberg *et al*., 2017). Every plot was equipped with three PVC collars 20 cm in diameter and 20 cm in height that were installed in September 2014, with a distance of at least 2 m from trees. The collars were driven 5 cm into the soil. Soil respiration measurements were performed twice a month from end of November 2014 until November 2015. For each measurement, the collars were manually closed with a plastic lid and connected to a portable infra-red gas analyzer (LI-8100, LI-COR, Lincoln, Nebraska, USA). Air was circulated in this closed system by a pump at a constant flow rate of 0.5 L $min^{-1}$, and the $CO_2$ concentration inside the chamber was logged every 10 s for a period of 5 min.

Soil surface $CH_4$ flux was measured using static chamber method and gas chromatography (GC; Lang *et al*., 2019). Fluxes were measured from November 2014 to December 2015 at monthly intervals. In each plot, three chambers were inserted into soil at 5 cm depth, covering soil surface area of 0.20 $m^2$ with total volume of 42.66 L. Four samples were taken in the 45 minutes closure time with 100 mL syringes and stored in airtight gas bags. $CO_2$ and $CH_4$ fluxes were calculated from linear regressions of increasing or decreasing $CO_2$ and $CH_4$ concentrations.

**2.4 DNA extraction, preparation of the amplicon libraries and paired-end Illumina sequencing**

Microbial DNA was extracted from approximately 0.5 g of soil using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The quality and quantity of DNA samples were evaluated through gel electrophoresis of 5 µl subsamples on 1.5% agarose gel and analyzed with Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington DE). The samples were submitted to the Research and Testing Laboratory (RTL, Lubbock, TX, USA) for paired end Illumina MiSeq sequencing.

Samples were amplified for sequencing in a two-step process. The forward primer was constructed with (5'-3') the Illumina i5 sequencing primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) and the ITS3F primer (GCATCGATGAAGAACGCAGC) (Schoch *et al*., 2012; White *et al*., 1990). The reverse primer was

constructed with (5'-3') the Illumina i7 sequencing primer (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) and the ITS4R primer (TCCTCCGCTTATTGATATGC) (Schoch *et al.*, 2012; White *et al.*, 1990). Amplifications were performed in 25 µl reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 µl of each 5 µM primer and 1 µl of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems, Carlsbad, California) under the following thermal profile: 95°C for 5 min, then 35 cycles at 94°C for 30 s, 54°C for 40 s, 72°C for 1 min, followed by one cycle of 72°C for 10 min and 4°C hold.

Products from first stage amplification were added to a second PCR based on qualitatively determined concentrations. Primers for the second PCR were designed based on the Illumina Nextera PCR primers as follows: Forward - AATGATACGGCGACCACCGAGATCTACAC[i5index]TCGTCGGCAGCGTC and Reverse - CAAGCAGAAGACGGCATACGAGAT[i7index]GTCTCGTGGGCTCGG. The second stage amplification was run the same as the first stage except that we used 10 cycles.

Amplification products were visualized with eGels (Life Technologies, Grand Island, New York). Products were then pooled equimolar, and each pool was size selected in two rounds using the SPRIselect reagent (Beckman Coulter, Indianapolis, Indiana) at a 0.75 ratio for both rounds. Size selected pools were then quantified using the Qubit 4 Fluorometer (Life Technologies) and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California) 2x300 flow cell at 10pM.

**2.5 Bioinformatics**

Bioinformatic analysis of the sequence data was performed to obtain high quality reads from the paired-end sequences using MOTHUR (Schloss *et al.*, 2009) and OBI Tools (Boyer *et al.*, 2016) software suits. Briefly, read pairs were extracted from raw libraries if the two reads held the expected primer (forward primer for forward library, reverse primer for reverse library) at its 5' end. Forward and reverse raw reads from the same sample were assembled by using a simple-Bayesian algorithm with a threshold of 0.6 and a minimum overlap of 15 nucleotides as implemented in pandaseq (Masella *et al.*, 2012). To extract the high-quality reads, all the assembled reads were trimmed using the following parameters: (i) minimum length of 50; (ii) minimum average Phred score of 26 on the trimmed length; (iii) no ambiguities in the sequence length; and (iv) maximum length of 10 homopolymers in the sequence. The reads were then pre-clustered using CD-HIT-EST, allowing a maximum

of 1% dissimilarity, and with only one base allowed per indel (Niu *et al.*, 2010) in order to merge the reads likely to have arisen from sequencing errors (Huse *et al.*, 2010). Chimeric sequences were detected using the UCHIME algorithm (Edgar *et al.*, 2011) as implemented in MOTHUR. After removal of chimeric sequences, reads from each sample were pooled together, dereplicated into unique sequences, and sorted by decreasing abundance. The resulting reads were then clustered into operational taxonomic units (OTUs) using the CD-HIT-EST algorithm (Fu *et al.*, 2012) at a threshold of 97% sequence similarity.

The OTU representative sequences (the most abundant sequence in each OTU) were taxonomically assigned against reference sequences from the unite database (version unite.v7) (Kõljalg *et al.*, 2013) using the naive Bayesian classifier (Wang *et al.*, 2007) as implemented in MOTHUR using the default parameters. The sequences identified as fungi were further classified against the full version of the unite.v7 database to improve their taxonomic annotation. Finally, all the sequences identified as fungi were again classified against fungal sequences of the UNITE database augmented with non-fungal eukaryotic sequences from NCBI (version 211, (Benson *et al.*, 2013) in order to detect sequences from non-target organisms.

In order to assess the effect of rare taxa (with < 3 read abundance per sample) that might potentially originate from sequencing errors (Kunin *et al.*, 2010), we performed a Mantel test using the Jaccard distance to measure and assess the correlations between the whole matrix and a matrix excluding the rare OTUs (presence/absence matrices). The result indicated that the removal of rare OTUs from the total community had no effect on the fungal community composition ($R$ = 0.97, $P$ = 0.001). The need for rarifying the abundant fungal matrix was determined by a Mantel test using Jaccard distance to measure and assess the correlations between the whole abundant fungal matrix (15,011 ± 238 reads per sample, mean ± SE) and a matrix rarified to 11,900 reads per sample. The results indicated that rarifying the abundant fungal matrix had no effect on the fungal community composition ($R$ = 0.99, $P$ = 0.001). Thus, we used the whole abundant OTU matrix (presence/absence) for further statistical analysis to address our hypothesis. The fungal OTUs were assigned to functional or ecological groups using FUNGuild (Nguyen *et al.*, 2016).

Abundances of potential metabolic functions in rhizosphere soils were predicted based on ITS sequence using the reference genome database of PICRUSt2 (Douglas *et al.*, 2020). We identified a total of 856 metabolic functions. Among these functions, 14 enzymes (acid phosphatase, alpha-amylase, beta-glucosidase, cellulase, endo-1,4-beta-xylanase, pectin lyase, xylan 1,4-beta-xylosidase, alpha-N-acetylglucosaminidase, amidase,

chitinase, urease, arylsulfatase, laccase, peroxidase) are considered important for soil health and fertility (Das & Varma, 2010).

**2.6 Statistical Analysis**

Composition of total and specific fungal functional group communities were visualized using Nonmetric Multidimensional Scaling (NMDS) based on presence-absence data and the Jaccard distance measure. The stress values from NMDS ordinations were lower than 0.20 in all cases. Factors were fit to NMDS ordinations using the 'envfit' function in the vegan package of R, and goodness-of-fit statistics ($R^2$) were calculated with $P$ values based on 999 permutations (Oksanen *et al.*, 2016). All significant factors based on the goodness-of-fit statistics ($P < 0.05$) were used for further analysis on the most important predictors of fungal community composition (total, saprotrophic, plant pathogenic, and mycorrhizal fungal communities) using distance-based redundancy analysis (dbRDA). Effects of forest conversion to rubber plantation on composition of total and specific fungal functional group communities (presence-absence data) were determined by PERMANOVA based on Jaccard distance measured using PAST software (Hammer *et al.*, 2001). $P$ values were based on 999 permutations and a Bonferroni correction was applied in all cases. The final model of PERMANOVA only considered the effect of forest conversion type, as site and season had no significant effect on fungal community composition (both total and specific functional group). Fungal richness was used as a measure of fungal diversity that refers to the number of fungal OTUs per sample. The effects of forest conversion to rubber plantation on total and specific fungal functional group richness were analyzed using repeated ANOVA implemented in SPSS. All fungal richness datasets were tested for normality and equality of variance using the Jarque–Bera test and Levene test, respectively. Data were $Log_{10}$ transformed when necessary. Correlations between fungal richness and soil ecosystem functions (soil respiration and $CH_4$ production) were analyzed using Pearson correlation. All soil ecosystem function datasets were tested for normality using the Jarque–Bera test. The validity of the linear regression was confirmed by the normality of unstandardized residues values analyzed with Shapiro-Wilk test, the absence of potential outliers analyzed with Cook's distance, and the absence of autocorrelation between regression variables analyzed by Durbin-Watson test. Correlations between soil respiration rate and nutrient content were analyzed using both Pearson correlation (respiration rate and C) and Spearman rank correlation (respiration rate and total N and P). Factors shaping the patterns of potential metabolic functions (in total 856 functions) in rhizosphere soils were analysed using goodness-of-fit statistics. Effects of forest conversion types

on proportion of the important potential metabolic functions in rhizosphere soils were analysed using one-way ANOVA. The datasets were tested for normality and equality of variances. Spearman's rank correlation was used to test the relationships between the important potential metabolic functions in rhizosphere soils and soil respiration rates.



## 3. Results

### 3.1 Bioinformatics processing of the sequence data sets

A total of 844,532 quality-filtered fungal ITS2 reads were obtained after removal of low-quality reads and chimeric sequences, which clustered into 27,689 fungal OTUs with 21,112 rare taxa. After checking the effect of removal of rare taxa ($R$= 0.97 and $P$ = 0.001) and the need for using a rarified data matrix containing 11,900 reads per sample ($R$= 0.99, $P$ = 0.001), we used a final abundance data set that contained 6,577 fungal OTUs.

### 3.2 Fungal taxonomy and functional groups

Overall, the 6,577 fungal OTUs belonged to six phyla and 653 genera. Members of the phylum Ascomycota (65%), Basidiomycota (23%), and Zygomycota (3%) were frequently detected and dominated the fungal community (Table 1, Tables S1 and S2, Fig. S2). Glomeromycota, Chytridiomycota, and Rozellomycota contributed little to the total fungal community (0.1 to 1%). Over 7% of fungal sequence mycorrhizae could not be assigned at the phylum level (Fig. S2). Ascomycota and Basidiomycota were OTU-rich phyla containing 58% and 21% of the total OTUs detected in this study, respectively (Fig. S3). Unclassified phyla were also rich, consisting of 15% fungal OTUs (Fig. S3). A wide range of functional groups were detected in the rhizosphere soils, including animal pathogens (56 OTUs, 1%), endophytes (20 OTUs, 0.3%), fungal parasites (23 OTUs, 0.3%), lichen (5 OTUs, 0.1%), plant pathogens (166 OTUs, 2.5%), mycorrhizae (arbuscular mycorrhiza, ectomycorrhiza, orchid mycorrhizae) (142 OTUs, 2.2%), and saprotrophs (1410 OTUs, 21.4%) (Table 1). However, 72.3% fungi had unknown functions (4,356 OTUs) and uncertain functional assignments (399 OTUs) (Table 1). The three richest functional groups (saprotrophs, plant pathogens, mycorrhizae) were further analyzed for the effect of forest conversion type on their community composition.

### 3.3 Effect of forest conversion to rubber plantation on fungal community composition: overall significant effects and specific fungal functional groups.

NMDS ordinations and PERMANOVA tests showed a significant effect of forest conversion to rubber plantation on fungal community composition ($P$ = 0.003), and these effects were consistent across different fungal functional groups (Table 2, Fig. 1). Although all forest conversion types were significantly different from each other, mature rubber plantations were the most different from natural rainforests (Table 2, Fig. 1).

### 3.4 Driving factors for fungal community composition changes

Conversion from natural tropical rainforests to rubber plantations caused significant changes in soil physicochemical properties, which in turn significantly corresponded to changes in fungal community composition. Specifically, in immature rubber plantations, total soil N and $NH_4$ were significantly reduced. $NH_4$ in immature rubber plantations was 47% lower than in natural rainforest (Fig. 2). Other factors, including CEC, OC, OM and P, were slightly different (8–15%) compared to the natural rainforest. At the mature plantation stage, significant changes were detected for most measures of soil physicochemical properties. Soil became more acidic, and N and P were significantly reduced as compared to natural rainforest (Fig. 2). Although both forms of N ($NH_4$ and $NO_3$) were low, only $NO_3$ was significantly lower than in natural rainforest (55% reduction). Most of the factors that were significantly altered by forest conversion to rubber plantation (pH, TN, TP, $NH_4$, CEC, forest conversion type) corresponded to the shift in the fungal community composition (Fig. 3). These factors, however, were not auto-correlated ($\rho < 0.70$) except for pH and total P ($\rho = 0.83$, $P < 0.001$). The factors that were most strongly associated with fungal community composition change were further analyzed using distance-based redundancy analysis (db-RDA). We found that pH (and thus, also total P) and forest conversion from natural rainforest to rubber plantation were the most important factors in determining changes in total and all specific fungal functional groups (Table 3). In addition, for total and saprotrophic fungal communities, concentrations of total N and $NH_4$ were influential factors. pH values in each forest conversion types were provided in Table S4. The values of all soil physicochemical properties have been provided in our previous study (Monkai et al. 2018).

### 3.5 Effect of forest conversion to rubber plantation on fungal richness: the overall richness was not affected, but the fungal taxa in natural rainforest disappeared significantly

Seasonal changes played a more important role than forest conversion type in determining the richness of overall and most specific fungal functional groups, with the exception of plant pathogens (Fig. 4). Fungal richness values for total, saprotrophic, and mycorrhizal fungi were significantly higher in the dry season than in the rainy season. For plant pathogenic fungi, the effects of season were only marginal ($P = 0.06$), but the effects of forest conversion type were highly significant ($P< 0.001$). Post-hoc tests showed that immature rubber plantations had significantly enriched plant pathogenic fungi compared to natural rainforests and mature rubber plantations. Although fungal richness for total and most fungal functional groups were not significantly affected, fungal OTUs found in natural rainforests largely disappeared from both immature rubber plantations (31.6% = 2,080 OTUs) and from mature rubber plantations (36.0% = 2,367 OTUs). Saprotrophs were the main functional group that disappeared from both immature (538 OTUs, 38.2 % of the total detected saprotrophic fungi) and mature (589 OTUs, 41.8% of the total detected saprotrophic fungi) rubber plantations. In immature rubber plantations, we found a gain of 2,041 OTUs, accounting for 31.0% of saprotrophic (352 OTUs, 25.0% of the total detected saprotrophic fungi) and plant pathogenic fungi (67 OTUs, accounting 40.4% of the total detected plant pathogenic fungi). In mature rubber plantations, however, we detected a total of 1,686 OTUs gained, accounting for 25.6% of saprotrophic fungi (270 OTUs, 19.2% of the total detected saprotrophic fungi), with relatively low abundance of plant pathogenic fungal OTUs (29 OTUs, 17.5% of the total detected plant pathogenic fungi). The net balance of gain and loss of OTUs between natural rainforests and immature and mature rubber plantations was modest, with the exception of fungal plant pathogens (+16.9% of the total detected plant pathogenic fungi) and fungi with unknown functions (+4.0 of the total detected fungi with unknown function) in immature plantations. The highest net losses of 186 (immature rubber plantation) and 319 (mature rubber plantation) fungal OTUs were detected for saprotrophs, accounting for 13.2 and 22.6% of total detected fungi with saprotroph functions. The net balance of beneficial functional groups (i.e., mycorrhiza) was also negative in both immature (-11.3% of the total detected mycorrhizal fungi) and mature (-22.5% of the total detected mycorrhizal fungi) rubber plantations. All information on gains and losses of fungal OTUs are presented in Table S1.

**3.6 The consequences of changes in fungal community composition on soil biological activity**

Following the significant changes in soil fungal community composition in natural rainforest as compared with mature rubber plantations, the respiration rate was significantly reduced by 24%. This pattern was consistent in both wet and dry seasons: 18% and 30%, respectively. Soil respiration rate was significantly positively

correlated with soil organic C ($R$ = 0.36, $P$ = 0.030), total N ($\rho$ = 0.54, $P$ < 0.001) and total P ($\rho$ = 0.34, $P$ = 0.042) (Fig. S4). Furthermore, the soil respiration rate was significantly negatively correlated with fungal richness (both total ($R$ = 0.-0.67, $P$ = 0.002) and specific ecological functional groups ($R$ = -0.56 – -0.63, $P$ = 0.015 –0.005)) in mature rubber plantations (Fig. 5). In the soils of the natural rainforest, we found no such negative correlations ($R$ = 0.03 – 0.21, $P$ = 0.295 - 0.913) (Fig. 5). Soil $CH_4$ production was not significantly affected by forest conversion. Specifically, in the dry season, all soil samples from both natural rainforest and mature rubber plantations functioned as a $CH_4$ sink while in the wet season, a few samples of both land use types became a source of $CH_4$ (Fig. S5). All soil samples with fungal OTU richness higher than 419 were identified as a $CH_4$ sink (Fig. S5).

Apart from soil respiration, forest conversion types were also the most important factor shaping the patterns of potential metabolic functions derived by fungi in this study, followed by TP and $NH_4$ (Table S3). Forest conversion types, TP and $NH_4$, were also listed as significant factors corresponding to the shift in the fungal community composition. Among the important metabolic functions in rhizosphere soils, cellulase, alpha-N-acetylglucosaminidase, chitinase, laccase, and peroxidase were significantly affected by forest conversion types (Fig. S6). Specifically, potential fungal cellulase was negatively affected by forest conversion types from natural forest to rubber plantations. Potential fungal chitinase was found to have declined in the mature rubber plantations. Potential alpha-N-acetylglucosaminidase and laccase derived by fungi were highest in immature rubber plantations but did not differ between natural forest and mature rubber plantations. Potential fungal peroxidase was highest in mature rubber plantations but did not differ between natural forest and immature rubber plantations.

**4. Discussion**

This study represents the first attempt to evaluate the effects of land use conversion from rainforests to rubber plantations on the fungal community in the rhizosphere using high throughput sequencing. We produced a new database for fungal taxonomic composition in the rhizosphere soil of tropical rainforests and rubber plantations (Table S2) and detected a total of 856 metabolic functional compositions in the rhizosphere soil, predicted based on ITS sequence using the reference genome database of PICRUSt2 (Table S5). Moreover, our study employs

unique criteria for selecting the two main types of converted plantations (immature and mature rubber plantations) for observing changes the soil mycobiome after forest conversion over time. The developmental stages of rubber plantations (immature vs. mature) were not simply based on plantation age but also combined the effects of time and management practices at different stages in the development of plantation. We also found that sampling sites did not significantly correspond with fungal community composition, indicating that rhizosphere fungi are homogeneously distributed in this ecosystem. Furthermore, sample rarefaction curves also reveal that our sampling efforts are sufficiently good quality to infer fungal richness and community composition in this region (Fig. S1 b-e). Therefore, the sample size is enough to accurately represent the soil mycobiome and is sufficient for the evaluation of the impact of forest conversion in this region.

The land use conversion from rainforests to rubber plantations results in changes in rhizosphere fungal community compositions. Our findings indicated that the community composition of specific functional groups (saprotrophic fungi, plant pathogenic fungi, mycorrhizal fungi) were significantly altered by rainforest conversion to rubber plantations (Table 2, Fig. 1). The most drastic change was found in mature rubber plantations (Table 2, Fig. 1). The decrease in plant diversity and increase in agricultural practice intensities across rubber plantations has led to changes in soil physicochemical properties that are the main drivers of soil microbial diversity and community composition (Creamer *et al*., 2016; Thomson *et al*., 2015). In this study, we suggest that the mechanism underlying the effects of forest type conversion on changes in fungal communities is related to reductions of soil pH, total N and $NH_4$ in rubber plantations (Fig. 2 and 3). Among these factors, pH (highly correlated with P) was found to be the most important determinant for soil fungal community compositions across all fungal functional groups (including saprotrophs, pathogens and mycorrhiza) analyzed in this study (Table 3). The different management strategies in immature and mature rubber plantations (fertilization and herbicide application) could also explain changes in soil physicochemical properties and fungal community compositions between these land cover types.

Fertilization treatments were different among natural rainforests, immature rubber, and mature rubber plantations; thus, these differences could lead to the changes in fungal community, nutrient availability, and soil respiration. In immature rubber plantations, fungal community composition in the rhizosphere soil was significantly different from those in the natural rainforests (Table 2, Fig. 1). We identified TN and $NH_4$ as the main drivers of these differences (Table 3, Fig. 3). This result is in line with Paungfoo-Lonhienne *et al.* (2015), who showed that N

fertilization alters the community structure of fungi in rhizosphere soil, which may be due to changes in the secretion of root exudates and N mineralization rates (Phillips & Fahey, 2007; Yoneyama *et al.*, 2013).

Fungal community composition in mature rubber plantations was also significantly different from that of natural rainforests (Table 2, Fig. 1). However, reductions in soil pH, TN and TP played an important role in shaping fungal community composition at this stage (Fig. 2 and 3). The impact of latex-tapping and fertilization are associated with reductions in base cations, which leads to soil acidification (Zhang & Zhang, 2005; Zhang *et al.*, 2007). Moreover, a previous study showed that a significant decrease in pH in mature rubber plantations was associated with the accumulation of sulfur (used as a fungicide) (Li *et al.*, 2016). Hence a combination of factors may cause mature rubber plantations to have low pH, compared to natural rainforests and immature rubber plantations. Herbicide applications in mature rubber plantations have contributed to soil carbon loss and increased soil erosion (de Blécourt *et al.*, 2013; Li *et al.*, 2012; Liu *et al.*, 2016), which may affect soil fungal diversity and functions. Although a higher rate of N and P fertilizers were applied in mature rubber plantations, this was insufficient to meet the nutrient requirements of rubber trees and nutrient losses through increased erosion, which resulted in the reduction of TN and TP in the soil. These results indicate that application of chemicals (fertilizers, herbicide, fungicide) and latex-tapping were probably important factors behind the strong shift of fungal communities in rubber plantations compared to rainforests.

Saprotrophic fungi in natural rainforests largely disappeared from rubber plantations (Table 2, Fig. 1). Although there were some gains in novel saprotrophic fungal OTUs, and hence the richness of saprotrophic fungi was not significantly different among natural rainforest, immature, and mature rubber plantations ($P< 0.05$), the performance of these newly detected saprotrophic fungal OTUs differed greatly from that in natural rainforests (Table 1 and S1). In this study, we clearly show that new fungal communities detected in the mature rubber plantation were associated with a decline in soil respiration rates and with less potential for cellulase and chitinase productions (Table S3, Fig. 5 and S6). These ultimately negatively impact soil fertility and nutrient content in rubber plantations. Specifically, a reduction in soil respiration in mature rubber plantations was significantly linked with a reduction in soil organic C, total N, and total P (Fig. S4). Cellulase and chitinase are important enzymes for C and N cycling (Das & Varma 2010). Positive correlation between soil respiration and C, N, and P contents in soil is consistent with the results from other studies (Fan *et al.*, 2015; Guo *et al.*, 2016; Wang *et al.*, 2013). In addition, other studies have also shown the negative effects on soil ecosystem functions

resulting from the conversion of natural forest via the reduction of soil enzyme activities (Diniz *et al*., 2020; Nurulita *et al*., 2016). Specifically, C (β-glucosidase and cellobiohydrolase) and P (acid phosphatase) acquisition enzymes as well as a lignin-modifying enzyme (laccase) significantly decline in rubber plantations compared to natural forests (Fig. S6). Furthermore, we found that conversion from natural rainforest to rubber plantations can alter interactions among fungi living in each ecosystem. Significant negative correlations between fungal richness (total and other major ecological functional groups) and soil respiration detected in mature rubber plantations are a sign of severe competition. In such situations, fungi may use their energy and resources for competition (i.e., producing secondary metabolites) rather than decomposition (Fukami *et al*., 2010; Hoppe *et al*., 2015). Such negative correlations were not detected at all in natural rainforests, which may indicate less competition among fungi in this system(Hoppe *et al*., 2015).

A further concern is the loss of mycorrhizal fungi in rubber plantations (Fig. 4, Table S1). Rubber trees are associated with arbuscular mycorrhizal fungi, but native forest tree species are associated with both arbuscular mycorrhizal and ectomycorrhizal fungi. Conversion from natural rainforest to rubber plantations causes a decline in ectomycorrhizal fungi (e.g., *Amanita*, *Boletus*, *Clavulina*, *Coltricia*, *Gyrodon*, *Hymenogaster*, *Inocybe*, *Lactarius*, *Sebacina*, *Russula*), and gain of arbuscular mycorrhizal fungi (Glomeromycota) (Table S1 and S2). This species shift away from native ectomycorrhizal fungi could severely reduce tree growth after replanting and forest regeneration in any future effort to convert rubber plantations back to natural rainforests (Hawkins *et al*., 2015).

Another finding of this study was that fungal pathogens were enriched within the rhizosphere of immature rubber plantations (Fig. 4, Table S1). This result is consistent with a study by Shi *et al*., (2019), who suggested that the increase of pathogenic fungi following deforestation may reflect changes in plant-fungal relationships that normally suppress these fungi. Rubber trees in northern Southeast Asia are vulnerable to many fungal diseases, including powdery mildew and rubber anthracnose (Li *et al*., 2016; Liyanage *et al*., 2016). Sulfur-based fungicides are used to prevent powdery mildew disease, causing reduced latex yield (Liyanage *et al*., 2016). In our study, these were only applied in mature rubber plantations. As pathogenic fungi were fewer in areas where sulfur-based fungicides had been applied, it would appear that application of fungicides in mature rubber plantations could be an effective method to reduce plant pathogenic fungi in rhizosphere soils. In contrast, the rhizosphere soil of immature rubber plantations is a potential source of fungal pathogens, which could later

infect rubber trees (Ho *et al.*, 1984). Consequently, we advise that screening and the prevention of fungal diseases (especially root diseases) should be carried out during the immature stage of rubber trees.

Our finding is similar to that of a recent study in bulk soils that showed a significant decrease of symbiotrophic fungi and enrichment of saprotrophic and pathotrophic fungi in rubber plantations (Brinkman *et al.* 2019). Differences in rhizosphere fungal communities are not only due to differences in host plants or planting structures, but also have a greater relationship with soil properties. We show that changes in soil properties from our study also partly come from the conversion to rubber plantations, especially management activities in rubber plantation. Plant host plays an important role in structuring the fungal richness and community composition of specific functional groups of fungi that are known to have biotrophic strategies and strongly interact with plant hosts (e.g., plant pathogens, mycorrhizal fungi) (Peay *et al.*, 2013). A recent study also demonstrates that even saprotrophs can be highly dependent on their host plants (Purahong *et al.*, 2018).

**5. Conclusion**

This study provides a deeper insight into the impact of the conversion of natural rainforests to rubber plantations on soil fungal communities and their associated soil biological activities. Our study documented shifts in fungal communities in rubber plantation that had been converted from rainforest 25 years ago. Many fungi are specialized to the rhizosphere of rainforest and rubber plantations and may play a particular role in organic matter decomposition, plant nutrition, and productivity. Plantations may also have different ecosystem services if suitable initiatives of sustainable management are implemented. Therefore, it is essential to maintain and restore native rhizosphere fungal communities in rubber plantation ecosystems. Rainforest restoration strategies, including agroforestry and understory regeneration, should be implemented in order to increase plant diversity and establish hosts for ectomycorrhizal fungi and saprobic fungi in rubber plantations. Future studies are needed to determine how current rubber plantation management strategies will impact the long-term dynamics of soil fungal communities and whether a reduction in harmful management practices could recover original fungal community compositions. Future study should apply advanced sequencing analysis methods (e.g., metagenome and metabolome) to detect changes in functional components and metabolic functions due to conversion of

rainforest to rubber plantations. Soil enzyme activities should be measured from the same samples and linked to the microbial taxonomic and functional compositions and metabolic functions in rhizosphere soils.



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**Table legends**

**Table 1** Fungal OTUs detected in this study (NF = natural rainforest; IR = immature rubber plantation; MR = mature plantation.) These were classified on the basis of their ecological functions.

**Table 2** Effects of forest conversion to rubber plantation on composition of total and specific fungal functional group communities, as determined by PERMANOVA test. *P* values were based on 999 permutations and Bonferroni correction was applied in all cases.

**Table 3** Significant predictors in the distance-based redundancy analysis (dbRDA) model of total, saprotrophic, plant pathogenic and mycorrhizal fungal community composition.

**Figure legends**

**Figure 1** Non-metric multidimensional scaling (NMDS) ordinations of rhizosphere soil fungal community composition: total (a) and saprotrophic (b) fungal communities. The amounts of variance explained by coordinate1 and 2 were 43 and 18% with stress value = 0.159 for NMDS of total fungal communities and 44 and 24% with stress value = 0.198 for NMDS of saprotrophic fungal communities. NF (green) = natural rainforest; IR (red) = immature rubber plantation; MR (blue) = mature rubber plantation. Light and dark colors = dry and wet season, respectively. The number in the circle indicates the study site (1 = Mandian, 2 = Manlu and 3 = Manfei).

**Figure 2** Experimental set-up and factors changed by the conversion of natural rainforest to rubber plantations. pH; pH, OM; organic matter, OC; organic carbon, N; total nitrogen, P; total phosphorus, CEC; Cation-exchange capacity, $NH_4$; ammonium nitrogen, $NO_3$; nitrate nitrogen.

**Figure 3** Goodness-of-fit statistics ($R^2$) for factors fitted to the non-metric multidimensional scaling (NMDS) ordinations of fungal community compositions (total, saprotroph, plant pathogen, mycorrhizal fungal communities). Significant corresponding factors with the fungal communities are indicated with an asterisk ($P<$ 0.05*, $P<$ 0.01**, $P<$ 0.001***). Sand; sand content, Silt; silt content, Clay; clay content, pH; pH, OC; organic carbon, TN; total nitrogen, TP; total phosphorus, CEC; Cation-exchange capacity, $NH_4$; ammonium nitrogen, $NO_3$; nitrate nitrogen.

**Figure 4** Effect of seasons (D = dry and W = wet (rainy season)) and forest conversion types (NF (green) = natural rainforest, no conversion, IR (red) = immature rubber plantation; MR (blue) = mature rubber plantation) on fungal OTU richness of total (a), saprotroph (b), plant pathogen (c) and mycorrhizal (d) fungi.

**Figure 5.** Relationships between soil respiration rate, fungal OTU richness and community composition: a = total richness, b = community composition, c = richness of total fungi (all, black) and saprotrophs (sap, purple) detected in rainforest, d = richness of total fungi and saprotrophs detected in mature rubber plantation, e = richness of mycorrhizal (myc, green) and plant pathogenic fungi (pat, orange) detected in rainforest, and f = richness of mycorrhizal and plant pathogenic fungi detected in mature rubber plantation. Dash line indicates the mean respiration rate. Light green triangle = rainforest in dry season, dark green triangle = rainforest in rainy season, Light red triangle = mature rubber plantation in dry season, dark red triangle = mature rubber plantation in rainy season. Up-pointing triangle (no filled) = respiration above the average, down-pointing triangle (filled) = respiration below the average.

**Supporting Information**

**Table S1** Fungal OTUs detected in this study (NF = natural rainforest; IR = immature rubber plantation; MR = mature rubber plantation.) These were classified on the basis of their ecological functions. Gain, loss and net balance were also calculated.

**Table S2** New database of fungal taxonomic composition in the rhizosphere soil of tropical rainforests and rubber plantations

**Table S3** Factors shaping the patterns of potential metabolic functions (in total 856 functions) in rhizosphere soils predicted based on ITS sequence using the reference genome database of PICRUSt2. Goodness-of-fit statistics ($r^2$) for factors fitted to the non-metric multidimensional scaling (NMDS) (stress = 0.13) potential metabolic functional patterns. *P* values were based on 999 permutations.

**Table S4** The soil pH value in natural rainforest, immature and mature rubber plantations (values are means ± SE, n = 9).

**Table S5** Metabolic functional composition in rhizosphere soils (856 functions) predicted based on ITS sequence using the reference genome database of PICRUSt2

**Figure S1** The study sites and sampling plots in Nabanhe Nature Reserve, Xishuangbanna, China (a) and sample rarefaction curve (with 95% confidence) for all natural rainforest and rubber plantation plots (b), for all natural rainforest plots (c), for all immature rubber plantation plots (d) and for all mature rubber plantation plots (e).

**Figure S2** Total fungal community composition based on relative abundance data

**Figure S3** Total fungal community composition based on presence/absence data

**Figure S4** Correlations between soil respiration rate and organic carbon content (Pearson's $r = 0.36$, $P = 0.0030$) (a), total nitrogen (Spearman's rank $\rho = 0.54$, $P < 0.001$) (b, purple) and phosphorus (Spearman's rank $\rho = 0.34$, $P = 0.0418$) (b, red).

**Figure S5** Relationships between soil respiration rate and fungal OTU richness and community composition: a = total richness, b = community composition, c = richness of total fungi (all, black) and saprotrophs (sap, purple) detected in rainforest, d = richness of total fungi and saprotrophs detected in mature rubber plantation, e = richness of mycorrhizal (myc, green) and plant pathogenic fungi (pat, orange) detected in rainforest and f = richness of mycorrhizal and plant pathogenic fungi detected in mature rubber plantation. Dash line indicates the mean respiration rate. Light green triangle = rainforest in dry season, dark green triangle = rainforest in rainy season, Light red triangle = mature rubber plantation in dry season, dark red triangle = mature rubber plantation in rainy season. Up-pointing triangle (no filled) = respiration above the average, Down-pointing triangle (filled) = respiration below the average.

**Figure S6.** Effect of forest conversion types on proportion of the important potential metabolic functions in rhizosphere soils predicted based on ITS sequence using the reference genome database of PICRUSt2. The number before the enzyme's name indicate the groups of potential metabolic functions (1 = phosphorus cycle, 2 = carbon cycle, 3 = nitrogen cycle, 4 = sulfur cycle and 5 = lignin modification/degradation). The number after the enzyme's name indicate the forest conversion types (1 (green) = natural rainforest, no conversion, 2 (red) = immature rubber plantation; 3 (blue) = mature rubber plantation. NS = not significant. Different letters above the bars indicate significant differences among mean using one-way ANOVA ($P < 0.05$).

**Data accessibility**

Data and material availability: most relevant data are within the paper and in the electronic supplementary material. The raw sequence datasets are available in NCBI under the study number SRP133659 (https://www.ncbi.nlm.nih.gov/sra/SRP133659).

**Authors' contributions**

K.H., R.H. and P.M. conceived and designed the experiments. J.M. and S.G. performed the field experiments and the laboratory work for molecular fungal community data. T.W. and A.N. performed bioinformatics. W.P.

and T.W. analysed and interpreted the results. W.P. and J.M. wrote the manuscript. J.X. and R.H. obtained funding. All authors contributed to revisions of the manuscript and gave approval for submission.

**Competing interests**

We have no competing interests.

Table 1. Fungal OTUs detected in this study (NF = natural rainforest; IR = immature rubber plantation; MR = mature plantation.) These were classified on the basis of their ecological functions.

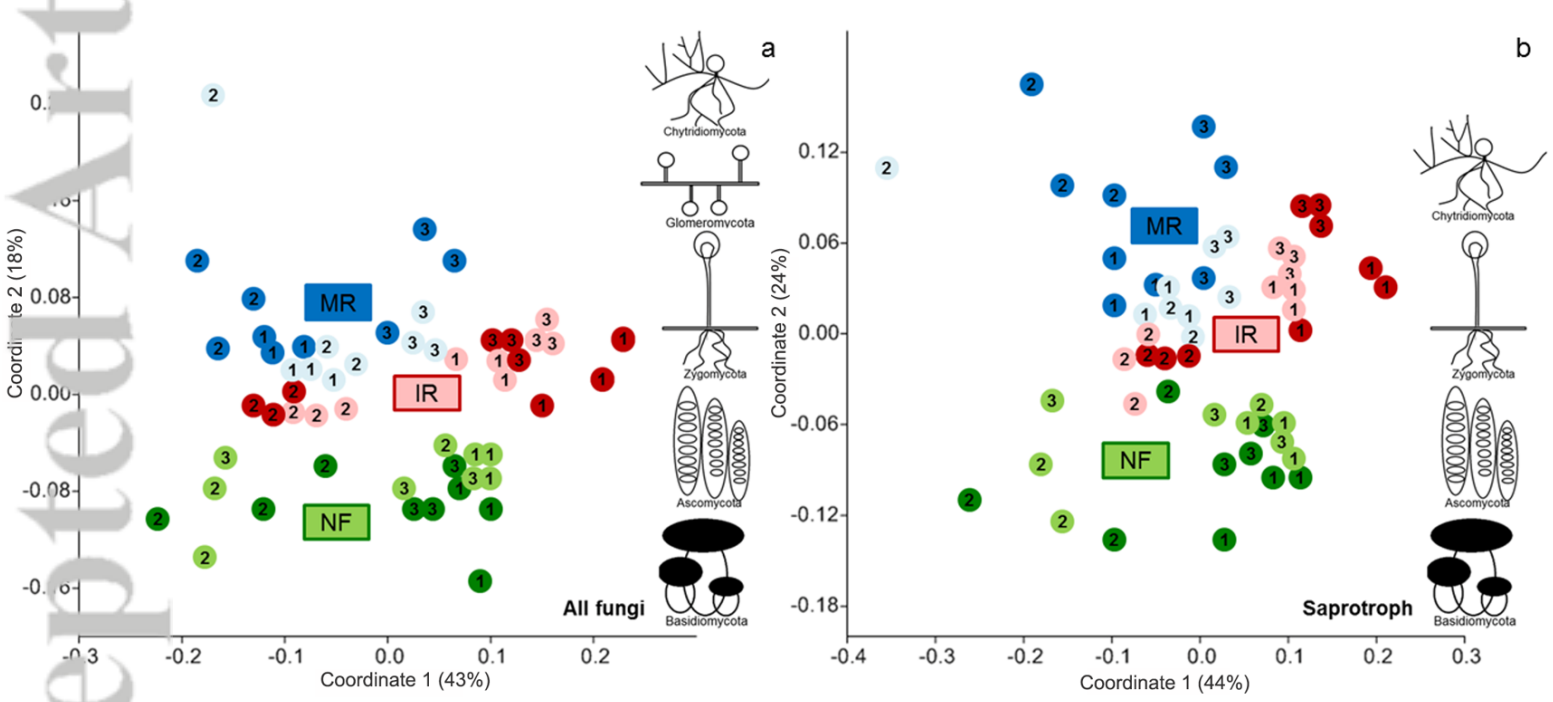
| Fungi | Total Fungi | Animal Pathogen | Endophyte | Fungal Parasite | Lichenized | Mycorrhizal | Plant Pathogen | Saprotroph | Uncertain | Unknown |
|---|---|---|---|---|---|---|---|---|---|---|
| Core Mycobiome (NF, IR, MR) | 825 | 16 | 3 | 7 | 1 | 12 | 23 | 216 | 59 | 488 |
| Fungi in natural rainforest undetected in IR | 383 | 6 | 2 | 4 | 0 | 10 | 9 | 111 | 26 | 215 |
| Fungi in natural rainforest undetected in MR | 670 | 5 | 4 | 1 | 0 | 14 | 26 | 162 | 58 | 400 |
| Fungi in natural rainforest absent in both IR and MR | 1,697 | 21 | 2 | 7 | 2 | 50 | 30 | 427 | 100 | 1,058 |
| Newly detected fungi from IR | 1,316 | 5 | 3 | 1 | 2 | 24 | 49 | 224 | 81 | 927 |
| Newly detected fungi from MR | 961 | 2 | 5 | 2 | 0 | 12 | 11 | 142 | 41 | 746 |
| Newly detected fungi from both IR and MR | 725 | 1 | 1 | 1 | 0 | 20 | 18 | 128 | 34 | 522 |
| *Sum NF* | *3,575* | *48* | *11* | *19* | *3* | *86* | *88* | *916* | *243* | *2,161* |
| *Sum IR* | *3,536* | *27* | *11* | *10* | *3* | *70* | *116* | *730* | *232* | *2,337* |
| *Sum MR* | *2,894* | *25* | *11* | *14* | *1* | *54* | *61* | *597* | *160* | *1,971* |
| **Total Sum** | **6,577** | **56** | **20** | **23** | **5** | **142** | **166** | **1410** | **399** | **4,356** |

Table 2. Effects of forest conversion to rubber plantation on composition of total and specific fungal functional group communities, as determined by PERMANOVA test. *P* values were based on 999 permutations and Bonferroni correction was applied in all cases.

| Treatment | Total fungi | Saprotrophic fungi | Plant parasitic fungi | Mycorrhizal fungi |
|---|---|---|---|---|
| Overall | $F = 2.81$, $P = 0.003$ | $F = 2.81$, $P = 0.003$ | $F = 3.25$, $P = 0.003$ | $F = 2.13$, $P = 0.003$ |
| Natural rainforest *vs*. Immature rubber plantation | $F = 2.62$, $P = 0.003$ | $F = 2.69$, $P = 0.003$ | $F = 3.17$, $P = 0.003$ | $F = 2.09$, $P = 0.003$ |
| Natural rainforest vs. Mature rubber plantation | $F = 3.01$, $P = 0.003$ | $F = 3.03$, $P = 0.003$ | $F = 3.26$, $P = 0.003$ | $F = 2.10$, $P = 0.003$ |
| Immature rubber plantation vs. Mature rubber plantation | $F = 2.79$, $P = 0.003$ | $F = 2.71$, $P = 0.003$ | $F = 3.34$, $P = 0.003$ | $F = 2.20$, $P = 0.003$ |

Table 3. Significant predictors in the distance-based redundancy analysis (dbRDA) model of total, saprotrophic, plant pathogenic and mycorrhizal fungal community composition.

| **Factors** | ***F*** | ***P*** |
|---|---|---|
| **Total fungi** | | |
| Soil pH | 4.57 | 0.001 |
| Conversion type | 2.37 | 0.001 |
| TN | 2.24 | 0.001 |
| $NH_4$ | 1.90 | 0.002 |
| **Saprotrophic fungi** | | |
| Soil pH | 3.81 | 0.001 |
| Conversion type | 2.34 | 0.001 |
| $NH_4$ | 2.11 | 0.001 |
| TN | 2.02 | 0.001 |
| **Plant pathogenic fungi** | | |
| Soil pH | 4.90 | 0.001 |
| Conversion type | 4.24 | 0.001 |
| **Mycorrhizal fungi** | | |
| Soil pH | 3.53 | 0.001 |
| Conversion type | 2.36 | 0.001 |

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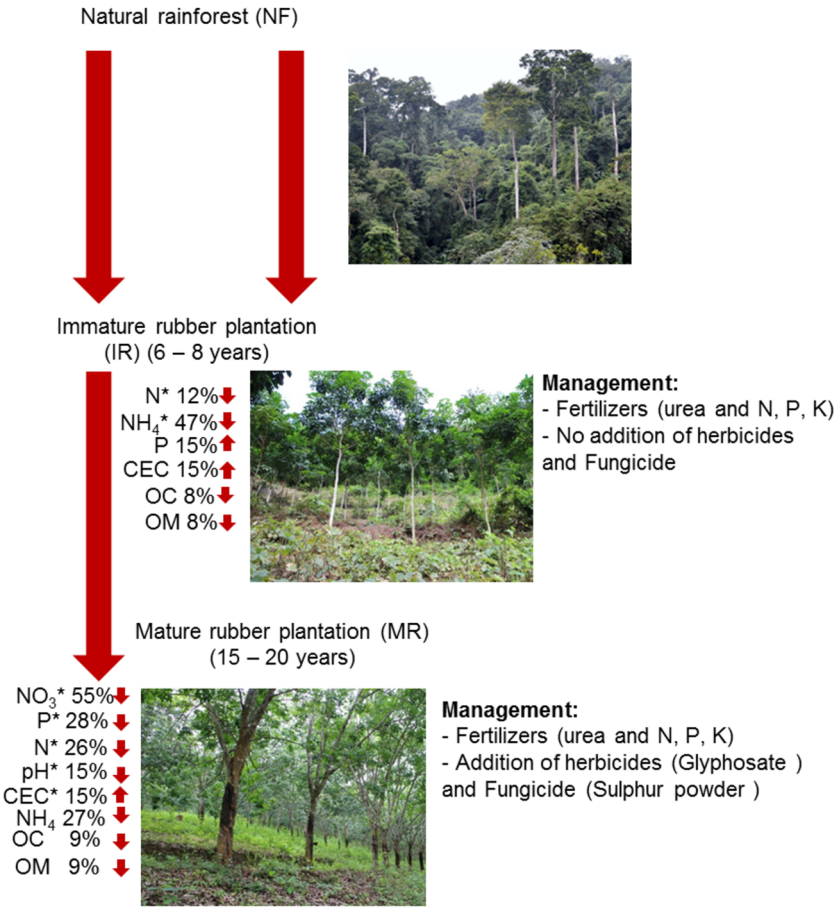


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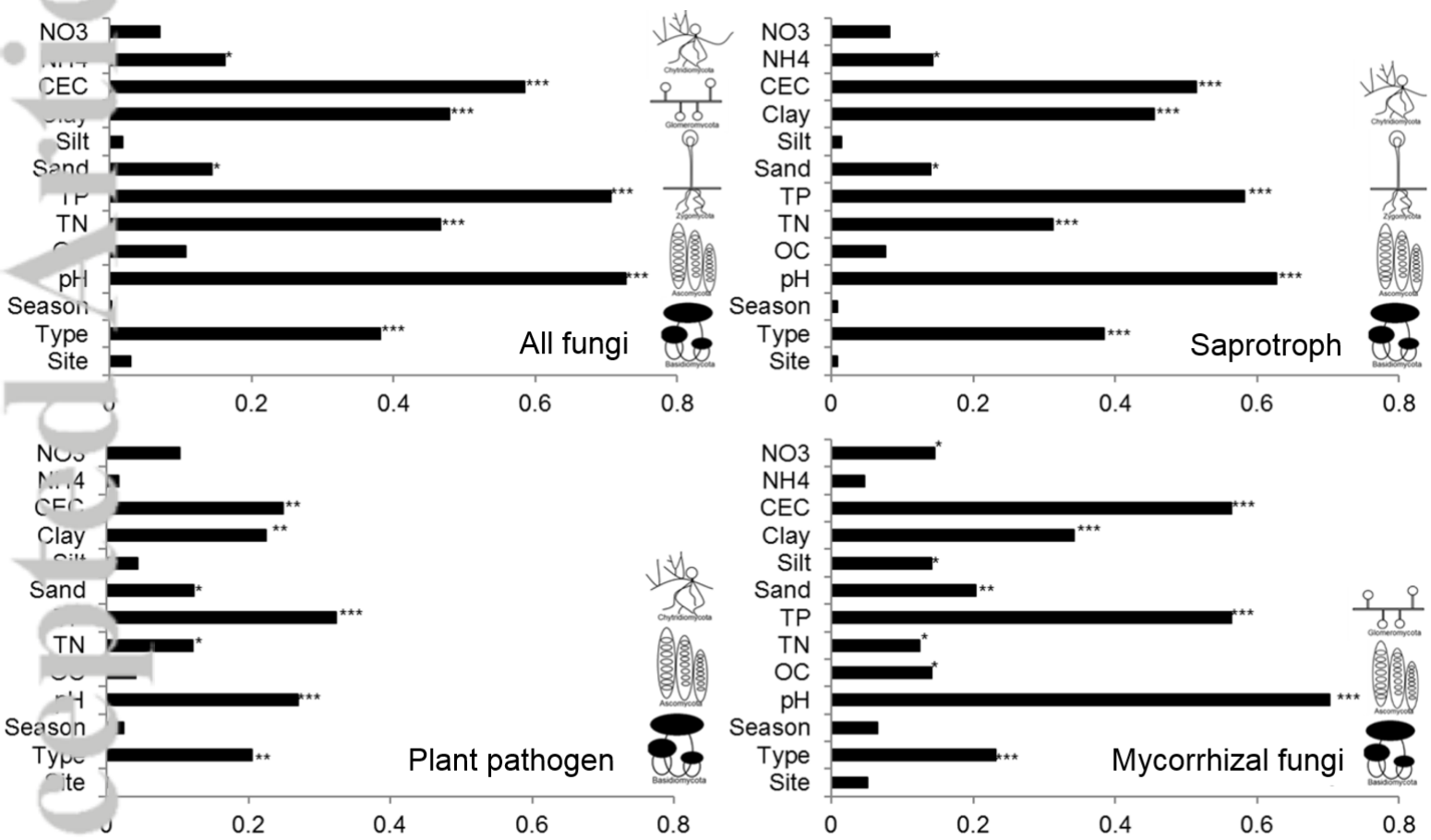


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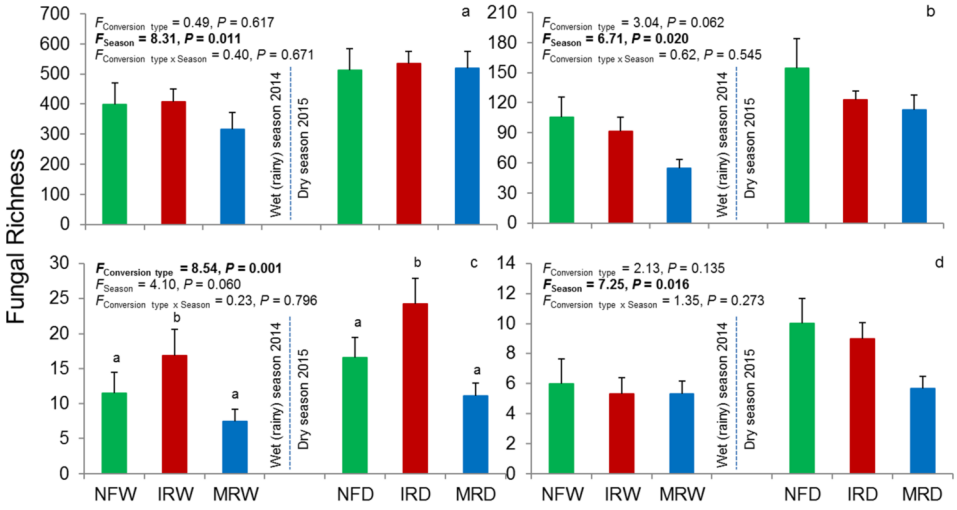


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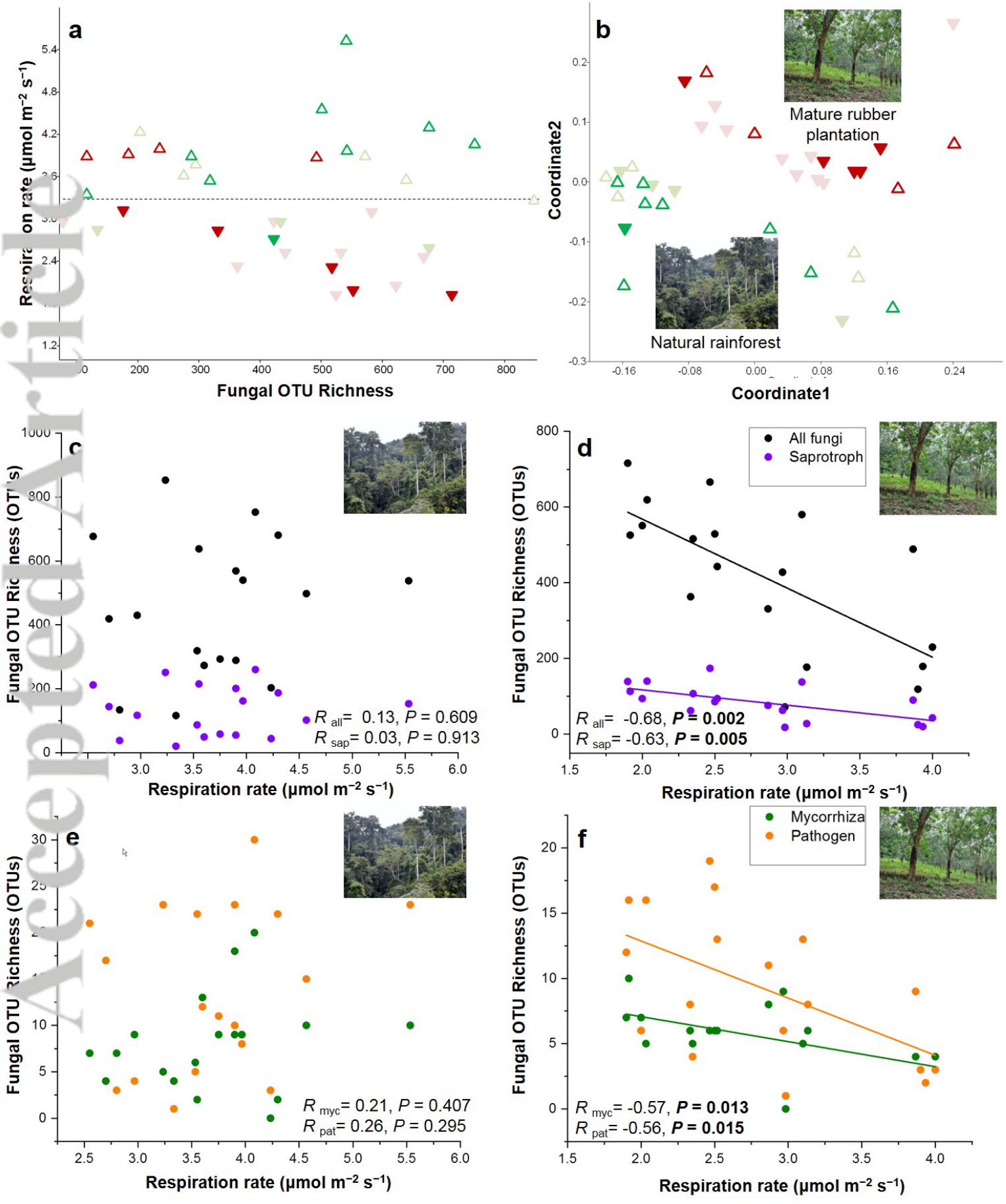


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