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Drivers of fungal succession in three different woody species in a tropical forest fragment

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ABSTRACT
Wood-inhabiting fungi are important in forest ecosystems and play an essential role in nutrient and carbon recycling. However, the drivers of fungal succession are poorly understood. We assessed the fungal succession of three tree species in a fragment of Atlantic forest in the state of São Paulo, Brazil. Decomposing branches from different locations were sampled every four months for two years. This study identified 2 309 Operational Taxonomic Units (OTUs), predominantly from the phyla Ascomycota and Basidiomycota, in freshly cut branch fragments of tropical angiosperm trees. The richness exceeded comparable studies in non-tropical ecosystems, attributed to the unique substrate and sequencing methodology. Despite variations in OTU abundance across time points, no significant differences were observed between the three tree species studied. Decomposition

KEY WORDS
Wood decay, decomposition, neotropical fungi, fungal community structure, ITS2, lignin, branches, next-generation sequencing, community assemblage, diversity.

rates exhibited temporal variations, with more intense activity during summer and reduced rates in winter. Nitrogen content increased over time, associated with microbial action. Cellulose degradation predominated, followed by hemicelluloses and lignin. Fungal community structure showed a shift from Ascomycota to Basidiomycota, indicative of a natural decomposition pattern. Factors influencing decomposition included substrate specificity, individual branch history, and the fungal community's resilience and competitiveness. The study emphasizes the complex interplay between biotic and abiotic factors in shaping fungal communities during wood decomposition in tropical forests. Overall, the findings contribute to understanding the intricate dynamics of fungal succession, nutrient cycling, and substrate specificity in tropical wood decay processes.

MOTS CLÉS
Pourriture du bois, décomposition, champignons néotropicaux, structure des communautés fongiques, ITS2, lignine, branches, séquençage de nouvelle génération, assemblage des communautés, diversité.

RÉSUMÉ
Facteurs de succession fongique chez trois espèces ligneuses différentes dans un fragment de forêt tropicale.
Les champignons vivant dans le bois sont importants dans les écosystèmes forestiers et jouent un rôle essentiel dans le recyclage des nutriments et du carbone. Cependant, les facteurs responsables de la succession fongique sont mal compris. Nous avons évalué la succession fongique de trois espèces d'arbres dans un fragment de forêt atlantique de l'État de São Paulo, Brésil. Des branches en décomposition provenant de différents endroits ont été échantillonnées tous les quatre mois pendant deux ans. Cette étude a identifié 2 309 unités taxonomiques opérationnelles (OTU), principalement issues des phyla Ascomycota et Basidiomycota, dans des fragments de branches fraîchement coupées d'angiospermes tropicaux. La richesse a dépassé les études comparables dans les écosystèmes non tropicaux, attribuée au substrat unique et à la méthodologie de séquençage. Malgré les variations de l'abondance de l'OTU au fil du temps, aucune différence significative n'a été observée entre les trois espèces d'arbres étudiées. Les taux de décomposition présentaient des variations temporelles, avec une activité plus intense en été et des taux réduits en hiver. La teneur en azote a augmenté avec le temps, associée à l'action microbienne. La dégradation de la cellulose prédominait, suivie par les hémicelluloses et la lignine. La structure de la communauté fongique a montré un changement d'Ascomycota vers Basidiomycota, indiquant un modèle de décomposition naturelle. Les facteurs influençant la décomposition comprenaient la spécificité du substrat, l'histoire de chaque branche ainsi que la résilience et la compétitivité de la communauté fongique. L'étude met l'accent sur l'interaction complexe entre les facteurs biotiques et abiotiques dans la formation des communautés fongiques lors de la décomposition du bois dans les forêts tropicales. Dans l'ensemble, les résultats contribuent à comprendre la dynamique complexe de la succession fongique, du cycle des nutriments et de la spécificité du substrat dans les processus de décomposition du bois tropical.

INTRODUCTION

The decomposition of deadwood plays an essential role in the global carbon and nutrient cycling in forest ecosystems (Stokland *et al.* 2012). Deadwood is a vast store of carbon, and only a few organisms have evolved to digest its main structural components into simple compounds that are accessible to other organisms (Purahong *et al.* 2018). Fungi are the primary decomposers of deadwood (Bradford *et al.* 2014), and fungal communities change over the course of deadwood decomposition in a sequential occupation called fungal succession (Rayner & Todd 1979). Fungal succession varies depending on the forest biome, tree species and among different coarse woody debris components like bark, sapwood or heartwood (Rajala *et al.* 2012; Purahong *et al.* 2018; Leonhardt *et al.* 2019). Previous studies from temperate and boreal zones demonstrated that macroclimate, resource quality and tree species are diversity drivers of wood-inhabiting fungi (Stokland *et al.* 2012; Heilmann-Clausen *et al.* 2014; Baber *et al.* 2016; Krah *et al.* 2018). While the structuring of fungal communities are well studied in temperate and boreal zones, only a few studies address the ecology and structure of fungal community, and the mechanisms involved in the wood decomposition dynamics in the tropics (Chambers *et al.* 2000, 2004; Blanc *et al.* 2009; Hérault *et al.* 2010; Abril *et al.* 2013; Fearnside 2015).

Mycology studies in the tropics are mainly focused on taxonomy and systematics, however, there are a few studies regarding fungal communities in the tropics. For example, Vaz *et al.* (2017) published the first study using next-generation sequencing analyzing the diversity of wood-decaying fungi in tropical forests, but no correlations to environmental factors were performed. Also, Olou *et al.* (2019) used traditional inventory and identification of polyporoid fungi to conclude that richness of wood-inhabiting fungi is affected by resource quality, and fungal community composition is strongly affected by macroclimate.

Despite the importance of dead wood for the carbon balance of forests, factors involved in wood decomposition are poorly understood, particularly in tropical forests (Bradford *et al.* 2014). Thus, a deeper knowledge of the fungal diversity in deadwood should be considered, in order to maximize our understanding of forest ecosystem functions and biodiversity conservation strategies. The focus of the present study was to assess the composition and distribution patterns of wood-inhabiting fungal communities in the early phase of

decomposition (two years) in three tree species, distributed in four locations within an Atlantic forest fragment. Our objectives were: 1) to describe the succession of the fungal taxa during wood decay; 2) identify the biochemical constitution of the substrate and its variation over time; and 3) study the role of microclimate, biochemical and tree substrate variables on the fungal communities in space and time.

MATERIAL AND METHODS

Study area

The study was conducted in Parque Estadual das Fontes do Ipiranga (PEFI, 23°38'08"S, 46°36'48"W), an Atlantic Forest fragment located within the city of São Paulo, São Paulo state, Brazil (Appendix 1A). With a total area of 495 ha, the park has a mesothermal humid climate and annual average temperature of 19.1°C (Plano de Manejo do PEFI 2008).

Experimental design and sampling

We selected three tree species for this study: *Alchornea sidifolia* Müll. Arg. (Euphorbiaceae, pioneer), *Piptadenia gonoacantha* (Mart.) J.F.Macbr. (Fabaceae, late pioneer) and *Calyptranthes grandifolia* O.Berg. (Myrtaceae, secondary). We selected these species because they are native common species (Tanus *et al.* 2012). Branches, 3 to 5 cm in diameter and ± 80 cm long, were cut from the trees and carefully placed within plots of 1 m^2, in four different locations within the forest fragment (Appendix 1B). Two points were located in the interior of the forest fragment (P1 and P3) and two at the forest edge (P2 and P4). Each site contained three branches of each tree species (nine branches in total). Branches were sampled every four months (from March 2016) for two years (six times). We cut approximately 2 cm from the terminal portion of each branch at each sample for biochemical tests and DNA extraction (246 samples in total because six samples went missing).

Fungal community analysis

We used an electric drill with a flame-sterilized, with a 4 mm wood auger drill bit, before DNA extraction to extract sawdust from each wood sample taking care not to contaminate the samples with exogenous DNA (Hoppe *et al.* 2015; Kubart *et al.* 2016; Hu *et al.* 2017). We stored approximately 1.5 to 3 g of sawdust material in a sterile tube per sample. We extracted sawdust DNA with DNeasy PowerLyzer PowerSoil Kit™ (QIAGEN). The initial sawdust was between 50 and 120 mg. DNA concentration was determined with a Qubit™ Fluorometer (Life Technologies). Before PCR reactions, DNA extracts were normalized to 1 ng μl^{-1}. The fungal ITS2 region was amplified using tagged versions of the primers gITS7 (Ihrmark *et al.* 2012) and ITS4 (White *et al.* 1990). Tags were eight to nine nucleotides and differed in at least three positions from each other, as described by Frøslev *et al.* (2017). PCR reactions contained 14.6 µl of ultrapure water, 1 U/µl of AmpliTaq Gold® DNA Polymerase, 1X Gold buffer (10x), 2.5 mM of $MgCl_2$, 0.83 mg/ml of bovine serum albumin (BSA) (20 mg ml^{-1}), 0.08 mM of each dNTP, 0.625 µM of each tagged primer and 1 µl of DNA sample in a 25 µl total reaction volume. PCR parameters used were an initial denaturation step at 95°C for 5 min, followed by 31 cycles of amplification at 95°C for 30 s, 55°C for 30 s and 72°C for 1 min, and final elongation step at 72°C for 7 min. PCR products were visualized on 1% agarose gels. PCR products were pooled for a total of seven pools, each pool included at least 4 negative PCR controls. PCR pools were purified with MinElute PCR purification kit (QIAGEN GmbH) and the length of PCR amplicons was verified on Bioanalyzer High-Sensitivity Chip (Agilent Technologies, Inc., Santa Clara, California, Unites States). Each of the seven pools was built into separate sequencing libraries. Libraries were built using the TruSeq DNA PCR-Free Library Preparation Kit (Illumina), replacing all the manufacturer suggested clean up step (sample purification beads) with MinElute purification (MinElute PCR purification kit, QIAGEN GmbH). A final library purification was carried out to remove adapter dimers with Agencourt AMPure XP beads (Beckman Coulter, Inc., CA, United States). Sequencing was carried out on MiSeq (Illumina Inc., San Diego, CA, Unites States), at the Danish National High- throughput DNA Sequencing Centre, using one full 300 bp paired-end run.

Biochemical analysis of wood

We assessed basic wood density for each sample (Trugilho *et al.* 1990), by chopping and grinding the dry wood in a knife mill until it could pass a 0.75 mm mesh. We made an extraction for 6 hours in a Soxhlet apparatus with 95% ethanol. We calculated the content of ethanol-soluble components (extractives) by the difference between initial mass and post extraction mass. We also determined the content of structural components (cellulose, hemicelluloses and insoluble lignin) from the acid hydrolysis of samples free of extractives with H_2SO_4 72% (Ferraz *et al.* 2000). We determined the acid-soluble lignin content by spectroscopy of a 1:10 dilution of the hydrolysate at 205 nm, with an absorbance of 105 nm as standard (Dence 1992). We performed a chromatographic analysis on a high performance liquid chromatography using a BIORAD HPX-87H column heated to 45°C and H_2SO_4 5 mM at 0.6 ml/min as eluent and a refractive index detector (SHIMADZU). We determined sugar concentration by calibration curves prepared to analytical grade standards, dried under silica and vacuum. Cellulose was obtained by detection in its monomeric form in glucose units. Hemicellulose (polyoses) was obtained by detecting its monomeric forms: xylose (from xylan chains), arabinose and acetic acid (pendant groups present in xylan chains in arabinosyl and acetyl forms).

We homogenized lyophilized wood samples to quantify nitrogen by using a ball mill (Fritsch, Idar-Oberstein, Germany). We measured the nitrogen contents (% N) of the samples and their isotopic signature by weighing 10 mg subsamples in tin combustion cups for elemental analysis. We measured the Isotopic ratios of $^{15}N/^{14}N$ by the combustion of Dumas (1,020°C) in an elemental analyzer (EA Flash 2000; Thermo Scientific, Bremen, German y) coupled in continuous flow mode to a Delta V Advantage isotope ratio mass

spectrometer (Thermo Scientific). We converted all the results of the biochemical analyzes (lignin, cellulose, hemicellulose, extracts, nitrogen), obtained in percentage, into grams, and then considering a standard volume of 1 ml of sample, all masses were standardized in order to compare the mass loss of a standard volume of sample wood.

Microclimatic analysis

We measured the microclimate characterization (temperature, absolute air humidity and relative humidity) using autonomous digital temperature and relative humidity recorders with ± 0.2°C and ± 2.5% accuracy, respectively. The equipment was installed at the 4 sampling sites near the branches, approximately 20 cm from ground level and the sampling interval for temperature and humidity data was every hour. The sensors were installed in passive ventilation mini-enclosures developed by Armani & Galvani (2006). We collected data every four months using the BoxCar® Pro program (version 4.3.1.1). In addition to the temperature and humidity variation data, we obtained the rainfall data from the meteorological station of the Astronomical and Geophysical Institute of the University of São Paulo (IAG-USP), located within PEFI.

The role of biochemical, microclimate and tree substrate on fungal communities

To investigate the key drivers of fungal community composition, we calculated richness and β diversity (βsor: Sorensen dissimilarity, Baselga, 2010) as an operational response variable for the underlying structure of fungal communities, to further test it as a function of variables of tree biochemistry, microclimate, tree species, edge or interior, and time.

Bioinformatics

Sequence analyses were performed using LULU pipeline (Frøslev *et al.* 2017). Briefly, sequencing output was demultiplexed and combined tag and primer sequence were removed using CUTADAPT v1.17 (Martin 2011). Demultiplexed reads were subsequently processed and assessed for quality by following the main steps outlined in the high-resolution DADA2 pipeline. Matching of forward and reverse reads was ensured before applying the following DADA2 steps: learn the error rates (*learnErrors*), dereplication (*derepFastq*), sample inference (*dada*), merge paired reads (*mergePairs*), construction of sequence table (*makeSequenceTable*) and chimera removal (*removeBimeraDenovo*). This was followed by ITS extraction with ITSx (Bengtsson-Palme *et al.* 2013) and clustering with VSEARCH software (Rognes *et al.* 2016) at 98.5% – the consensus clustering level used to delimit species hypotheses (SHs) in the UNITE database (Kõljalg *et al.* 2013), and subsequent post-clustering curation with LULU (Frøslev *et al.* 2017) to eliminate remaining redundant sequences. Taxonomic assignment of the Operational Taxonomic Units (OTUs) was done by matching the OTUs with VSEARCH against the 2019 UNITE general FASTA release. Documentation of the steps for analyzing the sequence data can be found on GitHub along with necessary files and links to the raw data.

Statistical analyses

For the ecological statistical analyses R software (R Core Team 2017) was used. A cluster analysis with the Bray-Curtis dissimilarity index was performed using the vegan package version 2.5-1 (Oksanen *et al.* 2018), cophenetic distance = 0.876. Studies involving the richness of OTUs were performed with analysis of variance (ANOVA) when data were normal, and the Kruskal-Wallis test when they did not follow normal distribution. In abiotic factors, repeated measures ANOVA was used because the data collected referred to the same samples in a temporal sequence. In addition, linear regression and correlation were also performed to compare microclimatic data and percentage of mass loss of the samples. Species richness among wood samples were calculated and plotted based on the microbiomeSeq, phyloseq and vegan R packages.

For multivariate analysis, the vegan package (Oksanen *et al.* 2018) was used. Venn diagram was produced with the OTUs distribution per tree species with the Limma package (Ritchie *et al.* 2015). A stacked barplot was built with the phyloseq package (McMurdie & Holmes 2013) to aid the visualization of trends in the fungal community between the different tree species, time of decomposition and locations.

For selection of fungal taxa with significant changes in its abundance accordingly to tree species, time and location, a global negative binomial model (nbGLM) was applied to the dataset at genus level (mvabund package) (Wang *et al.* 2012) and analysis of deviance ($p < 0.01$) was used. The selected taxa and their abundance were graphically displayed in a heatmap based on centered-scaled $\log_{10}$-transformed counts for each taxon.

The variance in the fungi composition was analyzed by redundancy analysis (RDA). Before the analyses, OTUs relative abundance was Hellinger transformed to avoid problematic Euclidean distances used in the RDA (Legendre & Gallagher 2001). Wood and climatic parameters were log-transformed to avoid bias caused by differences in unit scales. A forward selection of variables ("forward.sel" function, "vegan" R package) was used to select the environmental variables and spatial factors that best explained variation in the fungal community composition. Potential collinearity between the selected predictors was examined by analyzing variance inflation factors (VIF) using the "vif.cca" function. Variables with VIF >10 were excluded if any. The final selected variables were subjected to variation partitioning to determine the proportion of variation explained by the environment and the space (Borcard *et al.* 1992) using the function "varpart". The significance of each partition was obtained by applying a partial RDA after controlling for the other factors ("anova.cca" function). These analyses were carried out using the "vegan" R package (Oksanen *et al.* 2018). For representation purposes, an RDA ordination including every selected variable was performed using "ggplot2" R package (Wickham 2008).

To investigate how the biochemical constitution of the substrate, the microclimate and other environmental variables influence the composition of the fungal community (species richness and beta diversity) during the decomposition process, we used generalized linear models (GLM) with appropriate error distributions (poisson for richness γ and *beta* for β-diversity).

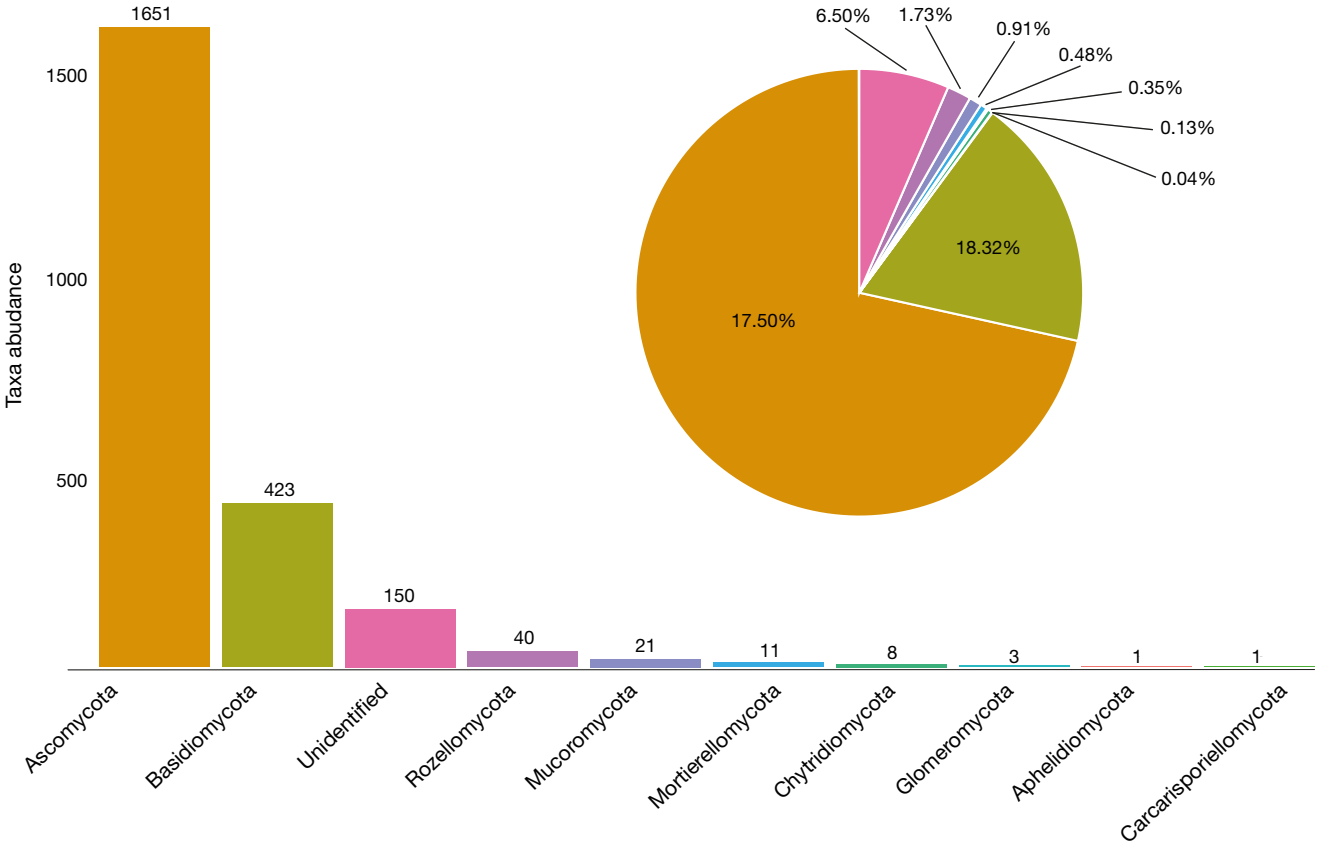

Fig. 1. — Operational Taxonomic Units (OTUs) abundance among the phyla, across all samples.

We modeled the two response variables measured in each of the seven time intervals (between t0 and t6), see separately as a function of biochemical constitution of the substrate, the microclimate and other environmental variables (habitat). We also examined the quadratic forms of these predictors to account for potentially non-linear relationships with forest cover, as well as a reference model without predictors. We used an AICc model selection approach to select the best model (including the spatial scale of landscape-level forest cover) for each dependent variable, considering models with $\Delta AICc \leq 2$ as having the strongest empirical support. To understand how habitat association modulates biodiversity response, we ran the same analyses separately for both forest specialists (FS) and non-forest specialists (NFS). We ran all analyses in R version 3.6.1 with the packages: bbmle (Bolker & R Development Core Team 2017), car (Fox & Weisberg 2019), DHARMa (Hartig 2022) and MASS (Venables & Ripley 2002).

RESULTS

Fungal community composition

Sequencing of 246 samples from seven times points across three tree species resulted in a total of 1, 861, 279 reads. After clustering and exclusion of non-fungal sequences, the full dataset contained 2,309 OTUs. Of these, 10 phyla, 33 classes, 88 orders, 177 families, and 319 genera were identified, considering "unidentified" as a category at all taxonomic ranks (Fig. 1). Within Ascomycota which was the most abundant phyla (71.5%), Sordariomycetes was the most abundant class followed by Dothideomycetes, Eurotiomycetes and Leotiomycetes. Of the *Basidiomycota phylum* (18.3%) Agaricomycetes was the most abundant class, followed by Tremellomycetes (Fig. 1).

We found that there is a high number of exclusive UTUs in each tree species and less than 35% of shared OTUs (Appendix 2). Another remark is a closer relationship between *Alchornea* and *Piptadenia* species, and *Calyptranthes* as the most different. Species vary in wood density and *Calyptranthes* has the highest density, which can make the compounds more inaccessible to fungi.

Wood decomposition

Initially (T0), *Calyptranthes* showed the highest wood density with 0.61 g/ml, followed by *Piptadenia* with 0.40 g/ml and *Alchornea* with the lowest density of 0.26 g/ml. The wood density decreased throughout the experiment, when comparing among the tree species from Times 0 to 6 ($W = 92$, p-value = 0.0001905 in *Alchornea*; $W = 108$, p-value = 0.008625 in *Piptadenia*; and $W = 114$, p-value = 0.002185 in *Calyptranthes*), indicating that there was mass loss due to decomposition (Fig. 2).
We observed that *Alchornea* and *Piptadenia* lost 30-35% of wood mass between points 1 and 2 with respect to points 3 and 4 ($p = 0.001$) for, while *Calyptranthes* showed an average loss

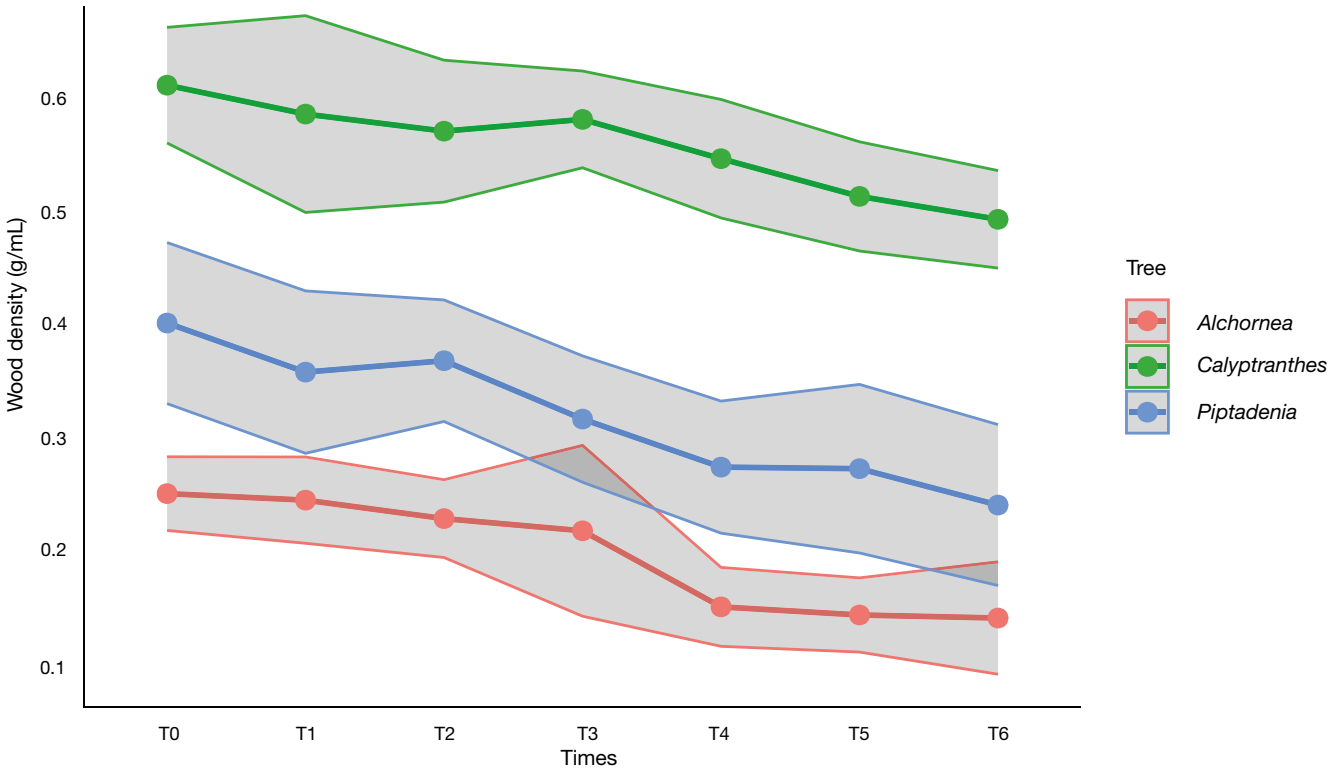

Fig. 2. — Mean and confidence interval of wood density over time per tree species.

between 15 and 25% in the four sampled points. We also observed a higher degradation rate in *Alchornea* and *Piptadenia* between sequential times (p = 0.001) (Fig. 3).

A low correlation between the percentages of density loss and the abiotic factors measured in this study (temperature, rainfall, absolute humidity and relative humidity), was observed (Appendix 6). In a regression test, although the data were significant (except for relative humidity), the value of r^2 was low, no more than 5% of the correlation's explanatory power, yet all curves were positive, namely temperature (p = 0.012 and r^2 = 0.029), rainfall (p = 0.001 and r^2 = 0.049), absolute humidity (p = 0.017 and r^2 = 0.027) and relative humidity (p = 0.25 and r2 = 0.006).

We observed a slight increase in the mass of nitrogen from *Calyptranthes* branches over time (p <0.001) (Fig. 4). In *Piptadenia*, a decrease in nitrogen values at Times 1 and 2 and a subsequent increase was noted. *Alchornea* twigs with the lowest nitrogen values showed a slight increase in concentration but still within the confidence interval.

To understand how the Time and Tree factors affect nitrogen variability – considering the specific repetition structure of the data, i.e., the substrates (samples) were the same over time –, a Repeated Measures ANOVA combined with a Two-Way ANOVA analysis was performed. The results revealed a significant interaction between the factors Time and Tree (F = 2.020, p = 0.0244) in the variation of Nitrogen, indicating that the response over time differs between the tree species considered. Furthermore, the analysis within each substrate showed that the Tree factor had a significant impact on the observed variations (F = 10.432, p = 0.000364), while the Time factor was not statistically significant (F = 1.672, p = 0.1941). These results suggest that the tree species has a more pronounced influence on the variation of Nitrogen than the time itself, especially when we consider the different interactions in the substrates.

Lignin, cellulose, hemicelluloses (Xylan, Arabinose and Acetyl) and wood extractive contents were also analyzed throughout the experiment with the same statistical analyses (Fig. 5). The analysis aimed to understand how the factors Time and Tree affect the variability of the constituent compounds of wood, considering the specific structure of data repetition (Substrate). The results suggest that the sample collection time have a significant impact on the variability of the response variable lignin (F value = 12.18, p <0.001), cellulose (F value = 8.45, p <0.001), xylan (F value = 11.63, p <0.001), arabinose (F value = 6.44, p <0.01), acetyl (F value = 14.67, p <0.001) and extratives (F value = 6.32, p <0.01). Tree species also has a significant influence on the amounts of the wood compounds, lignin (F value = 65.33, p <0.001), cellulose (F value = 35.91, p <0.001), xylan (F value = 54.30, p <0.001), arabinose (F value = 57.44, p <0.001), acetyl (F value = 94.07, p <0.001) and extratives (F value = 60.36, p <0.001).

When evaluating the effect of the "Time" factor and its interaction with the "Tree" factor on the response variable within each substrate separately, cellulose (F value = 1.97, p <0.05), arabinose (F value = 7.33, p <0.001), acetyl (F value = 1.87, p <0.05) and extratives (F value = 8.48, p <0.001) had a significant variation; except for lignin (F value = 0.51, p = 0.907)

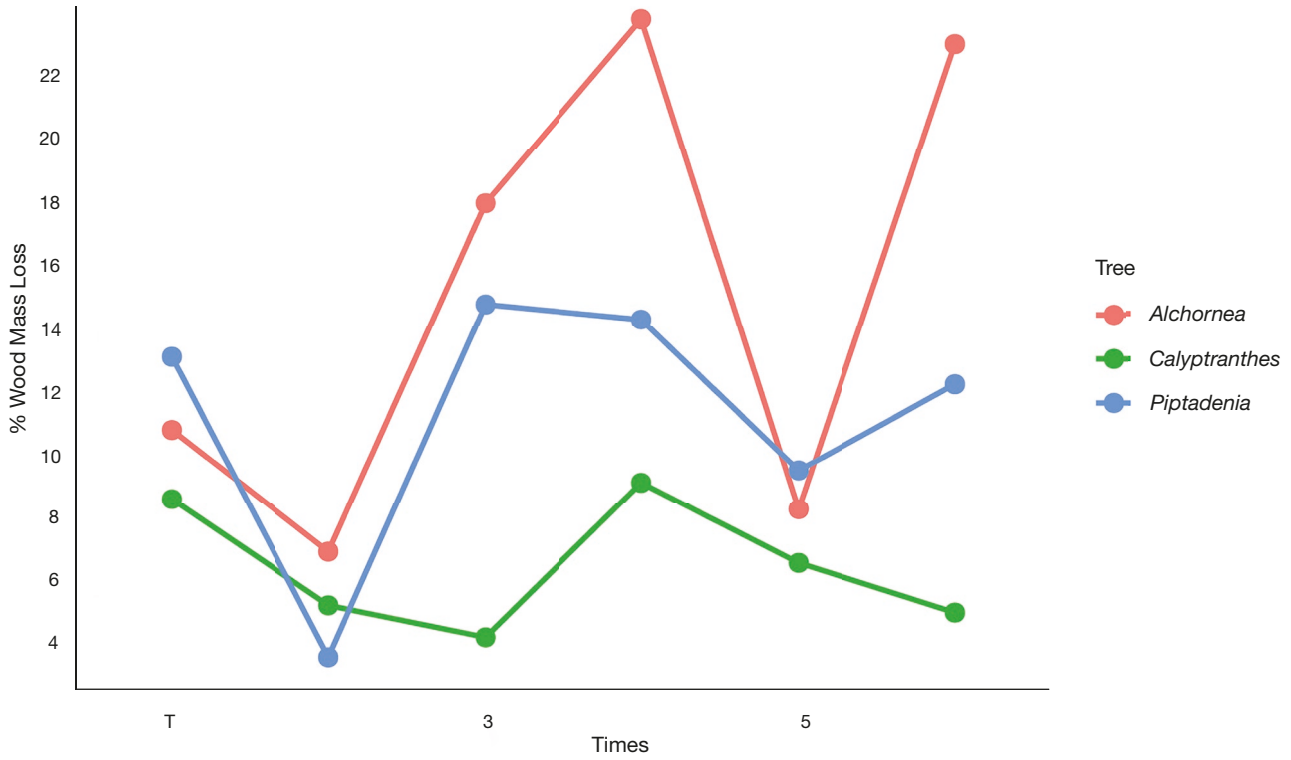

Fig. 3. — Percentage of mass loss between one time and the next of the three tree species (p = 0.001).

and xylan (F value = 1.78, p = 0.0529). This result suggests that the effect of time on the response variable may depend on the type of tree.

As well as density loss, the percentage of decay of the molecules at the end of the experiment was higher in *Alchornea* and *Piptadenia* samples and lower in *Calyptranthes* (Appendix 3). When calculating the lignin/cellulose ratio including all tree species together (Appendix 4), it was possible to find significant values of differences between the mean ratios between the times in the nonparametric Kruskal-Wallis analysis (p <0.001). And also the lignin/hemicellulose ratio, with p = 0.025.

The role of biochemical, microclimate and tree substrate on fungal communities

We found that the best fitted model (Table 1) for species richness is the interaction between time and tree species (R2 = 0.74). While the same model was selected also for beta-diversity (R2 = 0.431, AIC = -318.9, dAIC <2), the models where time interacted independently with substrate biochemistry and microclimate where also selected (R2 = 0.393 for both models) (Table 1).

Species richness

According to the selected model, the richness of fungal species in the studied community is better explained by the interaction between time and the species of tree (Fig. 6; Appendix 9).

The best fitted models were the ones containing time interacting individually with microclimate, substrate biochemistry and tree species (Fig. 7; Appendix 10).

Table 1. — Summary of the model selection performed to investigate the variables affecting the richness and beta diversity in a community of fungi. Selected models presented values of dAIC <2.

Models	AIC	dAIC	df
Richness			
Time * tree	2020	0	22
Beta diversity			
Time * biochemistry	-339.6	0	13
Time * microclimate	-337.9	0	19
Time * tree	-318.9	1.8	7

Redundancy analysis (RDA) explains a small percentage of the variation in fungal community composition (total 8.7% for environmental variables). The ordination plot shows that the fungal community composition is shaped by both wood properties (e.g. nitrogen, carbon, lignin), explaining 3% of the variation and climate variables (e.g. rain, relative humidity), explaining 2% of the variation (Appendix 5).

DISCUSSION

In this study we found 2,309 OTUs, mostly from phyla Ascomycota and Basidiomycota, a pattern similar to that found by Vaz *et al.* (2017), also in the Brazilian Atlantic forest. This is more OTUs than it was found in other comparative studies using next generation sequencing techniques in other non-tropical ecosystems (e.g. Fukasawa & Matsuoka 2015; Runnel *et al.* 2015; Mäkipää *et al.* 2017). However, our study dealt with freshly cut branch fragments of tropical angiosperm trees, and

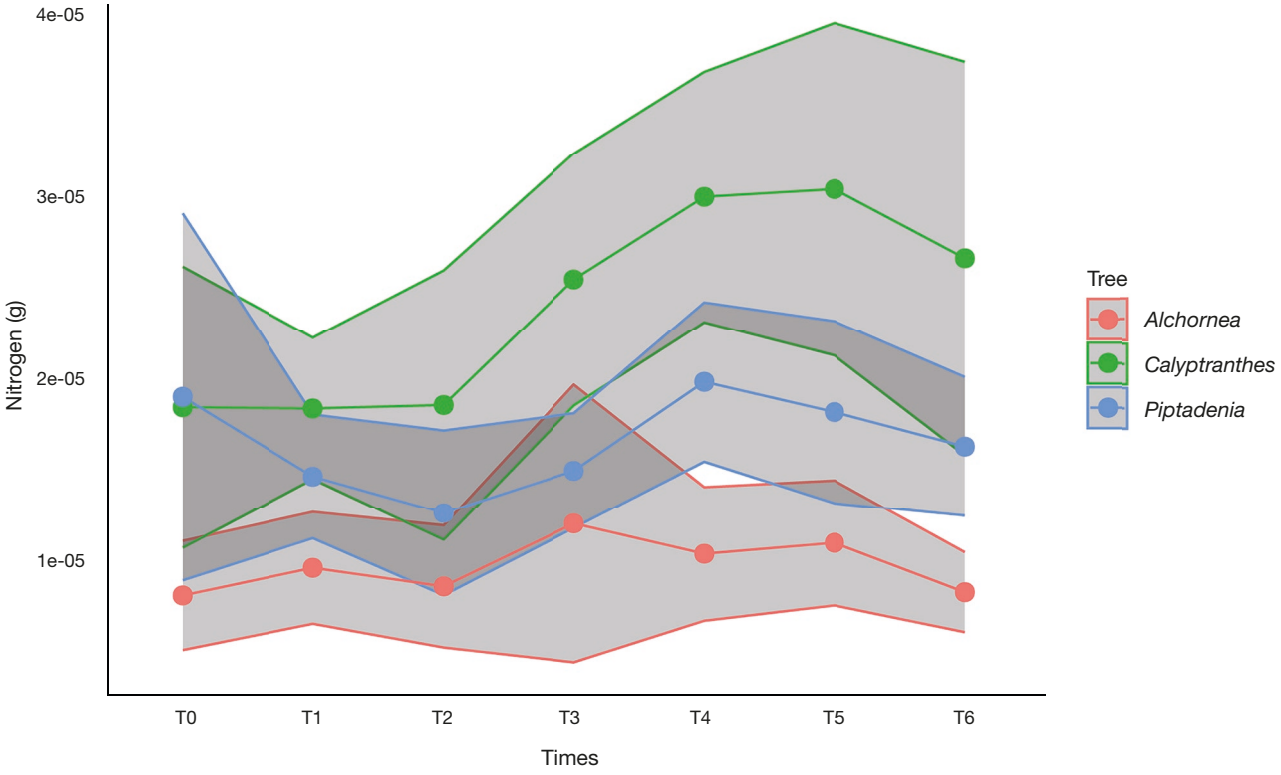

Fig. 4. — Mean and confidence interval of nitrogen quantity over time.

most of the Nordic studies with large logs of gymnosperms, often at the later stages of decay. Another factor that complicates comparisons by total OTU numbers is differences in sequence processing and clustering pipelines. Dead standing trees and fallen logs from temperate zones also present differences in degradation rates with time (Mäkinen *et al.* 2006; Angers *et al.* 2011) because microclimatic conditions influence the presence and activity of the decomposer community and the mineralization rates.

Considerable differences in decomposition rates were observed between individual branches and no differences were found between branches of the three tree species investigated. The percentages of mass loss is not uniform over time, and is dependent on other factors, corroborating the findings of other studies (Means *et al.* 1985; Romero *et al.* 2005; Mäkinen *et al.* 2006; Harmon *et al.* 2011; Fravolini *et al.* 2018). Even presenting the greatest richness and abundance of OTUs at Time 1 and 2, this condition was not the moment of greatest mass loss of the samples. In contrast, Time 2 and Time 5 showed the lowest loss rates; these samples refer from July to November 2016 and from July to November 2017, respectively. July in São Paulo, Brazil is the winter period, where temperature and humidity are lowest. Thus, two periods of wood decomposition rate can be observed in a tropical forest, a more intense moment during the summer (hot and humid) and a moment of lower rates during the winter (cold and dry).

In a correlation analysis between the percentages of loss and abiotic factors, it can be inferred that what determined the largest differences between mass losses is more related to biotic factors, since the four points sampled showed little variation in terms of temperature, relative and absolute humidity and rainfall (which were the same for all four points). For a better understanding of abiotic factors in the decomposition process it would be necessary to choose much more geographically distant sites with less similar phytogeographic characteristics. Similar results were found by Mäkinen *et al.* (2006) and Yatskov *et al.* (2003), as the weather conditions in the sampled areas were quite similar, they had no profound effect on the decomposition rate when compared with each other.

We found that nitrogen increases in time. Wood is poor in nitrogen, but its contents increased due to the action of mycorrhizal fungi and rhizomorphic forming, as well as nitrifying bacteria (Romero *et al.* 2005; Fukasawa *et al.* 2014; Mäkipää *et al.* 2017). In the early times of the experiment, nitrogen values did not increase, because nitrogen was used by fungi (mineralization) (Romero *et al.* 2005). From Time 3, nitrogen increases – immobilization of nitrogen. This increased nutrient use may be associated with the richness and abundance of OTUs in the early days.

In the three substrates, cellulose was the most consumed compound, followed by hemicelluloses and, finally, lignin, a more recalcitrant molecule that is responsible for retain carbon longer in its immobilized form, which is more difficult to mineralize (Sutherland *et al.* 1979; Harmon *et al.* 1986; Santhanam *et al.* 2012). In the study by Harmon *et al.* (1986), the values found in the lignin/cellulose ratio for undecayed wood logs ranged from 0.6 to 1.2 for angiosperms. The data obtained in the present study are slightly

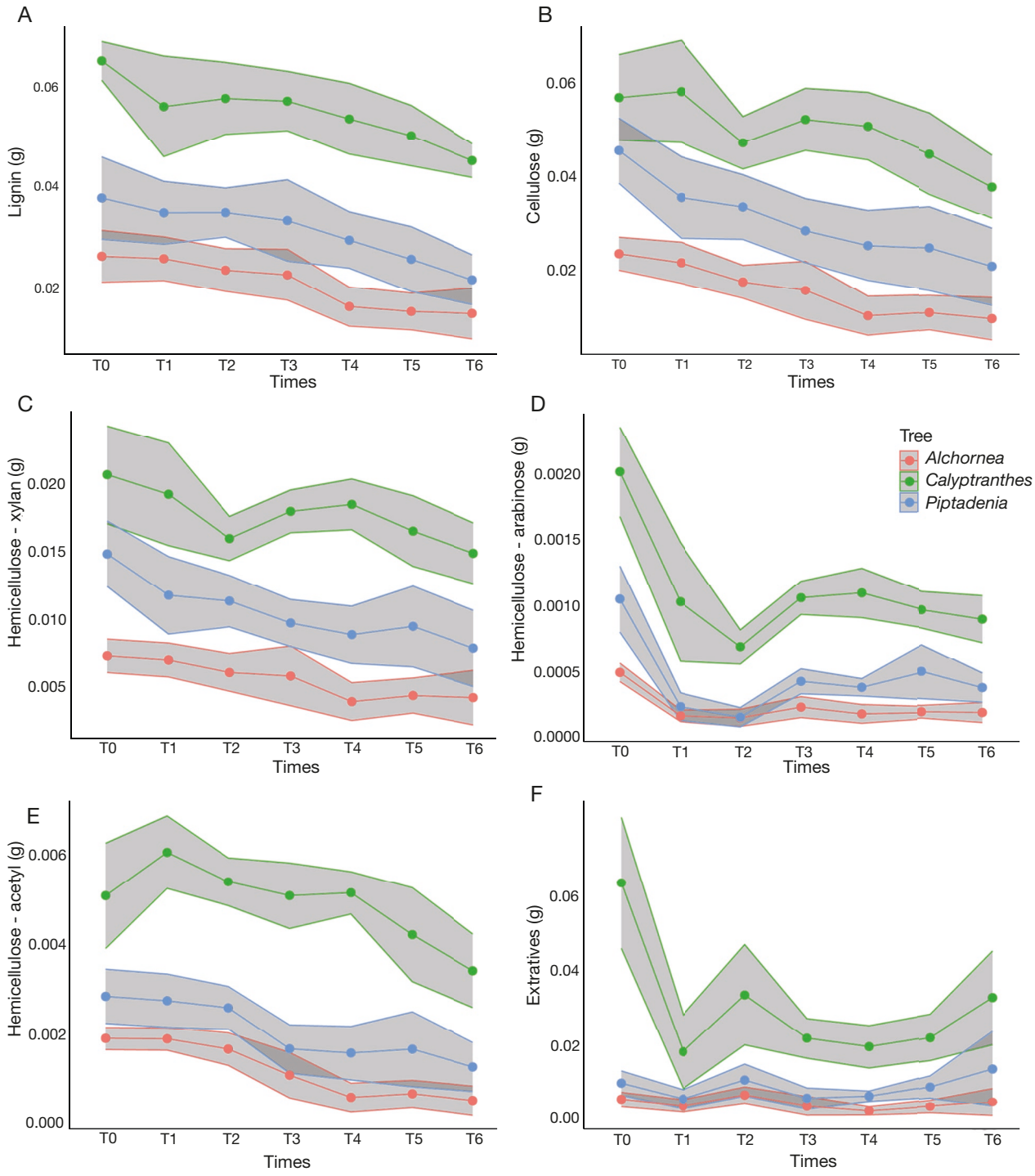

FIG. 5. — Mean and confidence interval of the concentration of **A**, lignin; **B**, cellulose; **C**-**E**, hemicelluloses; (**C**, xylan; **D**, arabinose; **E**, acetyl); **F**, wood extractives over time.

above that presented, but refer to branches, smaller in size and wood structure. And in the lignin/cellulose and lignin/hemicellulose ratio, it is possible to notice an increase in the ratios until Time 4, that is, the celluloses and hemicelluloses are more degraded than lignin and, from Time 5 this ratio decreases, indicating the appearance of fungi more specialized in lignin degradation.

Based on this data exploration of phyla abundance along the experiment time, a trend of inversion between Ascomycota phylum species being replaced by Basidiomycota species and greater species abundance in the early months is indicative of a natural pattern in wood decomposition. Time 0 would be expected to have dissimilarities, as each branch has an independent natural origin and history. Data of great species

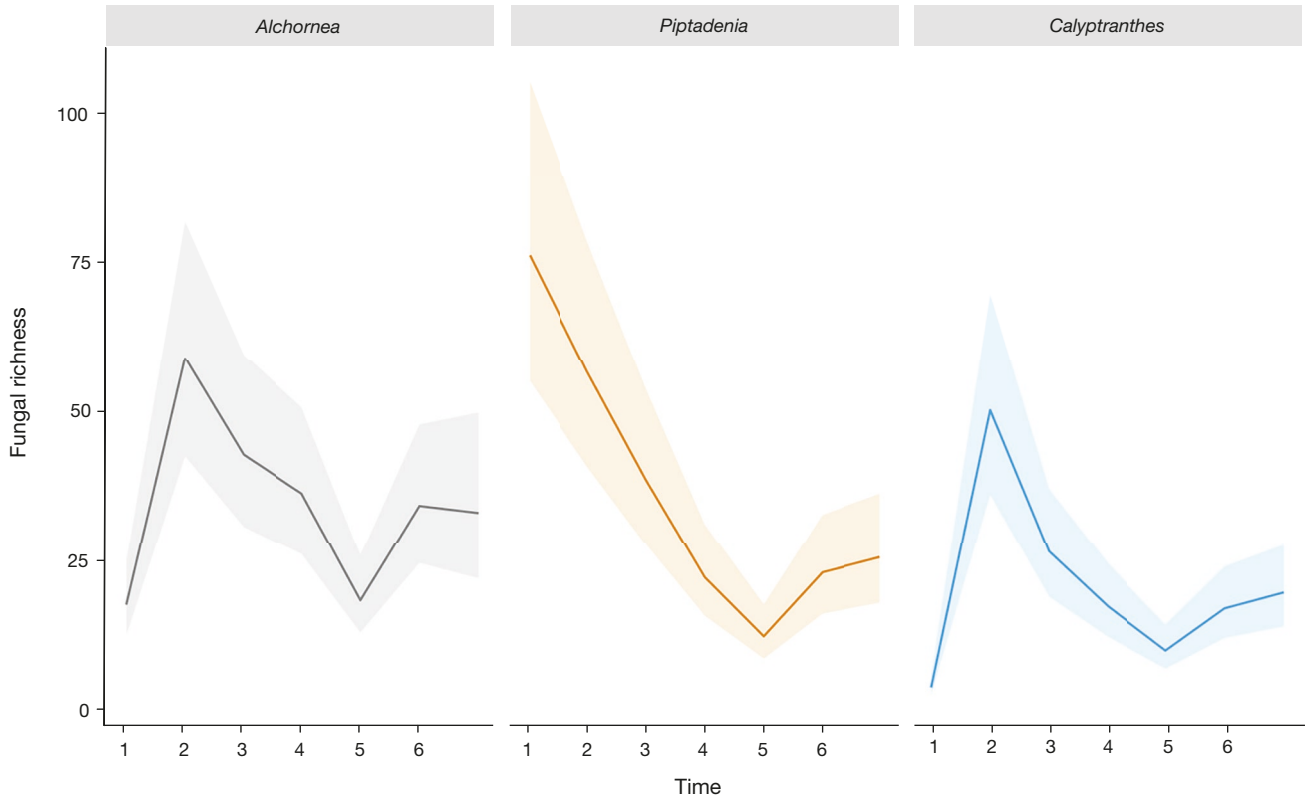

FIG. 6. — Temporal variation of fungal richness on three different tree species.

richness at the beginning of the succession are corroborated by the studies of Kubartová *et al.* (2015), where, working with fir trunks, they also find that less decomposed trunks have greater diversity and more decomposed trunks have less diversity. Precisely at Time 3, which we call the transition phase, there is an increasing trend of the Basidiomycota phylum compared to the amount in Times 1 and 2.

In the Permutational multivariate analysis of variance (PERMANOVA), all Times, Points and Trees behaved very similarly to each other, that is, the effect of each variable on the fungal community showed a very similar trend. When comparing the four collection points over time, 12-15% of the community's difference in location is explained by the data. Thus, it can be understood that the position of a branch in the studied forest has a not very high influence on the community structure, as pointed out by Hiscox *et al.* (2016) who, studying the pattern of fungal colonization on wood disks in the southern United Kingdom, observed different community compositions in the same environment.

When the weight of the different tree species was observed, the explanatory power was around 7-12%, indicating that there was a selectivity for the substrate, but this is not very expressive. The same happens with the positioning of the samples in the fragment (edge or interior), where the predictive power of the test varied between 3 and 5% only. The work of Brischke & Rapp (2008) studied the relationship between decomposition of two tree species (pine and spruce) in 23 locations in Europe, and as the data presented here, there was no significant correlation between different sites and decomposition speed.

On the other hand, when one observes the role of each substrate in community organization, the explanatory power of this variable is 40-50%. The community of each sample is more determined by the substrate itself, i.e., each branch has a history of unique community composition, which depends more on its own individual development. The study of Van der Wal *et al.* (2015) was the first to indicate that the most important factors that explain the variation in wood decomposition rates may change over time and that this variation, as time goes on, is more determined by the fungal community. Thus, the factors that would explain these variations would be the presence/absence of more or less potent species at the enzymatic level and also the degree of resilience and competitiveness to remain present in the environment (Fukami *et al.* 2010; Fukami 2015; Hiscox *et al.* 2015; Van der Wal *et al.* 2015).

If the data is organized according to the Points, the time variation explained between 14 and 16%. The tree species explained between 7 and 11% and, again, the different substrates correspond to 19-26% of the variation. This is indicative of the importance of the individual fungal community on each branch, that is, within each fungal community (the one existing on each branch), there is a dynamic process at play. This process involves the existing fungal community influencing or exerting pressure on itself (the community) to allow new species to settle and thrive

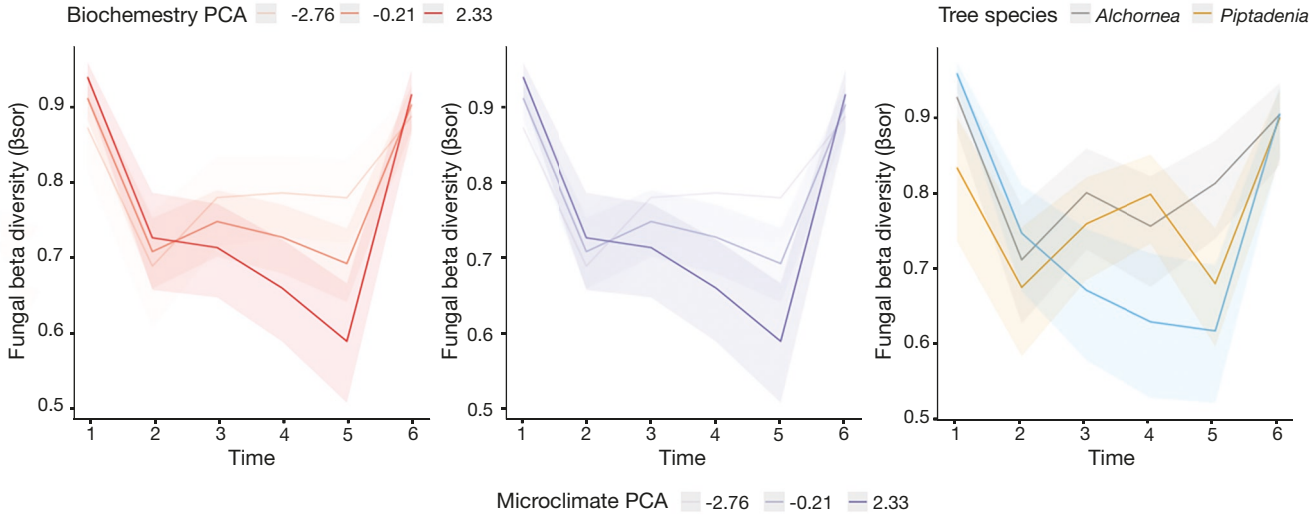

Fig. 7. — Temporal variation of the effect of biochemistry and microclimate on fungal beta diversity in three different wood substrates.

on the substrate. In other words, there's a competition or interaction within the fungal community itself, where it facilitates the colonization of new species on the substrate. Thus, it can be indicated that the priority effect may be one of the main factors responsible for the structuring of the fungal community over time (Fukami *et al.* 2010; Hiscox *et al.* 2015).

CONCLUSION

This work aimed to molecularly identify the fungal species that occur over time in the branches of three decaying tree species to characterize the community. The levels of lignin, cellulose, hemicellulose and nitrogen in the decaying branches were quantified over time, and the microclimate of the different sampling points was characterized. It was possible to notice the gradual reduction of wood density, as well as its main components, demonstrating that there is an intense process of nutrient cycling, in some samples the mass loss over the two years reached 70%. In the denser wood samples, this loss was between 15 and 20%.

Overall, all branches were decomposed and had a significant loss of mass. However, this loss was not constant among all samples, in which the percentage of loss varied over time, demonstrating that the decomposition responds to the present fungal community and is not constant during the process. Since the experiment was carried out in the same area – all four Points within the Parque Estadual das Fontes do Ipiranga –, variation in temperature, humidity or precipitation was not detected among the Points, meaning that these factors were the same for all community assemblages. In this sense, all the differences in the community were driven by the influence of the substrates and the community itself, e.g. priority effects.

When correlated fungal community patterns and wood biochemistry as a function of the factor "edge vs. interior of the forest fragment", it appears that this latter factor did not affect the decomposition process, which contradicts one of the hypotheses that the fragment edge would affect the decomposition process. In terms of richness of OTUs, there was a large increase at the beginning of decomposition, when simple sugars were still present in the substrate and niches were not yet defined. Over time, the more specialized species (enzymatically and competitively) became more dominant, but the species richness declined sharply.

There was a variation in the fungal community over time, which corroborates our hypothesis of fungal succession and species alternation. Substrate specificity, similar to that found by works in temperate and boreal forests (Prewitt *et al.* 2014; Purahong *et al.* 2018), was not observed. Competition between fungal species may be greater in tropical forests, notably because of the greater biodiversity. Geographical influence had some relevance, demonstrating that, when very geographically close, species tend to "choose" one or the other substrate, and that the species pool and priority effects were largely responsible for these variations.

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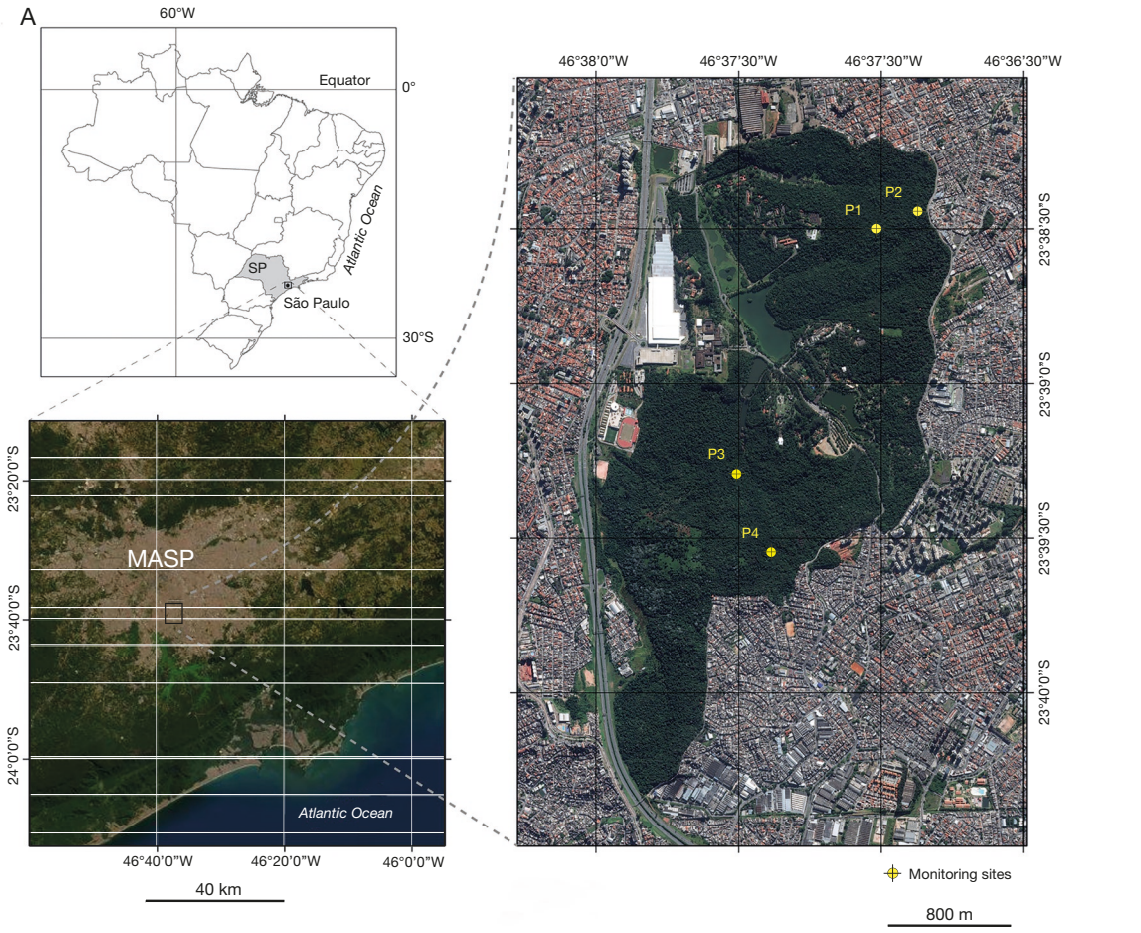
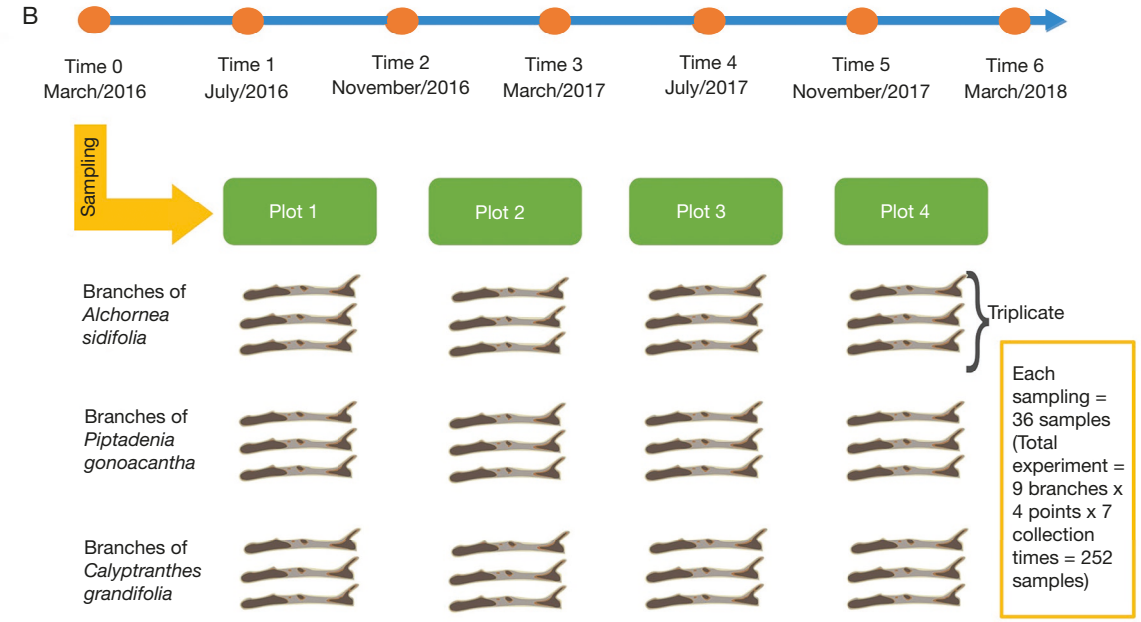
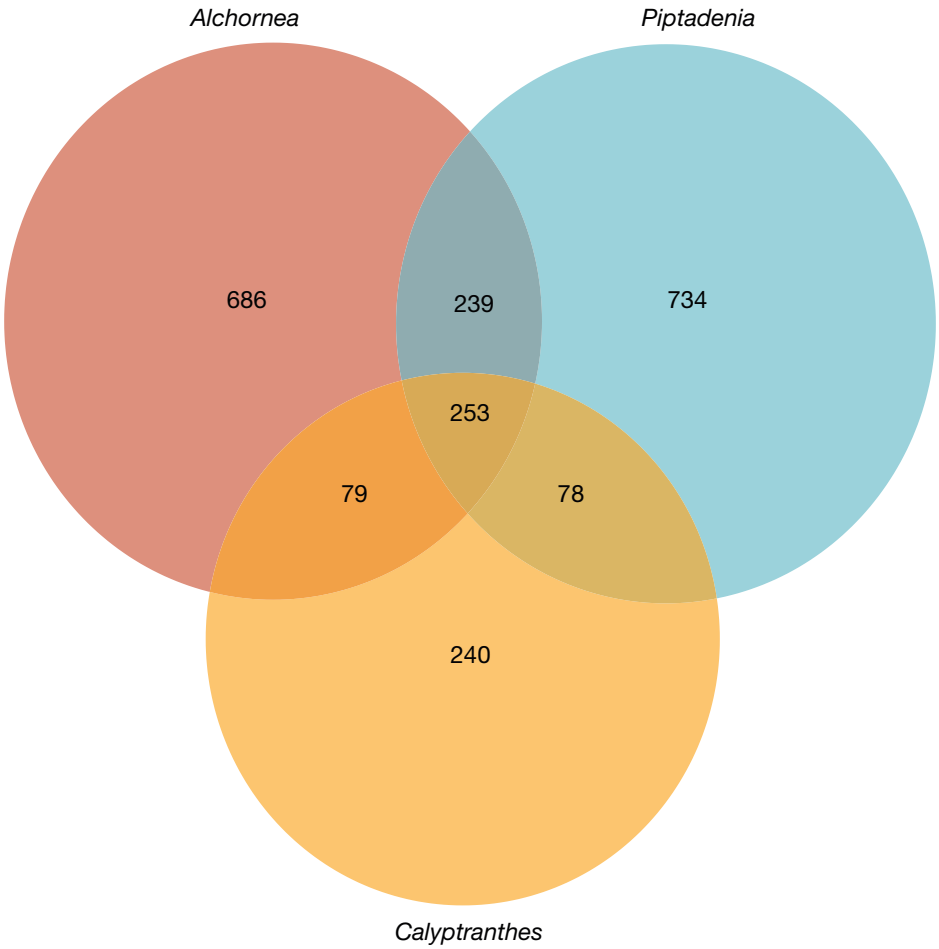
APPENDICES

Appendix 1.— **A**, Locations of the sampling points at the Parque Estadual das Fontes do Ipiranga (**PEFI**), in the metropolitan area of São Paulo (**MASP**). P1 and P3 are points in the interior of the forest fragment, while P2 and P4 are points at the border of the forest; **B**, experimental design and sampling scheme. Coordinate System: WGS 1984 Web Mercator Auxiliary Sphere. Projection: Mercator Auxiliary Spher. Date: WGS 1984. Image source: Global Imagery, DigitalGlobe, GeoEye, ESRI.

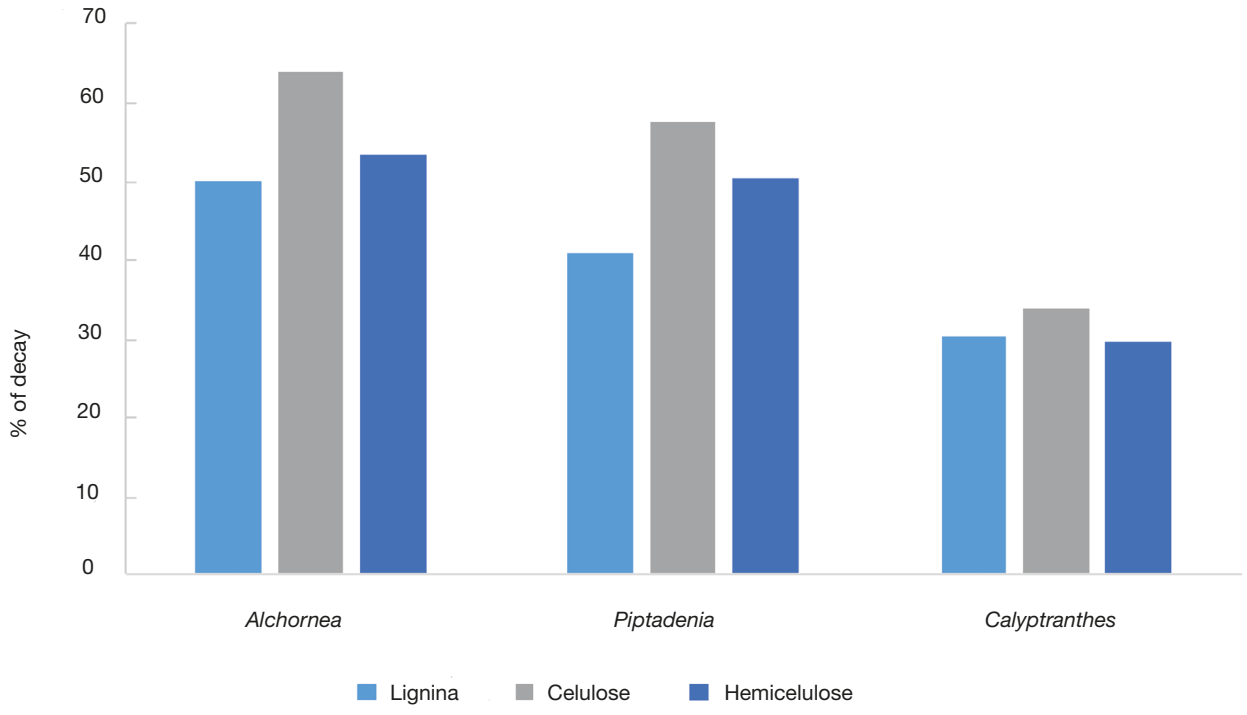




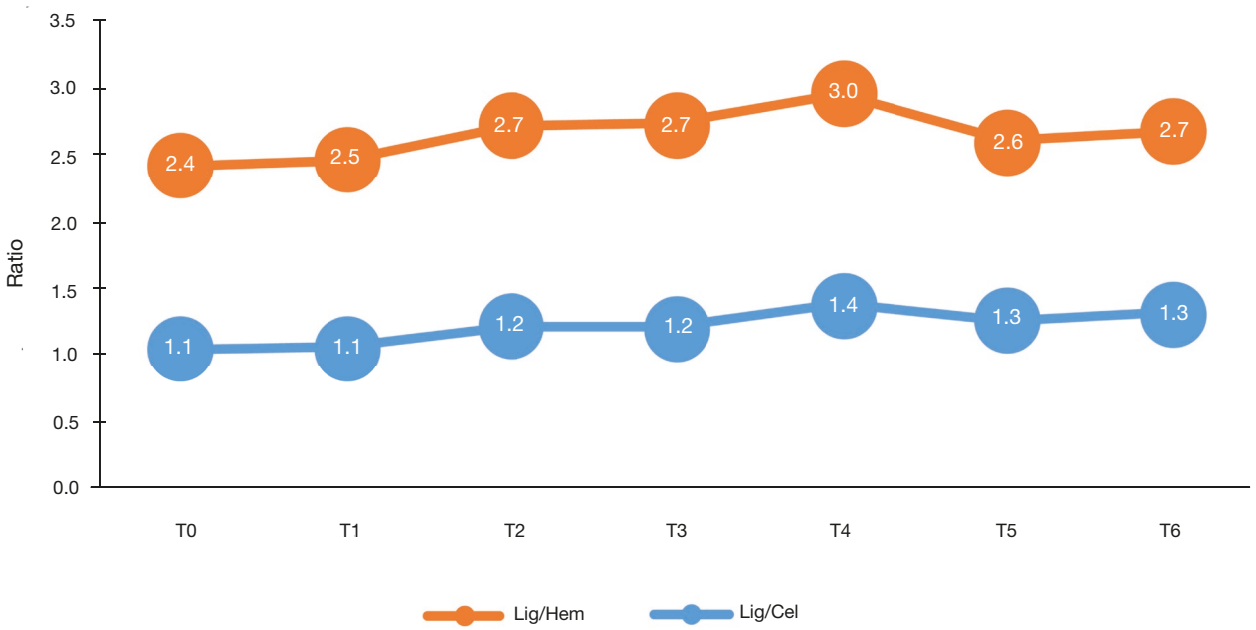
Appendix 2. — Venn diagram for the presence of Operational Taxonomic Units (OTUs) in tree species.



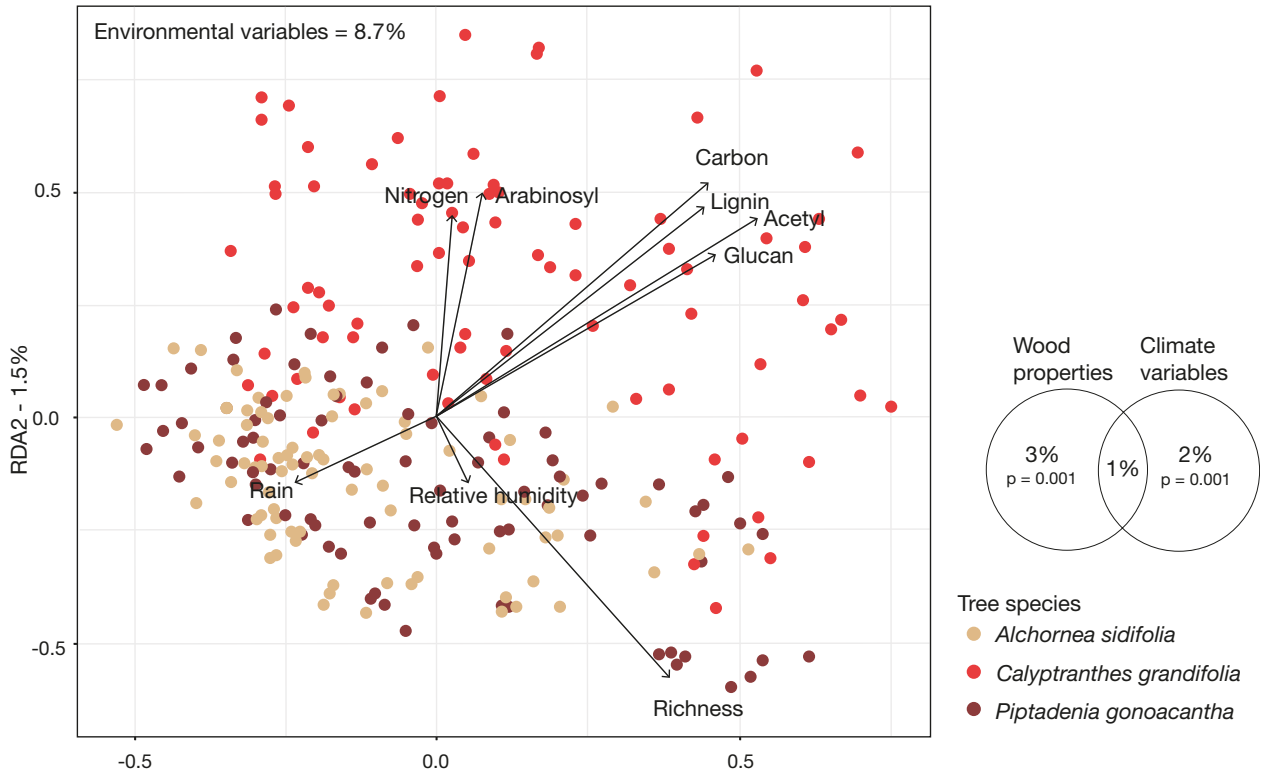
Appendix 3. — Percentage of decay of wood components in the end of the experiment (from Time 0 to Time 6).



Appendix 4. — Lignin/cellulose (p <0.001) and lignin/hemicellulose (p <0.025) ratio over time among all samples.



Appendix 5. — Redundancy analysis (**RDA**) ordination of all the wood samples fungal community, showing the relation between significant environmental and climatic variables. Nitrogen, carbon, arabinosyl, acetyl, rain and richness were used as explanatory factors. Significance of the model, factors and axes was determined by ANOVA. Variance partitioning for the fungal diversity based on distance-based redundancy analysis. Variation in the species data matrix is explained by wood properties, climate variables and unexplained variation.



Appendix 6. — Spearman correlation between loss percentages and abiotic factors.

	Temperature	Absolute humidity	Relative humidity	Rainfall	Loss
Temperature	1	0.93	0.35	0.90	0.17
Absolute humidity	0.93	1	0.67	0.90	0.16
Relative humidity	0.35	0.67	1	0.48	0.08
Rainfall	0.90	0.90	0.48	1	0.22
Loss	0.17	0.16	0.08	0.22	1

Appendix 7. — Results of NMDS analysis ordered by Times. P values: *** p <0.001; ** p <0.01; * p <0.05; . p >0.05.

	Time 0		Time 1		Time 2		Time 3		Time 4		Time 5		Time 6	
	R2	p	R2	p	R2	p	R2	p	R2	p	R2	p	R2	p
Nitrogen	0.09	.	0.04	.	0.26	*	0.02	.	0.27	**	0.33	**	0.08	.
Carbon	0.01	.	0.11	.	0.34	***	0.21	*	0.30	**	0.44	***	0.01	.
Density	0.01	.	0.13	.	0.37	**	0.28	**	0.30	***	0.43	***	0.00	.
Extratives	0.02	.	0.20	*	0.14	.	0.22	*	0.13	.	0.50	***	0.01	.
Lignin	0.01	.	0.18	*	0.36	***	0.38	**	0.29	**	0.42	***	0.01	.
Glucan	0.00	.	0.08	.	0.26	**	0.31	**	0.30	**	0.30	**	0.00	.
Xylan	0.00	.	0.08	.	0.34	**	0.30	**	0.28	**	0.37	***	0.01	.
Arabinosis	0.07	.	0.25	**	0.40	**	0.33	**	0.22	**	0.44	***	0.07	.
Acetyl	0.02	.	0.12	.	0.33	**	0.31	**	0.29	**	0.30	**	0.02	.
Temperature	0.06	.	0.14	.	0.01	.	0.13	.	0.05	.	0.04	.	0.08	.
Absolute humidity	0.40	***	0.22	*	0.01	.	0.23	*	0.03	.	0.07	.	0.39	**
Relative humidity	0.31	**	0.24	*	0.01	.	0.21	*	0.04	.	0.07	.	0.32	**

Appendix 8. — Results of NMDS analysis ordered by Points. P values: *** p <0.001; ** p <0.01; * p <0.05; . p >0.05.

	P1		P2		P3		P4	
	R2	p	R2	p	R2	p	R2	p
Nitrogen	0.2873	***	0.0043	.	0.1179	*	0.1641	**
Carbon	0.5556	***	0.3078	***	0.1686	**	0.2129	**
Density	0.5904	***	0.3720	***	0.3225	***	0.1879	**
Extratives	0.2679	**	0.4965	***	0.3454	***	0.0307	.
Lignin	0.5458	***	0.4077	***	03181	***	0.1664	**
Glucan	0.5184	***	0.4137	***	0.2875	***	0.1792	**
Xylan	0.5815	***	0.4029	***	0.2506	***	0.1685	*
Arabinosis	0.4546	***	0.4197	***	0.2681	***	0.0402	.
Acetyl	0.5064	***	0.3939	***	0.3562	***	0.1613	**
Temperature	0.0943	.	0.1179	*	0.1856	**	0.1367	*
Absolute humidity	0.0923	.	0.2160	**	0.1865	**	0.1592	*
Relative humidity	0.0262	.	0.2700	***	0.0543	.	0.1097	*

Appendix 9. — Summary of the statistical model used to explain species richness in a community of fungi. The results are presented as a mean estimate and 95% credible interval (**CI**) and significance value (**p**).

Predictors	**Estimates**	**CI**	**p**
(Intercept)	12.40	7.38 – 20.81	**<0.001**
Time 2	0.20	0.10 – 0.38	<0.001
Time 3	0.32	0.17 – 0.62	0.001
Time 4	0.25	0.13 – 0.47	<0.001
Time 5	0.35	0.18 – 0.67	0.002
Time 6	0.75	0.34 – 1.64	0.469
Tree [Calyptranthes]	1.78	0.83 – 3.81	0.140
Tree [Piptadenia]	0.40	0.21 – 0.78	0.007
Time 2 × Tree [Calyptranthes]	0.67	0.26 – 1.72	0.406
Time 3 × Tree [Calyptranthes]	0.29	0.11 – 0.74	0.010
Time 4 × Tree [Calyptranthes]	0.31	0.12 – 0.79	0.014
Time 5 × Tree [Calyptranthes]	0.21	0.08 – 0.54	0.001
Time 6 × Tree [Calyptranthes]	0.57	0.19 – 1.68	0.306
Time 2 × Tree [Piptadenia]	2.11	0.90 – 4.94	0.087
Time 3 × Tree [Piptadenia]	1.97	0.82 – 4.74	0.131
Time 4 × Tree [Piptadenia]	3.18	1.32 – 7.66	0.010
Time 5 × Tree [Piptadenia]	1.23	0.51 – 2.96	0.647
Time 6 × Tree [Piptadenia]	2.38	0.85 – 6.65	0.098
Observations		209	
R2		0.431	

Appendix 10. — Summary of the statistical model used to explain beta diversity in a fungi community for 3 selected models. The results are presented as a mean estimate and 95% credible interval (**CI**) and significance value (**p**).

Predictors	**Estimates**	**CI**	**p**	**Estimates**	**CI**	**p**	**Estimates**	**CI**	**p**
(Intercept)	10.56	7.77 – 14.33	<0.001	10.56	7.77 – 14.33	<0.001	12.40	7.38 – 20.81	<0.001
Time 2	0.23	0.16 – 0.34	<0.001	0.23	0.16 – 0.34	<0.001	0.20	0.10 – 0.38	<0.001
Time 3	0.28	0.19 – 0.40	<0.001	0.28	0.19 – 0.40	<0.001	0.32	0.17 – 0.62	0.001
Time 4	0.25	0.17 – 0.36	<0.001	0.25	0.17 – 0.36	<0.001	0.25	0.13 – 0.47	<0.001
Time 5	0.21	0.14 – 0.30	<0.001	0.21	0.14 – 0.30	<0.001	0.35	0.18 – 0.67	0.002
Time 6	0.89	0.57 – 1.37	0.592	0.89	0.57 – 1.37	0.592	0.75	0.34 – 1.64	0.469
Biochemistry	**1.17**	**1.04 – 1.32**	**0.008**	–	–	–	–	–	–
Time 2*biochemistry	0.88	0.76 – 1.03	0.116	–	–	–	–	–	–
Time 3* biochemistry	0.80	0.69 – 0.92	0.002	–	–	–	–	–	–
Time 4 * biochemistry	0.75	0.65 – 0.87	<0.001	–	–	–	–	–	–
Time 5* biochemistry	0.71	0.62 – 0.83	<0.001	–	–	–	–	–	–
Time 6* biochemistry	0.91	0.76 – 1.09	0.291	–	–	–	–	–	–
Microclimate	–	–	–	**1.17**	**1.04 – 1.32**	**0.008**	–	–	–
Time T2 * microclimate	–	–	–	0.88	0.76 – 1.03	0.116	–	–	–
Time 3* microclimate	–	–	–	0.80	0.69 – 0.92	0.002	–	–	–
Time 4* microclimate	–	–	–	0.75	0.65 – 0.87	<0.001	–	–	–
Time 5* microclimate	–	–	–	0.71	0.62 – 0.83	<0.001	–	–	–
Time 6* microclimate	–	–	–	0.91	0.76 – 1.09	0.291	–	–	–
Tree [Calyptranthes]	–	–	–	–	–	–	1.78	0.83 – 3.81	0.140
Tree [Piptadenia]	–	–	–	–	–	–	0.40	0.21 – 0.78	0.007
Tempo [T2] * Tree [Calyptranthes]	–	–	–	–	–	–	0.67	0.26 – 1.72	0.406
Tempo [T3] * Tree [Calyptranthes]	–	–	–	–	–	–	0.29	0.11 – 0.74	0.010
Tempo [T4] * Tree [Calyptranthes]	–	–	–	–	–	–	0.31	0.12 – 0.79	0.014
Tempo [T5] * Tree [Calyptranthes]	–	–	–	–	–	–	0.21	0.08 – 0.54	0.001
Tempo [T6] * Tree [Calyptranthes]	–	–	–	–	–	–	0.57	0.19 – 1.68	0.306
Tempo [T2] * Tree [Piptadenia]	–	–	–	–	–	–	2.11	0.90 – 4.94	0.087
Tempo [T3] * Tree [Piptadenia]	–	–	–	–	–	–	1.97	0.82 – 4.74	0.131
Tempo [T4] * Tree [Piptadenia]	–	–	–	–	–	–	3.18	1.32 – 7.66	0.010
Time 5 * Tree [Piptadenia]	–	–	–	–	–	–	1.23	0.51 – 2.96	0.647
Time 6 [T6] * Tree [Piptadenia]	–	–	–	–	–	–	2.38	0.85 – 6.65	0.098
Observations	209			209			209		
R2	0.393			0.393			0.431		