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USING Next-Generation Sequencing (NGS) TO UNCOVER DIVERSITY OF WOOD-DECAYING FUNGI IN NEOTROPICAL ATLANTIC FORESTS

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Abstract

A targeted amplicon-based metagenomics approach (metabarcoding) provides detailed access to the diversity of the mycobiome in any substrate in distinct environments on Earth. Fungi are the main decomposers of lignocellulosic woody debris in terrestrial forested ecosystems, contributing significantly to the global carbon cycle. The main objectives of this study were to assess the fungal taxonomic diversity in fallen woody debris samples from two Neotropical forest fragments (rainforest and seasonal forest), to analyze the qualitative and quantitative components of the taxonomic diversity, and to investigate the functional diversity of the ecological groups detected. Our study comprised three main methodological steps: (i) sampling in the field; (ii) extraction of DNA, amplification of targeted segments and massively parallel sequencing; and (iii) data analysis and interpretation. A total of 110 molecular operational taxonomic units showing sequence similarity of 95% or more across the two collection sites using two DNA metabarcoding markers (*ITS1* and *ITS2*) were assigned to putative fungal genera in 59 families, 27 orders, and 3 phyla. The number of putative fungal genera and the relative abundance of reads for each genus are higher in the tropical rainforest site than in the tropical seasonal forest site. Most of the identified genera are ligninolytic and cellulolytic and/or hemicellulolytic Basidiomycota (Agaricomycetes) and Ascomycota (Sordariomycetes), but "sugar fungi" and fungi associated with plants and detritivorous insects were also detected. This is the first study using NGS as a rapid and large-scale useful strategy to uncover the diversity of wood-decaying fungi in tropical forests.

Key words: fungal metabarcoding, rainforest, seasonal tropical forest, lignocellulosic residues

Introduction

The plant litter in terrestrial forest ecosystems consists of dead plant parts, including both non-woody and woody plant residues, with the latter comprising twigs, stems, branches and trunks, which are collectively referred to as woody debris (WD) (Cannon & Sutton 2004). Woody material refers not only to proper wood but also to bark (outer and inner) and sap (from inner bark and sapwood) at any stage of decay. Saproxylic organisms include any species that depend, at least during some portion of their lifecycle, upon wounded or decayed woody material from living, weakened or dead trees (Stokland *et al*. 2012). Saproxylic biota degrade woody material by fragmentation or physical destruction (microfauna) and by mainly aerobic, but also anaerobic, enzymatic action (fungi and bacteria) (Hattaka 2001, Stokland *et al.* 2012). The predominant mycelial habit, along with the ability of fungi to produce extracellular lignocellulolytic enzymes that degrade dead lignified plant residues, make fungi the most important group associated with wood degradation (Zhou & Ingram 2000).

The initial decay process involves fungal communities of endophytes, pathogens and saprotrophs, that are present in plant tissues (Boddy 2001). The ecological functional groups of saproxylic fungi include the following: (a) "sugar

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and staining fungi", which degrade simple carbon compounds found in cell contents and saps in the initial stages of wood decomposition (mainly ascomycotan and basidiomycotan yeasts and a number of mycelial ascomycotan groups, but also Mucoromycotina); (b) structural wood-decaying fungi, which have full enzymatic ability to degrade cellulose, hemicelluloses and lignin (mainly Basidiomycota and certain groups of Ascomycota); (c) residual wood-decaying fungi, which use the products resulting from the decomposition of wood by structural wood-decaying fungi; (d) fungi associated with detritivorous or saprophagous animals (mainly insects of the orders Coleoptera, Isoptera and Diptera, as well as Acari), which can be sap feeders, inner bark consumers (phloemophagous) and wood consumers (xylophagous, xylemophagous); (e) animal-predatory (nematode-trapping) fungi; (f) mycoparasites (both biotrophic and necrotrophic) and fungicolous fungi; and (g) mycorrhizal fungi (Stokland *et al.* 2012).

Structural wood-decaying fungi are the main decomposers of wood and are usually categorized as white-, brown- and soft-rot fungi according to the mode of wood decay (Hattaka 2001). White-rot fungi degrade all of the structural components of wood (cellulose, hemicelluloses and lignin), either simultaneously or sequentially (selective delignification), whereas brown-rot fungi degrade cellulose and hemicelluloses, leaving the lignin fraction nearly unaltered (Morgenstern *et al.* 2008). All known white- and brown-rot wood-decaying fungi are basidiomycetes, and recent evidence based on phylogenomics suggests a continuum rather than a dichotomy between the white- and brown-rot modes of wood decay in Basidiomycota (Riley *et al.* 2014). Soft-rot fungi, which are restricted to Ascomycota, degrade the cellulose and hemicellulose fractions, but only in the central layer (S2) of the secondary cell walls, and they have limited capacity to modify lignin (Schwarze 2007).

The targeted amplicon-based metagenomics approach of massively parallel sequencing, a molecular, culture-independent approach, theoretically provides the most detailed access to the diversity of the mycobiome of any substrate. Nevertheless, possible methodological biases, limitations of the markers and bioinformatic analysis may lead to incorrect conclusions (Lindahl *et al*. 2013). In general, high fungal taxonomic diversity has been observed in various environmental compartments (air, soil, continental and oceanic sediments, and surface and ground water), as well as on the external and internal surfaces and microenvironments within macroorganisms. These studies typically consider molecular operational taxonomical units (MOTUs) to differentiate the environmental sequences at the species or genus level (Cuadros-Orellana *et al.* 2013). The internal transcribed spacer and 5.8S region of the nuclear ribosomal repeat unit have been used as the primary DNA fungal barcoding marker for species delimitation (Schoch *et al.* 2012). Both the ITS1 and ITS2 spacer regions can be used as DNA metabarcodes in fungal metagenomic studies (Blaalid *et al.* 2013). However, most of the NGS platforms commonly used (Roche, Life Technologies, Illumina) generate short reads (Goodwin *et al*. 2016), preventing the reliable identification at species level.

To date, studies specifically investigating the mycobiomes of decaying wood using targeted, amplicon-based metagenomics are few, and all of these studies were conducted strictly in palearctic boreal and temperate forests (Ovaskainen *et al.* 2010, Kubartova *et al.* 2012, Ovaskainen *et al.* 2013, Ottosson *et al.* 2015, Van der Wal *et al.* 2015, Hoppe *et al.* 2015, Jang *et al.* 2015, Runnel *et al.* 2015, Yamashita *et al.* 2015). Thus, as far as we know, there has not been a similar metabarcoding study in tropical, and more specifically, neotropical forests. Although there are many studies comprising collection, direct observation and/or culturing of fungi from decaying wood in the Atlantic Forest biome, (see Maia *et al*. 2015 for an extensive review of the literature), a metabarcoding study theoretically allows the most comprehensive access to the fungal diversity in any kind of substrate. Thus, the objective of our study is to characterize the fungal diversity in fallen woody debris from two climatically and vegetationally distinct, neotropical forest fragments using a targeted, amplicon-based (nrITS) metagenomics approach.

Material & Methods

Study areas

There are two main types of tropical broadleaf forests distributed across both the Neotropical and Paleotropical phytogeographical zones: (i) tropical rainforests, with high temperatures and high rainfall conditions throughout the year, and (ii) tropical seasonal forests, with year-round high temperatures but a clearly seasonal drought (Evert & Eichorn 2013). In this work two study areas were selected as representatives of each main type of tropical broadleaf forest: Rio Doce State Park (RDSP) and Serra do Conduru State Park (SCSP). The permissions for the field studies were obtained from the Instituto Estadual de Florestas (IRF): COL:045/12 for RSDP park and Instituto do Meio Ambiente e Recursos Hídricos (INEMA): 2013-009569/TEC/PESQ-0036 for SCSP park.

RDSP is situated in the state of Minas Gerais, Brazil (19°29'S–19°48'S and 42°28'W–42°38'W, in the interior

sub-region according to Ribeiro *et al*. 2009), with an altitude ranging from 230–515 m and a total area of 36,113 ha. The main vegetation is seasonally dry tropical forest, and the regional climate is classified as tropical wet and dry climate, Aw in the Köppen climate classification system, with a mean annual temperature of 23°C and a mean annual rainfall of 1300 mm. This area is characterized by a marked seasonality, with a humid season with intense precipitation in the summer and a dry season in the winter (Lopes *et al.* 2002). The surrounding areas near our sampling collection site are often visited by tourists leading to some anthropogenic impact.

SCSP is situated in the state of Bahia, Brazil (14°20'–14°30' S; 39°02'–39°08' W, in the Bahia sub-region according to Ribeiro *et al*. 2009), with an altitude ranging from 60–500 m and a total area of 9,275 ha. The main vegetation is tropical rainforest, and the regional climate is classified as tropical rainforest climate, Af in the Köppen climate classification system, with a mean annual temperature of 24°C and a mean annual rainfall of 2000 mm, which is well distributed throughout the year, i.e., without a dry season (Martini *et al.* 2007). In both study areas, the sample collection sites consisted of the main vegetation type: an open canopy environment near the lacunar system in RDSP and a dense canopy environment in SCSP.

Sample Collection

A total of 20 samples (10 per collection site) of fallen woody debris (FaWD) was randomly collected. The FaWD samples were collected in accordance to the following exclusion and inclusion criteria: (i) larger than 2 cm in diameter, (ii) including wood and bark, (iii) without visible epixylic lichens or bryophytes, (v) at least 5 m far apart from each other, and (vi) representing all stages of decay, according to Keller *et al*. (2004). Five subsamples were taken using a sterilized scalpel from different parts of each FaWD, and subsequently pooled forming one compound sample, which were placed in sterilized plastic bags, transported to the laboratory within 4 h, and stored at -20ºC until processed.

DNA extraction, amplification, and massively parallel sequencing

Subsamples (1 g) from each wood sample were ground under liquid nitrogen, and metagenomic DNA was extracted from 100 mg samples according to Góes-Neto *et al.* (2005) with the following modifications: salt extraction buffer [(0.05 M Tris-HCl (pH 9), 0.005 M EDTA, 0.1 M NaCl, 1% SDS, 3% β-mercaptoethanol 3% polyvinyl-pyrrolidone (PVP)] and Proteinase K (50 µg/ml). The internal transcribed spacer (ITS) region of the rRNA gene was amplified using the primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (Schoch *et al.* 2012). Each PCR reaction contained the following components: 1× PCR reaction buffer (100 mM Tris-HCl), 2.5 mM $MgCl_2$ 0.2 mM dNTP, 15 pmol of each oligunucleotide primer, 5 units Taq polimerase (Kappa, USA), 1 M betaine, 1 µg of BSA, 2% of DMSO and approximately 1 ng genomic DNA, and sterilized, deionized water. Cycle parameters were as follows: 1 initial denaturation cycle at 94ºC for 2 min, followed by 35 denaturation cycles at 94ºC for 1 min, and annealing at 60ºC for 1 min, and extension at 72ºC for 3 min, with a final extension cycle at 72ºC for 5 min. At least three independent amplification reactions were performed from the same DNA extract. The PCR products were purified, quantified and combined until 100 ng of purified PCR product was obtained for each sample. Samples from the same collection site (RDSP or SCSP) were pooled in an equimolar proportion to produce a composite sample with a final content of 1 µg of purified PCR product, which was quantified using a DNA fluorescence assay.

An Ion Torrent adapter-ligated library was constructed following the Ion Fragment Library Kit (Life Technologies) protocol (Part #4467320 Rev. A). Briefly, fragmentation of 1 µg of each composite sample was performed using Adaptive Focused Acoustics™ (AFA; Covaris). After fragmentation, samples were end-repaired, and the Ion Torrent adapters P1 and A were ligated using DNA ligase. Following AMPure bead purification (Beckman Coulter, Brea, CA, USA), adapter-ligated products were nick-translated and PCR-amplified for a total of 10 cycles. The resulting library was also purified using AMPure beads, and the concentrations and sizes of fragmentation products were determined using an Agilent BioAnalyzer DNA 1000 Kit (Agilent Technologies). Sample emulsion PCR, emulsion breaking, and enrichment were performed using the Ion Xpress Template Kit (Part #4467389 Rev. B) according to the manufacturer's instructions. Briefly, an input concentration of one DNA template copy/Ion Sphere Particle (ISP) was added to the emulsion PCR master mix, and the emulsion was generated using an IKADT-20 mixer (Life Technologies). Next, the ISPs were recovered, and Dynabeads MyOne Streptavidin C1 beads (Life Technologies) were used to enrich for template-positive ISPs. ISP enrichment was confirmed using the Qubit 2.0 fluorometer (Life Technologies), and the sample was prepared for sequencing using the Ion Sequencing Kit protocol (Part #4467391 Rev. B). Each composite sample was loaded onto an Ion 316 chip and sequenced on the PGM (Personal Genome Machine) system for 110 cycles (Rothberg *et al*. 2011).

Data analyses

All of the generated sequences were deposited in (a) NCBI BioProject: PRJNA255944, BioSample: SAMN02934078, SAMN02934079 and (b) MG-RAST Locality 2 ITS1: 4576861.3 ITS2: 4576862.3, Locality 3 ITS1: 4576871.3 ITS2: 4576872.3. This study is part of the Brazilian Microbiome Project (BMP): http://www.brmicrobiome.org/ (Pylro *et al*. 2013). The datasets were analyzed on a CentOS release 6.6 system. Sequences of short length (< 50 bp) and low quality (mean Phred score of Q < 20) with ambiguous character states (non-IUPAC) (maxambig = 0) and homopolymers longer than 8 bp (maxhomop = 8) were filtered out using PRINSEQ v0.15 (Schmieder & Edwards 2011). PCR amplification biases (overrepresented fragments) were also removed using USEARCH v.7.0.1090 (Edgar 2010). Subsequently, the FungalITSextractor was used for splitting ITS1 and ITS2 sequences (Nilsson *et al.* 2010), which were then clustered employing 97% stringency and filtered using a reference-based chimera filtering by using USEARCH v.7.0.1090 (Edgar 2010). Reads matching the internal transcribed spacers ITS1 or ITS2 were independently compared to the UNITE database (Köljalg *et al*. 2013), using BLASTn v. 2.2.27 (E-value ≤ 1e-10, minimum overlap length ≥ 90%, minimum identity ≥ 95%, Camacho *et al.* 2008), assigned to putative genera (Jumpponen & Jones 2009), and a fungal abundance table was generated. Non-informative names in the taxonomy dependent assignment were excluded prior to the diversity analyses (uncultured, fungal, fungi, fungus, isolate, *mycota, *mycetidae, leaf litter, unassignment, endophytic, endophyte, *mycetes, *mycete, *aceae, *ales, cf., symbiont, mycorrhiza, aff.) The number of fungal genera and the relative abundance of reads at the phylum, class and order levels were compared using barplots. All analyses were performed using R software (R Development Core Team 2016).

The ecological functional roles of the identified genera were investigated based on evidence at the gene and biochemical levels using the presence or absence of genes and/or enzymes related to lignocellulosic decomposition (degradation of cellulose, hemicelluloses and lignin), based on the CAZY (Carbohydrate-Active Enzymes: http://www.cazy.org), BRENDA (www.brenda-enzymes.org), NCBI (www.ncbi.nih.gov) databases, and the literature (Zhao *et al.* 2013, Riley *et al.* 2014). A majority consensus of these databases and literature was used to assign the ecological functional roles of putative fungal genera.

Results

The sequencing resulted in a total of 2.55 Mb, of which 687,662 and 1,861,279 reads were obtained for RDSP and SCSP, respectively (Table 1). Using both metabarcodes, a total of 110 genera was identified, 71 in RDSP and 85 in SCSP. In RDSP, 19.7% of genera were identified with ITS1, 43.7% with ITS2, and 36.6% with both markers. In SCSP, 18.8% of genera were identified with ITS1, 37.7% with ITS2, and 43.6% with both genomic regions.

TABLE 1. Summary of descriptive statistics.

	RDSP	SCSP	Total
Raw reads	687,662 (26.98%)	1,861,279 (73.02%)	2,548,941
Reads passing quality filter	628,970 (25.91%)	1,798,479 (74.09%)	2,427,449
Median read length (bp)	116	199	-
% G+C	43.48 ± 8.61	47.06 ± 5.58	-
Reads after 97% similarity clustering	259,138 (17.25%)	1,243,319 (82.75%)	1,502,547
Reads after FungalITSExtractor	114,696 (9.98%)	1,034,128 (90.02%)	1,148,824
ITS1	46,144 (10.76%)	382,758 (89.24)	428,902
ITS2	67,985 (10.38%)	587,037 (89.62%)	655,022
Reads assigned to any MOTUs			
ITS1	39,915 (30.32%)	91,720 (69.68%)	131,635
ITS2	59,999 (37.22%)	101,190 (62.78%)	161,189
Both markers	36,567 (30.3%)	84,109 (69.7%)	120,676
MOTUs (genus level) after all filters			

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TABLE 1. (Continued)

	RDSP	SCSP	Total
ITS1	41	54	-
ITS2	58	70	-
Both markers	26	37	-

Note: Percentage values were calculated by line.

Regardless of the collection site or metabarcode used, a higher number of genera of Ascomycota than Basidiomycota were detected, and Zygomycota only in the SCSP (Fig. 1). The same pattern was observed when the relative abundance of reads was considered (Fig. 2).

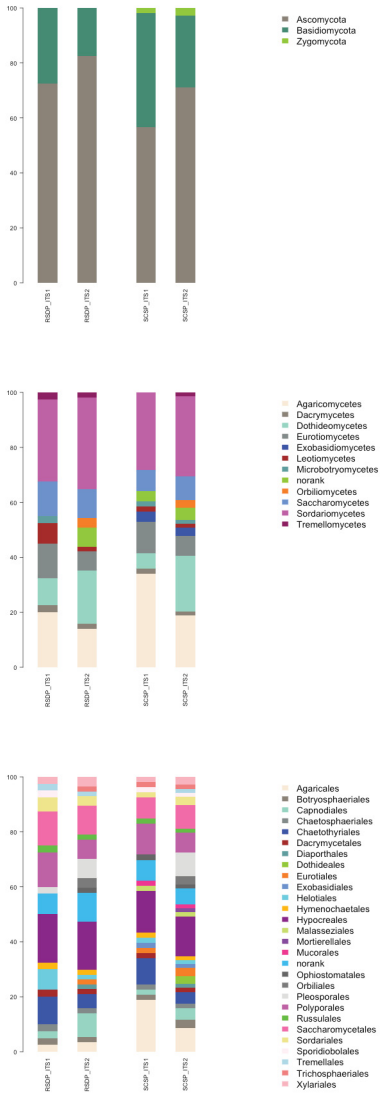

FIGURE 1. Genera richness at the levels of phylum, class and order per study area and metabarcode using 95% sequence similarity with reference sequences in the UNITE database as surrogate for traditional taxonomic generic concepts.

Sordariomycetes (Ascomycota) and Agaricomycetes (Basidiomycota) exhibited the highest number of genera in the two collection sites. However, the relative abundances of reads in Saccharomycetes were considerably higher than all the other fungal classes.

At the ordinal level, Hypocreales (Ascomycota) showed the highest number of genera regardless of the collection site or metabarcode. Agaricales (Basidiomycota) showed the highest number of genera in the SCSP whereas Polyporales (Basidiomycota) was the highest in RDSP. However, when analyzing the total number of sequences, Saccharomycetales (Ascomycota) was the most abundant.

The genera that showed the highest number of sequences in RDSP were *Meyerozyma* (34%), *Scytalidium* (23%), *Resinicium* (16.7%), *Vertexicola* (4.79%), *Pichia* (4.48%) and *Candida* (1.85%) whereas in SCSP were *Meyerozyma* (34.3%), *Pichia* (10.58%), *Asterostroma* (9.84%), *Resinicium* (8.35%), *Scytalidium* (7.48%), *Candida* (6.1%), and *Vertexicola* (3.50%). Furthermore, when considering only ITS1, *Meyerozyma* and *Resinicium* were those with the highest number of reads, and for ITS2 were *Scytalidium* and *Vertexicola*. All together, these seven genera (*Meyerozyma*, *Scytalidium, Resinicium, Pichia, Asterostroma, Candida, Vertexicola*) represented 86.4% and 80.2% of the relative abundances of the reads for RDSP and SCSP, respectively (Fig. 2, and Table 2).

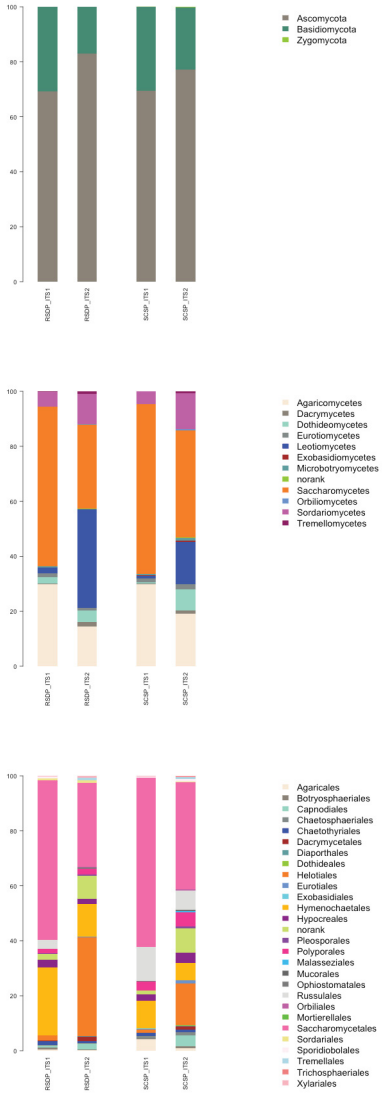

FIGURE 2. Relative abundances of reads of fungal genera per study area and metabarcode using 95% sequence similarity with reference sequences in the UNITE database as surrogate for traditional taxonomic generic concepts.

TABLE 2. Relative abundances of reads of fungal genera per study area and metabarcode. Generic names were selected using a BLAST search of 95% sequence identity and names are based on the taxonomic concepts applied in the UNITE database.

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Acanthostigma	0	0	0	0	0.148	0.148	Ascomycota	Dothideomycetes	Pleosporales	Tubeufiaceae	HC	saprotroph
Acremonium	0.12	0.218	0.338	0.496	1.33	1.826	Ascomycota	Sordariomycetes	Hypocreales	norank	HC	saprotroph
Agaricus	**0**	**0**	**0**	**0.062**	**0**	**0.062**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Agaricaceae**	**LHC**	**saprotroph**
Antrodia	0	0	0	0.062	0	0.062	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	HC	saprotroph
Arthrobotrys	0	0.073	0.073	0	0.074	0.074	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	HC	saprotroph
Arthrographis	0.723	1.16	1.883	0.186	1.996	2.182	Ascomycota	Dothideomycetes	norank	Eremomycetaceae	HC	saprotroph
Aspergillus	0	0	0	0	0.148	0.148	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	HC	saprotroph
Asterostroma	**3.133**	**0.145**	**3.278**	**12.392**	**6.8**	**19.192**	**Basidiomycota**	**Agaricomycetes**	**Russulales**	**Lachnocladiaceae**	**LHC**	**saprotroph**
Auerswaldia	0	0	0	0	0.074	0.074	Ascomycota	Dothideomycetes	Dothideales	Dothideaceae	HC	saprotroph
Aureobasidium	0	0	0	0	0.148	0.148	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	HC	saprotroph
Beauveria	0	0	0	0	0.074	0.074	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	HC	parasitic or mutualistic symbiont of animals
Calocera	0.12	1.668	1.788	0.124	1.183	1.307	Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	HC	saprotroph
Candida	2.651	1.378	4.029	7.373	4.582	11.955	Ascomycota	Saccharomycetes	Saccharomycetales	norank	HC	parasitic or mutualistic symbiont of animals
Chaetosphaeria	0.723	0.725	1.448	0	0.148	0.148	Ascomycota	Sordariomycetes	Sordariales	Chaetosphaeriaceae	HC	saprotroph
Chaunopycnis	0	0	0	0.062	0.074	0.136	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	HC	parasitic or mutualistic symbiont of plants

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Chloridium	0.361	0.218	0.579	0.991	1.33	2.321	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	HC	saprotroph
Cladophialophora	0	0	0	0	0.074	0.074	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	HC	parasitic or mutualistic symbiont of animals
Cladosporium	0.602	0.943	1.545	0.062	1.33	1.392	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	HC	parasitic or mutualistic symbiont of plants
Clitopilus	**0**	**0**	**0**	**0.062**	**0**	**0.062**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Entolomataceae**	**LHC**	**saprotroph**
Clonostachys	0	0.073	0.073	0	0	0	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	HC	parasitic or mutualistic symbiont of plants
Collybia	**0**	**0**	**0**	**0**	**0.074**	**0.074**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Tricholomataceae**	**LHC**	**saprotroph**
Cordana	0	0.073	0.073	0	0	0	Ascomycota	norank	norank	norank	HC	saprotroph
Cordyceps	0	0.073	0.073	0	0.074	0.074	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	HC	parasitic or mutualistic symbiont of animals
Cryptococcus	0.12	1.015	1.135	0	0.739	0.739	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	HC	parasitic or mutualistic symbiont of animals
Curvularia	0	0.073	0.073	0	0	0	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	HC	parasitic or mutualistic symbiont of plants
Cyphellophora	0.12	0.218	0.338	0.186	0	0.186	Ascomycota	Eurotiomycetes	Chaetothyriales	Chaetothyriaceae	HC	parasitic or mutualistic symbiont of animals

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Dactylella	0	0.145	0.145	0	0.443	0.443	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	HC	parasitic or mutualistic symbiont of animals
Debaryomyces	0	0.218	0.218	0	0.074	0.074	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	HC	saprotroph
Devriesia	0	0	0	0	0.074	0.074	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	HC	saprotroph
Diplodia	0	0	0	0	0.148	0.148	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	HC	parasitic or mutualistic symbiont of plants
Epicoccum	0.241	0	0.241	0	0.222	0.222	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	HC	parasitic or mutualistic symbiont of plants
Exophiala	0	0	0	0.248	0	0.248	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	HC	parasitic or mutualistic symbiont of animals
Fimetariella	0	0.145	0.145	0	0	0	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	HC	saprotroph
Flavodon	**0.361**	**0.29**	**0.651**	**2.664**	**4.065**	**6.729**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Meruliaceae**	**LHC**	**saprotroph**
Fomitopsis	0	0	0	0.062	0	0.062	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	HC	saprotroph
Fonsecaea	0.361	0	0.361	0.124	0.517	0.641	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	HC	parasitic or mutualistic symbiont of animals
Fusarium	0.482	0.145	0.627	0.248	0.443	0.691	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	HC	parasitic or mutualistic symbiont of plants

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Galerina	**0.482**	**0.073**	**0.555**	**0.434**	**0.074**	**0.508**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Strophariaceae**	**LHC**	**saprotroph**
Ganoderma	**0.602**	**0**	**0.602**	**0.062**	**0.074**	**0.136**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Ganodermataceae**	**LHC**	**saprotroph**
Gerronema	**0**	**0**	**0**	**0.31**	**0**	**0.31**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Marasmiaceae**	**LHC**	**saprotroph**
Gliocladium	0.241	0	0.241	0	0	0	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	HC	saprotroph
Gloeotinia	0.241	0	0.241	0	0	0	Ascomycota	Leotiomycetes	Helotiales	norank	HC	parasitic or mutualistic symbiont of plants
Grammothele	**0.241**	**0**	**0.241**	**0**	**0**	**0**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Polyporaceae**	**LHC**	**saprotroph**
Greeneria	0	0	0	0	0.148	0.148	Ascomycota	Sordariomycetes	Diaporthales	Gnomoniaceae	HC	saprotroph
Gymnopus	**0**	**0**	**0**	**1.425**	**0.296**	**1.721**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Omphalotaceae**	**LHC**	**saprotroph**
Hansfordia	0	0.218	0.218	0	0	0	Ascomycota	Sordariomycetes	Xylariales	norank	HC	saprotroph
Henningsomyces	**0**	**0**	**0**	**0.062**	**0**	**0.062**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Schizophyllaceae**	**LHC**	**saprotroph**
Hyphoderma	**0**	**0**	**0**	**0.124**	**0.148**	**0.272**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Meruliaceae**	**LHC**	**saprotroph**
Hypocrea	0	0.218	0.218	0.372	0.074	0.446	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	HC	saprotroph
Isaria	0.12	0.145	0.265	0	0.296	0.296	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	HC	parasitic or mutualistic symbiont of animals
Kuraishia	0	0	0	0	0.074	0.074	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	HC	saprotroph
Lasiodiplodia	0.723	0.218	0.941	0.186	0.517	0.703	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	HC	parasitic or mutualistic symbiont of plants
Lasionectria	0	0	0	0	0.074	0.074	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	HC	saprotroph

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Lecanicillium	0	0.073	0.073	0	0	0	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	HC	parasitic or mutualistic symbiont of animals
Lepiota	**0**	**0**	**0**	**0.062**	**0**	**0.062**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Agaricaceae**	**LHC**	**saprotroph**
Letendraea	0	0.073	0.073	0	0	0	Ascomycota	Dothideomycetes	Pleosporales	Tubeufiaceae	HC	parasitic or mutualistic symbiont of plants
Leucocoprinus	**0**	**0**	**0**	**0.062**	**0.074**	**0.136**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Agaricaceae**	**LHC**	**saprotroph**
Malassezia	0	0	0	0.062	0.517	0.579	Basidiomycota	Exobasidiomycetes	Malasseziales	Malasseziaceae	HC	parasitic or mutualistic symbiont of animals
Megacollybia	**0**	**0**	**0**	**0**	**0.222**	**0.222**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Marasmiaceae**	**LHC**	**saprotroph**
Meira	0	0	0	0.062	0.074	0.136	Basidiomycota	Exobasidiomycetes	Exobasidiales	Brachybasidiaceae	HC	parasitic or mutualistic symbiont of plants
Metacordyceps	0	0.073	0.073	0	0	0	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	HC	parasitic or mutualistic symbiont of animals
Metarhizium	0	0.29	0.29	0	0.148	0.148	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	HC	parasitic or mutualistic symbiont of animals

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Metulocladosporiella	0	0.87	0.87	0	2.513	2.513	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	HC	parasitic or mutualistic symbiont of plants
Meyerozyma	52.771	23.06	75.831	47.77	18.256	66.026	Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	HC	saprotroph
Monascus	0.12	0	0.12	0	0	0	Ascomycota	Eurotiomycetes	norank	Monascaceae	HC	saprotroph
Mortierella	0	0	0	0	0.074	0.074	Zygomycota	norank	Mortierellales	Mortierellaceae	S	saprotroph
Munkovalsaria	0	0	0	0	0.074	0.074	Ascomycota	Dothideomycetes	Pleosporales	Dacampiaceae	HC	saprotroph
Mycosphaerella	0	0.218	0.218	0	0	0	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	HC	parasitic or mutualistic symbiont of plants
Myrothecium	0	0	0	0.062	0	0.062	Ascomycota	Sordariomycetes	Hypocreales	norank	HC	saprotroph
Nigrospora	0	0.073	0.073	0.062	0.37	0.432	Ascomycota	Sordariomycetes	Trichosphaeriales	norank	HC	saprotroph
Ochroconis	0	0	0	0	0.074	0.074	Ascomycota	norank	norank	norank	HC	parasitic or mutualistic symbiont of animals
Paraconiothyrium	0	0.363	0.363	0	0.148	0.148	Ascomycota	Dothideomycetes	Pleosporales	Montagnulaceae	HC	saprotroph
Paradictyoarthrinium	0	0.073	0.073	0	0	0	Ascomycota	norank	norank	norank	HC	saprotroph
Penicillium	0	0.145	0.145	0.372	0.887	1.259	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	HC	saprotroph
Perenniporia	**0**	**0.073**	**0.073**	**0**	**0**	**0**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Polyporaceae**	**LHC**	**saprotroph**
Pestalotiopsis	0	0.363	0.363	0	0.074	0.074	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriaceae	HC	saprotroph

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Phanerochaete	**0**	**0**	**0**	**0.31**	**0.148**	**0.458**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Phanerochaetaceae**	**LHC**	**saprotroph**
Phialemonium	0	0	0	0.062	0.222	0.284	Ascomycota	Sordariomycetes	Sordariales	Cephalothecaceae	HC	parasitic or mutualistic symbiont of animals
Phialophora	0.723	0.218	0.941	0.496	0	0.496	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	HC	parasitic or mutualistic symbiont of animals
Phlebia	**0.241**	**0.073**	**0.314**	**0**	**0**	**0**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Meruliaceae**	**LHC**	**saprotroph**
Phoma	0	0.073	0.073	0	0.074	0.074	Ascomycota	Dothideomycetes	Pleosporales	norank	HC	parasitic or mutualistic symbiont of plants
Pichia	2.41	5.729	8.139	6.258	15.743	22.001	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	HC	saprotroph
Pluteus	**0**	**0**	**0**	**0.31**	**0**	**0.31**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Pluteaceae**	**LHC**	**saprotroph**
Prosthecium	0	0.145	0.145	0	0	0	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	HC	parasitic or mutualistic symbiont of plants
Pseudocercospora	0	0.073	0.073	0	0	0	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	HC	parasitic or mutualistic symbiont of plants

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Ramichloridium	0	0.073	0.073	0	0	0	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	HC	saprotroph
Resinicium	**24.578**	**11.965**	**36.543**	**10.037**	**6.356**	**16.393**	**Basidiomycota**	**Agaricomycetes**	**Hymenochaetales**	**Rickenellaceae**	**LHC**	**saprotroph**
Resupinatus	**0**	**0.145**	**0.145**	**1.363**	**0.148**	**1.511**	**Basidiomycota**	**Agaricomycetes**	**Agaricales**	**Tricholomataceae**	**LHC**	**saprotroph**
Rhinocladiella	0.12	0.29	0.41	0.062	0.37	0.432	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	HC	parasitic or mutualistic symbiont of animals
Rhodotorula	0.602	0	0.602	0.434	0.813	1.247	Basidiomycota	Microbotryomycetes	Sporidiobolales	norank	HC	saprotroph
Scheffersomyces	0.12	0	0.12	0	0	0	Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	HC	saprotroph
Scytalidium	1.687	35.968	37.655	0.991	15.225	16.216	Ascomycota	Leotiomycetes	Helotiales	norank	HC	saprotroph
Seimatosporium	0.12	0	0.12	0	0	0	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriaceae	HC	saprotroph
Simplicillium	1.325	0	1.325	0.496	0	0.496	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	HC	saprotroph
Sporothrix	0	0.29	0.29	0.062	0.296	0.358	Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	HC	saprotroph
Sugiyamaella	0.12	0.073	0.193	0.186	0	0.186	Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	HC	saprotroph
Symbiotaphrina	0	0.073	0.073	0	0	0	Ascomycota	norank	norank	norank	HC	parasitic or mutualistic symbiont of animals
Teratosphaeria	0	0	0	0	0.148	0.148	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	HC	parasitic or mutualistic symbiont of plants

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TABLE 2. (Continued)

Genus	RSDP			SCSP			Phylum	Class	Order	Family	Biochemical Activity[1]	Main Ecological Group
	ITS1	ITS2	Total	ITS1	ITS2	Total						
Thielavia	0.12	0	0.12	0	0	0	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	HC	saprotroph
Torulaspora	0	0	0	0	0.074	0.074	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	HC	saprotroph
Trametes	**0.241**	**1.668**	**1.909**	**0**	**0.665**	**0.665**	**Basidiomycota**	**Agaricomycetes**	**Polyporales**	**Polyporaceae**	**LHC**	**saprotroph**
Trichoderma	0.12	0.508	0.628	0.434	0.961	1.395	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	HC	saprotroph
Tricladium	0.12	0	0.12	0	0	0	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	HC	saprotroph
Umbelopsis	0	0	0	0.062	0.222	0.284	Zygomycota	norank	Mucorales	Umbelopsidaceae	S	saprotroph
Veronaea	0	0.073	0.073	0.124	0	0.124	Ascomycota	norank	norank	norank	HC	parasitic or mutualistic symbiont of plants
Vertexicola	1.325	6.889	8.214	0.929	6.578	7.507	Ascomycota	Sordariomycetes	norank	Annulatascaceae	HC	saprotroph
Verticillium	0	0	0	0.062	0.222	0.284	Ascomycota	Sordariomycetes	norank	Plectosphaerellaceae	HC	parasitic or mutualistic symbiont of plants
Villosiclava	0.361	0	0.361	0.31	0	0.31	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	HC	parasitic or mutualistic symbiont of plants
Wickerhamomyces	0	0.073	0.073	0	0	0	Ascomycota	Saccharomycetes	Saccharomycetales	Wickerhamomyceteae	HC	saprotroph
Xylaria	0	0	0	0.124	0.074	0.198	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	HC	saprotroph

[1]Note: LHC: able to degrade lignin, cellulose and hemicelluloses (white-rot) in bold; HC: able to degrade hemicelluloses and/or cellulose, S: unable to degrade hemicelluloses and/or cellulose

Discussion

The internal transcribed spacer (ITS1 and ITS2) and 5.8S regions of the nuclear ribosomal repeat unit have been used as the primary fungal barcode markers for species delimitation (Schoch *et al.* 2012). The full-length ITS region comprises approximately 599 ± 113 bp; the ITS1 region contributes approximately 208 ± 57 and ITS2, 310 ± 67 (Porras-Alfaro *et al.* 2014). "Ion torrent 316" generates reads of approximately 200 bp in length; thus, it is important to determine the best metabarcodes for fungal metagenomics research. The use of ITS1 and/or ITS2 in the identification of fungal species has been previously evaluated but there is no consensus as to which of them, ITS1 (Wang *et al*. 2015), ITS2 (Bazzicalupo *et al*. 2013) or both (Porras-Alfaro *et al*. 2014) capture the highest number of fungal taxa. In our study, the full-length ITS region was amplified and submitted to NGS, randomly generating sequences. The sequence data from ITS1 and ITS2 were treated separately, and the obtained read lengths were sufficient to discriminate MOTUs at the genus level at 95% similarity level.

In this study we identified a higher number of genera in SCSP than in RSDP. The first area is located in a region known for a high level of species richness and a high degree of endemism of trees and did not show evidence of recent disturbance, representing the best state of conservation of the original vegetation (Martini *et al.* 2007). By contrast, RDSP showed lower species richness of trees (Lopes *et al.* 2002) and showed several signs of anthropogenic impacts. According to Boddy (2001), a higher species diversity of trees represents increased variability in the amount of woody debris available for decomposition by saproxylic fungi.

The majority of the ascomycotan genera retrieved in our study are directly or indirectly related to the decomposition of plant debris (Zhao *et al.* 2013), and Sordariomycetes exhibited the highest number of genera, as also reported in a global biodiversity study of soil mycobiome (Tedersoo *et al.* 2014). We identified four genera (*Pestalotiopsis*, *Xylaria*, *Hansfordia*, *Seimatosporium*) of Xylariales that comprise the most effective group of wood decomposers in the Sordariomycetes (Pointing *et al.* 2003, Eichlerová *et al.* 2015). Furthermore, many of the ascomycotan saproxylic species belonging to the Hypocreales (*Trichoderma, Acremonium, Fusarium, Simplicillium, Hypocrea, Villosiclava, Gliocladium, Myrothecium, Chaunopycnis, Clonostachys, Lasionectria*), Eurotiales (*Aspergillus* and *Penicillium*), Diaporthales (*Greeneria* and *Prosthecium*), Chaetosphaeriales (*Chloridium*) and the Sordariales (*Chaetosphaeria, Phiallemonium, Fimetariella, Thielavia*) were also retrieved. The genus *Sporothrix* (Ophiostomatales), which has been associated with bark and ambrosia beetles, as a causal agent of sap stain in freshly cut wood, and also as a ubiquitous saprobe on rotting leaves, wood, soil (Meyer *et al.* 2008) was also detected. In contrast to the Hypocreales, the majority of which are related to wood decomposition, the genera *Isaria*, *Beauveria*, *Cordyceps*, and *Lecanicillium* are composed of typical insect pathogens (Cannon & Kirk 2007). Moreover, several genera of Eurotiomycetes (*Phialophora*, *Fonsecaea*, *Rhinocladiella*, *Exophiala*, *Cyphellophora*, and *Cladophialophora*) can occur as saprobes or facultative pathogens of animals that cause opportunistic infections (Cannon & Kirk 2007).

Saccharomycetes are mainly related to sugar decomposition and are present in live and dead plant tissues, including wood (Stokland *et al.* 2012). Furthermore, Saccharomycetes are especially associated with the guts of saproxylic insects involved in lignocellulosic decomposition (Calderon & Berkov 2012). The majority of the genera of this class (*Pichia*, *Candida*, *Sugiyamaella*, *Debaryomyces*, *Kuraishia*, *Scheffersomyces*, *Wickerhamomyces*, *Torulaspora*, and *Meyerozyma*) have also been detected in previous studies of wood-decaying fungi from tropical forests that employed culture-dependent methods (Urbina *et al.* 2013, Davis 2014). Orbiliomycetes (*Arthrobotrys*, *Dactylella*) are typically found as saprotrophs on wood and can also present a nematophagic habit (Cannon & Kirk 2007), which represents a strategy for survival in low-nitrogen environments such as wood (Berg & McClaugherty 2008).

There were proportionally many more genera of white-rot fungi than brown-rot fungi as expected for wood-decaying Basidiomycota in tropical biomes (Ryvarden 1991) (in our study approx. 87:13%). With a few exceptions, the majority of the retrieved genera belonged to Agaricomycetes, a pattern similar to that reported in Tedersoo *et al.* (2014) for soil fungi. This basidiomycotan class includes saprotrophs, pathogens and mutualists, and all of the species are capable of lignin decomposition (Riley *et al.* 2014). Numerous representatives of white-rot genera of Hymenochaetales (*Resinicium*), Polyporales (*Flavodon*, *Ganoderma*, *Phanerochaete, Trametes*, *Hyphoderma*, *Phlebia*, *Perenniporia*, *Grammothele*), Russulales (*Asterostoma*) and Agaricales (*Pluteus, Resupinatus, Gymnopus, Leucocoprinus, Galerina, Lepiota, Gerronema, Megacollybia, Collybia, Clitopilus*, *Agaricus*) were detected. Furthermore, brown-rot genera of Polyporales such as *Fomitopsis* (Yoon *et al*. 2005) and *Antrodia* (Clausen & Green 2003) were also present, as well as typical brown-rot taxa of Dacrymycetes (*Calocera*) (Seifert, 1983) and hemicellulolytic taxa associated with saproxylic insects in the order Tremellales (*Cryptococcus*) (Urbina *et al.* 2013). Moreover, two genera (*Meira* and *Malassezia*) belonging to the subphylum Ustilaginomycotina, which are not directly related to wood decomposition,

were also retrieved. *Meira* is typically reported as an endophyte associated with mites (Rush & Aime 2013), and it has been detected in orchid roots (Huang *et al.* 2014). *Malassezia* has been detected in the gut of basidiomata-feeding beetles (Blackwell *et al.* 2007), in addition to occurring in the skin of animals as a commensal and facultative pathogen (Wang *et al.* 2014).

A total of 20% of the genera identified was unequivocally composed of lignin decomposers as the species of these genera possess high-oxidation potential peroxidases, such as LiP, MnP, and VP, based on genetic and/or biochemical evidence according to the CAZY (Lombard *et al.* 2013) and BRENDA (Schomburg *et al.* 2013) databases, as well as specialized literature. Moreover, the majority of the genera presented evidence of bearing hemicellulytic and/or cellulytic enzymes or causing brown and soft rot in wood, excluding some genera of typical "sugar fungi", e.g., *Mortierella* and *Umbelopsis*.

Nine studies have already specifically investigated the mycobiomes of decaying wood using targeted, amplicon-based metagenomics, five in palearctic boreal forests in northern Europe (Ovaskainen *et al.* 2010, Kubartova *et al.* 2012, Ovaskainen *et al.* 2013, Runnel *et al.* 2015) and four in palearctic temperate forests in Europe and Asia (Van der Wal *et al.* 2015, Hoppe *et al.* 2015, Jang *et al.* 2015, Yamashita *et al.* 2015). With the exception of Yamashita *et al.* (2015), which used the V9 region of 18S rDNA, all the other works used the complete ITS (Ovaskainen *et al.* 2010, Kubartova *et al.* 2012, Ovaskainen *et al.* 2013, Hoppe *et al.* 2015, Otosson *et al.* 2015) or the ITS2 (Jang *et al.* 2015, Runnel *et al.* 2015, Van der Wal *et al.* 2015) as the targeted region. Furthermore, all the analyses of these studies were mainly focused on Basidiomycota, limiting a fully global comparison with our study. Nevertheless, some general patterns could be evidenced taking into account the Phylum Basidiomycota. Some genera were retrieved in all forest types (boreal, temperate and tropical) such as *Resinicium*, *Pluteus*, *Ganoderma*, *Calocera*, *Cryptococcus*, *Phanerochaete*, *Galerina*, *Phlebia*, *Megacollybia*, *Fomitopsis* and *Antrodia*, while other genera, such as *Asterostroma*, *Flavodon*, *Resupinatus, Gymnopus*, *Leucocoprinus*, *Lepiota*, *Meira*, *Gerronema*, *Collybia*, *Grammothele* and *Agaricus* were only identified in our study in the tropical sites and were not reported in any of the other aforementioned studies based on large-scale DNA sequencing in boreal or temperate forests. The fungal genera identified in all forest types have a wide geographic distribution, occurring in all major climatic zones (Ryvarden 1993), whereas some of the genera detected only in the neotropical forests usually have known geographic distribution restricted to or mainly in tropical and subtropical regions (Suhara *et al.* 2010, Velinga 2004, Johnson & Vilgalys 1998).

There are at least twice as many Ascomycota than Basidiomycota taxa (Hibbett, 2014), and this pattern was also retrieved in our work. In a recent and comprehensive review of the diversity of Brazilian fungi, Maia *et al*. (2015) reported that almost half of the fungal taxa recorded in Brazil was Basidiomycota (47.9%), followed by Ascomycota (32.9%). The same authors suggested that this inverted ratio could be explained by the low number of experts in Ascomycota taxonomy in Brazil.

The number of fungal genera retrieved in our work was little more than half of the fungal genera presented in the continuously updated list of Plants and Fungi of Brazil (Canhos *et al*. 2015) when we filtered this general database for the parameters: Atlantic forest biome and dead wood. Furthermore, approximately 77% of the fungal genera retrieved in our work were not recorded in this trimmed dataset. Nevertheless, some of them are yeasts or microfungi that could not be represented in this database.

Conclusion

This is the first study using NGS to uncover the diversity of wood-decaying fungi in tropical forests. The higher diversity of trees and lower disturbance in the tropical rainforest site could explain the higher number of fungal genera obtained in the tropical rainforest site when compared with the seasonal tropical forest. All genera belonging to the ecological functional groups of saproxylic fungi, except for mycorrhizal fungi, were retrieved. Most prevalent genera were ligninolytic and/or cellulolytic Ascomycota (Sordariomycetes) and Basidiomycota (Agaricomycetes) but plant pathogens, endophytes and facultative or obligatory animal pathogenic fungi were also found. Moreover, the great majority of Basidiomycota genera are white-rotters as expected for tropical regions. As the metabarcoding approach is not dependent on collection, direct observation and/or culturing, this methodology theoretically permits the most thorough access to the diversity of the mycobiome of any kind of substrate. Thus, the metabarcoding as a rapid, large-scale, and theoretically taxonomically unbiased strategy, proved useful to perform comprehensive surveys, however, the clustering and taxonomic identification steps are still challenging. We recommend to test different percentage values for these steps and compare the results with a comprehensive and reliable database.

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