11-2020

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Main text: 4473 words

Figures: 6

Supplemental Information: Figure S1, Tables S1-S4.

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Abstract

Hurricanes impact forests by damaging trees and altering multiple ecosystem functions. As such, predicting which individuals are likely to be most affected has crucial economic importance as well as conservation value. Tree stem-inhabiting fungal communities, notably rot-causing agents, have been mentioned as a potential factor of tree predisposition to hurricane damage, but this assumption remains poorly explored. To examine this relationship, we sampled the stem wood of intact and damaged trees shortly after Hurricane Maria in a Puerto Rican dry tropical forest in 2017. We categorized samples depending on two types: trees with intact stems and trees in which stems were snapped. We extracted fungal environmental DNA of wood from 40 samples consisting of four different tree species. Fungal community taxonomic and functional richness and composition was assessed using high-throughput DNA metabarcoding. We found that snapped trees harbored significantly higher fungal operational taxonomic unit (OTU) richness than the intact trees and that the composition of the stem-inhabiting fungal communities diverged consistently between intact and snapped trees. On average, snapped trees' fungal communities were relatively enriched in "other saprotrophs" guild category and depleted in endophytes. Conversely, intact trees had high relative abundances of *Clonostachys*, a mycoparasitic endophyte, suggesting that endophytic fungi might act as biocontrols in tree stems. Overall, our results support the hypothesis that stem-inhabiting fungal communities could represent a predisposition factor of tree damage caused by hurricanes in tropical dry forests.

Keywords: Tropical cyclone, predisposition factor, tree holobiont, Caribbean Island, tropical forest.

Introduction

High-speed winds associated with hurricanes can cause massive tree falls (Van Bloem et al., 2005; 2006; Holm al., 2017, Parker et al., 2018) and have been described as important determinants of tropical forest properties (i.e. stem density, height, canopy size) as well as tree species composition (Zimmerman et al., 1994; Van Bloem et al., 2005; 2006). Disturbance associated with hurricanes frequently has cascading effects on forest ecosystem carbon and nutrient cycling by reducing CO_2 fixation due to leaf and stem damage and increasing CO_2 emissions associated with wood decomposition (McNulty et al., 2002; Holm et al., 2017). Additionally, windthrow can shift tree species composition because some species are more prone to windthrow while others are more likely to regenerate by basal sprouts or in gaps (Brokaw and Grear, 1991; Bellingham and Tanner, 1995). Given these impacts, having the ability to determine which tree individuals are most likely to be damaged by hurricanes may provide land managers better informed forest management.

High winds from hurricanes generally cause either whole trees to fall over due to uprooting or for tree stems to be broken (Tanner et al., 1991; Van Bloem et al., 2005; 2006). Stem breakage caused by wind generally depends on the tree diameter, the tree height, and the wood resistance (Gardiner et al., 2016; Jimenez-Rodríguez et al., 2018; Paz et al. 2018). Additionally, microbes infecting wood of living trees have been described as a potential predisposition factor of stem breakage (Putz et al., 1983; Hennon 1995; Lewis and Lindgren, 1999). Specifically, the presence of rot in tree trunks has been mentioned as a component of tree sensitivity to wind damages (Gardiner et al., 2016). The effect of stem rot on wood strength, however, has rarely been quantified and this biotic component is generally not included in forest wind risk models (Ciftci et al., 2014). At the same time, the development of detection tools of infected trees for landscape management has received considerable attention

(Guglielmo et al. 2010; Robles et al., 2015). In general, living trees infected by pathogenic fungi are considered a threat for humans and infrastructure in regions subject to hurricanes due to falling risk and are often subject to preventive cutting (Duryea and Kampf, 2017).

Pathogenic fungi cause different types of rot depending on the type of wood they inhabit (Vasaitis, 2013). Fungal infection of stem heartwood causes heart rot (Vasaitis, 2013), which can be quite common in moist and wet tropical forests, sometimes infecting as much as 60% of trees (Heineman et al., 2015; McDowell et al., 2018). By contrast, infection by pathogenic fungi of sapwood is called sap rot and affects the functional tissues of the tree stem (Terashima, 2013). Gilbert et al. (1994) found that pathogenic fungi creating "cankers" were also common, infecting up to 70% of trees in a moist tropical forest. Both of these types of rot might alter wood structural properties in ways that facilitate stem breakage (Vasaitis, 2013). For example, rot causing agents might decompose the lignin to get access to holocellulose as a carbon source (Schwarze et al., 2003; Schwarze, 2007; Santini et al., 2019). This decomposition phenomenon is often associated with a decrease of the wood density in the diseased zone and an increase of the wood density in the barrier zone created by the tree to limit the infection (Santini et al., 2019).

There is growing appreciation that the stems of living trees are not exclusively colonized by pathogenic fungi but also by endophytes, defined as fungi living in tree tissues producing asymptomatic infections (Rodriguez et al., 2009). Recent studies concluded that fungal endophytes might protect trees against pathogenic microbes and insects as well as improving the environmental stress tolerance of plants (Porras-Alfaro and Bayman, 2011; Rho et al., 2017). Saprotrophic fungi have also been detected in woody tissues of living trees (Pellitier et al., 2019; Skaltas et al., 2019). Some studies showed that saprotrophic fungi are present in a

latent form in the trunk and then initiate the decomposition of wood after the death of the tree (Boddy and Grifith 1989; Oses et al., 2008; Song et al., 2017; Cline et al., 2017). Other studies found that saprotrophic fungi living in wood possessed an opportunistic pathogenic trophic status when the tree health status was low (Body 2001; Persson et al., 2009; Parfitt et al., 2010; Slippers et al., 2012). High functional versatility among fungal taxa living in plant tissues switching from mutualism or commensalism to parasitism interactions was recently highlighted (Lofgren et al., 2018; for a synthesis see Selosse et al., 2018). Collectively, all of these different fungal guilds are the main component of the tree holobiont, with likely important consequences on both tree phenotype and response to environmental disturbances (Vandenkoornhuyse et al., 2015). However, to our knowledge, the identification of stem-associated fungal communities and their potential roles in tree predisposition to hurricane damage has not been investigated in tropical dry forests.

The goal of this study was to compare the structure of the fungal communities inhabiting stems of intact and snapped trees caused by a strong hurricane in a tropical dry forest. Hurricane Maria, which was a category 1 storm, hit the island of Puerto Rico in September 2017, causing widespread forest damage (Hu and Smith, 2018; Uriarte et al., 2019). Three weeks after the main event of the hurricane, we sampled intact and snapped stems from four abundant tree species, *Colubrina arborescens* (Rhamnaceae), *Exostema caribaeum* (Rubiaceae), *Leucaena leucocephala* (Fabaceae) and *Pithecellobium unguis-cati* (Fabaceae), in the Guánica Forest and Biosphere Reserve. We identified the fungal communities inhabiting the tree stems using high-throughput DNA metabarcoding of the ITS2 fungal marker. Additionally, we evaluated the wood properties by measuring wood density and lignin content. We hypothesized that snapped trees would contain fungal communities with (H1) higher OTU richness and (H2) greater relative abundances of pathogenic and

123 saprotrophic fungi by comparison with intact trees. We further hypothesized (H3) that
124 snapped trees would have different wood properties than intact trees.

125 **Materials and methods**

126 *Experimental site*

127 Guánica Forest is located in Southwestern Puerto Rico (18°N 66°55'W). The central part of
128 the forest is dominated by mature tropical dry forest that has been protected from extensive
129 cutting since 1919 when it was designated a State Forest (Murphy and Lugo 1986). The forest
130 boundaries were expanded in 1948, adding stands that were previously farmed or housing and
131 are currently dominated by secondary forests with some stands dominated by non-native
132 species including *Leucaena leucocephala* (Colón and Lugo 2006). Thus minimum stand ages
133 range from 60-120 or more years. Annual rainfall from 1931-2018 has averaged 824 mm,
134 average annual temperature is 25.1°C, and average potential evapotranspiration is >1200mm
135 (Murphy and Lugo 1986, Wolfe, Macchiavelli, and Van Bloem 2019). Typically, rainfall is
136 bimodally distributed into a spring wet and a fall wet season, but timing and amount of rains
137 is highly variable. Soils are mollisols developed from limestone parent material with pH >7.5
138 and low available P (Lugo and Murphy 1986). Dry conditions with low soil fertility result in
139 tree diameter growth averaging <1mm/yr (Van Bloem, unpublished data) which leads to
140 many species having dense wood (0.8-1.2) but a few species having light wood (<0.4) (Colón
141 and Lugo 2006). Hurricane Maria buffeted the forest with 105 km/hr winds (Category 1)
142 September 20, 2017. The previous hurricane to affect the forest was Georges in 1998 with 180
143 km/hr winds (Van Bloem et al. 2005).

144 *Sampling*

145 Approximately one month after Hurricane Maria, fallen and intact trees were sampled along
146 roads and trails in Guánica Forest. To minimize colonization of the fallen stems by soil fungi
147 we only sampled snapped trees standing vertically against other trees. Stem sections were cut

from snapped trees with a chainsaw. Sections were 1-1.5 m in length and included the snap point. Sections of intact stems were cut from undamaged trees found nearby snapped trees. We selected stems with diameters varied from 2.5-15 cm, a diameter range which comprises >99% of the trees in the forest (Murphy and Lugo 1986). We focused on four tree species which provided sufficient sample size of both snapped and intact stems: *Colubrina arborescens, Exostema caribaeum, Leucaenea leucocephala,* and *Pithecellobium unguis-cati* (see Table 1 for the number of samples for each tree species depending on the treatment). *L.leucocephala* is a naturalized legume that is commonly found in secondary forest stands in Puerto Rican dry forests. The other species are native to the island. Wood discs of ~2 cm thickness were cut from stem sections, either at the mid-sections of boles or at the first clean section to yield intact discs near the site of snaps. Samples were stored at 4°C in Puerto Rico after sampling, shipped overnight to Minnesota in freezer packs, and stored frozen until analyses.

Fungal community analyses

We collected shavings from each frozen wood disc from three spots randomly chosen using a drill bit sterilized by autoclaving. Total genomic DNA was extracted from the wood shavings for each sample with the DNEasy Powerlyzer PowerSoil Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. Prior to the first step in the protocol, all samples were bead-beat for 15 sec (BioSpec Products, Bartlesville, OK, USA) to facilitate sample homogenization. Fungal DNA from each sample was amplified for high throughput sequencing using a two-step PCR process. For the first PCR, the ITS 5.8-Fun and ITS4-Fun primer pair (Taylor et al., 2016), which targets the ITS2 region, was used. Samples, including a synthetic mock community (Palmer et al., 2018) and negative controls, were amplified in individual 20 ul reactions containing 10 ul of Phusion Hot Start II High-Fidelity PCR Master

Mix (Thermo Scientific, Waltham, MA, USA), 0.5 ul of each 20 mM primer, 1 ul of DNA template and 8 ul of PCR-grade water. Thermocycling conditions were as follows: 1. 98°C for 30 seconds, 2. 98°C for 10 seconds, 3. 55°C for 30 seconds, 4. 72°C for 30 s, repeat steps 2-4 34 times, 5. 72°C for 10 minutes and 6. infinite hold at 4°C. For the second PCR, a second set of forward and reverse primers with unique Golay barcodes and Illumina adaptors were used. Reaction and thermocycling conditions were identical to the first PCR. Following the second PCR, all samples were cleaned and normalized using the Charm Just-A-Plate kit (Charm, San Diego, CA, USA) following manufacturer's instructions. Samples were then quantified on a Qubit fluorimeter (Thermo Scientific, Waltham, MA, USA), mixed at an equimolar concentration (3 nM) into a single sequencing library, and sequenced using Illumina MiSeq 2 x 300 bp v3 chemistry at the University of Minnesota Genomics Center.

The raw demultiplexed .fastq files were processed using the 'AMPtk' pipeline outlined in Palmer et al. (2018). Briefly, primers were removed and sequences to trimmed to 250 bp. Sequences were then denoised using UNOISE3 (Edgar 2016) and clustered into operational taxonomic units (OTUs) at 97% similarity. Read counts in the OTU x sample matrix were adjusted by accounting for index bleed present in the synthetic mock community. Finally, taxonomy was assigned using a hybrid algorithm that integrates results from a USEARCH global alignment against the UNITE database (v8, Nilsson et al., 2018) and both UTAX and SINTAX classifiers. Sequence read counts of any OTUs present in PCR and DNA controls were summed and then subtracted from counts of those same OTUs in all other samples. Fungal data were rarefied to 1298 counts (Figure S1). Seven samples presenting a low number of counts were removed.

Based on the consensus taxonomic assignments, fungal OTUs were assigned to saprotrophic, pathogenic, and symbiotrophic trophic modes using FUNGuild (Nguyen et al., 2016). Saprotrophic fungi were further parsed between soft rot and white rot fungi, with the remaining OTUs were classified as other saprotroph. No brown rot fungi were detected. Pathogenic fungi were parsed between plant pathogen and animal pathogen fungi. All the OTUs without FUNGuild assignment were blasted against the NCBI database. When a taxonomic identification was found, we matched this information against FUNGuild to reassign the OTUs to a potential guild following the protocol described previously. If no taxonomic identification was found we used a potential NCBI guild information.

Wood properties analyses

Using one cut half (through the pith) of frozen discs, wood density (g cm^{-3}) was measured at fresh volume (cm^3) using water displacement, followed by oven drying for 48 h at 103 °C and weighing (g). The dried half was then ground to a powder through 20-mesh in a Wiley mill and chemically analyzed. Wood lignin was measured as the acid-insoluble (Klason) fraction using the standard gravimetric ASTM procedure (2001).

Data analyses

Statistical analysis and data visualization were performed using R (R Core Team 2016) and considered significant at $P \leq 0.05$. Statistical analysis concerning the fungal community data was performed on rarefied sequence read counts. Fungal OTU richness (N0) and Shannon's diversity (H) and Shannon's evenness (E) were calculated in Vegan package (Oksanen et al., 2013). The effect of the tree species and treatment (i.e. intact versus snapped) on each of these three alpha diversity metrics was tested using two-way analyses of variance (ANOVA). Variance homoscedasticity was tested using Cochran's test and data were log-transformed if

necessary. Differences in fungal OTU composition were visualized with non-metric multi-dimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity matrix using the metaMDS function in Vegan package. Permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity for fungal OTUs were applied to determine the independent and interactive effects of tree species and treatment. Effect of treatment on fungal genera was tested using Kruskal-Wallis test by ranks. Differences in stem diameters, wood density, and wood lignin content by tree species and treatment (intact and snapped stems) were assessed using a two-way factorial analysis of variance (ANOVA). Variance homoscedasticity was tested using Cochran's test and data were log-transformed if necessary.

Results

Fungal community structure

From the 40 samples, there were a total of 51 920 sequences and 381 fungal OTUs present in the final quality-controlled dataset. Fungal OTU richness and diversity varied significantly depending on tree species (richness: $F_{3,39} = 6.34$, $P = 0.017$; diversity: $F_{3,39} = 6.14$, $P = 0.002$) (Table S1), with *C. arborescens*, *E. caribaeum* and *P. unguis-cati* harboring higher OTU richness than *L. leucocephala* (Figure 1). When grouped across tree species, fungal communities inhabiting stems of snapped trees had higher OTU richness (48% higher, on average) by comparison with intact trees ($F_{1,39} = 3.64$, $P = 0.023$). Fungal OTU diversity was also higher in snapped than intact trees, but this difference was not significant ($F_{1,39} = 1.74$, $P = 0.20$). There was also no significant interaction between tree species and treatment for either fungal OTU richness or diversity (richness: $F_{3,39} = 0.43$, $P = 0.73$; diversity: $F_{3,39} = 0.30$, $P = 0.83$).

Both tree species ($F_{1,39} = 3.73$, $P = 0.001$) and treatment ($F_{1,39} = 1.76$, $P = 0.036$) significantly influenced the taxonomic composition of stem-inhabiting fungal communities (Table S2), explaining respectively 22.3% and 3.6% of variability in fungal OTU composition, respectively. NMDS analysis based on OTU composition also showed that fungal communities clustered by treatment (Figure 2a), but also by tree species within treatment (Figure 2b). OTU-based hierarchical clustering showed that communities generally clustered by tree species first and then by treatment (Figure 2c), with the exception of *E. caribaeum*. Across treatments, the fungal communities consisted primarily of taxa in the phylum Ascomycota, with members of the Sordariomycetes, Dothiodeomycetes and Eurotiomycetes together representing 65-95% of relative sequence abundances (Figure 2c). In contrast, fungi in the Basidiomycota, represented by the classes, Agaricomycetes and Tremellomycetes, had lower relative abundances, ranging from 3% to 22%. Many of the most abundant fungal genera were host generalists (i.e. *Lasiodiplodia*, *Neofusicoccum*, *Phaeoacremonium*, *Albonectria, Schizophyllum*), although some genera displayed notable host preferences (Figure 3). *Clonostachys*, *Neofusicoccum*, *Neonectria*, *Bisporella* and *Diaporthe* were significantly more abundant in intact trees, while *Phaeoacremonium*, *Kwoniella*, *Eutypella*, *Fusicolla*, *Cryptococcus* and *Exophiala* were significantly more abundant in snapped trees.

Overall fungal guild composition was dominated by saprotrophic, plant pathogenic and endophytic fungi (Figure 2c). While there was no significant tree species effect on the relative abundances of other saprotroph, soft rot, plant pathogenic and animal pathogenic fungal guilds (Table S3), the relative abundance of the endophytic fungi was significantly impacted by tree species ($F_{3,32} = 5.61$, $P < 0.001$), being notably high for *L. leucocephala* (Figure 4). The relative abundance of white rot fungi was significantly affected by the interaction between tree species and treatment ($F_{3,32} = 3.53$, $P = 0.026$), being higher for intact *C.*

arborescens and *L. leucocephala* trees. The relative abundances of other saprotrophic fungi were significantly higher in snapped trees (76% on average) than in intact trees ($F_{1,32} = 4.19$, $P = 0.049$). This effect was mainly driven by *C. arborescence*, *E. caribaeum* and *L. leucocephala*. Animal pathogenic fungi also had significantly higher relative abundances in snapped trees ($4.8 \pm 6.5\%$) than in intact trees ($\sim 0 \pm 0.2\%$) ($F_{1,32} = 10.24$, $P = 0.003$).

3.2 Tree and wood properties

The 40 trees sampled ranged from 3.5 to 11 cm in diameter (Figure 5). There were no significant differences in diameter across either species ($F_{3,32} = 1.29$, $P = 0.29$) or treatment ($F_{1,32} = 0.096$, $P = 0.76$) (Table S4). Similarly, lignin content was not different among the four tree species ($F_{3,30} = 0.23$, $P = 0.88$) or between intact and snapped trees ($F_{1,30} = 1.05$, $P = 0.32$). Wood density, however, varied significantly by species ($F_{3,30} = 5.71$, $P = 0.003$), with *L. leucocephala* having the lowest wood density, *E. caribaeum* the highest, and *C. arborescens* and *P. unguis-cati* intermediate values. Wood density was not significantly different between snapped and intact trees ($F_{1,30} = 2.16$, $P = 0.15$).

Discussion

In our study, wood-associated fungal communities were dominated by Ascomycota fungi belonging to the Sordariomycetes, the Dothideomycetes and the Eurotiomycetes classes, as well as Basidiomycota fungi, notably the Agaricomycetes class. These observations are consistent with other studies from temperate and tropical forests (Thomas et al., 2008; Durand et al., 2017; Singh et al., 2017; Cregger et al., 2018; Sadeghi et al., 2019; Skaltas et al., 2019). Also consistent with previous studies, we found that the plant pathogenic, other saprotroph and endophytic fungal guilds were most abundant (Singh et al., 2017; Skaltas et al., 2019). Despite the similarities with other studies at the fungal class and guild level, our results

297 strongly differed at a lower taxonomic level. In tropical forests, stem-associated fungal
298 communities have typically been shown to be dominated by a few genera such as *Diaporthe*,
299 *Trichoderma* and *Collelotrichum* (Samuels et al., 2006; Gazis and Chaverri 2010; Sing et al.,
300 2017; Skaltas et al., 2019), but in our study, these three genera were almost absent. It is
301 possible that this relates to tree host phylogeny distinctions, although this has not been shown
302 a strong relationship in other, similar studies (Lee et al. 2019). Instead, we found an
303 interesting parallel between many abundant genera we identified (e.g., *Lasidioplodia*,
304 *Neofusicoccum*, *Phaeoacremonium* and *Schizophyllum*) and those described from stems of
305 grapevine (*Vitis vinifera*) (Essakhi et al., 2008; Casieri et al., 2009; Bruez et al., 2014; 2016;
306 Rezgui et al., 2018). Given the extremely dry climate and correspondingly slow growth, this
307 parallel might be due to a similarity in low stem diameter and/or stem old age (Van Bloem et
308 al., 2006; Rezgui et al., 2018).

309

310 In support of our first hypothesis, we found that snapped trees had significantly higher fungal
311 richness than intact trees across all four of the tree species studied. These results correspond
312 well with other non-hurricane-related studies that have found that the wood of unhealthy trees
313 typically have higher fungal richness than healthy trees (Ragazzi et al., 2003; Sun et al.,
314 2015). While our sampling did not specifically target diseased areas of the stems, Sun et al.
315 (2015) found that diseased zones in stem harbored a higher fungal richness than the intact
316 zones. Unhealthy trees, usually presenting stem wound lesions, were often described as
317 subject to a higher rate of co-infections (Sun et al., 2015). Collectively, these results suggest
318 that the stress of hurricane winds snapped trees that were in early infection stages and
319 unhealthy relative to their intact counterparts (Giordano et al., 2009; Kovalchuk et al., 2018).

320

In partial agreement with our second hypothesis, we found that the taxonomic and functional guild composition of the stem-inhabiting fungal communities was different between intact and snapped trees. Specifically, at the guild level, snapped trees harbored fungal communities enriched in other saprotrophic fungi. In Japan, bark stripping caused enhanced trunk decay by saprotrophic fungi and increased the risk of stem breakage due to typhoon winds (Hanada et al. 2008). Additionally, Larson et al. (2010) proposed that wood decay fungi were a cause of stem breakage and associated tree mortality in a temperate forest by changing the mechanical properties of the tree stem. These results might indicate that in our study other saprotrophic fungi started the decomposition of the tree stems and made them more sensitive to hurricane winds (Deflorio et al., 2008). Nevertheless, we did not find a significant difference in wood properties between intact and snapped trees, which contradicts our third hypothesis. Linking fungal community composition and wood properties, however, can be difficult. For example, even by targeting specifically the diseased zones and by comparing them with healthy parts of the stem, Santini et al. (2019) found only very slight changes in wood properties. Additionally, in our study, all the radial section of the stem was collected and wood was randomly sampled across the stem section. That might have attenuated wood properties differences by mixing healthy and unhealthy zones. Further efforts are needed to link the stem inhabiting fungal communities, their effect on wood properties and the impact of the modified wood properties on the resistance of the stem to physical stress caused by wind.

Contrary to our hypothesis, we did not find differences in plant pathogenic fungal relative abundance between fallen intact and snapped trees. Interestingly, however, we found that members of the fungal genus *Phaeoacremonium*, which causes wood decay symptoms, were significantly more abundant in snapped trees across all four of the tree species studied (Damm et al., 2008). *Phaeocremonium* has been found as a frequent agent of trunk dieback in almond

346 trees, olive trees and grapevines (Essakhi et al., 2008; Carlucci et al., 2015; Olmo et al.,
347 2015), suggesting the presence of this genus might serve as a specific predisposition factor of
348 trees to hurricane damage. *Eutypella*, a pathogenic fungal genus causing canker (Kliejunas et
349 al., 1974; Ogris et al., 2008), was exclusively detected in the snapped stems of *C. arborescens*
350 and *P. unguis-cati.* While less generalistic than *Phaeocremonium, Eutypella* might also
351 represent a candidate to tree predisposition to hurricane damages. Unexpectedly,
352 *Neofusicoccum*, another trunk canker and dieback pathogenetic genus (Slippers et al., 2007),
353 was abundant in samples of all four of the tree species with intact stems. *Neofusicoccum* has
354 been previously described as a latent pathogen that waits for stressful conditions to start an
355 infection (Slippers et al., 2007). By occupying stem tissues without pathogenic activity, this
356 fungus could act as a defensive mutualist by limiting the infections of the intact tree stems by
357 other actives pathogens or opportunistic saprotrophs. Support for this kind of multi-functional
358 nature of fungal-plant interactions was recently demonstrated by Robles et al. (2019), who
359 found that the same OTU might have both endophytic and pathogenic trophic modes. This
360 result shows the limitation of using only DNA metabarcoding followed by guild classification
361 based on taxonomic identification (Nguyen et al., 2016). Metatranscriptomics represents a
362 promising approach to identify the active part of the tree holobiont as well as the trophic
363 status of the taxa composing the fungal community in the plant tissues by comparing the
364 expressed genes repertoires involved in the pathogenicity and in the wood decomposition.
365
366 Perhaps most intriguingly, we found that fungi characterized by an endophytic lifestyle tended
367 to be more abundant in intact trees by comparison with snapped trees. In particular,
368 *Clonostachys*, which was the second most abundant fungal genus in our study, is a well-
369 characterized endophyte having biocontrol properties (Evans et al., 2003; Nygren et al.,
370 2010). The presence of fungi having mycoparasitic abilities in tree stems has been previously

371 reported in tropical forests, suggesting this may be a widespread phenomenon (Evans et al.,
372 2003; Skaltas et al., 2019). Additionally, in temperate forest, fungal endophytes were
373 described has having strong antagonism against the helm disease-causing stem dieback
374 (Weber et al., 1981; 1986). Based on these results, endophytes in general and *Clonostachys* in
375 particular might represent key taxa involved in tree resistance to hurricane tree damage
376 through their ability to limit infections by pathogenic and opportunistic saprotrophic fungi
377 (Arnold et al., 2003).
378
379 Wood samples were harvested 1 month after Hurricane Maria. This lag time was unavoidable
380 due to the unpredictable timing of hurricanes, and travel, safety, and funding limitations. It
381 does, however, introduce caveats, primarily in any colonization by new fungi in the snapped
382 samples. We took precautions to avoid this phenomenon by selecting only those snapped
383 stems that were standing vertically and by cutting the wood disks away from the broken area.
384 Additionally, wood decomposition in the Guanica tropical dry forest is slow (Torres et al.,
385 2005), and the precipitation was very low during this month (172.97 mm), concentrated just
386 before initial sampling. Consequently, we believe that our data mirror the fungal communities
387 initially inhabiting the stems at the time of Hurricane Maria. It does imply that a manipulative
388 experiment cutting live trees and then submitting whole, never-dried stem portions to
389 resistance stress (e.g. 3-point bending, or similar) combined with the identification of the
390 fungal communities might represent a complementary approach to explore the role of the
391 fungal communities in the wood properties of the living trees.
392
393 It is important to note that tree stem breakage was described as having a limited impact on
394 overall tree mortality in same Puerto Rican dry forest where this study was conducted (Van
395 Bloem et al., 2006; 2007). Tree resprouting after stem breakage was described as a very

frequent phenomenon after hurricanes and makes the Puerto Rican dry forest more resistant to damage by comparison with Puerto Rican tropical wet forests (Van Bloem et al., 2006; Holm al., 2017). We suggest that the fungal communities inhabiting tree wood may not only be an important factor related to tree predisposition to wind damage, but might also have an important impact on tree survival after hurricanes. Specifically, stem breakage creates a major wound facilitating the entry of pathogenic and opportunistic saprotrophic fungi, which represents a threat for the new stems during the tree regeneration process. The presence of stem-inhabiting fungal communities in stems may limit deleterious infections, although it is certainly possible that latent fungal pathogens and opportunistic saprotrophs might also cause more damage to storm-damaged trees as well. Assessing the richness and composition of fungal communities in snapped but recovering stems will provide greater clarity on the functional roles of tree stem-inhabiting fungal communities identified in this study.

Acknowledgements

We would like to thank the reviewers for their insightful comments on the manuscript. Funding provided by National Science Foundation (NSF) RAPID awards 1822065 to J.S.S. and 1822081 to S.J.V.B. focusing on post-hurricane early response research. We thank the E. Martínez, T.A.P. Allerton, A. Acevedo Morales, and the Guánica Forest staff for their help in harvesting stem sections, and J.A. Sanchez Dominguez for providing lodging during the post-Maria recovery period.

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666 **Table**

667 Table 1. Summary of the number of replicates for each tree species (*C. arborescens*, *E.*
668 *caribaeum*, *L. leucocephala* and *P. unguis-cati*) depending on the treatment (intact and
669 snapped stem).

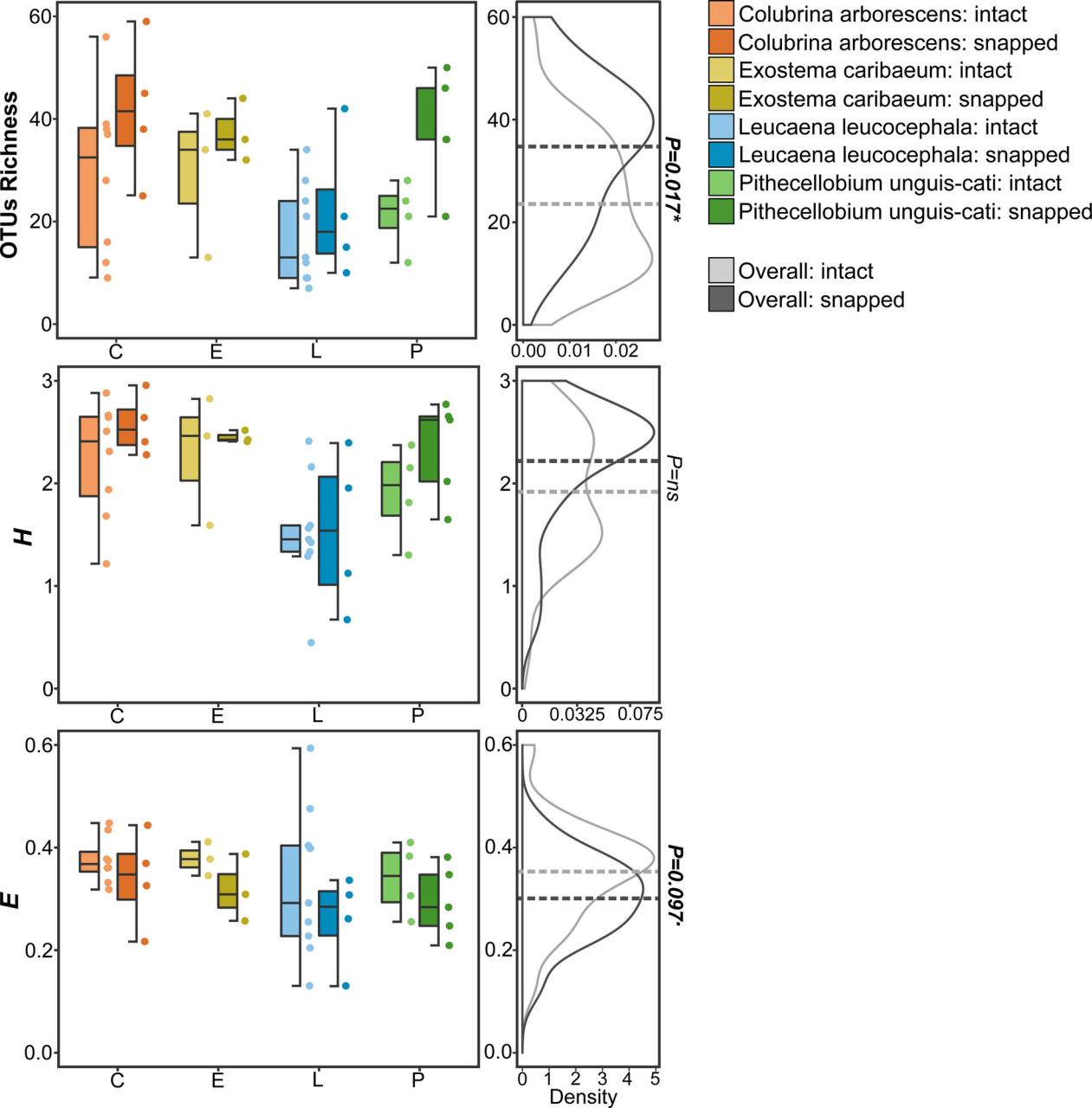
670 **Figures**

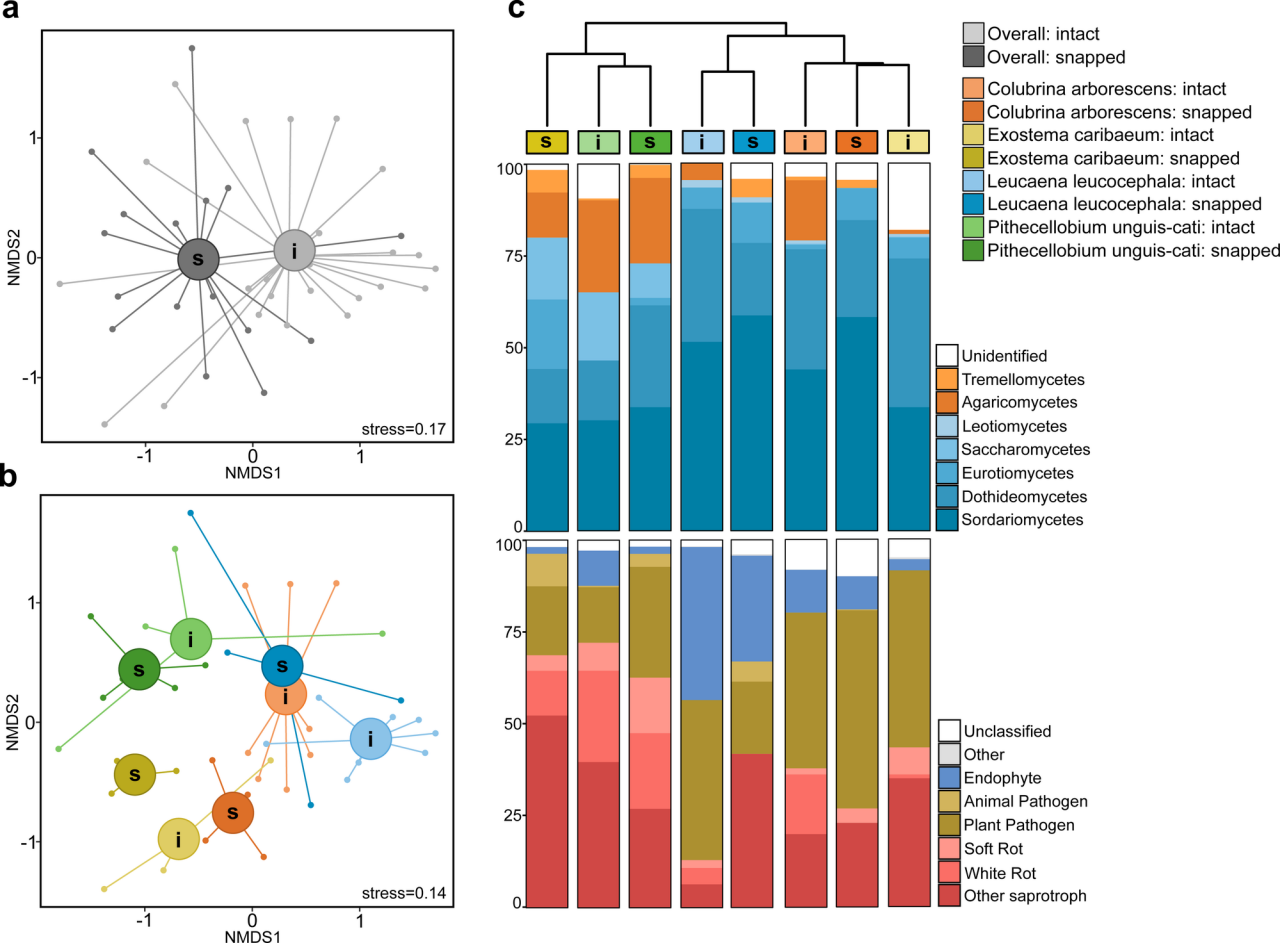
671 Figure 1. OTU richness (N0), Shannon's diversity index (H) and Shannon's evenness (E) of
672 fungal communities depending on the tree species (*C. arborescens*, *E. caribaeum*, *L.*
673 *leucocephala* and *P. unguis*) and the treatment (intact and snapped stem). Density plot
674 summarizes the overall treatment effect on alpha diversity indices. Treatment effect was
675 evaluated using ANOVA (*P≤0.05; **P≤0.01; ***P≤0.001).

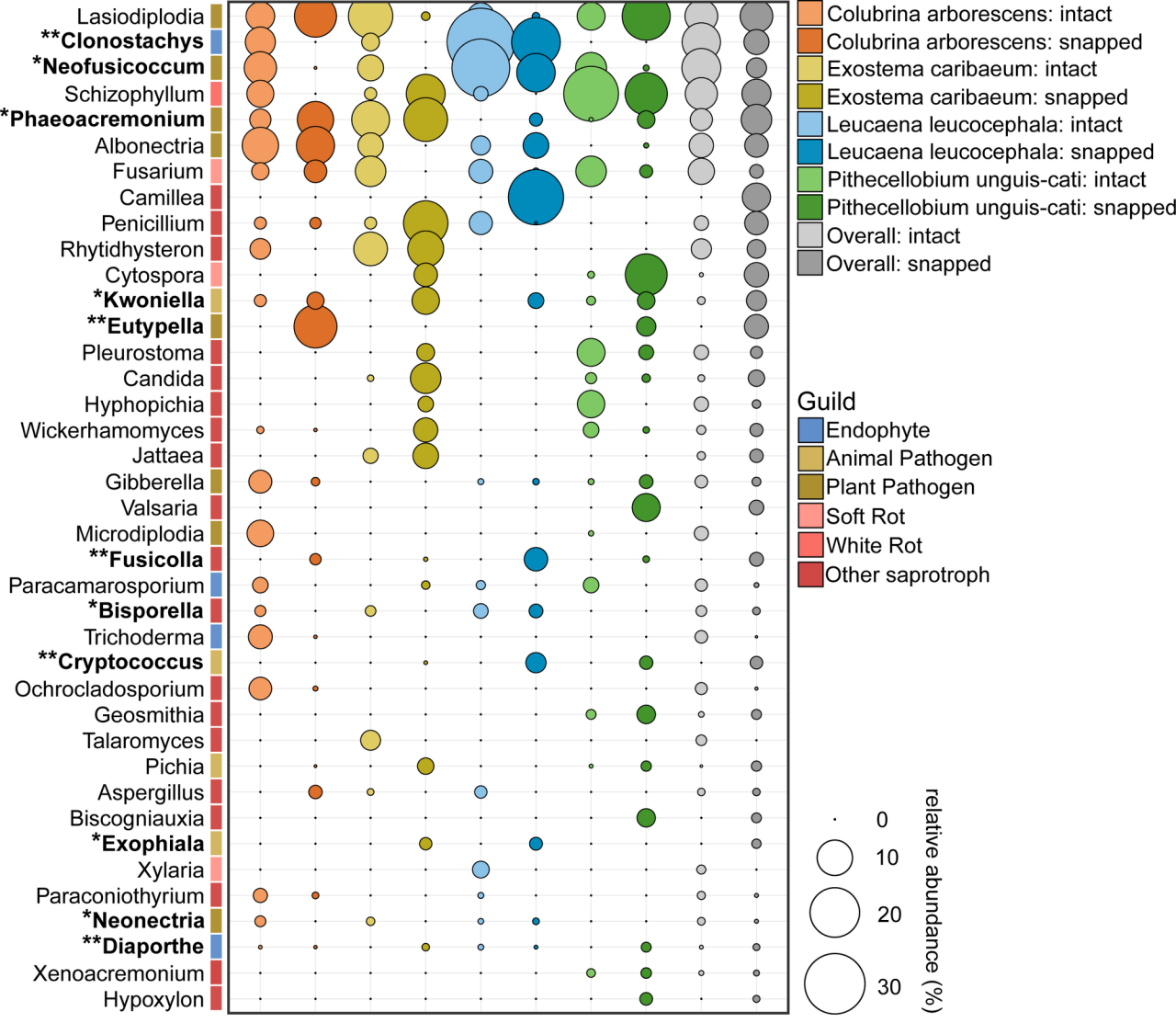
676 Figure 2. Non-metric multidimensional scaling (NMDS) analysis of the fungal communities
677 based on OTUs composition depending on (a) the treatment only (intact and snapped stem)
678 and depending on (b) the treatment and the tree species (*C. arborescens*, *E. caribaeum*, *L.*
679 *leucocephala* and *P. unguis-cati*). Large circles represent the cendroids and small circles
680 represent individual samples for each treatment or each combination of treatment and tree
681 species. (c) Hierarchical clustering of the fungal communities based on OTUs composition
682 depending on the treatment (intact and snapped stem) for each tree species (*C. arborescens*, *E.*
683 *caribaeum*, *L. leucocephala* and *P. unguis-cati*). Bar plots summarize the relative abundance
684 of fungal classes and guilds depending on the treatment for each combination of treatment and
685 tree species.

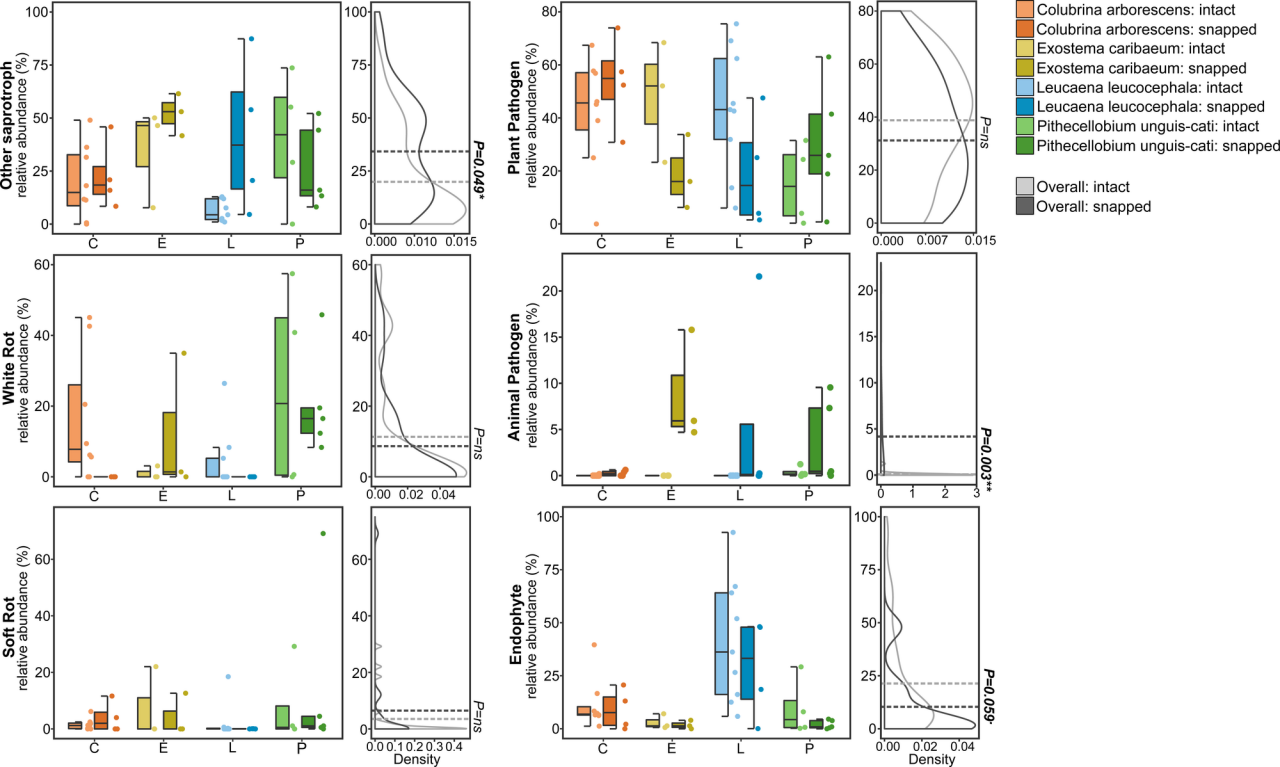
686 Figure 3. The relative abundance of the fungal genera depending on the treatment (intact and
687 snapped stem) for each tree species (*C. arborescens*, *E. caribaeum*, *L. leucocephala* and *P.*

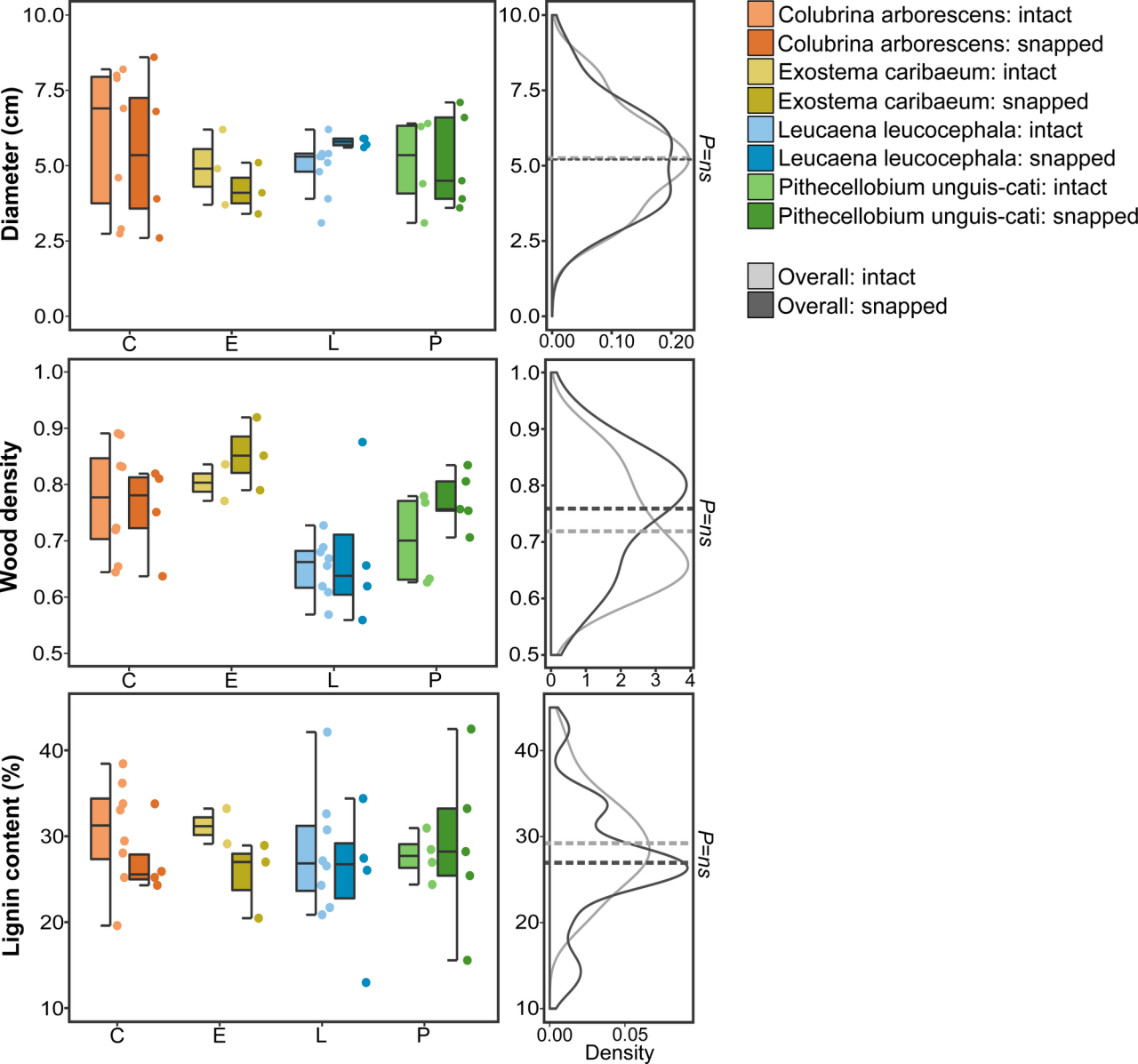
688 *unguis-cati*). Size of the circles is proportional to the relative abundance of the fungal genera.
689 Treatment effect was assessed using Wilcoxon rank-sum test for each fungal genus (*P≤0.05;
690 **P≤0.01; ***P≤0.001).
691 Figure 4. The relative abundance of the fungal guilds depending on the treatment (intact and
692 snapped stem) for each tree species (*C. arborescens*, *E. caribaeum*, *L. leucocephala* and *P.*
693 *unguis-cati*). Density plot summarizes the overall treatment effect on guilds relative
694 abundance. Treatment effect was evaluated using ANOVA (*P≤0.05; **P≤0.01;
695 ***P≤0.001).
696 Figure 5. Tree stem diameters, wood density and wood lignin content depending on the tree
697 species (*C. arborescens*, *E. caribaeum*, *L. leucocephala* and *P. unguis-cati*) and the treatment
698 (intact and snapped stem). Density plot summarizes the overall treatment effect on tree stem
699 diameters. Treatment effect was evaluated using ANOVA (*P≤0.05; **P≤0.01; ***P≤0.001).


OTUs Richness
H
E
C
E
L
P
Density
P=0.017*
P=ns
P=0.097.
Colubrina arborescens: intact
Colubrina arborescens: snapped
Exostema caribaeum: intact
Exostema caribaeum: snapped
Leucaena leucocephala: intact
Leucaena leucocephala: snapped
Pithecellobium unguis-cati: intact
Pithecellobium unguis-cati: snapped
Overall: intact
Overall: snapped


a
b
c
NMDS1
NMDS2
stress=0.17
stress=0.14
s
i
Overall: intact
Overall: snapped
Colubrina arborescens: intact
Colubrina arborescens: snapped
Exostema caribaeum: intact
Exostema caribaeum: snapped
Leucaena leucocephala: intact
Leucaena leucocephala: snapped
Pithecellobium unguis-cati: intact
Pithecellobium unguis-cati: snapped
Unidentified
Tremellomycetes
Agaricomycetes
Leotiomycetes
Saccharomycetes
Eurotiomycetes
Dothideomycetes
Sordariomycetes
Unclassified
Other
Endophyte
Animal Pathogen
Plant Pathogen
Soft Rot
White Rot
Other saprotroph


Lasiodiplodia
**Clonostachys
*Neofusicoccum
Schizophyllum
*Phaeoacremonium
Albonectria
Fusarium
Camillea
Penicillium
Rhytidhysteron
Cytospora
*Kwoniella
**Eutypella
Pleurostoma
Candida
Hyphopichia
Wickerhamomyces
Jattaea
Gibberella
Valsaria
Microdiplodia
**Fusicolla
Paracamarosporium
*Bisporella
Trichoderma
**Cryptococcus
Ochrocladosporium
Geosmithia
Talaromyces
Pichia
Aspergillus
Biscogniauxia
*Exophiala
Xylaria
Paraconiothyrium
*Neonectria
**Diaporthe
Xenoacremonium
Hypoxylon
Colubrina arborescens: intact
Colubrina arborescens: snapped
Exostema caribaeum: intact
Exostema caribaeum: snapped
Leucaena leucocephala: intact
Leucaena leucocephala: snapped
Pithecellobium unguis-cati: intact
Pithecellobium unguis-cati: snapped
Overall: intact
Overall: snapped
Guild
Endophyte
Animal Pathogen
Plant Pathogen
Soft Rot
White Rot
Other saprotroph
0
10
20
30
relative abundance (%)


Other saprotroph
relative abundance (%)
Plant Pathogen
relative abundance (%)
White Rot
relative abundance (%)
Animal Pathogen
relative abundance (%)
Soft Rot
relative abundance (%)
Endophyte
relative abundance (%)
C
E
L
P
Density
P=0.049*
P=ns
P=0.003**
P=0.059
Colubrina arborescens: intact
Colubrina arborescens: snapped
Exostema caribaeum: intact
Exostema caribaeum: snapped
Leucaena leucocephala: intact
Leucaena leucocephala: snapped
Pithecellobium unguis-cati: intact
Pithecellobium unguis-cati: snapped
Overall: intact
Overall: snapped


Diameter (cm)
Wood density
Lignin content (%)
C
E
L
P
P=ns
Density
Colubrina arborescens: intact
Colubrina arborescens: snapped
Exostema caribaeum: intact
Exostema caribaeum: snapped
Leucaena leucocephala: intact
Leucaena leucocephala: snapped
Pithecellobium unguis-cati: intact
Pithecellobium unguis-cati: snapped
Overall: intact
Overall: snapped

	Intact	Snapped
Colubrina arborescens	8	4
Exostema caribaeum	3	3
Leucaena leucocephala	9	4
Pithicellobium unguis-cati	4	5