

## Stem-inhabiting fungal communities differ between intact and snapped trees after hurricane Maria in a Puerto Rican tropical dry forest



François Maillard<sup>a,b,c</sup>, Erin Andrews<sup>a</sup>, Molly Moran<sup>a</sup>, Peter G. Kennedy<sup>b,c</sup>, Skip J. Van Bloem<sup>d</sup>, Jonathan S. Schilling<sup>a,\*</sup>

<sup>a</sup> Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN, USA

<sup>b</sup> Department of Plant & Microbial Biology, University of Minnesota, St. Paul, MN, USA

<sup>c</sup> Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA

<sup>d</sup> Baruch Institute of Coastal Ecology and Forest Science, Clemson University, PO Box 596, Georgetown, SC 29442, USA

### ARTICLE INFO

#### Keywords:

Tropical cyclone  
Predisposition factor  
Tree holobiont  
Caribbean Island  
Tropical forest

### 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.

### 1. Introduction

High-speed winds associated with hurricanes can cause massive tree falls (Van Bloem et al., 2005, 2006; Holm et 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<sub>2</sub> fixation due to leaf and stem damage and increasing CO<sub>2</sub> emissions associated with wood decomposition (McNulty, 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

\* Corresponding author.

E-mail address: [schillin@umn.edu](mailto:schillin@umn.edu) (J.S. Schilling).

<https://doi.org/10.1016/j.foreco.2020.118350>

Received 11 March 2020; Received in revised form 8 June 2020; Accepted 18 June 2020

Available online 12 August 2020

0378-1127/ © 2020 Published by Elsevier B.V.

models (Ciftci et al., 2014; Hale et al., 2015). 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., 2011, 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 (Rodríguez 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 (Porrás-Alfaro and Bayman, 2011; Rho et al., 2017). Saprotrophic fungi have also been detected in woody tissues of living trees (Pellitier et al., 2019; Skaltsas 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 Griffith 1989; Oses et al., 2008; Song et al., 2017; Cline et al., 2018). Other studies found that saprotrophic fungi living in wood possessed an opportunistic pathogenic trophic status when the tree health status was low (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 (Vandenkoornhuys 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 saprotrophic fungi by comparison with intact trees. We further hypothesized (H3) that snapped trees would have different

wood properties than intact trees.

## 2. Materials and methods

### 2.1. Experimental site

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

### 2.2. Sampling

Approximately one month after Hurricane Maria, fallen and intact trees were sampled along roads and trails in Guánica Forest. To minimize colonization of the fallen stems by soil fungi 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 to 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*, *Leucaena 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.

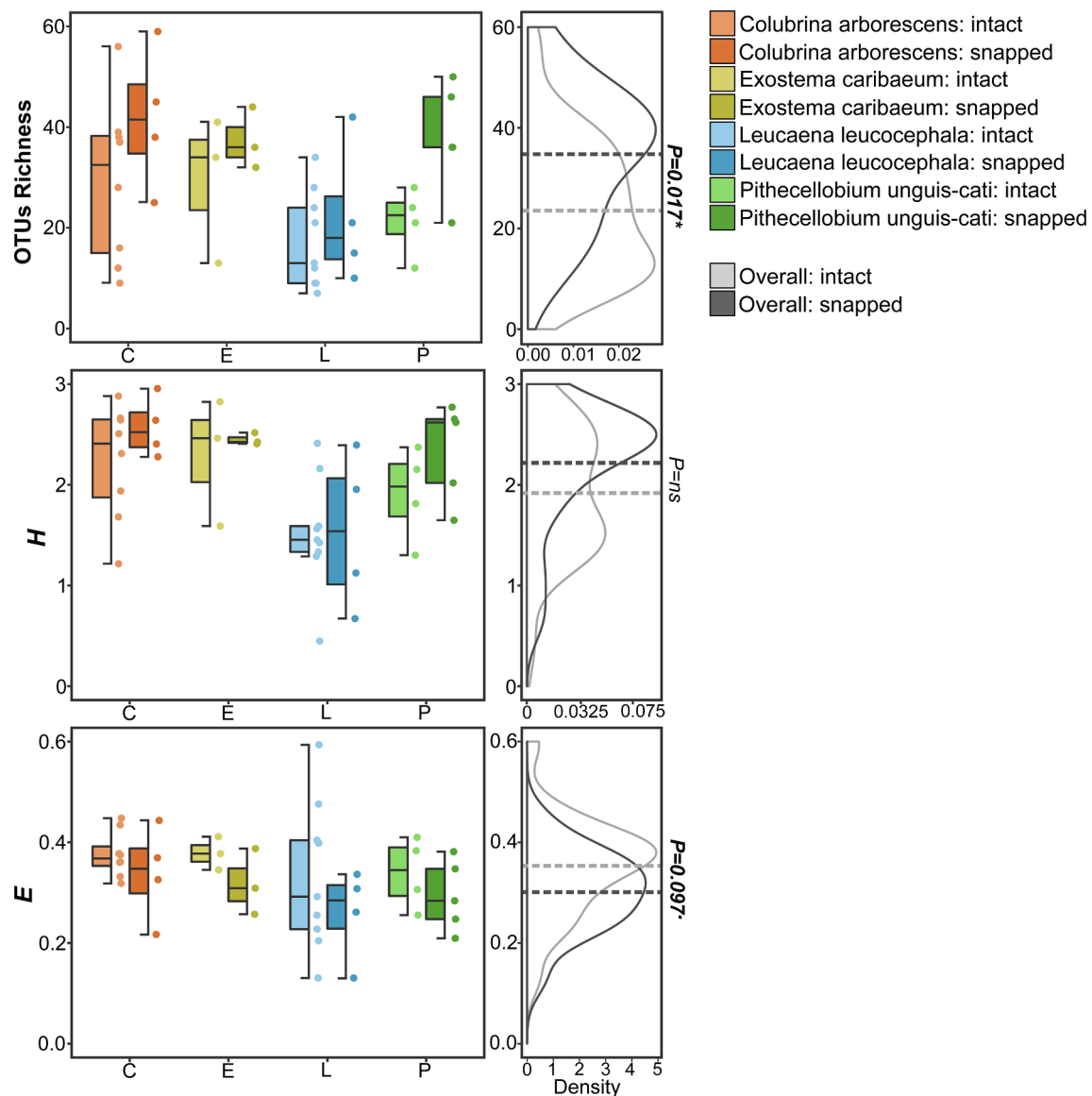
### 2.3. Fungal community analyses

We collected shavings from each frozen wood disc from three spots

**Table 1**

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

	Intact	Snapped
<i>Colubrina arborescens</i>	8	4
<i>Exostema caribaeum</i>	3	3
<i>Leucaena leucocephala</i>	9	4
<i>Pithecellobium unguis-cati</i>	4	5

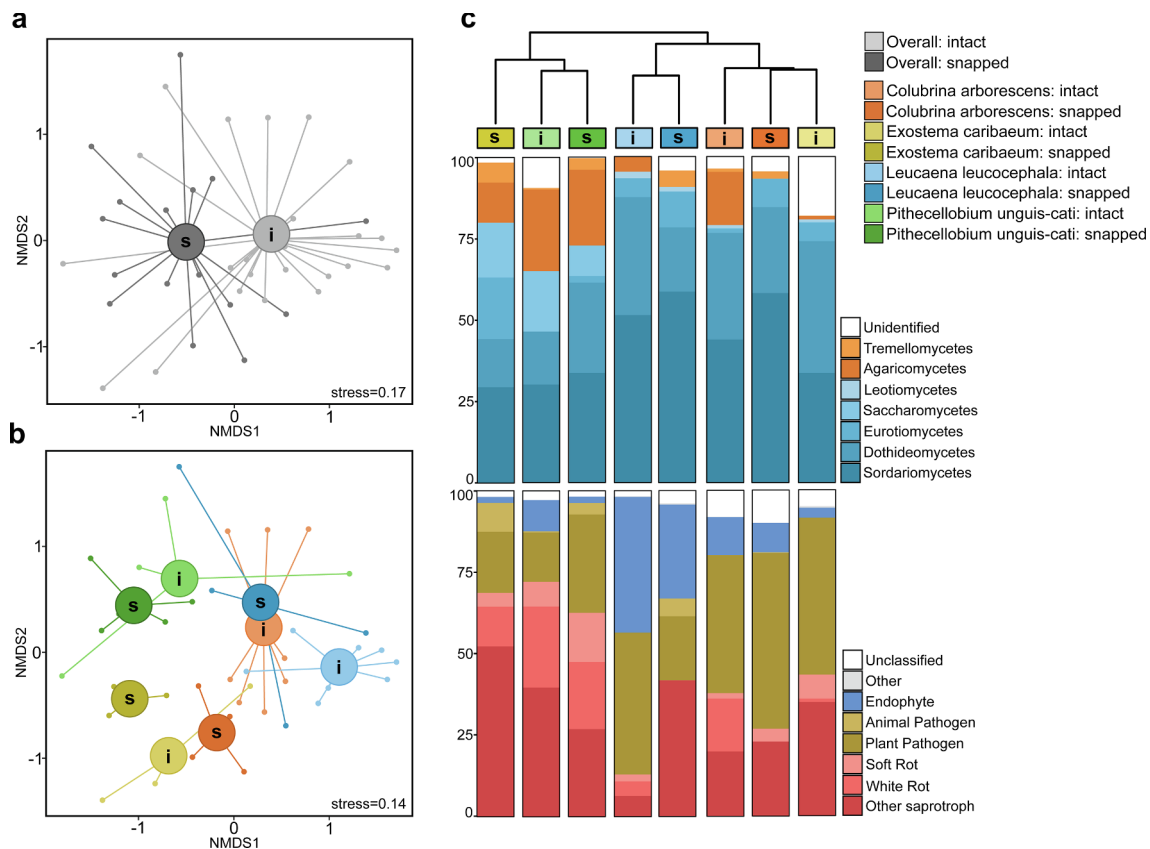


**Fig. 1.** OTU richness (N0), Shannon's diversity index (H) and Shannon's evenness (E) of fungal communities depending on the tree species (*C. arborescens*, *E. caribaeum*, *L. leucocephala* and *P. unguis*) and the treatment (intact and snapped stem). Density plot summarizes the overall treatment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

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., 2017) and negative controls, were amplified in individual 20  $\mu$ l reactions containing 10  $\mu$ l of Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Scientific, Waltham, MA, USA), 0.5  $\mu$ l of each 20 mM primer, 1  $\mu$ l of DNA template and 8  $\mu$ l of PCR-grade water. Thermocycling conditions were as follows: 1. 98 °C for 30 s, 2. 98 °C for 10 s, 3. 55 °C for 30 s, 4. 72 °C for 30 s, repeat steps 2–4 34 times, 5. 72 °C for 10 min 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  $\times$  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. (2017). Briefly, primers were removed and sequences were 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  $\times$  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 (Fig. S1). Seven samples presenting a low number of counts were removed.



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

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.

#### 2.4. Wood properties analyses

Using one cut half (through the pith) of frozen discs, wood density ( $\text{g cm}^{-3}$ ) was measured at fresh volume ( $\text{cm}^3$ ) 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).

#### 2.5. 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

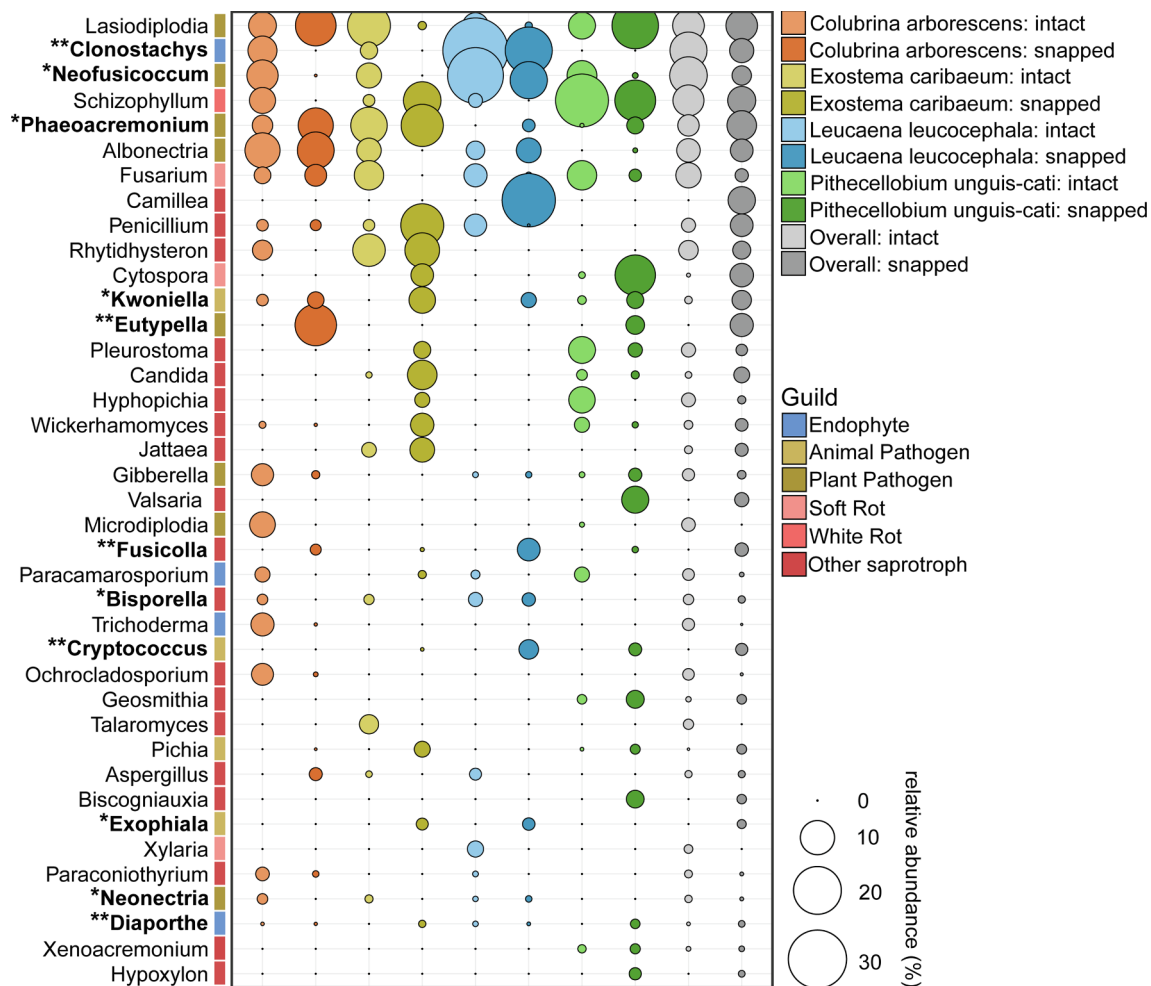
rarefied sequence read counts. Fungal OTU richness (NO) and Shannon's diversity (H) and Shannon's evenness (E) were calculated in Vegan package (Oksanen et al., 2013). The effect of the tree species and treatment (i.e. intact versus snapped) on each of these three alpha diversity metrics was tested using 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.

### 3. Results

#### 3.1. 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:  $F_{3,39} = 6.14$ ,





**Fig. 3.** The relative abundance of the fungal genera depending on the treatment (intact and snapped stem) for each tree species (*C. arborescens*, *E. caribaeum*, *L. leucocephala* and *P. unguis-cati*). Size of the circles is proportional to the relative abundance of the fungal genera. Treatment effect was assessed using Wilcoxon rank-sum test for each fungal genus (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ).

$P = 0.002$ ) (Table S1), with *C. arborescens*, *E. caribaeum* and *P. unguis-cati* harboring higher OTU richness than *L. leucocephala* (Fig. 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 (Fig. 2a), but also by tree species within treatment (Fig. 2b). OTU-based hierarchical clustering showed that communities generally clustered by tree species first and then by treatment (Fig. 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, Dothiodesomycetes and Eurotiomycetes together representing 65–95% of relative sequence abundances (Fig. 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 (Fig. 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 (Fig. 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* (Fig. 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. arborescens*, *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 (Fig. 5).

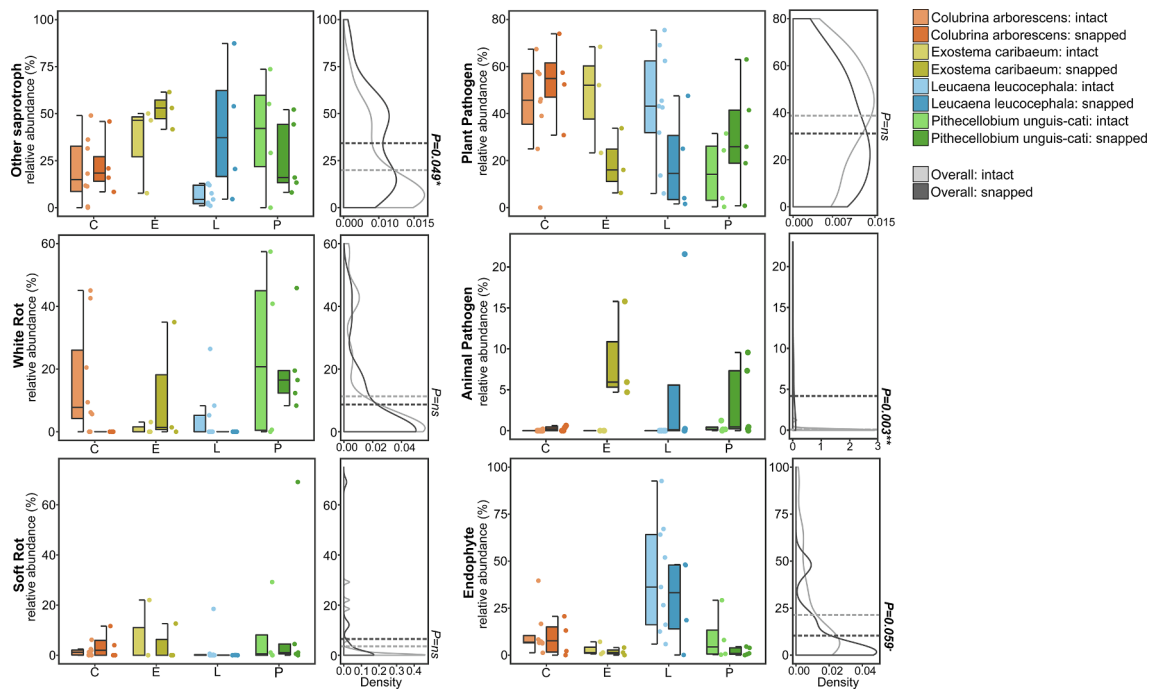


Fig. 4. The relative abundance of the fungal guilds depending on the treatment (intact and snapped stem) for each tree species (*C. arborescens*, *E. caribaeum*, *L. leucocephala* and *P. unguis-cati*). Density plot summarizes the overall treatment effect on guilds relative abundance. Treatment effect was evaluated using ANOVA (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ).

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 ( $F_{1,30} = 2.16$ ,  $P = 0.15$ ).

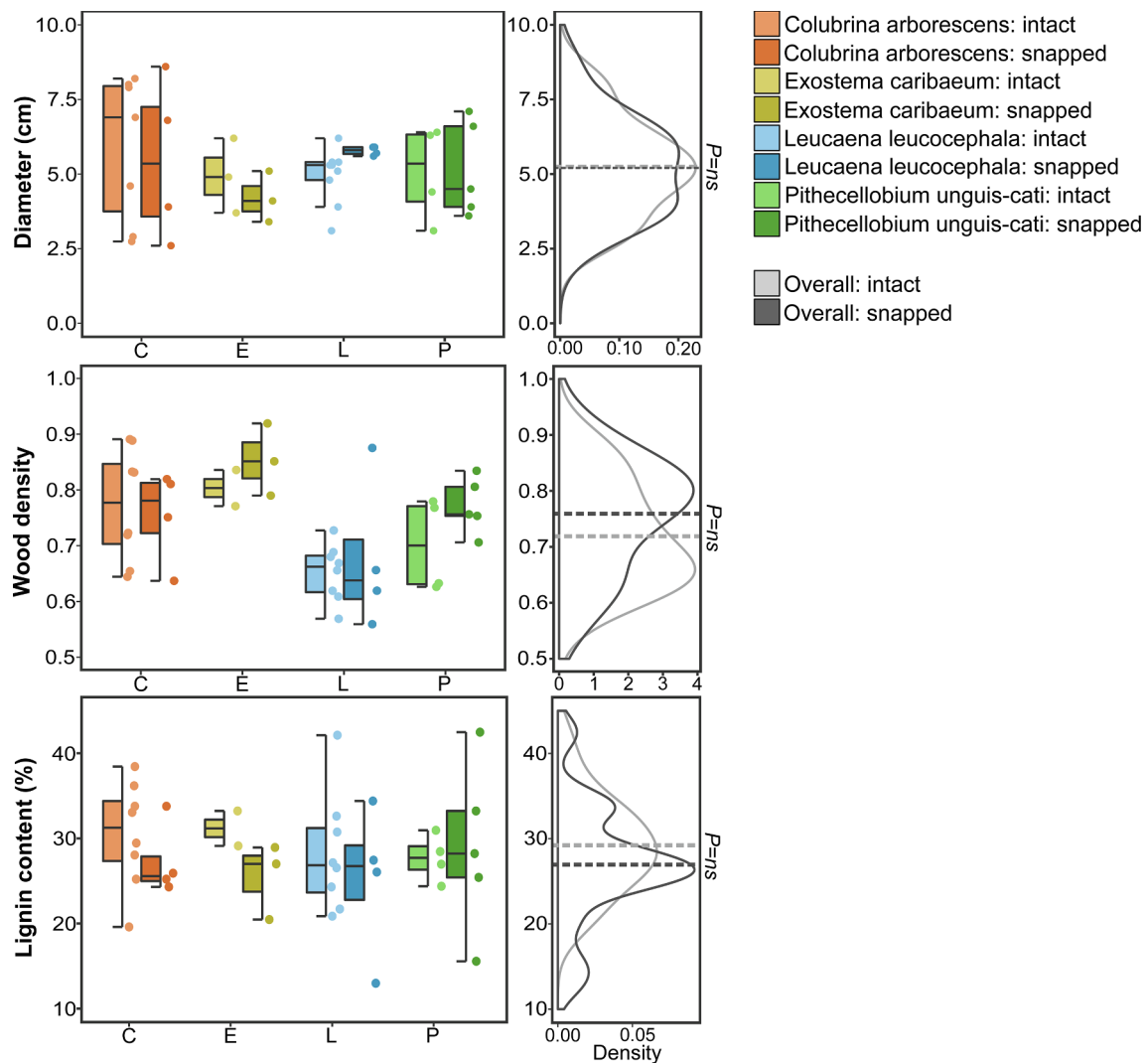
#### 4. Discussion

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

2006; Rezgui et al., 2018).

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

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., 2010). Additionally, Larson and Franklin (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



**Fig. 5.** Tree stem diameters, wood density and wood lignin content depending on the tree species (*C. arborescens*, *E. caribaeum*, *L. leucocephala* and *P. unguis-cati*) and the treatment (intact and snapped stem). Density plot summarizes the overall treatment effect on tree stem diameters. Treatment effect was evaluated using ANOVA (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ).

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). *Phaeoacremonium* has been found as a frequent agent of trunk dieback in almond trees, olive trees and grapevines (Essakhi et al., 2008; Olmo et al., 2015), suggesting the presence of this genus might serve as a specific predisposition factor of trees to hurricane damage. *Eutypella*, a pathogenic fungal genus causing canker (Kliejunas and Kuntz, 1974; Ogris et al., 2008), was exclusively detected in the snapped stems of *C. arborescens* and *P. unguis-cati*. While less generalistic than *Phaeoacremonium*, *Eutypella* might also represent a candidate to tree predisposition to hurricane damages. Unexpectedly, *Neofusicoccum*, another trunk canker and dieback pathogenetic genus (Slippers and Wingfield, 2007), was abundant in samples of all four of the tree species with intact stems. *Neofusicoccum* has been previously described as a latent pathogen that waits for stressful conditions to start an infection (Slippers and Wingfield, 2007). By occupying stem tissues

without pathogenic activity, this fungus could act as a defensive mutualist by limiting the infections of the intact tree stems by other active pathogens or opportunistic saprotrophs. Support for this kind of multifunctional nature of fungal-plant interactions was recently demonstrated by Robles et al. (2019), who found that the same OTU might have both endophytic and pathogenic trophic modes. This result shows the limitation of using only DNA metabarcoding followed by guild classification based on taxonomic identification (Nguyen et al., 2016). Metatranscriptomics represents a promising approach to identify the active part of the tree holobiont as well as the trophic status of the taxa composing the fungal community in the plant tissues by comparing the expressed genes repertoires involved in the pathogenicity and in the wood decomposition.

Perhaps most intriguingly, we found that fungi characterized by an endophytic lifestyle tended to be more abundant in intact trees by comparison with snapped trees. In particular, *Clonostachys*, which was the second most abundant fungal genus in our study, is a well-characterized endophyte having biocontrol properties (Evans et al., 2003; Nygren et al., 2017). The presence of fungi having mycoparasitic abilities in tree stems has been previously reported in tropical forests, suggesting this may be a widespread phenomenon (Evans et al., 2003; Skaltsas et al., 2019). Additionally, in temperate forest, fungal endophytes were described as having strong antagonism against the

helm disease-causing stem dieback (Webber, 1981; Webber and Hedger, 1986; Martin et al., 2013). Based on these results, endophytes in general and *Clonostachys* in particular might represent key taxa involved in tree resistance to hurricane tree damage through their ability to limit infections by pathogenic and opportunistic saprotrophic fungi (Arnold et al., 2003).

Wood samples were harvested 1 month after Hurricane Maria. This lag time was unavoidable due to the unpredictable timing of hurricanes, and travel, safety, and funding limitations. It does, however, introduce caveats, primarily in any colonization by new fungi in the snapped samples. We took precautions to avoid this phenomenon by selecting only those snapped stems that were standing vertically and by cutting the wood disks away from the broken area. Additionally, wood decomposition in the Guanica tropical dry forest is slow (Torres et al., 2005), and the precipitation was very low during this month (172.97 mm), concentrated just before initial sampling. Consequently, we believe that our data mirror the fungal communities initially inhabiting the stems at the time of Hurricane Maria. It does imply that a manipulative experiment cutting live trees and then submitting whole, never-dried stem portions to resistance stress (e.g. 3-point bending, or similar) combined with the identification of the fungal communities might represent a complementary approach to explore the role of the fungal communities in the wood properties of the living trees.

It is important to note that tree stem breakage was described as having a limited impact on overall tree mortality in same Puerto Rican dry forest where this study was conducted (Van 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 et 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.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### 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 logging during the post-Maria recovery period.

#### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2020.118350>.

#### References

- Arnold, A.E., Mejía, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., Herre, E.A., 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proc. Natl. Acad. Sci. USA* 100, 15649–15654.
- ASTM, 2001. Standard test method for determination of acid-insoluble residue in biomass E1721–01. ASTM International, West Conshohocken, PA.
- Bellingham, P.J., Tanner, E.V.J., 1995. Damage and responsiveness of Jamaican montane tree species after disturbance by a hurricane. *Ecology* 76, 2562–2580.
- Boddy, L., Griffith, G.S., 1989. Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. *Sydowia* 41, 41–73.
- Brokaw, N.V.L., Grear, J.S., 1991. Forest structure before and after Hurricane Hugo at three elevations in the Luquillo Mountains, Puerto Rico. *Biotropica* 23, 386–392.
- Bruez, E., Baumgartner, K., Bastien, S., Travadon, R., Guerin-Dubrana, L., Rey, P., 2016. Various fungal communities colonise the functional wood tissues of old grapevines externally free from grapevine trunk disease symptoms. *Aust. J. Grape Wine Res.* 22, 288–295.
- Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J.-P., Guerin-Dubrana, L., Rey, P., 2014. Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of esca leaf-symptomatic and asymptomatic vines. *PLoS ONE* 9, e95928.
- Casieri, L., Hofstetter, V., Viret, O., Gindro, K., 2009. Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. *Phytopathol. Mediterr.* 48, 73–83.
- Ciftci, C., Kane, B., Brena, S.F., Arwade, S.R., 2014. Loss in moment capacity of tree stems induced by decay. *Trees Struct. Funct.* 28, 517–529.
- Cline, L.C., Schilling, J.S., Menke, J., Groenhof, E., Kennedy, P.G., 2018. Ecological and functional effects of fungal endophytes on wood decomposition. *Funct. Ecol.* 32 (1), 181–191.
- Colón, Sandra Molina, Lugo, Ariel E., 2006. Recovery of a subtropical dry forest after abandonment of different land uses. *Biotropica* 38 (3), 354–364.
- Cregger, M.A., Veach, A.M., Yang, Z.K., Crouch, M.J., Vilgalys, R., Tuskan, G.A., Schadt, C.W., 2018. The *Populus* holobiont: dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* 6, 31.
- Damm, U., Mostert, L., Crous, P.W., Fourie, P.H., 2008. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. *Persoonia* 20, 87–102.
- Deflorio, G., Johnson, C., Fink, S., Schwarze, F.W.M.R., 2008. Decay development in living sapwood of coniferous and deciduous trees inoculated with six wood decay fungi. *For. Ecol. Manage.* 255, 2373–2383.
- Duryea, M.L., Kampf, E., 2017. Wind and trees: lessons learned from hurricanes. *Wind Trees* 16.
- Durand, A., Maillard, F., Foulon, J., Gweon, H.S., Valot, B., Chalot, M., 2017. Environmental metabarcoding reveals contrasting belowground and aboveground fungal communities from poplar at a Hg phytomanagement site. *Microb. Ecol.*
- Edgar, R.C., 2016. UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*, 081257.
- Essakhi, S., Mugnai, L., Crous, P.W., Groenewald, J.Z., Surico, G., 2008. Molecular and phenotypic characterisation of novel *Phaeoacremonium* species isolated from esca diseased grapevines. *Persoonia* 21, 119134.
- Evans, H.C., Holmes, K.A., Thomas, S.E., 2003. Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. *Mycol. Program* 2, 149–160.
- Gardiner, B., Berry, P., Moulia, B., 2016. Review: wind impacts on plant growth, mechanics and damage. *Plant Sci.* 245, 1–25.
- Gaziz, R., Chaverri, P., 2010. Diversity of fungal endophytes in leaves and stems of rubber trees (*Hevea brasiliensis*) in Tambopata, Peru. *Fungal Ecol.* 4, 94–102.
- Gilbert, G.S., Hubbell, S.P., Foster, R.B., 1994. Density and distance-to-adult effects of a canker disease in a moist tropical forest. *Oecologia* 98, 100–108.
- Giordano, L., Gonther, P., Varese, G.C., Miserere, L., Nicolotti, 2009. Mycobiota inhabiting sapwood of healthy and declining Scots pine (*Pinus sylvestris* L.) trees in the Alps. *Fungal Divers.* 38, 69–83.
- Guglielmo, F., Gonther, P., Garbelotto, M., Nicolotti, G., 2010. Sampling optimization for DNA-based diagnosis of wood decay fungi in standing trees. *Lett. Appl. Microbiol.* 51, 90–97.
- Hale, S., Gardiner, B., Peace, A., Nicoll, B., Taylor, P., Pizzirani, S., 2015. Comparison and validation of three versions of a forest wind risk model. *Environ. Modell. Software* 68 (27–41).
- Hanada, R.E., Pomella, A.W., Costa, H.S., Bezerra, J.L., Loguercio, L.L., Pereira, J.O., 2010. Endophytic fungal diversity in *Theobroma cacao* (cacao) and *T. grandiflorum* (cupuacu) trees and their potential for growth promotion and biocontrol of black-pod disease. *Fungal Biol.* 114, 901–910.
- Heineman, K.D., Russo, S.E., Baillie, I.C., Mamit, J.D., Chai, P.P., Chai, L., Hindley, E.W., Lau, B.T., Tan, S., Ashton, P.S., 2015. Influence of tree size, taxonomy, and edaphic conditions on heart rot in mixed-dipterocarp Bornean rainforests: implications for aboveground biomass estimates. *Biogeosci. Discuss.* 12, 6821–6861.
- Hennon, P.E., 1995. Are heart rot fungi major factors of disturbance in gap-dynamic forests? *Northwest Sci.* 69, 284–293.
- Holm, J.A., Van Bloem, S.J., Larocque, G.R., Shugart, H.H., 2017. Shifts in biomass and productivity for a subtropical dry forest in response to simulated elevated hurricane disturbances. *Environ. Res. Lett.* 12, 025007.
- Hu, T., Smith, R.B., 2018. The impact of Hurricane Maria on the vegetation of Dominica and Puerto Rico using multispectral remote sensing. *Remote Sens.* 10, 827.
- Jimenez-Rodriguez, D.L., Alvarez-Añorve, M.Y., Pineda-Cortes, M., Flores-Puerto, J.I., Benítez-Malvido, J., Oyama, K., Avila-Cabadilla, L.D., 2018. Structural and functional



- traits predict short term response of tropical dry forests to a high intensity hurricane. *For. Ecol. Manage.*
- Kliejunas, J.T., Kuntz, J.E., 1974. *Eutypella* canker, characteristics and control. *For. Chron.* 50, 106–108.
- Kovalchuk, A., Mukrimin, M., Zeng, Z., Raffaello, T., Liu, M.X., Kasanen, R., Sun, H., Asiogbu, F.O., 2018. Mycobiome analysis of asymptomatic and symptomatic Norway spruce trees naturally infected by the conifer pathogens *Heterobasidion* spp. *Environ. Microbiol. Rep.* 2018 (10), 532–541.
- Larson, A.J., Franklin, J.F., 2010. The tree mortality regime in temperate old-growth coniferous forests: the role of physical damage. *Can. J. For. Res.* 40, 2091–2103.
- Lee, M.R., Powell, J., Oberle, B., Cornwell, W.K., Lyons, M., Rigg, J.L., Zanne, A.E., 2019. Good neighbors aplenty: Fungal endophytes rarely exhibit competitive exclusion patterns across a span of woody habitats.
- Lewis, K.J., Lindgren, B.S., 1999. Influence of decay fungi on species composition and size class structure in mature *Picea glauca* x *engelmannii* and *Abies lasiocarpa* in sub-boreal forests of central British Columbia. *For. Ecol. Manage.* 123, 135–143.
- Lofgren, L.A., LeBlanc, N.R., Certano, A.K., Nachtigall, J., LaBine, K.M., Riddle, J., Broz, K., Dong, Y., Bethan, B., Kafer, C.W., et al., 2018. *Fusarium graminearum*: pathogen or endophyte of North American grasses? *New Phytol.* 217, 1203–1212.
- Lugo, A.E., Murphy, P.G., 1986. Nutrient dynamics of a Puerto Rican subtropical dry forest. *J. Trop. Ecol.* 2 (1), 55–72.
- Martin, J.A., Witzell, J., Blumenstein, K., Rozpedowska, E., Helander, M., Sieber, T.N., Gil, L., 2013. Resistance to Dutch Elm disease reduces presence of xylem endophytic fungi in Elms (*Ulmus* spp.). *PLoS ONE* 8, e56987.
- McDowell, N., Allen, C.D., Anderson-teixeira, K., Brando, P., Brienen, R., Chambers, J., et al., 2018. Drivers and mechanisms of tree mortality in moist tropical forests. *New Phytol.* 219, 851–869.
- McNulty, S.G., 2002. Hurricane impacts on US forest carbon sequestration. *Environ. Pollut.* 116, S17–S24.
- Murphy, P.G., Lugo, A.E., 1986. Structure and biomass of a subtropical dry forest in Puerto-Rico. *Biotropica* 18 (2), 89–96.
- Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* 20, 241–248.
- Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., et al., 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* 47, D259–D264.
- Nygren, K., Dubey, M., Zapparata, A., Iqbal, A., Tzelepis, G.D., Durling, M., Jensen, F., Karlsson, M., 2017. The mycoparasitic fungus *Clonostachys rosea* responds with both common and specific gene expression during interspecific interactions with fungal prey. *Evol. Appl.* 11, 931–949.
- Ogris, N., Diminic, D., Piškur, B., Kraigher, H., 2008. First report of *Eutypella parasitica* causing cankers on field maple (*Acer campestre*) in Croatia. *Plant Pathol.* 57, 785 (New Dis. Rep., 2008, 16, 39).
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., et al., 2013. *vegan: Community Ecology Package, version 2.0–7*. R package. Available: <http://CRAN.R-project.org/package=vegan>.
- Olmo, D., Gramaje, D., Armengol, J., Leon, M., 2015. Pathogenicity testing of lesser-known fungal trunk pathogens associated with wood decay of almond trees. *Eur. J. Plant Pathol.* 143, 607–611.
- Oses, R., Valenzuela, S., Freer, J., Sanfuentes, E., Rodriguez, J., 2008. Fungal endophytes in xylem of healthy Chilean trees and their possible role in early wood decay. *Fungal Divers.* 33, 77e86.
- Parker, G., Martínez-Yrizar, A., Álvarez-Yépez, J., Maass, M., Araiza, S., 2018. Effects of hurricane disturbance on a tropical dry forest canopy in western Mexico. *For. Ecol. Manage.*
- Palmer, J.M., Jusino, M.A., Banik, M.T., Lindner, D.L., 2017. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ* 6, e4925.
- Parfitt, D., Hunt, J., Dockrell, D., Rogers, H.J., Boddy, L., 2010. Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. *Fungal Ecol.* 3, 338–346.
- Paz, H., Vega-Ramos, F., Arreola-Villa, F., 2018. Understanding hurricane resistance and resilience in tropical dry forest trees: a functional traits approach. *For. Ecol. Manage.*
- Pellitteri, P.T., Zak, D.R., Salley, S.O., 2019. Environmental filtering structures fungal endophyte communities in tree bark. *Mol. Ecol.* 28 (23), 5188–5198.
- Persson, Y., Vasaitis, R., Långström, B., Öhrn, P., Ihrmark, K., Stenlid, J., 2009. Fungi vectored by the bark beetle *Ips typographus* following hibernation under the bark of standing trees and in the forest litter. *Microb. Ecol.* 58, 651–659.
- Porras-Alfaro, A., Bayman, P., 2011. Hidden fungi, emergent properties: Endophytes and microbiomes. *Annu. Rev. Phytopathol.* 49, 291–315.
- Putz, F.E., Coley, P.D., Lu, K., Montalvo, A., Aiello, A., 1983. Uprooting and snapping of trees: structural determinants and ecological consequences. *Can. J. For. Res.* 13, 1011–1020.
- R Core Team. 2016. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Ragazzi, A., Moricca, S., Capretti, P., Dellavalle, I., Turco, E., 2003. Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. *Forest Pathol.* 33, 31–33.
- Rho, H., Hsieh, M., Kandel, S.L., Cantillo, J., Doty, S.L., Kim, S.H., 2017. Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. *Microb. Ecol.* 1–12.
- Robles, C.A., Carmaran, C.C., Lopez, S.E., 2011. Screening of xylophagous fungi associated with *Platanus acerifolia* in urban landscapes: Biodiversity and potential biodegradation. *Landscape Urban Plan.* 100, 129–135.
- Robles, C.A., Ceriani-Nakamurakare, E.D., Pereira, S., Carmaran, C.C., 2019. Relationships between Endophytic and Pathogenic Strains of *Inonotus* (Basidiomycota) and *Daldinia* (Ascomycota) from Urban Trees. *Mycol. Progr.* 18 (9), 1155–1171.
- Robles, C.A., Lopez, S.E., McCargo, P.D., Carmaran, C.C., 2015. Relationships between fungal endophytes and wood-rot fungi in wood of *Platanus acerifolia* in urban environments. *Can. J. For. Res.* 45 (7), 929–936.
- Rodriguez, R.J., White Jr, J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes: Diversity and functional roles. *New Phytol.* 182 (2), 314–330.
- Selosse, M.A., Schneider-Maunoury, L., Martos, F., 2018. Time to re-think fungal ecology? Fungal ecological niches are often prejudged. *New Phytol.* 217, 968–972.
- Sadeghi, F., Samsampour, D., Seyahooei, M.A., Bagheri, A., Soltani, J., 2019. Diversity and Spatiotemporal Distribution of Fungal Endophytes Associated with *Citrus reticulata* cv. Siyahoo. *Curr. Microbiol.* 76, 279.
- Samuels, G.J., Suarez, C., Solis, K., Holmes, K.A., Thomas, S.E., Ismael, A., Evans, H.C., 2006. *Trichoderma theobromicola* and *T. paucisporum*: two new species from South America. *Mycol. Res.* 110, 381–392.
- Santini Jr, L., Ortega Rodriguez, D.R., Trindade Quintilhan, M., Brazolin, S., Tommasiello, Filho M., 2019. Evidence to wood biodegradation of tropical species revealed by non-destructive techniques. *Sci. Total Environ.* 672 (357), 69.
- Skaltsas, D.N., Badotti, F., Martins Vaz, A.B., Ferreira da Silva, F., Gazis, R., Wurdack, K., Castlebury, L., Góes-Neto, A., Priscila Chaverri, P., 2019. Exploration of stem endophytic communities revealed developmental stage as one of the drivers of fungal endophytic community assemblages in two Amazonian hardwood genera. *Sci. Rep.* 9 (1), 12685.
- Schwarze, F.W.M.R., 2007. Wood decay under the microscope. *Fungal Biol.*
- Schwarze, F.W.M.R., Fink, S., Deflorio, G., 2003. Resistance of parenchyma cells in wood to degradation by brown rot fungi. *Mycol. Prog.* 2, 267–274.
- Singh, D.K., Sharma, V.K., Kumar, J., Mishra, A., Verma, S.K., Sieber, T.N., Kharwar, R.N., 2017. Diversity of endophytic mycobiota of tropical tree *Tectona grandis* Linn.f.: Spatiotemporal and tissue type effects. *Sci. Rep.* 7, 3745.
- Slippers, B., Wingfield, M.J., 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biol. Rev.* 21, 90–106.
- Slippers, B., de Groot, P., Wingfield, M.J., 2012. The Sixx Woodwasp and its Fungal Symbiont. *Research and Management of a Worldwide Invasive Pest*. Springer, Dordrecht, The Netherlands.
- Terashima, Y., 2013. Physiological characteristics of the trunk sap rot pathogen *Fomitopsis* sp. on the “Sanbu-sugi” cultivar of *Cryptomeria japonica*. *Mycoscience* 54, 171–177.
- Rezgui, A., Vallance, J., Ben Ghnaya-Chakroun, A., Bruez, E., Dridi, M., Djidjou Demasse, R., Rey, P., Sadfi-Zouaoui, N., 2018. Study of Lasiodiplodia *Pseudotheobromae*, *Neofusicoccum Parvum* and *Schizophyllum Commune*, three pathogenic fungi associated with the grapevine trunk diseases in the North of Tunisia. *Eur. J. Plant Pathol.* 152 (1), 127–142.
- Song, Z., Kennedy, P.G., Liew, F.J., Schilling, J.S., 2017. Fungal endophytes as priority colonizers initiating wood decomposition. *Funct. Ecol.* 31, 407–418.
- Sun, S.S., Zeng, X., Zhang, D.W., Guo, S.X., 2015. Diverse fungi associated with partial irregular heartwood of *Dalbergia odorifera*. *Sci. Rep. UK* 5.
- Taylor, D.L., Walters, W.A., Lennon, N.J., Bochicchio, J., Krohn, A., Caporaso, J.G., Pennanen, T., 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. *Appl. Environ. Microbiol.* 82, 7217–7226.
- Tanner, E.V.J., Kapos, V., Healey, J.R., 1991. Hurricane effects on forest ecosystems in the Caribbean. *Biotropica* 23, 513–521.
- Thomas, S.E., Crozier, J., Aime, M.C., Evans, H.C., Holmes, K.A., 2008. Molecular characterization of fungal endophytic morphospecies associated with the indigenous forest tree, *Theobroma gileri*, in Ecuador. *Mycol. Res.* 112, 852–860.
- Uriarte, M., Thompson, J., Zimmerman, J.K., 2019. Hurricane María tripled stem breaks and doubled tree mortality relative to other major storms. *Nat. Commun.* 10, 1–7.
- Van Bloem, S.J., Murphy, P.G., Lugo, A.E., Ostertag, R., Rivera Costa, R., Ruiz Bernard, I., Molina Colon, S., Canals, Mora M., 2005. The influence of hurricane winds on Caribbean dry forest structure and nutrient pools. *Biotropica* 37, 571–583.
- Van Bloem, S.J., Lugo, A.E., Murphy, P.G., 2006. Structural response of Caribbean dry forests to hurricane winds: a case study from Guánica Forest, Puerto Rico. *J. Biogeogr.* 33, 517–523.
- Van Bloem, S.J., Murphy, P.G., Lugo, A.E., 2007. A link between hurricane-induced tree sprouting, high stem density and short canopy in tropical dry forest. *Tree Physiol.* 27, 475–480.
- Vandenkoornhuise, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. *New Phytol.* 206 (4), 1196–1206.
- Vasaitis, R., 2013. Heart rots, sap rots and canker rots. In: Gonthier, P., Nicolotti, G. (Eds.), *Infectious Forest Diseases*. CAB International, Wallingford, pp. 197–229.
- Webber, J.F., 1981. A natural biological control of Dutch elm disease. *Nature* 292 (5822), 449–451.
- Webber, J.F., Hedger, J.N., 1986. Comparison of interactions between *Ceratocystis ulmi* and elm bark saprobes in vitro and in vivo. *Trans. British Mycol. Soc.* 86 (1), 93–101.
- Wolfe, B.T., Macchiavelli, R., Van Bloem, S.J., 2019. Seed rain along a gradient of degradation in Caribbean dry forest: effects of dispersal limitation on the trajectory of forest recovery. *Appl. Veg. Sci.* 22 (3), 423–434.
- Zimmerman, J.K., Everham, E.M., Waide, R.B., Lodge, D.J., Taylor, C.M., Brokaw, N., 1994. Responses of tree species to hurricane winds in subtropical wet forest in Puerto Rico: Implications for tropical tree life histories. *J. Ecol.* 82, 911–922.