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Mechanical Branch Wounding Alters the BVOC Emission Patterns of *Ficus* Plants

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Abstract: Tree leaves emit biogenic volatile organic compounds (BVOCs) in response to mechanical wounding by insect folivores. However, BVOCs are also released from leaves in response to damage to other tree organs. In this study, we hypothesized that if trees utilize BVOCs to defend against leaf herbivory, BVOCs emitted in response to leaf wounding would be different from those emitted in response to other types of mechanical damage. To test this hypothesis, we measured BVOCs emitted from the leaves of four *Ficus* species in response to leaf-cutting, branch-cutting, leaf-branch-cutting, and control (constitutive BVOCs). We found that leaf-cutting triggered the emission of BVOCs, but their emission patterns were species-specific, and the overall BVOC composition did not significantly differ from that of constitutive BVOCs. In contrast, branch-cutting triggered the emission of many BVOCs, some known as parasitoid attractants and herbivore deterrents. Our study suggests that plant defense mediated by BVOCs is highly species-specific and not effective for attracting herbivore enemies when unrelated disturbances such as tree falls and windstorms occur. Additionally, we recommend avoiding ex situ BVOC sampling of cut plants, as this method alters BVOC emission patterns from both intact and damaged leaves.

Keywords: biogenic volatile organic compounds (BVOCs); branch-cutting; *Ficus*; leaf-cutting; mechanical damage

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1. Introduction

Biogenic volatile organic compounds (BVOCs) are omnipresent in the atmosphere and are released by the bioactivities of living life forms, primarily plants [1,2]. BVOCs are known to promote growth, flowering, and plant–pollinator interactions [3], as well as reduce competition [4]. Plants also release BVOCs in response to biotic interactions, including herbivory and pathogen attacks [5,6], as well as abiotic stress such as drought, increased salinity, temperature, windstorms, or other mechanical damage [6–10]. In response to these stresses, plants produce specific BVOCs for defense. BVOCs are also known as the precursors of ozone pollution, potentially influencing photosynthetic carbon uptake by plants [11]. Many studies have focused on BVOC emissions in response to herbivory [12,13]; however, plant BVOC responses to multiple biotic and abiotic stressors are poorly understood.

When leaves are damaged due to insect herbivory, the tissues that store BVOCs (e.g., secretory tissues, resin ducts, glandular trichomes, and mesophyll) are ruptured [14]. Leaf damage also activates the biosynthetic pathways that utilize photosynthesized carbon to generate BVOCs [15]. Consequently, BVOC emissions occur from both storage and de novo synthesis [6,14,16,17]. Similarly, other mechanical damage caused by windstorms, arboreal animals, and selective felling also stimulates BVOC emissions [7,8]. Unlike leaf herbivory caused by localized damage to leaves, other mechanical damage may sever branches. The functions of BVOCs may differ between herbivory and mechanical damage to other parts of a plant [18].

The diversity of BVOCs is distinctive in response to biotic and abiotic stresses [6,18,19]. Stress-induced BVOCs are generally categorized into different compound groups, namely green leaf volatiles (GLVs, C6 compounds of alcohols, aldehydes, and esters), terpenes (monoterpene, sesquiterpene, and homoterpene), isoprene, fatty acids (esters), alkanes, alkenes, ketones, and ethers [20,21]. The GLVs, terpenes, and isoprene are often released in response to herbivory and are reported to function as deterrents [22], noxious substances [23,24], attractants of herbivore enemies [25], and agents for plant–plant communications [26,27]. In response to mechanical damage, the same three compound groups contribute to stress resistance, healing wounds, and preventing pathogen infection in damaged areas [18]. The latter five compound groups are also found in the BVOC blends [28,29], but their specific roles are unknown. Therefore, exploring the roles of BVOCs in response to both biotic and abiotic stress will improve our understanding of their importance and ecological functions.

Plants' BVOC emission patterns differ depending on the nature and extent of damage [30]. However, differences in BVOCs released from localized plant damage caused by herbivory (leaf damage) and other mechanical damage are poorly understood. One of the unique functions of BVOCs released in response to herbivory is communication with other plants (to signal them to prepare for herbivory [26,27]) and to attract herbivore enemies [31]. Such BVOC-mediated communication would not be effective if the same BVOCs are released in response to non-herbivory damage. GLVs and terpenes are known to be synthesized in leaves mainly in response to herbivory [15]. Therefore, plants under herbivory may release a unique set of BVOCs to signal other plants and herbivore enemies. Current evidence supports this hypothesis, as herbivore predators are able to identify and follow certain BVOCs, including GLVs and terpenes [32,33]. Alternatively, predators may follow BVOCs regardless of the nature of the damage, although this behavior would be inefficient as non-herbivory damage is common in forests.

In this study, we conducted a manipulative field experiment to understand the BVOC emission patterns in response to mechanical damage to plants' leaves and branches under semi-natural conditions. We selected *Ficus*, one of the most diverse pantropical tree species, as the focal species. *Ficus* trees produce abundant fruits, attracting a wide range of animals, including over 1200 species of vertebrates and numerous invertebrates [34–36]. Therefore, a range of defense strategies (e.g., physical, chemical, tolerance) are utilized by *Ficus* for different animals [37,38]. Despite multiple defense strategies, insect herbivory is common in *Ficus* plants [38,39], suggesting that passive defense strategies such as BVOCs may be more important for defense against insects.

We hypothesized that BVOC emissions significantly increase when plants are exposed to mechanical damage, just as they do in response to other abiotic and biotic stresses. If certain BVOCs are utilized for specific purposes, such as plant–plant and plant–herbivore enemy communications, the composition of BVOCs released from plants with cut leaves will be different from those with severed branches. Our study has methodological implications, as some studies only measure BVOCs released in response to experimental manipulations from cut plant parts [40,41], whereas we tested differences in cut and uncut tree branches emitting BVOCs and compared these emissions to those of intact and damaged leaves.

2. Materials and Methods

2.1. Study Area

The study was conducted at Xishuangbanna Tropical Botanical Garden (XTBG, 101°25′ E, 21°41′ N, 630 m a.m.s.l), Xishuangbanna, Yunnan, Southwest China. The study area is characterized by a tropical monsoon climate with distinct wet (May–October), cool dry (November–January), and hot dry (February–April) seasons. The annual mean temperature ranges from 15.1 °C to 21 °C, and annual precipitation is 1493 mm, with 80% of rainfall occurring in the wet season [42]. Our study was conducted in April 2021, at the end of the dry season in this area.

2.2. Experimental Manipulations

We sampled in semi-natural habitat, using saplings of *F. virens* and *F. microcarpa*, and mature trees of *F. religiosa* and *F. benjamina*. These four species are all commonly grown in XTBG. We chose saplings and mature trees (hereafter referred to as trees) of 5–10 m and 18–25 m in height, respectively. Eight trees of each species were included (32 trees total). For each tree, the foliage of a branch tip with no signs of herbivore or pathogen infection was selected. Trees of the same species were spatially aggregated, and clusters of the four species were separated by approximately 100 m to 4 km. Trees within each cluster were separated by at least 1 m.

We applied four treatments to tree foliage: control, leaf-cutting, branch-cutting, and leaf-branch-cutting (Figure 1). Only one of these four treatments was applied to each tree as the localized damage can influence the whole plant [43]. The number of leaves used for the experiment varied depending on the leaf size of different species: 5–13 leaves per tree for the large-leaved species (*F. virens* and *F. religiosa*) and 7–22 leaves for the small-leaved species (*F. microcarpa* and *F. benjamina*). For the leaf-cutting treatment, a total of six squares or rectangles were cut in the lamina of each leaf. We avoided cutting mid ribs; each cut area was 1 cm^2 for small-leaved species and 4 cm^2 for large-leaved species. For the branch-cutting treatment, we cut branches immediately below the foliage used for the experiment. The lengths of cut branches ranged between 50–80 cm. The severed areas were covered by cotton soaked with water, and the branches were brought to the laboratory for BVOC sampling. Cut branches were brought to the laboratory, as many studies employ branch cutting and sample BVOCs under controlled environments in a laboratory [40,41,44]. For the leaf-branch-cutting treatment, branches were cut the same way as the branch-cutting treatment and then brought to the laboratory before leaves were cut the same way as the leaf-cutting treatment. For the control treatment, we did not manipulate the leaves before BVOC sampling.

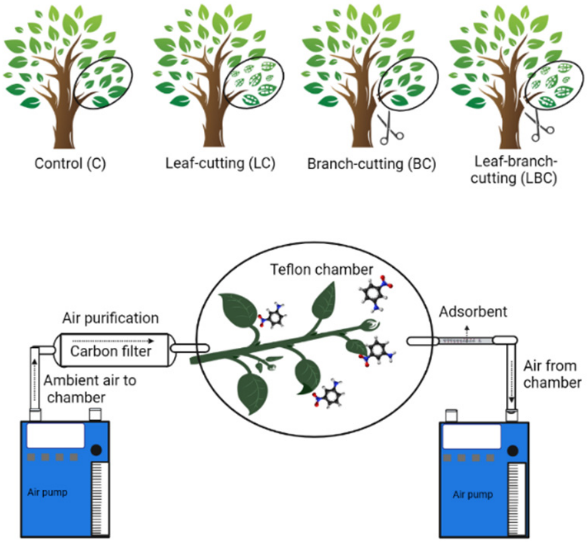

Figure 1. The experimental manipulations applied to four *Ficus* trees before biogenic volatile organic compound (BVOC) sampling (**top**), and a simplified illustration of the equipment setup used for the BVOC sampling (**bottom**).

2.3. BVOC Sampling and Identification

We sampled BVOCs in situ for the tree branches under the control and leaf-cutting treatments, whereas cut tree branches (the branch-cutting and leaf-branch-cutting treatments) were brought to the laboratory immediately after branch cutting. BVOCs were sampled between 15 and 30 min after experimental treatments were applied. We used the dynamic headspace method to sample BVOCs (Figure 1). The foliage was enclosed by the open-ended transparent Teflon bag (polytetrafluoroethylene (PTFE), 0.05 mm thick, 22 cm diameter, Beijing Haochen Tiancheng Environmental Protection Technology Co., Ltd., China) [45]. Two portable air pumps (QC-1B Air Sampler, Beijing Municipal Institute of Labour Protection, Beijing, China) set at the rate of 500 mL/min were connected by Teflon tubes to the two sides of the bag and sealed. The air was purified before pumping to the chamber using a carbon filter (silver-loaded activated carbon, Shenzhen Huiant Import & Export Co., Ltd., China). We used large carbon filters for indoor use (96 g of activated carbon), and small filters for outdoor use (0.3 g of activated carbon). The small filter was replaced for each BVOC sampling, whereas the large filter was used to process eight samples. The outlet of the Teflon chamber was connected to a sorbent tube containing Porapak-Q adsorbent (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) to trap BVOCs released from the foliage.

We sampled BVOCs for ten hours immediately after leaves were damaged to capture a wide array of BVOCs. We measured ambient temperature before and after BVOC sampling and used these data for statistical analyses described below. The temperature range was 20–35 °C in situ and 26–31 °C in the laboratory. We did not use PPFD for statistical analyses, as PPFD was highly variable in the field within and among the samples (18–1512 μmol/m^2/s) compared to the laboratory (2–5 μmol/m^2/s), making the data not amenable for statistical analyses. After sampling, adsorbents were immediately transported to the laboratory to extract the volatile compounds in n-hexane solution (n-hexane purity was HPLC level $\geq$ 99% and further purified by the heavy steam method). The solution samples (1 μL each) were analyzed with gas chromatography and mass spectrometry (GC-MS, 5977B GC/MSD, Agilent Technologies, Santa Clara, CA, USA) consisting of a HP-5 ms quartz capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness). GC-MS was run for 42 min to detect BVOCs [46,47]. We also carried out blank tests to distinguish contaminants derived from the sampling devices and ambient atmosphere in the sample. These potential contaminants were removed before subsequent data analyses.

BVOCs were identified using a retention index (based on retention times of n-alkanes) and information provided by MSD ChemStation (Agilent Technologies, Inc. Santa Clara, CA, USA) with the National Institute of Standards and Technology data (NIST17L). We also used the NIST library [4,48–50], NIST Webbook Library (https://webbook.nist.gov/chemistry/) (accessed on 12 November 2022), PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 12 November 2022), and The Pherobase (https://pherolist.org/) (accessed on 12 November 2022) to confirm our BVOC identification. We considered our identification of BVOCs "reliable" when the probability of identification by MSD ChemStation was greater than or equal to 70%, and considered it "verified" when the calculated retention index of the "reliable" BVOCs matched with the reference retention index (within ±10%). As the quantification of BVOCs was not useful due to field sampling conditions, we used the presence/absence of BVOCs in each sample instead of using peak areas to estimate relative abundance. Individual BVOCs were also categorized into six functional groups: green leaf volatiles (GLVs), isoprene, monoterpenes, sesquiterpenes, homoterpenes, and others (alcohols, aldehydes, alkanes, alkenes, esters, ethers, ketones, and uncategorized functional groups).

2.4. Statistical Analysis

Differences in BVOC richness among the four experimental treatments were analyzed using the generalized linear mixed model (GLMM) with Poisson distribution implemented by the glmer function in the lme4 package [51]. The experimental treatments and ambient temperature (mean of the temperatures measured before and after BVOC sampling) were considered as fixed factors, and the four plant species as a random factor. When the experimental treatment was found to be significant in the main test, post hoc pairwise comparisons using emmeans (emmeans::emmeans) were conducted to find significantly different treatment pairs. In addition, we visually investigated the six BVOC groups to identify how the richness differed among the experimental treatments and *Ficus* species. To this end, we counted the number of BVOCs in each of the six BVOC groups for each experimental treatment and each species.

To visualize the overall BVOC composition among samples, we used non-metric multidimensional scaling (NMDS) (vegan::metaMDS) [52]. Multivariate permutational ANOVA (PerMANOVA) (vegan::adonis2) using *Ficus* species as a block (equivalent to a random factor) was performed to investigate the BVOC compositional differences among the experimental treatment (based on Sørensen index and 9999 permutations). We used post hoc pairwise tests (999 permutations) (pairwiseAdonis::pairwise.adonis2) to identify significantly different treatment pairs. In addition, similarity percentage analysis (SIMPER) was used to identify BVOCs that contributed to the differences between the control and other mechanically damaged foliage to find BVOCs that are different from constitutive compounds (i.e., BVOCs released under control conditions). SIMPER calculates the contribution of individual BVOCs to between-group differences. We identified significant BVOCs based on 9999 constrained permutations with species as a block. All analyses were conducted in R software 4.2.1 [53].

3. Results

A total of 252 BVOCs were detected from the four *Ficus* species under the four experimental treatments. Among the four species, *F. benjamina* released the largest number of BVOCs (200), followed by *F. virens* (129), *F. microcarpa* (120), and *F. religiosa* (66) (Table S1). Identifications of 125 BVOCs were deemed “reliable” with greater than or equal to 70% identification probability, and 75 BVOCs were further “verified” with the reference retention index values. The plants under leaf-cutting, branch-cutting, and leaf-branch-cutting treatments released greater richness of BVOCs than the control, and the highest richness of BVOCs was released from the leaf-branch cutting treatment (Figure 2A). The results of GLMM on the total BVOC richness showed a significant effect of the experimental treatments (df = 3, $\chi^2 = 67.78$, $p < 0.0001$), but not of temperature (df = 1, $\chi^2 = 1.199$, $p = 0.274$). Pairwise comparisons revealed significant differences between treatments involving branch cutting (branch-cutting and leaf-branch-cutting) and others (control and leaf-cutting) (Figure 2A). Non-significant differences were found in BVOC richness between the control and leaf-cutting treatments, and between the branch-cutting and leaf-branch-cutting treatments.

Among the six BVOCs groups, GLVs, monoterpenes, sesquiterpenes, and others were found in all four *Ficus* species, whereas isoprene was found only in *F. religiosa* under the branch-cutting treatment, and homoterpenes found in all species except *F. microcarpa*. The richness of monoterpenes and sesquiterpenes was similar across the four treatments and the four *Ficus* species (Figure 2B). DMNT ((E)-4,8-dimethylnona-1,3,7-triene) was the only compound belonging to homoterpenes and was detected from: *F. religiosa* in response to all mechanical damage treatments (leaf-cutting, branch-cutting, and leaf-branch-cutting); *F. benjamina* under leaf-branch-cutting; and *F. virens* under all treatments except branch-cutting (Table S1). The richness of GLVs was highly variable across the *Ficus* species, but GLVs were only released when mechanical damage treatments were applied (Figure 2B).

The NMDS ordination showed similar BVOC composition between the control and leaf-cutting treatments and between the branch-cutting and leaf-branch-cutting treatments (Figure 3). These two clusters appear to be separated, except for *F. religiosa* where branch-cutting and leaf-branch-cutting treatments were within the cluster of control and leaf-cutting treatments of the other three species. PerMANOVA showed a significant difference in BVOC composition among the experimental treatments (df = 3, pseudo-F = 2.519, $p < 0.001$). Post hoc pairwise comparisons showed significant differences ($p < 0.05$) between all pairs, except between the control and leaf-cutting treatments ($p = 0.501$).

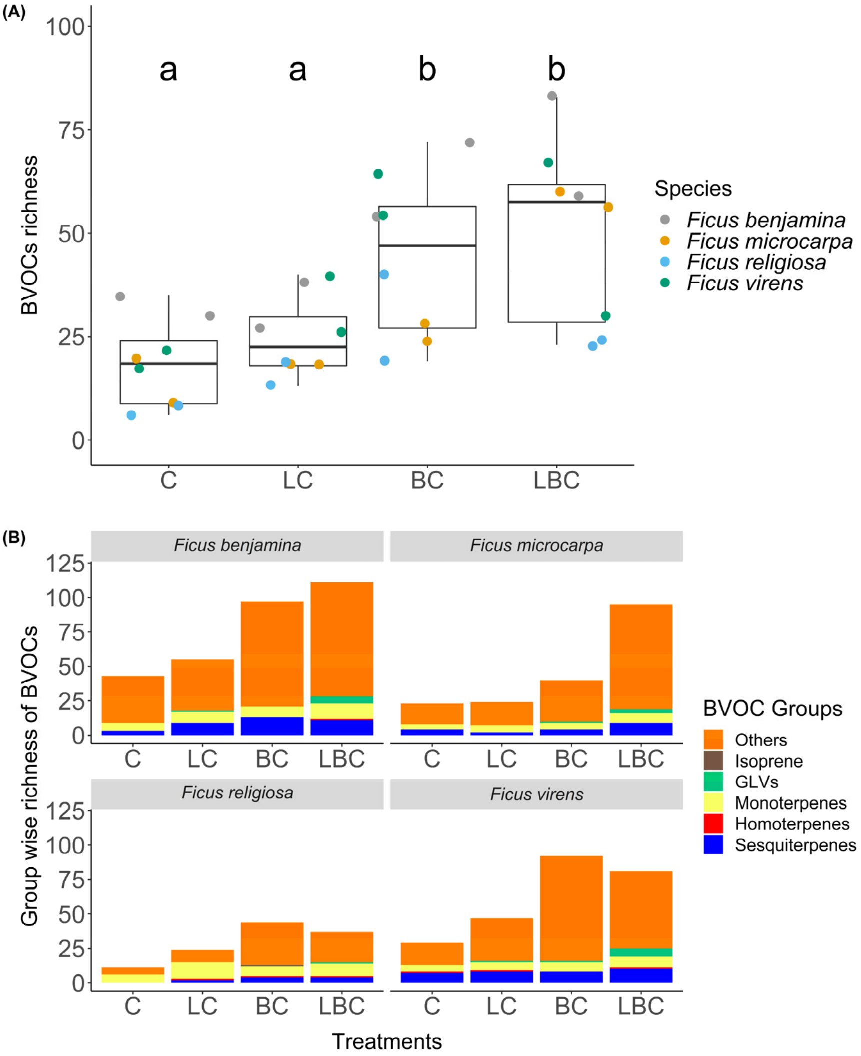

Figure 2. (**A**) Boxplots showing BVOCs richness with respect to the experimental treatments across four *Ficus* species. Letters in (**A**) represent statistically significant differences between the treatments based on post hoc pairwise tests. (**B**) The total richness of BVOCs categorized into six groups (see Methods for more details) among the experimental treatments for each species. C = control, LC= leaf-cutting, BC = branch-cutting, and LBC = leaf-branch-cutting.

Based on SIMPER analysis, we found many BVOCs that significantly differentiated between control and other mechanical damage treatments (Table S2). It should be noted, however, that our results were highly conservative as we detected significant BVOCs based on presence/absence instead of relative abundance. A total of four BVOCs were found to increase in response to the leaf-cutting treatment, and none of these were found to significantly increase in other treatments (Table S2). These four BVOCs were: one monoterpene (β-pinene); one homoterpene ((E)-4,8-dimethylnona-1,3,7-triene = DMNT); one alcohol (1,3,2-dioxaphosphorinane-2-methanol, 2-oxo-.alpha.-phenyl-), and one other BVOC (p-nitrosotoluene, but the identification of this compound was deemed unreliable based on our criteria). No GLVs, sesquiterpenes, alkanes, alkenes, esters, ethers, or ketones were found to be significantly greater in the leaf-cutting treatment. A large number of significant BVOCs, including GLVs, sesquiterpenes, alcohols, alkanes, alkenes, esters, and ethers, were detected to increase in response to the branch-cutting, and leaf-branch-cutting treatments (Table S2).

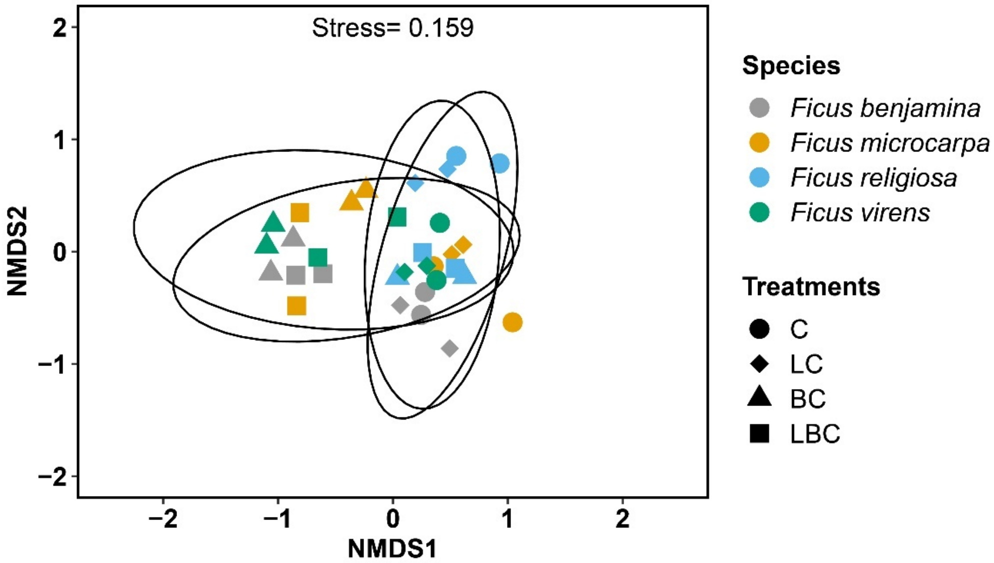

Figure 3. An non-metric multidimensional scaling (NMDS) ordination showing the BVOC compositional differences among the samples with respect to the experimental treatments and the four *Ficus* species. Each oval represents the 95% confidence interval from the centroid. C = control, LC= leaf-cutting, BC = branch-cutting, and LBC = leaf-branch-cutting.

4. Discussion

Our study applied mechanical damage by cutting leaf lamina and tree branches of four *Ficus* species. We found that locally inflicted leaf damage increased the richness of BVOCs; however, the difference between the leaf-cutting and control treatments was not significant. In contrast, branch cutting significantly altered the BVOC emission patterns of *Ficus* plants. For the plants that received the branch-cutting treatments, we sampled BVOCs under controlled laboratory conditions to compare our results to other studies that cut tree organs and measured BVOCs under controlled environments, e.g., [40,41]. As the emissions of BVOCs, especially monoterpenes and sesquiterpenes, are positively related to ambient temperature and light [54–56], we expected greater BVOC emissions from the control and leaf-cutting treatments for which BVOCs were sampled under the semi-natural environment with higher temperature and light. Nevertheless, the results showed the opposite pattern, and temperature did not explain BVOC emissions. Our results clearly suggest that branch cutting overwhelmed other environmental conditions and strongly influenced BVOC emission patterns.

Certain BVOCs, such as GLVs, are emitted immediately after leaves are damaged, whereas the emission of monoterpenes and sesquiterpenes can lag by 30–60 min or longer [57–59]. In addition, specific BVOC emissions are related to daytime temperature and light [54–56]. We therefore opted to sample BVOCs for an extended duration (10 h) to capture as many compounds as possible over time. However, the extensive duration of BVOC sampling may have reached break-through volumes for some compounds, and our results may have underestimated the BVOC emissions, especially for the plants that received the branch-cutting treatments.

Our results contrast with previous studies that reported significant changes in the richness and composition of BVOCs in response to leaf damage [40,60–62]. These studies, however, differ from our leaf-cutting treatments, as they used detached or cut parts of plants. Therefore, their leaf damage treatments are equivalent to our leaf-branch-cutting treatment, and their "control" treatments are equivalent to our branch-cutting treatment. It is important to note that leaves under our branch-cutting treatment did not emit the same BVOCs as those emitted from our control treatment, and BVOCs were also different between our leaf-cutting and leaf-branch-cutting treatments. Our study suggests that, when BVOCs are measured ex situ by cutting branches, trees no longer release constitutive BVOCs from their leaves, and the response patterns to leaf cutting are strikingly different from in situ leaf cutting (i.e., without branch cutting). One may argue that, if cut or detached plant parts are used for both control and experimental treatments, their relative differences in BVOC composition reflect the true response patterns of intact plants. However, we found BVOCs released in response to the leaf-branch-cutting treatment differed from those released in response to the leaf-cutting treatment (Supplementary Table S1), which is consistent with previous work suggesting that BVOC release in response to single and combined stresses can differ [63]. The results of our study have serious implications for BVOC study methods: BVOCs should be measured in situ without cutting.

Our leaf-cutting treatment did not trigger a significant increase in the number of BVOCs or alter overall BVOC composition compared with the control. Nevertheless, SIMPER identified a number of BVOCs that were emitted in response to leaf-cutting but not the control treatment. It should be noted that the significance of these compounds was tested using permutations that were constrained to the same species. Therefore, we detected the BVOCs that were specific to certain species. The results, however, should be interpreted with caution as the number of permutable samples are small ($n = 4$). Despite this caveat, the results of our study are in agreement with other studies that also reported high interspecific variability in the BVOC emission pattern for both *Ficus* and other taxonomic groups [46,64–66], suggesting that it is difficult to draw general patterns even if a study focuses on species of the same genus.

We found a number of compounds that may be used for defense against herbivory. For example, we found one GLV (3-hexen-1-ol, acetate, (E)-) from *F. religiosa* unique to the leaf-cutting treatment. This GLV compound was also detected from *Arabidopsis* plants when their plant leaves were damaged by *Pieris rapae* caterpillars, and attracted parasitic wasps, *Cotesia glomerata* [67,68]. Similarly, one homoterpene ((E)-4,8-dimethylnona-1,3,7-triene (DMNT)) from *F. religiosa* and *F. virens* was also found in the black poplar, *Poplus nigra*, in response to the herbivory by gypsy moth caterpillars, *Lymantria dispar*, and attracted parasitoid wasps, *Glyptapanteles liparidis* [69]. One monoterpene (β-Pinene) from *F. religiosa*, one sesquiterpene (alloaromadendrene) from *F. benjamina*, and one alcohol (benzyl alcohol) from *F. microcarpa* and *F. virens* were also found in *Lycopersicon esculentum, Veronica spicata* and *Trifolium pratense* plants in response to herbivory by *Spodoptera exigua, Melitaea cinxia,* and *Spodoptera littoralis*, respectively [58,70,71]. These studies suggested that β-pinene, alloaromadendrene, and benzyl alcohol could be associated with plant defense, although their specific roles are unknown. One of the monoterpenes (camphene) detected from *F. religiosa* and *F. benjamina* was found as a deterrent when applied artificially in alfalfa pellets and offered to sheep [72]. However, camphene did not alter the palatability of the herb, *Satureja douglasii*, to the molluscan herbivores, *Ariolimax dolichophallus* [73]. One monoterpene (β-pinene) from *F. religiosa* and one sesquiterpene (γ-cadinene) from *F. benjamina* are essential oils that are known as toxic to a number of beetle species [74]. Our study demonstrated that, although species-specific, mechanical wounding to leaf lamina triggered plants to release some BVOCs that are found to be effective in herbivory defense.

Isoprene was released only from *F. religiosa* under the branch-cutting treatment. Although isoprene is emitted in considerable amounts from tropical plants [75], we did not find it in the other three *Ficus* species. Our findings differ from other studies that measured constitutive BVOCs in several regions, including our study region (Xishuangbanna), and found isoprene emissions from all of the four *Ficus* species that we included in our study [46,64,65]. It is not clear why we did not detect isoprene from our *Ficus* trees except for *F. religiosa*. Tropical trees are known to emit less isoprene with increasing dry season length [76], and our study was conducted in the monsoonal tropics where the dry season duration is six months, which is longer than other tropical forests. The lack of isoprene in the three *Ficus* species may be related to this area's extensive dry season length and the timing of our sampling at the end of the dry season. Alternatively, it is likely that the adsorbents we used for this study could not detect isoprene, as isoprenoids are highly volatile with low molecular weight, requiring polymeric or graphitized carbon-based adsorbents [77].

We measured BVOCs induced by mechanical damage instead of using real herbivores. Although some of the BVOCs that we detected are also reported in other studies that employed real herbivores [67–69,74], mechanical leaf-cutting may not be the perfect imitation of natural herbivory, as plants perceive physicochemical properties of natural herbivory, such as saliva and chewing vibrations of herbivores [78–80]. Oral secretions from chewing herbivores elicit herbivore-associated molecular responses to defend against herbivory [81,82]. To precisely imitate natural herbivory, some studies have developed machines (MecWorm and Spitworm) able to simulate feeding time and apply larval saliva to leaves subjected for wounding [63,83]. These studies compared BVOCs induced by natural herbivory and those induced by the herbivory mimicking machines, and found the machines induced BVOCs similar to herbivore-induced BVOCs. Additionally, these studies found that the herbivory mimicking machines also elicited similar molecular responses to natural herbivory [81,82]. In our study, however, we neither used caterpillar mimicking machines nor applied real herbivores (e.g., caterpillars). We were unable to find a good number of natural herbivores feeding on *Ficus* plants. Furthermore, using herbivores from other host plants, or commercially available generalist herbivores, may alter BVOC emission patterns as herbivore-specific BVOCs are reported in other studies [78–80]. Nevertheless, overall BVOC composition has been reported to be similar between mechanical damage and herbivory by the caterpillars of several lepidopteran species [58,61]. These studies suggest that the BVOCs emitted from mechanical leaf cutting may represent the overall BVOC emission patterns induced by natural herbivory.

5. Conclusions

Although leaf-cutting triggered the emission of some species-specific BVOCs known as parasitoid attractants and herbivore deterrents, branch-cutting triggered the emission of more BVOCs with similar functions. Our study suggests that BVOC-mediated defense can be highly species-specific and may not be effective when mechanical wounding to tree branches occurs. The frequency and intensity of such disturbances may increase due to human activities such as logging and climate change [84]. Additionally, we recommend avoiding ex situ BVOC sampling using cut or detached plant parts, as branch-cutting alters BVOC emission patterns from intact and damaged leaves.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f13111931/s1: Table S1: The list of BVOCs emitted from the foliage of the four *Ficus* species under the four experimental treatments. Compounds that were not reliably identified (less than 70% identification probability) or lacked reference retention index (or calculated retention index mismatched with their reference values) are italicized; Table S2: BVOCs identified by SIMER analysis as significantly ($p < 0.0002$) contributing to the differences between control (C) and other mechanical damage treatments (LC, BC, and LBC). Values under the treatments are the average contribution of each compound to the pairwise difference, with the name of the experimental treatment in which the greater occurrence of that compound was found. Compounds that were not reliably identified (less than 70% identification probability) or lacked reference retention index (or calculated retention index mismatched with their reference values) are italicized.

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