UNDERSTANDING PATTERNS AND MECHANISMS OF FOREST CANOPY DIVERSITY AND ECOSYSTEM FUNCTIONS IN A CHANGING WORLD

EDITED BY: Akihiro Nakamura, Brett Scheffers, Roger Laurence Kitching
and Louise A. Ashton
PUBLISHED IN: Frontiers in Forests and Global Change

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-83250-066-8
DOI 10.3389/978-2-83250-066-8

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UNDERSTANDING PATTERNS AND MECHANISMS OF FOREST CANOPY DIVERSITY AND ECOSYSTEM FUNCTIONS IN A CHANGING WORLD

Topic Editors:
Akihiro Nakamura, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (CAS), China
Brett Scheffers, University of Florida, United States
Roger Laurence Kitching, Environmental Futures Research Institute, Griffith University, Australia
Louise A. Ashton, The University of Hong Kong, SAR China

Citation: Nakamura, A., Scheffers, B., Kitching, R. L., Ashton, L. A., eds. (2022). Understanding Patterns and Mechanisms of Forest Canopy Diversity and Ecosystem Functions in a Changing World. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-066-8

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frontiers | Frontiers in Forests and Global Change

TYPE Editorial
PUBLISHED 15 August 2022
DOI 10.3389/ffgc.2022.944981

OPEN ACCESS

EDITED AND REVIEWED BY
Jairo Patiño,
Spanish National Research Council
(CSIC), Spain

*CORRESPONDENCE
Akihiro Nakamura
a.nakamura@xtbg.ac.cn

SPECIALTY SECTION
This article was submitted to
Forest Growth,
a section of the journal
Frontiers in Forests and Global Change

RECEIVED 16 May 2022
ACCEPTED 03 August 2022
PUBLISHED 15 August 2022

CITATION
Nakamura A, Ashton LA, Scheffers BR and Kitching RL (2022) Editorial: Understanding patterns and mechanisms of forest canopy diversity and ecosystem functions in a changing world. *Front. For. Glob. Change* 5:944981. doi: 10.3389/ffgc.2022.944981

Editorial: Understanding patterns and mechanisms of forest canopy diversity and ecosystem functions in a changing world

Akihiro Nakamura[1,2]*, Louise A. Ashton[3], Brett R. Scheffers[4] and Roger L. Kitching[5]

[1]CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, China, [2]Core Botanical Gardens, Chinese Academy of Sciences, Mengla, China, [3]School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong SAR, China, [4]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL, United States, [5]School of Environmental and Natural Sciences, Griffith University, Brisbane, QLD, Australia

KEYWORDS

arboreality, forest canopy, vertical stratification, elevation, anthropogenic disturbance, biodiversity

Editorial on the Research Topic
Understanding patterns and mechanisms of forest canopy diversity and ecosystem functions in a changing world

Introduction

In this Editorial, we illustrate the ecological significance of the forest canopies and describe how a collection of papers in this Research Topic describes the importance of understanding forest canopy biodiversity and ecological processes in the age of the Anthropocene. We lastly address prospects and challenges in forest canopy ecology.

Forest canopies provide additional spatial dimensions

Traditionally, community assembly and ecosystem functions have been considered on a two-dimensional plane with reference points such as latitude, longitude, and elevation (or depth). Almost all terrestrial and aquatic habitats, however, include complex structures that create multiple, rather than just two, spatial dimensions (Basset et al., 2015; Oliveira and Scheffers, 2019). One of the most prominent multi-dimensional structures is found in forests (Ozanne et al., 2003), where the forest canopies function as climatic insulators that provide cool and moist climatic conditions in the understory compared with the hotter and drier climate of the more exposed upper canopy (Frenne et al., 2019; Figure 1). Despite their harsher and variable climatic conditions, forest canopies harbor high biodiversity by providing nesting and food resources and refugia to avoid predation and competition (Gámez and Harris, 2022). In addition, various microhabitats, such as bark, tree holes, lianas, fungi, and epiphytes, provide additional above-ground resources and thermal refugia (Frenne et al., 2021). Where each of these above-ground resources supports specialized consumers, then a whole dependent food web may be created (see, e.g., Kitching, 2000). Epiphytes, for example, provide unique microhabitats in the forest midstory and upper canopies, harboring a diverse array of trophic groups such as herbivores, detritivores, and predators (Stuntz et al., 2002b). Epiphytes also create microclimatic refugia with stratified climatic conditions from the epiphyte's core to their underlying substrates (Stuntz et al., 2002a). However, these patterns of microclimatic stratification may change depending on the vertical positions of the epiphytes and other microhabitats (Figure 1), causing complex interactions of vertical structures that occur at various spatial scales (Moffett, 2013).

Forest canopy diversity and functions

Previous studies have demonstrated that forest biodiversity is stratified vertically (Stork and Grimbacher, 2006; Oguri et al., 2014). In many instances, forest canopies harbor high diversity, with many species only found in the upper strata (Basset et al., 2015). Although vertical stratification of diversity seems to be universal regardless of the latitude and elevation of the forest systems (Ashton et al., 2016), studies to date are limited geographically or taxonomically, and much more effort is required to fully understand the spatiotemporal distribution of forest organisms. In this Research Topic, research on forest canopy biodiversity and vertical stratification targets a variety of taxonomic groups, namely mammals (Haysom et al.), invertebrates (Kuchenbecker et al.; Nakamura et al.; Sallé et al.; Yoshida et al.; Gossner and Petermann; Sagar and Devy), epiphytes (Hu et al.; Seshadri et al.), and pathogens (Lan et al.). The studies represent a wide geographical coverage, including tropical and subtropical forests in Brazil, Borneo, Indo-Burma, and the Western Ghats, and temperate forests in Japan, western Oregon, and Germany. Some of these studies present wholly novel results and unique aspects of forest biodiversity and functions. Gossner and Petermann, for example, describe vertical stratification of aquatic communities in water-filled tree holes and suggest that not only tree hole microhabitat properties but also ecological traits of the invertebrate species that utilize them are determined by vertical position. Yoshida et al. focus on vertical stratification of "arthropod rain" (arthropods falling from above ground, providing important food resources for ground-dwelling predators) and address the importance of "wandering" detritivores and herbivores falling to the forest floor.

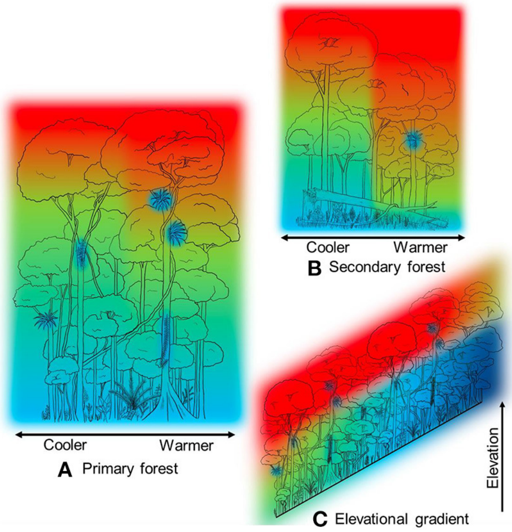

FIGURE 1
Hypothetical depictions of how vertical stratification created by forest canopies interact with anthropogenic disturbance and elevational patterns across time. Colors represent differences in microclimatic conditions (i.e., average and variability of temperature and humidity) and corresponding changes in biodiversity and ecosystem functions. In a primary forest **(A)**, canopies intercept a large amount of insolation, providing cool and stable microclimates in the understory. In addition, epiphytes in the canopy strata create and maintain cool and wet microhabitats. In a secondary forest **(B)**, the magnitude of thermal buffering is smaller than in primary forests due to more exposed canopy surfaces, creating hotter and more unstable conditions in the understory (Sagar and Devy). In addition, seasonal variations may be intensified in the secondary forest. Along an elevational gradient **(C)**, highly variable climatic conditions in the canopy may cause organisms to have greater elevational range distributions (analogous to Rapoport's rule), making elevational species' turnover smaller in the canopy than the understory (Leahy et al., 2021).

The exposed nature of the forest canopy surface also makes community assembly of canopy organisms more influenced by diel, seasonal, and annual variabilities in climatic conditions (Basham and Scheffers, 2020). Kuchenbecker et al., indeed, demonstrated that temporal beta diversity of herbivorous insects was much greater in the forest canopy than in the understory, implying that not only spatial but also temporal dimensions of forest structures must be considered to better understand community assembly and functions of forest ecosystems. Studies by Kuchenbecker et al. and Yoshida et al. both suggest weather conditions are a significant driver of temporal changes.

Anthropogenic impacts on forests and their canopies

Forest canopies are an interface between the land surface and the atmosphere (Ozanne et al., 2003); hence the organisms in the forest canopies are "at the forefront of major changes in response to both direct and indirect effects of climate change" (Sallé et al.). Impacts of climate change are likely to interact with other anthropogenic disturbances (e.g., selective logging and biological invasions, Sallé et al.) that impact forest canopy communities (Haysom et al., Seshadri et al.). Sagar and Devy show that canopies are more exposed and experience higher temperatures in secondary compared with primary forests (Figures 1A,B). This implies that forest disturbance may intensify the impacts of climate change on forest canopy diversity and ecological processes, as predicted by Frenne et al. (2021).

Arboreality, elevation, and latitude

Elevation and latitude are proxies for environmental conditions where temperature and other climatic conditions change spatially along with diversity and ecosystem processes. Nakamura et al. show a decrease in canopy leaf herbivory and an associated increase in tannins and phenolics (plants' defense against herbivores) with increasing elevation. Similarly, forest organisms show elevational stratifications of diversity (e.g., Wang et al., 2012) and ecosystem processes (e.g., Roslin et al., 2017). However, it should not be assumed that elevational or latitudinal patterns in diversity and ecological processes will be the same across forest canopies and the understory (Scheffers and Williams, 2018). Forest canopies present highly variable microclimatic conditions (Frenne et al., 2019, 2021). One recent study found that arboreal ants were indeed exposed to highly variable climatic conditions and presented their elevational range sizes greater than those found in the ground stratum (Leahy et al., 2021; Figure 1C). This suggests that the elevational Rapoport's rule (i.e., organisms present larger elevational range sizes with increasing elevation, Macek et al., 2021) may operate differently between the forest canopy and understory.

Current and future perspectives on forest canopy ecology

The papers published in this Research Topic add information and new insights to the body of work on the ecology of forest canopies. Clearly, however, further study is needed at multiple spatial and temporal scales to understand community assembly and the functioning of forest ecosystems in order to predict the impacts of climate change and other anthropogenic disturbances. As has long been pointed out, forest canopies pose challenging technical obstacles as accessibility to high canopy strata is often limited, making it difficult to produce much needed data (Cannon et al.). Current technological advances and infrastructure development have assisted the acquisition of data from forest canopies. The recent development of canopy cranes across the globe (Nakamura et al., 2017), for example, makes it possible to carry out spatially replicated intensive surveys and manipulative experiments that were not hitherto possible using conventional techniques such as single rope climbing and scaffolding. The development of mobile aerial platforms combined with advanced robotics (Cannon et al.) and next-generation sequencing, such as barcoding mass samples and environmental DNA (Ji et al., 2013; Ladin et al., 2021), provide additional research power that extends the capabilities of conventional surveys. Last, the establishment of multidisciplinary communication platforms (e.g., the Canopy Science Community Forum https://groups.google.com/g/canopy-science) and canopy-oriented conferences (e.g., the 8th International Canopy Conference in Xishuangbanna, China, October 2023) provide opportunities to share information and to collaborate with multidisciplinary communities for studying arboreality and vertical dynamics of forest ecosystems (Cannon et al.).

Author contributions

AN drafted the manuscript. LA, BS, and RK provided extensive edits and additional ideas. All authors contributed to the article and approved the submitted version.

Funding

AN was supported by the National Natural Science Foundation of China International (Regional) Cooperation and Exchange Project (32161160324), Strategic Priority Research Program of Chinese Academy of Sciences (XDB31000000), and the High-End Foreign Experts Program of the High-Level Talent Recruitment Plan of Yunnan Province.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers in Forests and Global Change

ORIGINAL RESEARCH
published: 09 July 2021
doi: 10.3389/ffgc.2021.673071

Life in the Canopy: Using Camera-Traps to Inventory Arboreal Rainforest Mammals in Borneo

Jessica Karen Haysom[1]*, Nicolas J. Deere[1], Oliver R. Wearn[2], Azniza Mahyudin[3], Jamiluddin bin Jami[4], Glen Reynolds[4] and Matthew J. Struebig[1]

[1] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury, United Kingdom, [2] Fauna & Flora International, Vietnam Programme, Hanoi, Vietnam, [3] Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia, [4] South East Asia Rainforest Research Partnership, Danum Valley Field Centre, Lahad Datu, Malaysia

Edited by:
Akihiro Nakamura,
Xishuangbanna Tropical Botanical Garden (CAS), China

Reviewed by:
William J. McShea,
Smithsonian Conservation Biology Institute (SI), United States
Laura Braunholtz,
Newcastle University, United Kingdom
Seth Wong,
Leibniz Institute for Zoo and Wildlife Research, Germany

****Correspondence:***
Jessica Karen Haysom
jkh27@kent.ac.uk

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *26 February 2021*
Accepted: *11 June 2021*
Published: *09 July 2021*

Citation:
Haysom JK, Deere NJ, Wearn OR, Mahyudin A, Jami J, Reynolds G and Struebig MJ (2021) Life in the Canopy: Using Camera-Traps to Inventory Arboreal Rainforest Mammals in Borneo. Front. For. Glob. Change 4:673071. doi: 10.3389/ffgc.2021.673071

Arboreal mammals form a diverse group providing ecologically important functions such as predation, pollination and seed dispersal. However, their cryptic and elusive nature, and the heights at which they live, makes studying these species challenging. Consequently, our knowledge of rainforest mammals is heavily biased towards terrestrial species, limiting our understanding of overall community structure and the possible impacts of human-induced disturbance. We undertook the first in-depth appraisal of an arboreal mammal community in Southeast Asia, using camera-traps set in unlogged and logged tropical rainforest in Sabah, Borneo. Using paired canopy and terrestrial camera-traps at 50 locations (25 in unlogged forest, 25 in logged), we assessed the effectiveness of camera-trapping at characterising the arboreal versus terrestrial community, and tested the influence of strata and forest type on community structure and composition. The paired design detected 55 mammal species across 15,817 camera-trap nights (CTNs), and additional canopy sampling in a subset of trees added a further two arboreal species to the inventory. In total, thirty species were detected exclusively by terrestrial camera-traps, eighteen exclusively by canopy camera-traps, and nine by units set at both heights, demonstrating significant differences between arboreal and terrestrial communities. This pattern was strongest in unlogged forest, reflecting greater structural diversity of this habitat, but held in logged forest as well. Species accumulation curves revealed that canopy camera-trapping significantly boosted species inventories compared to terrestrial-only sampling, and was particularly effective at detecting gliding mammals, rodents and primates. Canopy inventories took longer to reach an asymptote, suggesting that a greater sampling effort is required when deploying canopy camera-traps compared to those set on the ground. We demonstrate that arboreal mammals in Borneo's rainforest form a diverse and distinct community, and can be sampled effectively using canopy camera-traps. However, the additional costs incurred by sampling in the canopy can be substantial. We provide recommendations to maximise sampling effectiveness, while bringing down costs, to help encourage further study into one of the last frontiers of tropical forest research.

Keywords: camera-trap, biodiversity monitoring, tropical rainforest, disturbance, Southeast Asia, mammal survey

INTRODUCTION

Tropical rainforests support exceptional levels of biodiversity, but are highly threatened by anthropogenic activities such as logging (Barlow et al., 2018). Rainforests are structurally complex environments, comprising not only ground-level (i.e., terrestrial) vegetation, but also several interlinked above-ground strata [hereafter the canopy (Moffett, 2000)], and culminating in tree crowns that can reach 30–45 m in height (Dudley and De Vries, 1990). However, due mainly to the difficulties of canopy access (Lowman et al., 2013), most rainforest research is heavily biased towards terrestrial communities and processes (Whitworth et al., 2019a). Consequently, the canopy remains a largely unexplored ecological frontier (Godoy-Guinao et al., 2018). With only a limited understanding of canopies and the wildlife they support, we are missing key insights into the composition, dynamics and functioning of rainforest ecosystems as a whole.

The wildlife utilising the forest canopy plays essential roles in ecosystem functioning, for example by regulating biogeochemical and nutrient cycles, and facilitating forest regeneration via animal-mediated seed dispersal (Nakamura et al., 2017). An estimated 75% of rainforest vertebrates are arboreal or semi-arboreal, spending all or part of their lives in the canopy (Kays and Allison, 2001). Among them, mammals are one of the most diverse and numerous taxonomic groups, filling a wide variety of ecological roles including seed dispersal, pollination, herbivory, and predation (Kays and Allison, 2001; Nakabayashi et al., 2019; Whitworth et al., 2019a). Removal of these key vertebrates may affect the capability of rainforests to recover from disturbance, with potentially cascading consequences for ecosystem stability and resilience (Gardner et al., 2019).

In addition, arboreal mammals may be more vulnerable to the effects of logging than their terrestrial counterparts because the large, tall trees that constitute the main structure of their canopy habitat are often also those lost through logging. At present, most tropical research into the effects of logging on wildlife does not include targeted sampling for arboreal mammals, and it is not clear to what extent this group is affected, or whether populations can recover after the cessation of logging activities (Bowler et al., 2017). For many terrestrial taxa, species diversity in logged forest can return to approximately pre-logging levels within a few decades of the cessation of logging (Berry et al., 2010; Brodie et al., 2014). However, it has also been suggested that terrestrial mammal inventories in logged forest may be artificially inflated by increased detections of semi-arboreal species spending more time on the ground (Berry et al., 2010). While this has been demonstrated for some small-bodied arboreal mammals in some areas (Malcolm, 1997; Malcolm and Ray, 2000), other studies have shown post-logging reductions in the abundance of small arboreal rodents (Wells et al., 2007) and occupancy of medium- and large-bodied arboreal mammals (Whitworth et al., 2019a) without an apparent influx effect at ground-level. The current lack of monitoring of the canopy strata is a barrier to our understanding of whether this phenomenon occurs, and highlights the risk that we may be missing declines in arboreal species, and underestimating the true impact of logging.

Traditionally, arboreal mammals have been sampled using ground-based visual surveys, but these tend to be biased towards larger-bodied, diurnal species that can be readily observed and identified from below, and show some degree of tolerance to people (Whitworth et al., 2016; Bowler et al., 2017; Moore et al., 2020). Moreover, the heights at which arboreal species are detected present significant challenges for accurate identification from the ground (Jayasekara et al., 2007; Gregory et al., 2014; Whitworth et al., 2016). Canopy-based live-trapping has also been tested, but is labour-intensive, tends to exclude larger-bodied species, and can result in biased sampling, particularly as bait is used (Caravaggi et al., 2020).

Advances in camera-trapping technology have led to the widespread use of this survey method in the study of terrestrial mammals (Wearn and Glover-Kapfer, 2019), but it is yet to be commonly implemented at canopy-level. To date, applications of camera-trapping in the canopy have focused mainly on documenting animal presence (e.g., Suzuki and Ando, 2019), behaviour (e.g., Godoy-Guinao et al., 2018), or activity in relation to particular habitat features such as fruiting trees (e.g., Jayasekara et al., 2007) or canopy bridges (e.g., Gregory et al., 2017) (see also **Supplementary Table 1**). Published inventories of arboreal mammal communities based on camera-trap data are limited to five sites (Whitworth et al., 2016, 2019a; Bowler et al., 2017; Hongo et al., 2020; Moore et al., 2020), all in the Neotropics or Africa, and with four of five focusing on medium- and large-bodied mammals. Only three of these studies compared canopy inventories to those generated from camera-traps on the ground (Whitworth et al., 2019a; Hongo et al., 2020; Moore et al., 2020), limiting the inferences that can be made when describing arboreal versus terrestrial communities. Further, almost half of all published canopy-based camera-trap studies that recorded camera-trap height (26 of 54, **Supplementary Table 1**) placed camera-traps ≤ 10 m above the ground, missing a large portion of the vertical space from their sampling. While canopy camera-trapping has shown great potential as a sampling technique, it has yet to be employed as a standard tool for monitoring arboreal mammal communities. Understanding the advantages and limitations of this method is therefore essential if it is to be more widely adopted.

Here, we provide the first in-depth appraisal of the arboreal mammal community in Southeast Asia, using camera-traps set in unlogged and logged tropical rainforest of Sabah, Malaysian Borneo. The rainforests of Borneo are among the tallest in the world, and are renowned for their arboreal and semi-arboreal mammal fauna, comprising over 70 species and including 14 flying squirrel taxa, representing the global epicentre of gliding mammal diversity (Payne and Francis, 2007; Thorington et al., 2012). We compare the ability of camera-traps to define the arboreal and terrestrial mammal community, and extend our assessment from the medium- and large-sized mammals typically investigated in camera-trap studies to include the numerous smaller-bodied arboreal species, most of which can also be identified by this method (De Bondi et al., 2010). We evaluate the comparative costs of terrestrial versus canopy camera-trapping, and quantify the diversity missed or gained by each technique, revealing how studies of rainforest mammals that focus only

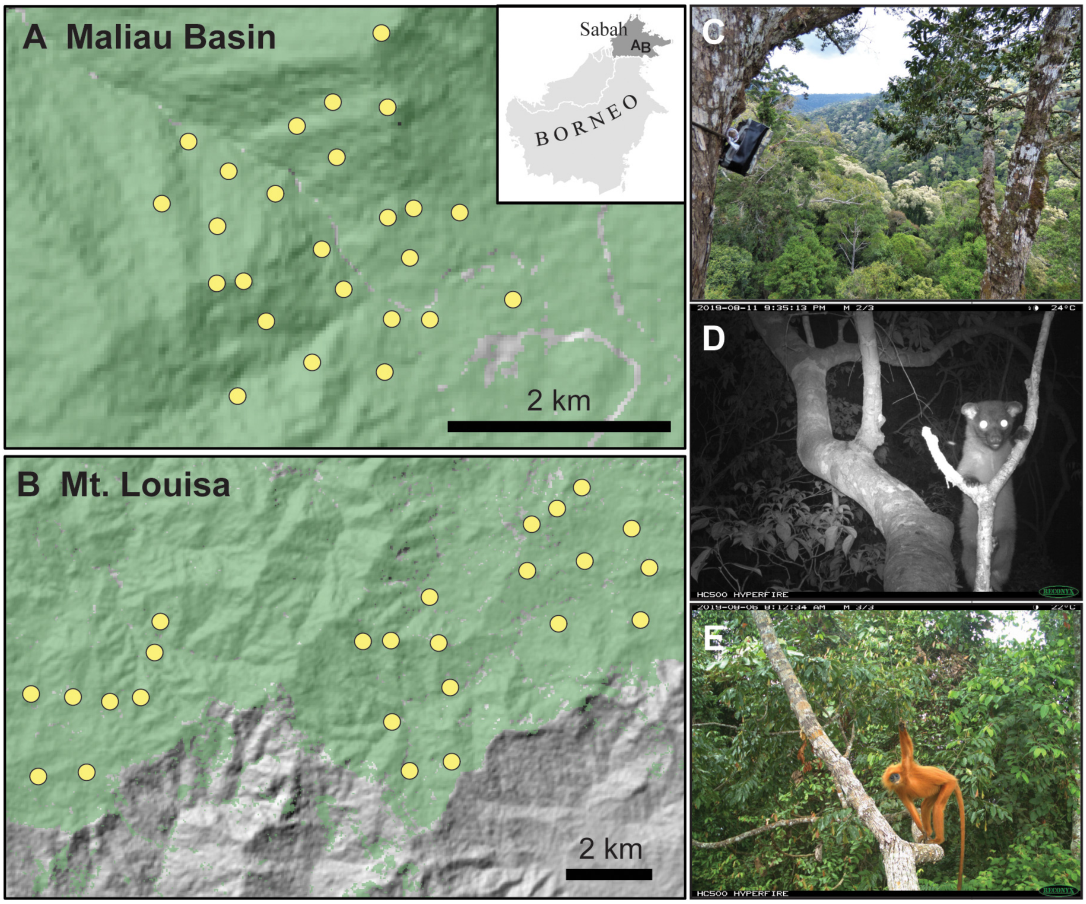

FIGURE 1 | Camera-trap locations in unlogged **(A)** and logged **(B)** forest of Sabah, Borneo, with the location of the sampling in relation to Borneo shown on the inset maps. Canopy camera-traps were set on the trunk of trees, facing focal branches **(C)**. Example arboreal species detected included small-toothed palm civet *Arctogalidia trivirgata* **(D)** and maroon langur *Presbytis rubicunda* **(E)**.

on terrestrial species may be overlooking a key component of ecosystem dynamics.

MATERIALS AND METHODS

Study System

Research was undertaken in and around the Stability of Altered Forest Ecosystems Project (Ewers et al., 2011; **Figure 1**) in Sabah, Malaysian Borneo. We sampled mammals in unlogged forest at Maliau Basin Conservation Area, and in logged forest in the Mt. Louisa Forest Reserve. These areas form part of an extensive contiguous block of dipterocarp forest covering approximately one million hectares in south-central Sabah (Reynolds et al., 2011). Mt. Louisa experienced multiple rounds of logging between 1978 and 2008, but has since been formally protected, whereas the unlogged forest at Maliau Basin has experienced very little disturbance. Our logged forest sampling area was characterised by lower canopy height and reduced canopy cover, with fewer canopy pathways and more canopy gaps than our unlogged forest sampling area (Deere et al., 2020). Camera-trap locations in both unlogged and logged forest covered similar elevations (average 482 m, range 225–933 m).

Camera-Trapping

Camera-traps (Hyperfire HC500, Reconyx, WI, United States) were deployed across 50 locations between October 2017 and September 2019. Locations were divided equally between unlogged and logged forest and identified in advance using a 1.5 km^2 grid, whereby every corner of each grid cell comprised a sampling location. Upon navigating to a location either via pre-existing trails or by creating new trails, the nearest tree to the marked point that could be safely climbed (hereafter the focal tree) was rigged with climbing ropes. We did not target a particular species, height or branch architecture type.

Accounting for accessibility and safety constraints, the average distance between sampling locations was 1.26 km (range: 0.5–4 km). Each location comprised one terrestrial camera-trap set approximately 0.3 m above the ground, paired with a canopy camera-trap in the mid- or upper-canopy of the focal tree, which was situated within a 10 m horizontal distance of the terrestrial placement. Canopy camera-traps were set at an average of 25.9 m above ground (range: 9.8–52.3 m), with the average height in unlogged forest (36.0 m) and logged forest (19.3 m) reflecting the differences in average canopy height between the two forest types. To reduce false triggers, camera-traps were attached to trunks or large, stable branches (**Figure 1**) and any leaves within the detection zone were removed (Gregory et al., 2014). Where possible, canopy camera-traps faced north or south to reduce the likelihood of overexposed images, which is a particular risk in the upper canopy (Otani, 2001). Most camera-traps faced branches of the same tree, were set approximately 0.2–0.3 m above the branch and were angled where necessary using a wooden wedge to account for slope of the branch. Three units faced trunks of adjacent trees (two in unlogged forest, one in logged) where these were judged to be within trigger distance (5–10 m away). Terrestrial camera-traps were also attached to medium-large, stable trunks and any vegetation within the detection zone deemed likely to result in false triggers (e.g., thin herb stems) was cleared. Vegetation disturbance was kept to a minimum in both strata, and canopy orchids and epiphytes in particular were left undisturbed. In order to reduce detection bias for terrestrial species which may either preferentially use or avoid trails (Wearn and Glover-Kapfer, 2017), and in line with our canopy protocol, terrestrial camera-traps did not target any particular habitat feature.

Camera-traps at each location were deployed for a total of 7–8 months. In unlogged forest this occurred continuously with one check mid-deployment to replenish batteries and SD cards, while due to scheduling practicalities, the logged forest deployment occurred in two separate phases. Each camera-trap was set to take three consecutive images per detection with no delay between triggers and no sleep delay before retriggering. The camera-traps we used were equipped with infrared flash for low light conditions to minimise disturbance. This is particularly important for nocturnal species, some of which suffer temporary blindness or may exhibit "trap shyness" when using white flashes (Schipper, 2007).

Sampling comprised a total of 100 camera-trap deployments and, after accounting for malfunction, we obtained data from 99, comprising 49 terrestrial camera-traps (24 in unlogged forest, 25 in logged) and 50 in the canopy (25 in unlogged forest and 25 in logged). Three functioning units did not obtain any mammal captures during deployment (all canopy, two in unlogged forest, one in logged). Thus in total, camera-traps were deployed for 15,817 camera-trap nights (CTNs): 6,661 terrestrial CTN (3,995 in unlogged forest and 2,666 in logged) and 9,156 canopy CTN (6,041 in unlogged forest and 3,115 in logged).

To evaluate whether placing more than one camera-trap in a tree simultaneously could maximise species detection, we set a second canopy camera-trap in 20 of our original focal trees over a period of approximately 3 months. Trees were randomly selected and additional camera-traps were deployed concurrently with the main canopy camera-trap, but positioned at different heights and facing different branches. These second camera-traps (10 in unlogged forest and 9 in logged after excluding one malfunctioning unit) resulted in an additional 1,409 CTN (903 in unlogged forest, 506 in logged).

Data Analysis

Mammal detections were summarised by camera-trap location (per CTN) and species using the R package gtools (R version 4.0.2). Capture events were considered independent if they were separated by a minimum period of 30 min, or if subsequent detections within this threshold contained different individuals or species (Laughlin et al., 2020).

To compare species accumulation between canopy and terrestrial strata, we generated sample-based rarefaction curves based on CTNs using the R package iNEXT (Hsieh et al., 2016). This approach accounts for differences in sampling effort between camera-trap locations (i.e., variation in deployment duration due to units being set and collected, or failing, at different times), without needing to discard data. Rather than reducing all locations to the lowest sampling effort, we interpolated species detections up to the maximum observed sample size and then extrapolated detections to a common sample size above this (S_{max}). Extrapolations were made to approximately double the maximum obtained sample size, as recommended by Hsieh et al. (2016). Where rarefaction results are referred to as statistically significant, this indicates non-overlapping confidence intervals of the relevant species accumulation curves.

Inventory comparisons were made between (a) arboreal and terrestrial communities and (b) unlogged and logged forest habitats, and rarefactions were repeated with subsets of the community data, with species assigned to groupings according to: arboreality, IUCN threat status, body size, and taxonomic group. Arboreality was defined according to the strata in which the species was detected (arboreal = exclusively on canopy camera-traps, terrestrial = exclusively on terrestrial camera-traps, semi-arboreal = on camera-traps in both strata); IUCN threat status was categorised as threatened = categories Vulnerable, Endangered or Critically Endangered; not threatened = Near Threatened, Least Concern or Data Deficient (International Union for Conservation of Nature, 2021); body size was defined as small $\leq$ 1 kg, medium = 1–5 kg, large $\geq$ 5 kg; and taxonomic group was divided into Carnivora (viverrids, mustelids, felids, and bear), gliding mammals (flying squirrels and Sunda colugo), non-gliding rodents (murid rodents, non-flying squirrels, and porcupines), Insectivora (treeshrews, moonrat, and Sunda pangolin), Primates (macaques, langurs, gibbon, and orangutan) and Ungulates (deer, mousedeer, pig, and banteng, plus elephant). Information on body size and taxonomic classifications was obtained from Payne and Francis, 2007 and the International Union for Conservation of Nature (2021).

Ordinations were used to explore variation in mammal community composition between terrestrial and canopy camera-trap locations, as well as unlogged versus logged forest. We standardised the species-camera data matrix by maximum values (a Wisconsin double standardisation) to improve detection of community patterns, and calculated pairwise Bray–Curtis dissimilarity coefficients using species detections pooled from the first 91 days of sampling at each camera-trap location (the maximum sampling effort common to a majority of locations, since units functioned for varying time periods). Using the vegan

TABLE 1 | Sampling effort, mammal species recorded and number of independent capture events for camera-traps set in terrestrial and canopy strata of unlogged and logged forest in Borneo.

Forest type and Strata	No. camera-traps	Camera-trap nights (CTN)	No. mammal species	Independent capture events	Capture events per 100 CTN
Main Dataset					
All	118	15,817	55*	8,008	50.6
Terrestrial camera-traps					
All	49	6,661	39	6,885	103.4
Unlogged forest	24	3,995	35	3,880	97.2
Logged forest	25	2,666	29	3,005	112.7
Canopy camera-traps					
All	50	9,156	25	1,123	12.3
Unlogged forest	25	6,041	18	705	11.7
Logged forest	25	3,115	20	418	13.4
Second Canopy Camera-traps					
All additional	19	1,409	18 (4)	253	17.9
Unlogged forest	10	903	15 (3)	169	18.7
Logged forest	9	506	10 (2)	84	16.6

Rarefaction indicated that sample completeness exceeded 0.98 in all cases. Parentheses denote number of species added to canopy records from experimental second canopy camera-traps. Note that the sum of species from terrestrial and canopy camera-traps is greater than the total number of mammal species detected (marked with an asterisk), because some species (9 overall: 6 in unlogged forest and 5 in logged forest) were detected by both terrestrial and canopy camera-traps and so are included in the figures for both strata.

package in R, we then generated a non-metric multidimensional scaling (NMDS) ordination to organise camera-trap locations by similarity in species composition. To examine compositional differences between the habitats and strata, we applied a permutational multivariate analysis of variance (PERMANOVA) using the ADONIS function in vegan. Last, we applied the envfit function with 999 permutations as well as the Pearson's coefficient function (akin to an indicator species analysis) to the species detections and ordination axis scores to identify the species that contributed the most to the variation in community structure between camera-trap locations.

Costs of Canopy Versus Terrestrial Camera-Trapping

Since we deployed camera-traps at both terrestrial and canopy levels, we calculated the additional costs incurred by our canopy deployments. Calculations assumed a fixed number of camera-traps available for use and compared the cost of setting all units on the ground versus half on the ground and half in the canopy. We assumed cost per camera-trap unit did not vary between strata (damage and repair bills during our study were similar between strata), but this will differ greatly depending on the camera-trap model and battery type used. For our study, cost per unit was USD $464, including one Reconyx Hyperfire HC500 ($450), one 16 GB memory card ($7) and 12 × AA batteries ($7 per camera-trap per deployment) – prices valid for January 2021.

RESULTS

Species Detections and Richness

We recorded 55 mammal species during 8,008 capture events across 15,817 CTNs (**Table 1**). Of these species, 30 were only detected on terrestrial camera-traps, 16 were restricted to canopy camera-traps, and 9 were detected by camera-traps in both strata (hereafter referred to as semi-arboreal species) (**Supplementary Table 2**). The 19 experimental second canopy camera-traps added a further 1,409 CTN, with 238 capture events of 18 species. These additional records included 2 arboreal species otherwise undetected (Bornean pygmy squirrel, *Exilisciurus exilis*, and Temminck's flying squirrel, *Petinomys setosus*, both in unlogged forest), and 1 semi-arboreal species otherwise recorded only on terrestrial cameras (banded civet, *Hemigalus derbyanus*, in logged forest), thus bringing the total number of species recorded exclusively on canopy camera-traps to 18.

There was substantial overlap of species recorded in unlogged and logged forest, although each forest type included records of a limited number of species not detected in the other (**Supplementary Table 2**). The number of CTN and capture events varied between unlogged and logged forest and between terrestrial and canopy strata (**Table 1**). Mammal detections on unlogged forest camera-traps totalled 47 species (29 terrestrial, 12 arboreal, 6 semi-arboreal), while those in logged forest totalled 44 (24 terrestrial, 15 arboreal, and 5 semi-arboreal), including one arboreal squirrel (*Callosciurus* sp.) whose appearance does not fit the description of any known Bornean species (**Supplementary Figure 4**). Twenty-one species potentially present within the landscape and likely detectable using camera-traps were not detected at all (**Supplementary Table 2**). Of these, nine are presumed terrestrial, six arboreal, and six semi-arboreal (Payne and Francis, 2007). Assumption of presence was based on known body size, geographic distribution, elevation range and habitat preferences (Payne and Francis, 2007; International Union for Conservation of Nature, 2021).

Any mammals that could not be identified to species level were excluded from analyses. Of a total of 8,276 capture events

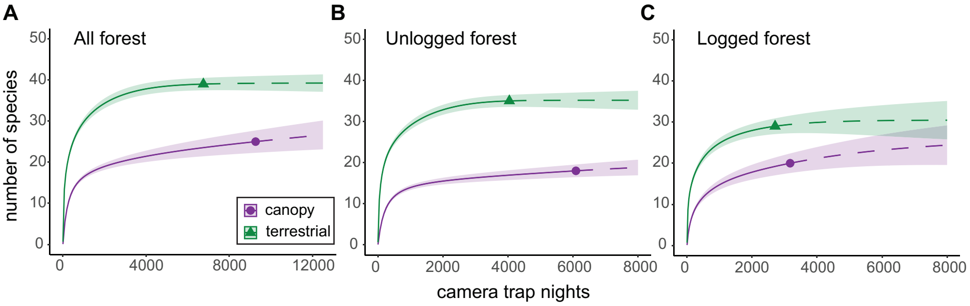

FIGURE 2 | Rarefied species accumulation curves for arboreal and terrestrial mammal communities in panel **(A)** both forest types combined, **(B)** unlogged forest only, and **(C)** logged forest only. Curves were extrapolated (dashed line) to approximately double the minimum observed sample size in each comparison. Confidence intervals (CIs) were set at 84%, which has been demonstrated equivalent to a p value of 0.05 significant differences (MacGregor-Fors and Payton, 2013). CIs are represented by shaded areas around the curves. Additional analyses with CIs at 95% are presented in **Supplementary Figure 1** for comparison.

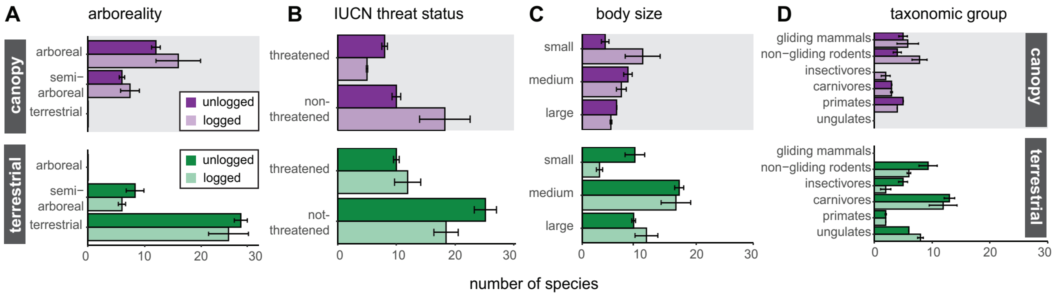

FIGURE 3 | Number of species detected by canopy and terrestrial camera-traps in logged and unlogged forest, split into groups according to panel **(A)** arboreality, **(B)** IUCN threat status, **(C)** body size and **(D)** taxonomic group. Richness data were extracted from rarefied species accumulation curves at a common sample size, together with 84% confidence intervals.

of mammal species across all camera-traps, 15 capture events were excluded on this basis, leaving 8,261 for analysis (8,008 from terrestrial and main canopy camera-traps, and 253 from second canopy camera-traps) (**Table 1**). The 15 exclusions comprised: 1 arboreal squirrel, 1 arboreal murid rodent, and 2 terrestrial squirrels where only a small portion of the body was visible; and 1 arboreal squirrel, and 10 terrestrial murid rodents (likely of 2 species) where identification to species level could be proposed with some confidence, but not with enough certainty to include in analyses.

Effectiveness of Sampling Across Forest Types and Strata

Rarefaction curves for terrestrial communities combined across both forest types reached an asymptote after 3,000–5,000 CTN, suggesting that terrestrial inventories were near complete for this method (**Figure 2**). In contrast, while canopy inventories also approached an asymptote at 3,000–5,000 CTN, curves still increased gradually (**Figure 2**), indicating that further sampling effort in the canopy would likely result in further detections of unique (i.e., not previously detected) species. This was corroborated by extrapolated accumulation curves, which predicted that arboreal communities may require more than double the sampling effort of terrestrial communities to generate complete or near-complete inventories (**Supplementary Figure 2**).

Arboreal mammal species diversity in both unlogged and logged forest types was significantly lower than that characterised at the terrestrial level (**Figure 2**). The signal was stronger in unlogged forest (35 species across terrestrial camera-trap locations versus 18 species across canopy camera-trap locations) than logged forest (29 species across terrestrial camera-trap locations versus 20 species across canopy camera-trap locations) and this was driven by lower detections of terrestrial species in logged forest. Arboreal communities in both forest types largely comprised different species than those found at ground level, with a majority of species (46 of 55; 84%) detected exclusively by camera-traps in one strata, and only nine species (16%) captured on camera-traps at both heights (**Supplementary Table 2**).

Canopy camera-traps were particularly effective at detecting gliding mammals and primates, with non-gliding rodents also well-sampled (**Figure 3**), reflecting the main taxa present in the canopy. Canopy camera-traps matched terrestrial camera-traps in their ability to detect semi-arboreal species (**Figure 3**). On the other hand, terrestrial camera-traps detected more viverrids, mustelids, and felids. Terrestrial camera-traps were also effective at sampling non-gliding rodents, although examination of species identity (**Supplementary Table 2**) reveals there to be little overlap with non-gliding rodent species detected in the canopy,

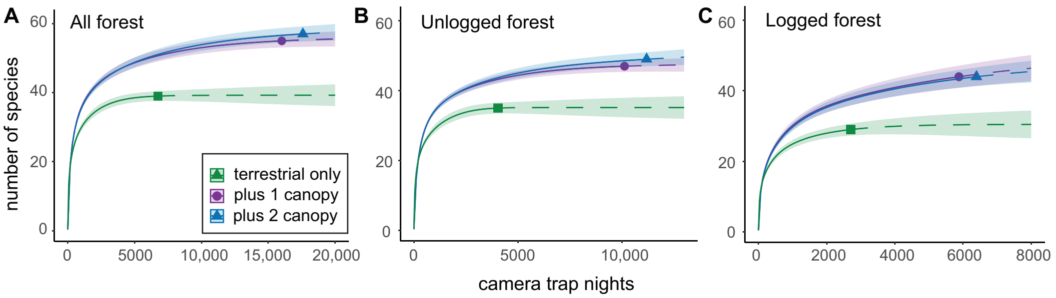

FIGURE 4 | Species accumulation curves comparing species numbers obtained from 19 camera-traps set solely at terrestrial-level, with those obtained by pooling data from terrestrial- plus one canopy camera-trap, and terrestrial- plus two canopy camera-traps. Inventories are shown for panel **(A)** both forest types combined, **(B)** unlogged forest, and **(C)** logged forest. Confidence intervals were set at 84% in line with **Figure 2** (MacGregor-Fors and Payton, 2013) and are represented by shaded areas under the curves. Analyses here represent data from the entire sampling period, totaling 6,661 CTN for terrestrial camera-traps, 9,156 CTN for single canopy camera-traps, and 1,409 CTN for experimental second canopy camera-traps. Analyses were repeated using a standardised subset of 1,409 CTN (the minimum trapping effort arising from the second canopy camera-traps), and these are presented in **Supplementary Figure 3** for comparison.

reflecting the high diversity within this group. Significantly more threatened arboreal mammals were detected in unlogged forest than in logged forest, although there was no significant difference in detection of threatened terrestrial mammals between forest types (**Figure 3**).

Rarefaction analyses based on comparisons of data from terrestrial-only versus terrestrial-and-canopy camera-traps showed that the inclusion of canopy sampling significantly improved inventories in both forest types (**Figure 4**). Canopy camera-traps added 12 unique species in unlogged forest, 15 unique species in logged forest, and 16 species overall, or 18 species if data from the experimental second canopy camera-traps is included (**Figure 4** and **Supplementary Table 2**). This difference was greatest in logged forest, driven mainly by the lower number of species detected by terrestrial-only camera-traps in this forest type. However, the addition of a second camera-trap elsewhere in the canopy did not significantly improve inventories (**Figure 4** and **Supplementary Figure 3**).

Variation in Mammal Community Between Habitats and Strata

The NMDS ordination utilised information from 79 camera-trap locations that photographed mammals, and was statistically robust (stress = 0.120). Canopy and terrestrial camera-traps formed distinct groupings in ordination space, highlighting significant differences in community structure between these strata (PERMANOVA, R^2 = 0.215, $p < 0.001$) (**Figure 5**). Terrestrial camera-trap locations were more similar to each other than canopy camera-trap locations, irrespective of forest type. Differences between the communities of unlogged and logged forest were much subtler (R^2 = 0.027, $p < 0.015$). Key species identified by both envfit and Pearson's coefficient tests to be driving community differences between strata were small-toothed palm civet *Arctogalidia trivirgata* and Prevost's squirrel *Callosciurus prevostii*, indicative of the canopy, and Malay porcupine *Thecurus crassispinis*, tufted ground squirrel *Rheithrosciurus macrotis*, pig-tailed macaque *Macaca nemestrina*, red muntjac *Muntiacus muntjak*, Bornean yellow muntjac *Muntiacus atherodes*, greater mousedeer *Tragulus napu*, bearded pig *Sus barbatus*, sun bear *Helarctos malayanus*, Malay civet *Viverra tangalunga*, banded civet *H. derbyanus* and leopard cat *Prionailurus bengalensis*, indicative of ground level (**Figure 5** and **Supplementary Table 3**).

Cost

Canopy camera-trapping was more expensive than terrestrial camera-trapping due to the following additional costs that sampling at height brings: (i) hire or purchase of climbing equipment, (ii) canopy-access training; (iii) increased salary costs for trained climbers, (iv) more personnel needed to carry climbing equipment; and (v) increased time needed to rig trees and access the canopy (**Table 2**). For both terrestrial and canopy camera-trap locations, setup took substantially longer than maintenance or collection due to the extra time necessary to cut trails, identify suitable locations and position camera-traps. Total costs will vary depending on study design, field conditions and resources already available.

In the context of our study, we estimated the implementation costs of canopy camera-trapping to be approximately double those of terrestrial-only sampling (approximately $5,000–$6,000 for terrestrial-and-canopy sampling versus approximately $2,500 for terrestrial-only sampling, per sampling site) (**Table 2**). However, we calculated that a terrestrial-only study under our sampling conditions would have taken longer to implement (an estimated 164 days vs. the 119 days for paired canopy-and-terrestrial locations, see **Table 2**). This is because terrestrial and canopy camera-traps are specifically targeting different elements of the mammal community (i.e., ground-dwelling vs. canopy-dwelling species), and thus are deliberately deployed very close to each other in order to fully sample as much of the mammal community as possible at each location. In contrast, terrestrial camera-traps target only ground-dwelling species, and are widely spaced in order to satisfy assumptions of independence. At our field site, two staff members had already been trained in canopy access techniques and climbing equipment was available, with the cost of equipment hire built into the increased daily salary of the climbing crew. Our additional one-off expenses therefore totalled $686 for canopy access training for one researcher (JKH,

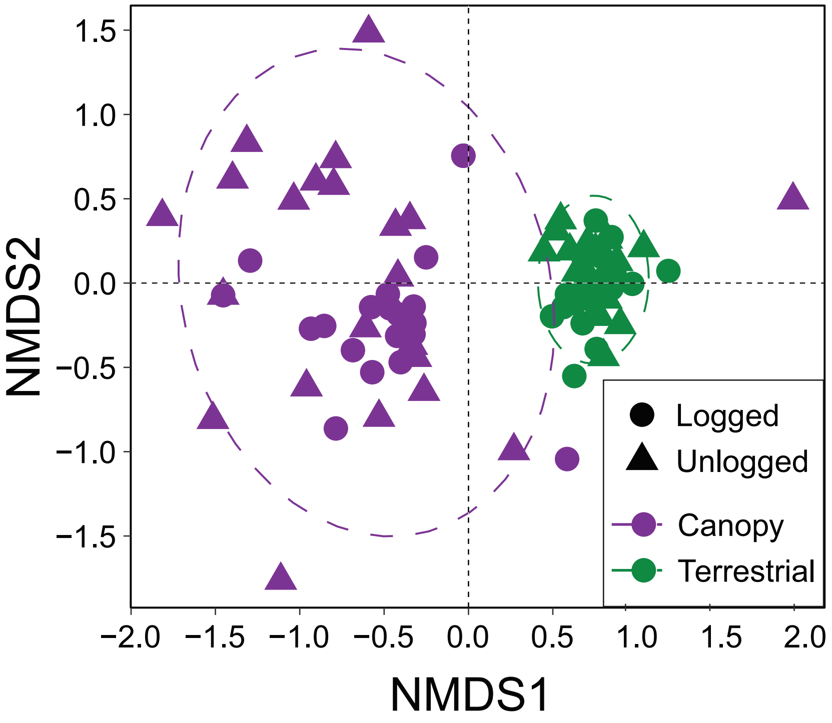

FIGURE 5 | Non-metric multidimensional scaling (NMDS) ordination showing variation in mammal community structure detected by camera-traps set in the canopy versus terrestrial strata of unlogged and logged forest.

undertaken in Malaysia). For studies where equipment is not already available, we provide prices from recognised online retailers, current as of January 2021, and based on two climbers ascending trees up to 70 m height using one main climbing rope and one safety rope, at an approximate additional cost of $4,317.

DISCUSSION

We describe the first canopy-based camera-trap inventory of arboreal mammals in Southeast Asia, and provide the first comparison of camera-trapping between terrestrial and canopy strata in this region. Our results reveal that arboreal mammals form a diverse community (**Figure 2**), comprising mainly species rarely or never detected at ground level (**Figure 4** and **Supplementary Table 2**). This corroborates earlier findings on arboreal mammal communities from Peru (Gregory et al., 2017; Whitworth et al., 2019a), Brazil (Malcolm, 1991), Rwanda (Moore et al., 2020) and Sri Lanka (Jayasekara et al., 2007), and emphasises the importance of effective monitoring protocols for arboreal species that are easily overlooked by traditional terrestrial-based sampling.

Contribution of Canopy Cameras to Mammal Inventories

Our results clearly indicate that a sizeable proportion of the mammal community is routinely missed if sampling is only conducted at ground-level. Species accumulation curves showed canopy camera-traps significantly increased the overall mammal inventory compared to using only terrestrial camera-traps, and this pattern held across unlogged and logged forest (**Figure 4**). Across all locations, canopy camera-traps recorded 18 species that were not detected at ground level, adding 32% more species to inventories than would have been recorded using terrestrial camera-traps alone (**Figure 4** and **Supplementary Table 2**). The mammal fauna detected on terrestrial camera-traps was more consistent across sampling locations than that documented by canopy camera-traps (**Figure 5**), reflecting the greater patchiness in detections and slower accumulation of arboreal species (**Supplementary Figure 2**). Differences between unlogged and logged forest were much weaker than those between terrestrial and canopy strata, and were largely driven by the greater number of species detected by terrestrial camera-traps in unlogged forest (**Figure 2**).

Despite high detections in both strata, twenty-one species known to be present and detectable in the landscape were not recorded (**Supplementary Table 2**): nine terrestrial, six arboreal and six semi-arboreal (i.e., potentially detectable in either strata) taxa. This finding is common to all survey methods, including camera-trapping in the canopy (Whitworth et al., 2016; Bowler et al., 2017; Moore et al., 2020) and terrestrial (e.g., Evans et al., 2016; Wearn et al., 2017) strata. Indeed, comparisons of the efficacy of visual surveys versus canopy camera-traps found both methods failed to detect some arboreal species that were picked up by the other (Whitworth et al., 2016; Bowler et al., 2017; Moore et al., 2020), and a similar pattern is reported for terrestrial versus

TABLE 2 | Costs (US dollars) of terrestrial-only versus terrestrial-and-canopy camera-trapping, based on our experience in Sabah, where the cost of equipment hire was incorporated into the increased daily salary for climbing-trained staff.

Item	Terrestrial-and-canopy sampling	Terrestrial-only sampling
Training expenses		
Canopy access course^	$686 per person (in Malaysia)	n/a
Climbing equipment		
Climbing rope 150 m × 2^	$1,457 ($728.50 per unit)	n/a
Rope 50 m × 1^	$135	n/a
Rope bag 55 liter × 3^	$515 ($171.60 per unit)	n/a
Harness × 2^	$659 ($ per unit)	n/a
Harness chest attachment × 2^	$110 ($55 per unit)	n/a
Chest ascender (“croll”) × 2^	$110 ($55 per unit)	n/a
Hand ascender (“jumar”) × 2^	$124 ($62 per unit)	n/a
Descender (“rig”) × 2^	$324 ($162 per unit)	n/a
Fall-arrest (“backup device”) × 2^	$384 ($192 per unit)	n/a
Foot ascender (“pantin”) × 2^	$117 ($58.50 per unit)	n/a
Footcord (“footloop”) × 2^	$55 ($27.50 per unit)	n/a
Helmet × 2^	$176 ($88 per unit)	n/a
Karabiners × 10^	$233 ($23.30 per unit)	n/a
Slings × 5^	$51 ($10.20 per unit)	n/a
Bigshot catapult × 1^	$165	n/a
Fishing rod × 1^	$96	n/a
Fishing line^	∼$14 to set 50 cameras	n/a
Fishing weights^	∼$14 to set 50 cameras	n/a
Pilot line 4 mm^	∼$93 to set 50 cameras	n/a
Total	$4,317	n/a
Labour		
Ground crew (×1 person)	$30 per person per day	$30 per person per day
Canopy-trained crew (×1 person)	$60 per person per day	n/a
Total	$90 per day	$30 per day
Implementation (100 cameras)	**Realised sampling effort**	**Estimated sampling effort**
Mt. Louisa sites (logged forest)	50 cameras, 25 locations	50 cameras, 50 locations
Setup 2017:	39 days × $90 = $3,510	25 days* × $30 = $750
Collection 2018:	10 day × $90 = $900	20 days* × $30 = $600
Re-set 2019:	11 days × $90 = $990	22 days* × $30 = $660
Collection 2019:	8 days × $90 = $720	16 days* × $30 = $480
Total	57 days, $6,120	83 days*, $2,490
Maliau Basin sites (unlogged forest)	**50 cameras, 25 locations**	**50 cameras, 50 locations**
Setup 2018: Full crew	22 days × $90 = $1,980	25 days* × $30 = $750
Ground crew, scouting for sites	+ 12 days × $30 = $360	n/a
Maintenance Check 2019:	15 days × $90 = $1,350	30 days* × $30 = $900
Collection 2019:	13 days × $90 = $1,170	26 days* × $30 = $780
Total:	62 days, $4,860	81 days*, $2,430
Total (all locations):	50 canopy-and-terrestrial locations = 119 days, $10,980	100 terrestrial-only locations = 164 days*, $4,920

*Items followed by ^ indicate one-off rather than recurring expenses. Costs are calculated based on a three-person team of one ground crew and two climbers, in line with tree-climbing safety protocols. In our study, fieldwork implementation was led by the lead author, who comprised one half of the two-person climbing team, and who received funding separately through a Ph.D. scholarship. Salary costs for local research assistants are therefore calculated for 1× ground crew and 1× climbing crew per day. For studies where implementation will be led entirely by local research assistants, salary cost estimates can be adjusted accordingly as we have given a per-day per-person estimate for ground and climbing crew. For studies where climbing equipment is not available at the field site, we provide prices from recognised online retailers, current as of January 2021, and based on two climbers ascending trees up to 70 m height using one main climbing rope and one safety rope. The "Implementation" comparison assumes a fixed number of camera traps (here, based on our experience, a 50-camera-trap survey implemented at two sampling sites) and so costs reflect the actual number of days we required to deploy terrestrial-and-canopy camera-traps at our sampling locations, versus the estimated number of days that would have been required to deploy the same number of camera-traps at double the number of locations, if terrestrial-only sampling had been conducted. Costs followed by * indicate estimates. Under our study conditions, locations were widely spaced across difficult terrain, largely without trails or roads, and one-third required multi-night camping trips for access. We therefore estimated an average setup rate of two locations per day under a terrestrial-only design. Likewise, for maintenance checks and collection under a terrestrial-only design, we allowed double the time necessary than for our terrestrial-and-canopy design due to the wide spacing of sampling locations and the fact that a terrestrial-only study would have double the number of locations than our terrestrial-and-canopy study. Studies in other regions with less challenging terrain, or where sampling locations are less widely spaced, may adjust budget projections accordingly in line with the per-person, per-day estimates provided below.*

canopy camera-traps elsewhere (Whitworth et al., 2019a; Moore et al., 2020). Possible reasons to explain this discrepancy in our study include low density and/or patchy distributions (e.g., bay cat *Catopuma badia*), preference for specific habitat conditions not represented in the survey (e.g., riverine areas for flat-headed cat *Prionailurus planiceps*), or population fluctuations linked to resource availability (e.g., rodents, Nakagawa et al., 2007).

Canopy camera-traps are most effective at detecting small- and medium-bodied species, gliding mammals and primates (**Figure 3**), reflecting the dominant arboreal taxa present in Borneo (Payne and Francis, 2007). Previous canopy camera-trap studies in the Neotropics have focussed on medium- and large-bodied mammals (Cassano et al., 2012; Whitworth et al., 2016; Bowler et al., 2017), as have most terrestrial camera-trap surveys, since small-bodied species are often fast-moving or obscured by vegetation, making identification difficult (Jayasekara et al., 2007; Glen et al., 2013). It is therefore encouraging to find that smaller mammals, including the highly diverse gliding mammals, can be readily identified from canopy camera-trap images, especially given the prevalence of these groups in Bornean rainforests (Thorington et al., 2012). Both canopy and terrestrial camera-traps recorded relatively high detections of non-gliding rodents (**Figure 3**), with little overlap in the identity of these species present in each strata (**Supplementary Table 2**), demonstrating the ability of canopy camera-traps to boost species inventories of often poorly-sampled taxa. Of the larger-bodied species, primates were well-sampled by canopy camera-traps, recording six of eight species known to be present, compared to only three species recorded by terrestrial camera-traps (**Supplementary Table 2**). Consistently higher detection frequencies in the canopy for all recorded primates (**Supplementary Table 3**) indicates that canopy camera-trapping may prove useful for studying the activity and behaviour of this group, which can be sensitive to human presence, in a manner that is minimally invasive and able to be deployed for long time periods across large spatial scales (see as example Whitworth et al., 2019b).

Conversely, terrestrial camera-traps were more effective at detecting medium- and larger-bodied species, particularly felids, civets, and mustelids. Although many of these species are classified as semi-arboreal and therefore potentially detectable by cameras in either strata, we found they were more often detected on terrestrial camera-traps in our study area, indicating that they may be more habitually terrestrial than arboreal. However, this may also be a result of lower detection probability in the canopy, linked to the overall greater three-dimensional area of this strata and the resultant slower accumulation of species. It is also possible that larger-bodied semi-arboreal species tend to utilise the lower canopy strata, or habitat features such as vine tangles (e.g., masked palm civet *Paguma larvata*, Chutipong et al., 2015) or tree holes (e.g., pangolin *Manis javanica*, Lim and Ng, 2008), whereas most of our canopy camera-traps were set in the mid- to upper-canopy facing large branches or trunks. These differences between presumed and detected arboreality serve to highlight that there is much we do not yet know about vertical space use by rainforest mammals.

Lower detections of threatened species by canopy camera-traps reflect the fact that fewer arboreal species in our sampling locations are categorised as threatened compared to terrestrial species (**Supplementary Table 2**): particularly among rodents. However, canopy camera-traps at unlogged forest locations detected significantly higher numbers of threatened arboreal mammals than those in logged forest. In contrast, there was no significant difference in numbers of threatened terrestrial species between logged and unlogged forest. This may have implications for survey design where the aim is to monitor threatened species.

Species-Specific Insights

For most of the arboreal species we recorded, little is known about their ecology, geographic distribution and tolerance to habitat modification (Meijaard et al., 2005; Payne and Francis, 2007). For example, our record of an arboreal squirrel not fitting any known description for Bornean species (*Callosciurus* sp., **Supplementary Table 2** and **Supplementary Figure 4**), as well as our putative record of smoky flying squirrel (*Pteromyscus pulverulentus*), for which no confirmed photographic image currently exists (Thorington et al., 2012; Clayton, 2016), suggests that much remains to be discovered about Borneo's little-known arboreal small mammals. Canopy camera-traps also documented interesting behavioural insights such as confirmed use of the canopy by plain treeshrews *Tupaia longipes*, a species previously considered strictly terrestrial (Payne and Francis, 2007); probable branch scent-marking in tufted ground squirrels *R. macrotis*, a threatened and poorly-known Borneo endemic; prevalence of "branch-walking" rather than brachiating locomotion in Bornean gibbons *Hylobates funereus*, especially in unlogged forest locations; and canopy mating behaviour of the strictly arboreal small-toothed palm civet *A. trivirgata* (**Supplementary Figure 4**).

Effects of Logging on Rainforest Mammals

Overall, our results suggest that the arboreal mammal community of logged forest is comparable in terms of species identity and diversity to that of unlogged forest (**Figure 2** and **Supplementary Table 2**). This is encouraging from the perspective of species conservation, and corroborates similar findings from terrestrial mammal research (e.g., Brodie et al., 2014; Jati et al., 2018). In contrast, we recorded lower terrestrial species diversity in logged versus unlogged forest (**Figure 2**), indicating that logged forest inventories are not artificially inflated by arboreal species more frequently descending to ground level in this forest type, as sometimes proposed in the literature (e.g., Lambert et al., 2005; Berry et al., 2010). However, these findings should be viewed in the context of the relative habitat quality of our logged forest locations, which underwent selective logging 10 years prior to sampling and have been regenerating since that time. It is possible that there is a threshold of disturbance beyond which most arboreal species cannot persist (Deere et al., 2020), and that this threshold had not been met in our study system. It is also critical to note that our logged forest sampling area is subject to hunting levels which are very low in the regional context (Wearn et al., 2017). More commonly, logging is associated with a substantial increase in the hunting of medium and large mammals, due to the increased access given to the forest (Brodie et al., 2014).

Results from other locations are inconclusive, with canopy camera-trapping in the Neotropics showing medium- and large-bodied arboreal species to be more susceptible to habitat disturbance than their terrestrial counterparts (Cassano et al., 2012; Whitworth et al., 2019a), whereas live-trapping studies of small mammals documented varying responses to logging (e.g., Pardini, 2004; Wells et al., 2007; Laurance et al., 2008). In our study, it is also notable that while arboreal species diversity was similar between forest types, the frequency of capture events for some arboreal primates and gliding mammals was substantially lower in logged forest, whereas that of more common species (e.g., Prevost's squirrel *C. prevosti*, pig-tailed macaque *M. nemestrina*) increased markedly in logged forest (**Supplementary Table 3**). The causes of these differences warrant further examination. However, they serve to highlight the fact that to fully understand the impacts of logging, we should not rely solely on species diversity. Changes in the relative abundance of mammals can impact ecosystem functioning, with declines in key species negatively affecting forest regeneration (Chazdon et al., 2009). What seems clear from the limited but increasing number of canopy-based studies is that habitat management strategies derived from terrestrial-only sampling risk overlooking the effect of logging on arboreal mammals, and thereby underestimating its true impact on rainforest ecosystems.

Methodological Considerations

Species accumulated more slowly in canopy inventories compared to those on the ground, as reported by other studies (Gregory et al., 2014; Whitworth et al., 2016, 2019a). While terrestrial communities in both forest types reached an asymptote at a sampling period of 3,000–5,000 CTN (**Figure 2**), this increased to an estimated 12,000–13,000 CTN for arboreal communities, or up to 57,000 CTN when data from forest types were combined (**Supplementary Figure 2**). Mammals utilising the terrestrial space are limited mostly to a horizontal distribution at ground level unless they exhibit arboreal tendencies. In the canopy space, however, mammals can be distributed both horizontally across branches and vertically across interlinked canopy strata (Nakamura et al., 2017; Hanya et al., 2020), which in Borneo can incorporate heights of up to 100 m in emergent trees (Shenkin et al., 2019). Mammal density in the canopy space is therefore much lower (i.e., animals per cubic kilometre). The passive infrared sensors on most camera-traps are designed to maximise the horizontal width of the detection zone (which may span 10+ m), but not the vertical height (typically spanning 1–2 m) (Wearn and Glover-Kapfer, 2017). In addition, while terrestrial camera-traps sample within their detection zone relatively evenly up to their maximum trigger distance, canopy camera-traps facing a focal branch or trunk are effectively sampling only the width and length of that feature, with the rest of the surrounding sampling area comprising empty space. It is therefore intuitive that species will accumulate more slowly in canopy inventories, and this should be factored into study design.

It has become increasingly common in terrestrial camera-trapping studies to deploy multiple camera-traps per location to boost detection probabilities when they are low (Pease et al., 2016; O'Connor et al., 2017; Wong et al., 2019). There has been little research into whether this method is useful in canopy settings, although one study in Brazil deploying arrays of 2–8 canopy camera-traps found detection probabilities of primates increased with the number of camera-traps per sampling location (Kaizer, 2019). Our trials deploying a second canopy camera-trap did not yield much improvement to new species detections (**Figure 4** and **Supplementary Figure 3**); however, they were limited to a small subset of trees and a shorter sampling period. It is worth noting that the gains in unique species detections from additional canopy camera-traps came from unlogged forest locations, which makes sense in the context of the greater height and structural complexity – and therefore larger potential sampling area – of unlogged forest canopies (Deere et al., 2020). Canopy camera-trapping as a sampling method is in its infancy, and forest canopies present a much more complex sampling space than the forest floor. While our results do not show strong support for prioritising additional canopy camera-traps per sampling location, we do not rule this out as an avenue for future research into maximising arboreal species detections, depending on study aims and resources available.

Costs

Research budgets are almost always a limiting factor in study design. Canopy camera-trapping is reported to be cost-effective when compared with alternative methods such as line transects (Whitworth et al., 2016; Kaizer, 2019). However, for studies where camera-trapping alone is the preferred sampling method, we could find no information evaluating the extra expenses incurred by deploying camera-traps in the canopy. We found canopy-and-terrestrial camera-trapping to be approximately twice as expensive as equivalent sampling effort at ground-level only (**Table 2**), although exact costs will vary according to study design and field conditions. At our field sites, the greatest cost arose from the additional personnel needed to carry climbing equipment and access the canopy. This was due in part to our study design, with camera-trap locations widely spaced across difficult, off-trail terrain and therefore requiring substantial time to access regardless of the forest type, or the strata in which our camera-traps were set. However, we note that for studies with easier access to sampling locations, the cost of canopy camera-trapping is likely to be proportionately higher in comparison to that of terrestrial-only sampling because once a location has been accessed, the greatest time cost of canopy camera-trapping arises from the process of canopy access itself. Selecting a suitable tree, rigging it with climbing ropes, ascending to the canopy and positioning a camera-trap took between 1.5 and 6 h per tree (although this will vary with field conditions and, to some extent, experience). In contrast, once a location has been accessed, a terrestrial camera-trap can generally be set within 0.5–1 h.

For these reasons, costs must be considered in the context of individual study aims and budget, and weighed against the value of the additional data likely to be obtained by canopy camera-traps (**Figure 4** and **Supplementary Table 2**). For researchers wishing to implement canopy camera-trapping, we recommend the following ways of reducing costs: (1) deploy camera-traps over one, longer period rather than two separate shorter periods, thus reducing the number of times locations need to be accessed to setup, check and collect camera-traps; (2) scout for suitable focal trees first with ground crew only and minimal equipment, marking positions and preparing access without the burden of heavy bags; (3) leave pilot line (thin, inexpensive rope running from canopy to ground) in place in focal trees so that climbing ropes can then be easily re-installed upon subsequent visits, avoiding the lengthy rigging process; (4) consider study longevity. Investment in climbing equipment and training is more cost-effective for multi-season studies as both last for several years; and (5) where possible, source equipment and training locally as this is often less expensive, reduces transport costs, and has the additional benefit of enabling familiarisation with location-specific climbing conditions (humidity, terrain, insects, etc.).

Recommendations

Based on our findings, we recommend that studies aiming to inventory the entire, non-flying mammal community of a given area include canopy camera-traps alongside paired units at ground-level (although detection of very small-bodied species and bats may require additional live-trapping). Canopy camera-traps have shown comparative efficiency to ground-based visual surveys for inventorying arboreal mammal communities (Bowler et al., 2017; Moore et al., 2020). We provide evidence corroborating the ability of canopy camera-traps to reliably detect strictly arboreal and semi-arboreal species, and suggest that studies focussing on primarily arboreal species may consider deploying canopy camera-traps as a stand-alone method. However, consideration should be given to specific study aims, target species and resources available, as both camera-traps and visual surveys may miss a small proportion of the mammal community (see for example Whitworth et al., 2016). On the other hand, for studies concentrating on terrestrial and larger-bodied semi-arboreal species, it may be more cost-effective to deploy camera-traps only at ground-level. Behavioural studies of larger-bodied semi-arboreal species known to use both strata (e.g., orangutan *Pongo pygmaeus*, binturong *Arctictis binturong*) may benefit from camera-trapping at both levels as species likely utilise each stratum differently (Ancrenaz et al., 2014; Chutipong et al., 2015; Whitworth et al., 2019b).

CONCLUSION

We provide compelling evidence that the inclusion of canopy camera-trapping significantly increases overall rainforest mammal inventories. However, this survey method can incur substantial additional costs, which should be factored into project planning alongside consideration of study aims and design. There is great scope for further study in this field, including increasing knowledge of arboreal species' geographic distributions, activity and vertical niche partitioning, tolerance to human-induced habitat disturbance, and optimal sampling methods for the canopy strata. Canopy camera-trapping also provides the potential for behavioural studies of species that are not easily observable from ground level. Our results add to the small but growing body of evidence that by overlooking arboreal communities, we are missing crucial insights into the true diversity and functional significance of rainforest mammals. Tropical rainforests are complex habitats covering vast horizontal and vertical areas, and with a high proportion of species utilising the canopy strata. In order to fully understand these ecosystems, and by extension effectively conserve them, it is vital that future research includes canopy-based sampling.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article has been made available by the authors (https://zenodo.org/record/5018280#.YNM-pehKiUk).

ETHICS STATEMENT

The animal study was reviewed and approved by the School of Anthropology and Conservation Research Ethics Committee, University of Kent.

AUTHOR CONTRIBUTIONS

JH, MS, ND, and AM conceived and designed the study. JH conducted the fieldwork and performed the statistical analysis under guidance from MS, ND, and OW. JH, ND, and MS wrote the manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

FUNDING

This study was funded by the UK Natural Environment Research Council (NERC) via the Human Modified Tropical Forests Programme (NE/K016407/1; https://lombok.nerc-hmtf.info/). JH was supported by a Ph.D. scholarship funded by NERC through the EnvEast DTP scholarship (Grant Number NE/L002582/1).

ACKNOWLEDGMENTS

We thank the Sabah Biodiversity Council, Sabah Forest Department, Yayasan Sabah, and Benta Wawasan Sdn. Bhd. for research permissions [access license number JKM/MBS.1000-2/2 JLD.5 (53)]. Henry Bernard advised on in-country logistics, and Mohd. Sabri bin Bationg, Almius Jupri, Fredino John, Arnold James, Masliadi bin Asri, Masdi bin Asri, Sabidi Mohd. Rizan, Anis-Ampat Siliwong, and David Paul were instrumental to the camera-trapping campaign.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.673071/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
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ORIGINAL RESEARCH
published: 23 July 2021
doi: 10.3389/ffgc.2021.672601

Vertical Distribution of Arthropod Assemblages and the Effects of Weather Conditions on Arthropod Rain in a Temperate Conifer Forest

Tomohiro Yoshida[1], Yasunori Kusunoki[2], Yuya Fukano[3] and Naoki Hijii[4]*

[1] Field Science Center, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Japan, [2] School of Agricultural Sciences, Nagoya University, Nagoya, Japan, [3] Graduate School of Agricultural and Life Sciences, University of Tokyo, Nishitokyo, Japan, [4] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

OPEN ACCESS

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***Correspondence:**
Tomohiro Yoshida
yoshitom@cc.tuat.ac.jp

Specialty section:
This article was submitted to
Forest Growth,
a section of the journal
Frontiers in Forests and Global
Change

Received: *26 February 2021*
Accepted: *28 June 2021*
Published: *23 July 2021*

Citation:
Yoshida T, Kusunoki Y, Fukano Y and Hijii N (2021) Vertical Distribution of Arthropod Assemblages and the Effects of Weather Conditions on Arthropod Rain in a Temperate Conifer Forest. Front. For. Glob. Change 4:672601. doi: 10.3389/ffgc.2021.672601

Vertical stratification of forests results in the occurrence of different arthropod assemblages between the vertical layers. Fallen arthropods from the canopy layers (i.e., “arthropod rain”) are additional food sources for predators thriving on the forest floor (FF). However, the abundances of arthropods are strongly affected by weather conditions and vertical stratification. Therefore, in this study, we investigated the vertical distribution of arthropod assemblages and effects of temperature and precipitation on the arthropod rain in a temperate conifer (*Cryptomeria japonica*) forest. Arthropods were collected by water-pan traps and trunk-sticky traps in the upper canopy (UC; 16 m), lower canopy (10 m), and FF (0.5 m). Among the fallen arthropods collected by water-pan traps, wandering detritivores, and herbivores were more abundant ranging from the FF to the UC, whereas the abundance of wandering predators (mainly spiders) was similar in the upper and lower canopies. However, detritivores, herbivores, and predators showed the highest abundance in the UC among the flying arthropods. Wandering arthropods moved upward from the FF toward the tree trunks more frequently than downward, indicating the importance of arthropod immigration from the ground to arboreal habitats. Temperature and precipitation had different effects on fallen and moving arthropods among different taxonomic groups. Flying arthropods were affected only by temperature, while wandering detritivores and herbivores were affected by precipitation and temperature. Thus, the abundance of wandering and flying arthropods differed among the vertical layers of a temperate conifer forest; additionally, arthropod rain was closely associated with weather conditions.

Keywords: canopy, arthropod abundance, trunk-sticky trap, water-pan trap, arthropod rain, weather conditions

INTRODUCTION

Forests exhibit vertical stratification, and the dendritic pattern (i.e., tree trunks and branches) of trees serves as suitable habitat patches for arthropods. Arboreal arthropods occupy habitat patches, based on their different niches, such as flying or wandering within such habitat structures (Davis et al., 1997; Simon and Linsenmair, 2001; Su and Woods, 2001; Tanabe, 2002;

Yoshida and Hijii, 2005b; Oguri et al., 2014); additionally, their population dynamics and community compositions are affected by the vertical stratification of forests (Basset et al., 2003; Nakamura et al., 2017). Wandering arthropods use arboreal habitat patches in the forest vertical layers by moving within the tree trunks (Hanula and Franzreb, 1998) or by aerial dispersal (Karasawa et al., 2005; Lindo, 2010; Bailey et al., 2018). Additionally, flying arthropods exhibit vertical distribution among different strata (Sutton and Hudson, 1980; Simon and Linsenmair, 2001; Tanabe, 2002; Leksono et al., 2005; Maguire et al., 2014).

Fallen arthropods from the canopy layers (i.e., "arthropod rain") are additional food sources for predators thriving on the ground (Pringle and Fox-Dobbs, 2008; Rozanova et al., 2019) and in the streams (Kawaguchi and Nakano, 2001; Kawaguchi et al., 2003; Chan et al., 2008). Arthropod rain varies among different forest vertical layers, forest types (conifer vs. broad-leaved, evergreen vs. deciduous), and seasons. For example, many herbivore arthropods (hereafter referred to as herbivores) fall from the upper canopy (UC), which is characterized by living foliage, while detritivore arthropods (hereafter referred to as detritivores) fall from the lower canopy (LC), which is characterized by dead foliage. Thus, understanding the composition of arthropod rain and its source from the different canopy layers can reveal potential roles of energy inputs from the canopy into soil food webs (Goncharov et al., 2016; Potapov et al., 2016; Rozanova et al., 2019).

Moreover, weather conditions are known to influence arthropod dispersal (Greenstone, 1990; Delattre et al., 2013). For example, precipitation may increase or decrease arthropod activity on the forest floor (FF) (Pedigo, 1970; Lensing et al., 2005). Previous studies have reported that collembolans move upward from the FF to arboreal habitats during precipitation (Bowden et al., 1976; Bauer, 1979; Farrow and Greenslade, 1992). Rainwater also washes out arboreal arthropods, inhabiting the substrates of trees. Additionally, temperature predominately determines the activity and growth of arthropods, which are ectotherms, and affects the aerial dispersal of some arthropods (Johnson, 1969; Richter, 1970; Bishop, 1990). Therefore, weather conditions play an important role in determining the vertical distribution of arthropods and the arthropod rain in the canopy layers of forests.

Understanding the vertical distribution of arboreal arthropods and the factors affecting their movement among the vertical layers helps clarify the mechanisms and dynamics of arthropod assemblages in forests. Although many studies have reported on the vertical distribution of arthropods, the knowledge about the upward and downward movements of arboreal arthropods in the vertical structure of forests is limited. In the present study, we investigated the abundances of wandering and flying arthropods in the vertical layers of a temperate conifer forest using water-pan traps and trunk-sticky traps; additionally, we examined the effects of weather conditions on the arthropod rain in the forest. Surveys were conducted in a Japanese cedar (*Cryptomeria japonica*) plantation plot with much dead foliage in the LC layers (Yoshida and Hijii, 2006b). Detritivores, such as oribatid mites and collembolans, colonize both living and dead foliage of Japanese cedar trees (Yoshida and Hijii, 2005a,b), and most of them move from the FF into the arboreal habitats (Yoshida and Hijii, 2005b, Yoshida and Hijii, 2006a). It is likely that such colonists will be easily washed out by rainwater from the arboreal substrates due to less morphological adaptation to arboreal life unlike truly arboreal species (Orivel et al., 2001; Karasawa and Hijii, 2008). Thus, we tested the following hypotheses in this study: (1) the wandering arthropods are more abundant at the lower traps that collect cumulatively fallen arthropods; (2) the flying arthropods (especially flying herbivores) are more abundant in the UC having much living foliage compared with the LC having much dead foliage; (3) the catch of wandering and flying arthropods, which are ectotherms, is influenced by temperature (i.e., the arthropod dispersal increases with increasing temperature in the temperate forest), while wandering detritivores, which are wingless and move from the FF, constitute a major component of arthropod rain and are strongly affected by precipitation since they are washed from the tree substrates by rainwater.

MATERIALS AND METHODS

Study Sites

The study was conducted in a 36-year-old (as of 2006) Japanese cedar (*C. japonica* D. Don) plantation in the experimental forest plot of Nagoya University in central Japan (35°11′N, 137°33′E; 980 m.a.s.l., **Supplementary Figure 1**). The meteorological equipment near the stand (within 200 m) showed that mean annual precipitation and mean annual air temperature were 2,327 mm and 9.2°C, respectively (1995–2007). The stand density was approximately 2,000 trees ha^{-1}, and the mean tree height of the trees in the stand was approximately 20 m (Yoshida and Hijii, 2006b). Since the tree canopy was closed, less understory vegetation grew on the FF, and thus the proportion of dead leaves and branches was high in the lower canopies (Yoshida and Hijii, 2006b). A 17-m observation tower was built to access the canopy in the stand. The weekly precipitation and mean air temperature observed during the study period are presented in **Figure 1**.

Arthropod Sampling

Water-pan traps and trunk-sticky traps were used for arthropod sampling. Plastic rectangular containers (0.073-m^2 opening and 110-mm depth) were used as water pans to capture canopy

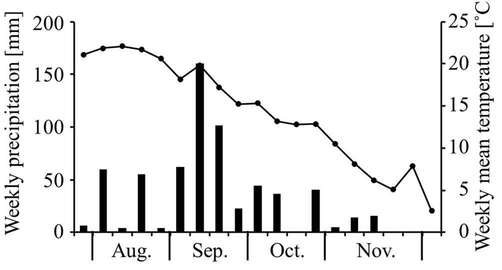

FIGURE 1 | Temporal changes in weekly precipitation (solid bars) and weekly mean air temperature (solid circles) during the study period.

arthropods along the vertical stratification of the stand. We placed five such water-pan traps, filled with 1 L of water and a small amount of detergent for preservation, at ca. 16 (UC), 10 (LC), and 0.5 m above the ground (near FF). The traps in the UCs and LCs were fixed with rubber bands on the edges of steel pipes protruding from the observation tower, and the traps near the FF were fixed to plastic boxes on the ground. We collected weekly arthropod samples through a 0.1-mm mesh sieved from July 25 to December 7, 2006. A total of 285 pan-trap samples [three vertical layers (UC, LC, and FF) × 5 replications × 19 sampling times] were collected.

A fly-catch sticky trap ribbon (Kamoi Kakoshi Co., Okayama, Japan) was used to collect arthropod samples from the tree trunks. The trap consisted of a strip of waterproof paper (270 mm × 50 mm) with adhesive on both sides. Similar to the water-pan traps, the sticky traps were fixed at ca. 10-m height (LC) using the footholds of steel pipes protruding from the observation tower and at 0.5-m height above the ground (near FF). The tree barks were stripped at each of the mentioned heights to provide a smooth surface where two sticky bands were wound, approximately 20 mm apart, around the trunk. This was done to differentiate between the arthropods moving downward from the upper trunk and those moving upward from the lower trunk. The traps were tightly fastened using tacks. We mounted a pair of sticky traps on the trunk at each height on each of the 10 different trees for a week of every month, i.e., August 10–17, September 21–28, October 19–26, and November 16–22, 2006. After the weekly period, we dissolved the adhesive on the sticky traps in kerosene to collect arthropods and segregated them by using a 0.1-mm mesh sieve. A total of 80 sticky-trap samples [2 vertical layers (UC and LC) × 2 band positions (upper and lower) × 5 replications × 4 sampling times] were collected.

Subsequently, we identified the arthropods sampled using the two traps to the order or suborder levels and counted them under a stereomicroscope (35× magnification). Furthermore, the arthropods were divided into four feeding groups (detritivores, predators, herbivores, and others) (following Moran and Southwood, 1982; Hijii, 1989; Stork and Blackburn, 1993). In our study, the detritivores included microbi-detritivores, fungivores, and epiphyte grazers since classification of the arthropods based on the feeding habits to the order and suborder level was difficult. We determined the feeding group of some orders and suborders that have multiple feeding groups based on the dominant family or species groups (e.g., leaf beetles for Coleoptera). Additionally, the arthropods were divided into "flying" and "wandering" groups with/without wings. Dipteran larvae were excluded from the water-pan trap samples since dipteran adults laid eggs in the traps. The density of arthropods collected by water-pan traps was expressed as the number of individuals per unit area per day (individuals m^{-2} day^{-1}), while the density from the trunk-sticky traps was expressed as the number of individuals per unit trap per day (individuals $trap^{-1}$ day^{-1}).

Data Analysis

Generalized linear mixed models (GLMMs) with Poisson's distributions and a log link were used to examine the effects of trap height (layer), temperature, and precipitation on the density of arboreal arthropods. We used the densities of the major taxonomic groups, feeding groups, and dispersal types (flying or wandering) as response variables; additionally, the type of layer, weekly mean temperature, and weekly precipitation were used as explanatory variables; and the replications of traps were treated as random effects. The *post hoc* comparisons between the layers (UC vs. LC, LC vs. FF) in terms of the densities of the major taxonomic groups of water-pan traps were made through estimated marginal means contrasts, using the emmeans package. For the analysis of the sticky trap, we used GLMMs with the density of arboreal arthropods as a response variable and the month of sampling, the layers, and the band position as the explanatory variables. We used likelihood ratio tests to evaluate the significance of the explanatory variables. Statistical analyses were conducted using the lme4 package for R v.3.6.2 (R Development Core Team, 2017).

RESULTS

The abundances of wandering and flying arthropods were 29,407 and 8,153, respectively. Collembola (detritivores) was the most dominant taxonomic group among the wandering arthropods (**Supplementary Table 1**), while winged arthropods belonging to the orders Diptera (detritivores) and Hymenoptera (predators) dominated the flying arthropods (**Supplementary Table 2**). Among the fallen (wandering) arthropods collected by water-pan traps, wandering detritivores and herbivores were more abundant in the LC layers than in the UC layers. The abundance of wandering predators was lower in LC than in UC, although the abundances were not significantly different between the two layers ($p > 0.05$; **Figure 2** and **Table 1**). Wandering spiders, which are the most dominant group among the wandering predators, were significantly more abundant in UC than in LC (**Supplementary Table 3**). Among the flying arthropods, detritivores, and predators had the highest abundance in UC, while the abundances of herbivores did not significantly differ between UC and LC ($p > 0.05$; **Figure 2** and **Table 1**).

The densities of wandering arthropods (7.4–65.4 individuals m^{-2} day^{-1} in the UC, 6.3–258.7 individuals m^{-2} day^{-1} in the LC, and 33.3–753.4 individuals m^{-2} day^{-1} near the FF) and flying arthropods (4.7–216.8 individuals in the UC, 1.6–43.1 individuals in the LC, and 5.1–134.6 individuals near the FF) collected by water-pan traps exhibited a similar temporal trend. The densities of both were maximum in September in the three layers (**Figure 2**). In contrast, among the arthropods collected by trunk-sticky traps, the densities of the wandering predators (0.1–1.9 individuals $trap^{-1}$ day^{-1}), wandering herbivores (0.0–3.8 individuals m^{-2} day^{-1}), and flying arthropods (0.0–9.2 individuals m^{-2} day^{-1}) decreased in September, and the density of wandering detritivores (1.6–37.0 individuals m^{-2} day^{-1}) decreased in November (**Figures 3**, **4**). Additionally, the abundances of both wandering and flying arthropods significantly differed between the LC and FF ($p < 0.05$; **Table 2** and **Figures 3**, **4**). Both wandering and flying arthropods (especially detritivores and herbivores) showed more upward migration (caught on the lower sticky bands) than

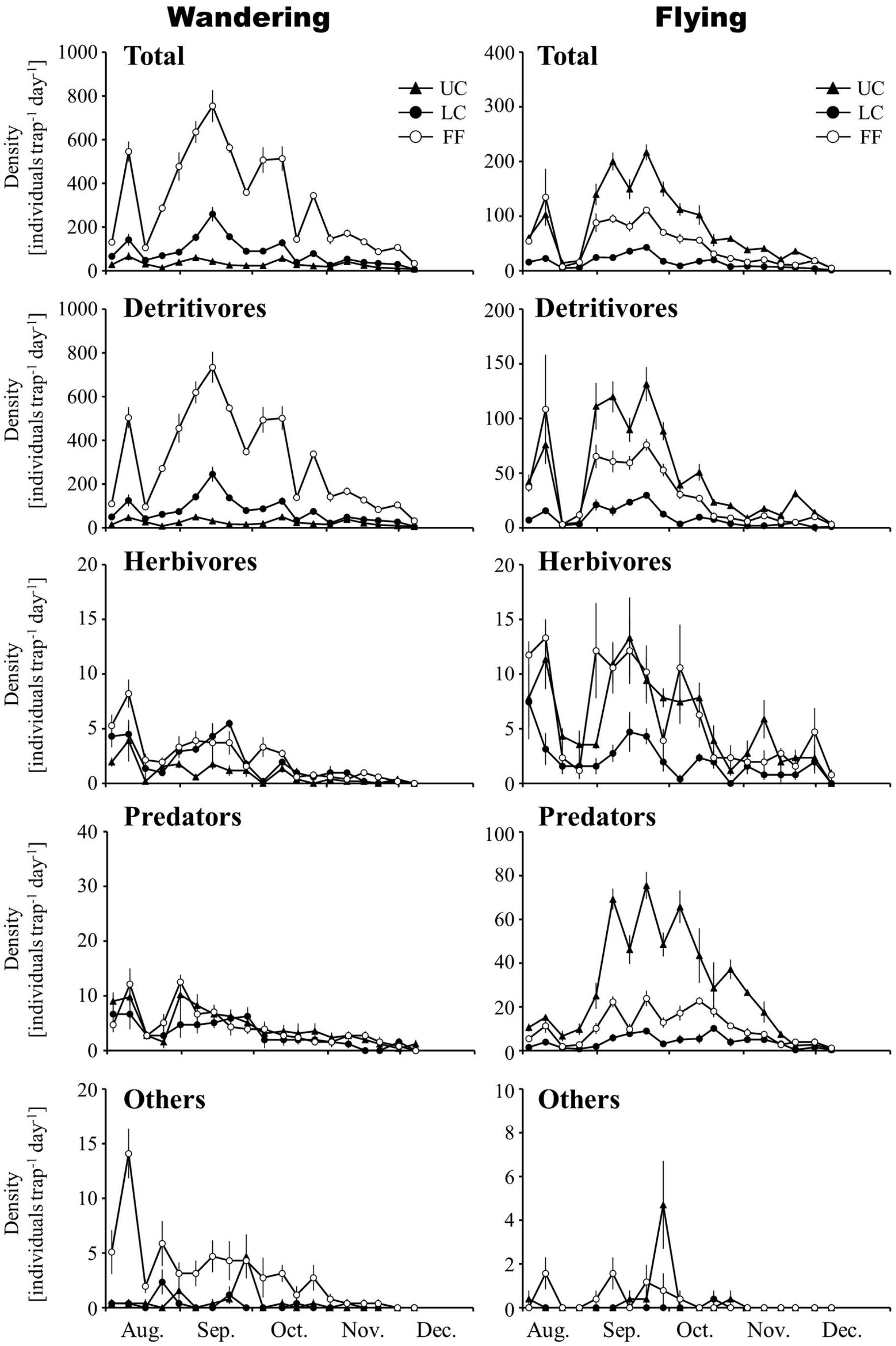

FIGURE 2 | Temporal changes in the density of arthropod groups collected by water-pan traps in the vertical strata of a Japanese cedar (*Cryptomeria japonica*) forest. Values represent mean ± standard error. UC, upper canopy; LC, lower canopy; FF, forest floor.

downward migration (caught on the upper bands) ($p < 0.05$; **Table 2** and **Figures 3**, **4**).

Temperature and precipitation had varying effects among the different taxonomic groups of arthropods collected by water-pan traps. Flying arthropods were affected only by temperature, while wandering detritivores and herbivores were affected by precipitation and temperature (**Table 1**).

DISCUSSION

The results of the water-pan trap method showed that the arthropod rain mainly consisted of wandering detritivores (collembolans and mites) in the studied temperate conifer forest. This was in agreement with the findings of the previous study in temperate forests (Rozanova et al., 2019). Furthermore,

TABLE 1 | Summary results of generalized linear mixed models (GLMMs) that incorporated the effects of trap height (Layer), different layers, temperature (Temp), and precipitation (Rain) on the abundance of feeding groups collected by water-pan traps (a total of 285 samples) in a Japanese cedar (*Cryptomeria japonica*) forest.

	Layer		FF vs. LC	LC vs. UC	Temp		Rain	
	Relationship	Deviance	t.ratio	t.ratio	Coef.	Deviance	Coef.	Deviance
Total arthropods (W)	FF = LC > UC	1,861.346***	0.952 n.s	5.762***	0.060	3.956*	0.009	4.207*
Detritivores (W)	FF = LC > UC	3,777.956***	0.958 n.s	5.761***	0.047	5.557*	0.012	19.113***
Predators (W)	FF = LC = UC	5.633*	0.1436 n.s	−0.760 n.s.	0.104	45.581***	0.003	2.3831 n.s.
Herbivores (W)	FF = LC > UC	15.072***	−1.571 n.s.	3.039**	0.143	45.855***	0.005	3.995*
Others (W)	FF = LC > UC	37.276***	0.184 n.s.	3.735***	0.161	32.286***	0.004	1.642 n.s.
Total arthropods (F)	FF > LC < UC	11,612.623***	5.045***	−4.463***	0.055	8.380**	0.010	13.113***
Detritivores (F)	FF > LC < UC	320.770***	4.428***	−3.391**	0.093	8.833**	0.004	0.787 n.s.
Predators (F)	FF > LC < UC	255.102***	4.185***	−5.192***	0.074	4.584*	0.003	0.504 n.s.
Herbivores (F)	FF > LC = UC	27.452***	3.632***	−1.227 n.s.	0.082	14.564***	0.004	2.0778 n.s.
Others (F)	FF = LC = UC	1.173 n.s.	0.442 n.s.	−0.743 n.s.	0.159	2.324 n.s.	0.009	0.6765 n.s.

W, wandering (wingless); F, flying (winged); t.ratio, test statistic used to calculate the p-value with Bonferroni correction; Coef., estimated coefficient of each explanatory variable in the GLMM model; Deviance, log-likelihood ratio statistics for each explanatory variable in the GLMM model; UC, upper canopy; LC, lower canopy; FF, forest floor. *$p < 0.05$; **$p < 0.001$; ***textit$p < 0.0001$; n.s., $p > 0.05$.

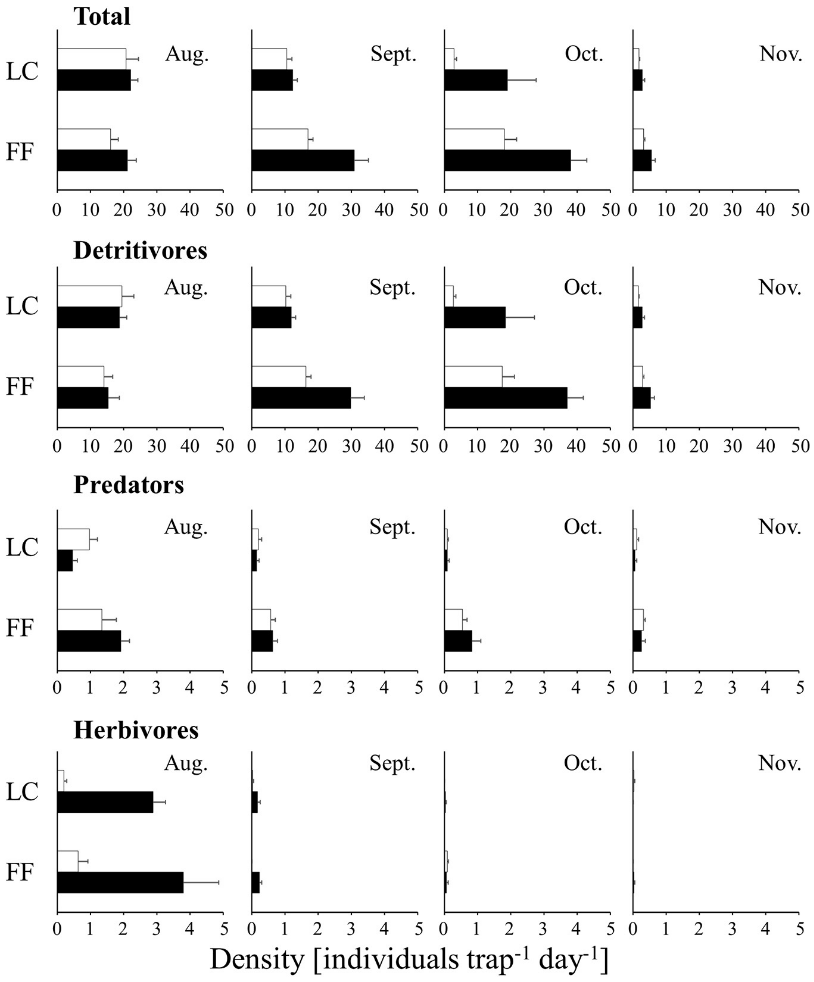

FIGURE 3 | Temporal changes in the density of wandering arthropods collected by trunk-sticky traps in the vertical strata of a Japanese cedar (*Cryptomeria japonica*) forest. The black and white bars indicate upper and lower sticky bands, respectively. Values represent mean ± standard error. LC, lower canopy; FF, forest floor.

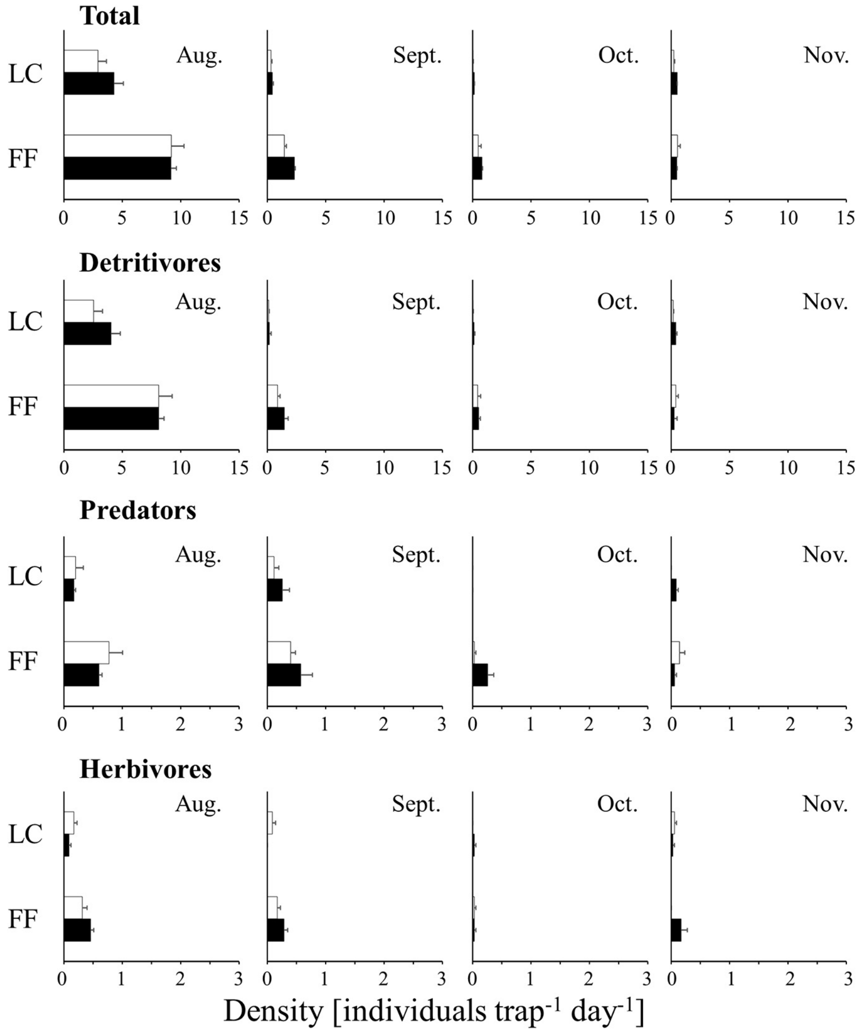

FIGURE 4 | Temporal changes in the density of flying arthropods collected by trunk-sticky traps in the vertical strata of a Japanese cedar (*Cryptomeria japonica*) forest. The black and white bars indicate upper and lower sticky bands, respectively. Values represent mean ± standard error. LC, lower canopy; FF, forest floor.

wandering detritivores and herbivores were more abundant in the lower two layers, thus proving that our first hypothesis, which stated that the abundance of wandering arthropods is more at the lower traps that collects fallen arthropods, is true. However, the density of wandering predators (spiders) was lower in the LC layer than the UC layer. A previous study, which used foliage beating method in the same stand, showed that spider densities at foliage level did not differ between the two layers (Oguri et al., 2014). The variability in the results could be attributed to the dispersal activity (ballooning) because spiders living in the higher strata of vegetation and depending on web-building to catch their prey are suggested to have more opportunities to ballooning dispersal due to more frequent silk production (Bonte et al., 2003).

Flying detritivores (winged arthropods belonging to the order Diptera) and predators (winged arthropods belonging to the order Hymenoptera) were more abundant in the UC than the other two layers, while the abundance of flying herbivores did not significantly differ between UC and LC. Therefore, the results of flying detritivores and predators except for the flying herbivores support the second hypothesis that flying arthropods would be more abundant in UC than in the LC. A similar tendency was observed in the previous studies, which proved that dipterans and hymenopterans exhibited higher activity in the upper canopies compared with the lower canopies in tropical and temperate forests (Sutton and Hudson, 1980; Simon and Linsenmair, 2001; Maguire et al., 2014). For example, some dipteran and hymenopteran species mate and swarm near tree crowns (Morgan, 1968; Downes, 1969). The reason dipteran adults were less abundant in the LC with much dead foliage is unknown. However, a foliage washing survey in the same stand found that dipteran larvae were more abundant in the dead foliage of the conifer canopies (Yoshida and Hijii, 2005a). This suggests that flying dipterans change the habitat use among their developmental stage vertically.

TABLE 2 | Summary of the results of the generalized linear mixed models (GLMMs) that incorporated the effects of trap height (Layer), trap parts (Part), and seasons (Season) on the abundance of feeding groups collected by trunk-sticky traps (a total of 80 samples) in a Japanese cedar (*Cryptomeria japonica*) forest.

	Layer		Part		Season
	Relationship	**Deviance**	**Relationship**	**Deviance**	**Deviance**
Total arthropods (W)	FF > LC	471.47**	Lower > upper	537.58**	1,363.33**
Detritivores (W)	FF > LC	419.92**	Lower > upper	453.82**	1,254.98**
Predators (W)	FF > LC	68.641**		0.216 n.s.	127.67**
Herbivores (W)	FF > LC	9.4068**	Lower > upper	120.9439**	237.988**
Others (W)	FF < LC	4.2791**	Lower > upper	12.7482**	17.2961**
Total arthropods (F)	FF > LC	234.8818**	Lower > upper	9.2554*	1,042**
Detritivores (F)	FF > LC	185.1574**	Lower > upper	7.0834*	949.826**
Predators (F)	FF > LC	33.8999**		1.1247 n.s.	50.4048**
Herbivores (F)	FF > LC	16.3666**		1.2016 n.s.	28.2264 **
Others (F)	–	–	–	–	–

Others (F) had no individuals.

W, wandering (wingless); F, flying (winged); Deviance, log-likelihood ratio statistics for each explanatory variable in the GLMM model; LC, lower canopy; FF, forest floor.

**$p < 0.05$; **$p < 0.001$; n.s., $p > 0.05$.*

Conversely, fewer herbivores were collected by water-pan traps in UC than expected despite the presence of large quantities of living foliage. However, previous studies that used chemical knockdown and foliage clipping methods reported that many herbivores belonging to orders Hemiptera and Thysanoptera were observed in the canopy of Japanese cedar forests (Hijii, 1989; Hijii et al., 2001). High abundance of predators in UC was due to the presence of sufficient herbivores (preys). Thus, the low abundance of flying herbivores observed in UC in this study would be either because they are majorly sessile (e.g., Coccoidea) or because they were dispersed on a smaller scale within the living foliage of a single layer, rather than between the layers.

Furthermore, our findings indicated that the number of wandering arthropods moving upward was more than that moving downward toward the FF, indicating the importance of immigration by arthropods from the FF to arboreal habitats. Some species of wandering arthropods are known to lay eggs on the FF and then move into trees during the larval and adult stages (Yoshida and Hijii, 2005b, Yoshida and Hijii, 2006a, 2014 for Collembola). In addition to this migration behavior, dispersal for colonizing new habitats might be another factor driving upward movement toward trees. For example, Lindo and Winchester (2007) reported that many ground-dwelling arthropods expand their habitat ranges to several meters above ground level on tree trunks. This immigration tendency of wandering arthropods and flying arthropods from the FF to arboreal habitats might increase the similarity of arthropod assemblages in the arboreal and forest-floor strata. Additionally, this tendency may sustain the arboreal food-web structure through the presence of ground-dwelling predators (e.g., spiders and harvestmen) and prey (e.g., collembolans) (Miyashita et al., 2003; Haraguchi et al., 2013).

Arthropod rain (falling of wandering detritivores and herbivores) was closely associated with precipitation and temperature fluctuations. The findings supported our third hypothesis that the wandering herbivores and detritivores are influenced by precipitation, whereas flying arthropods are influenced only by temperature. Additionally, the ballooning of wandering spiders is associated with air temperature (Richter, 1970; Bishop, 1990). Bishop (1990) indicated that spider ballooning occurred after a decline in air temperature after the warming of the ground, which provides upward air currents. Moreover, wandering arthropods, such as Acari and Collembola, can be washed out of the tree substrates by rainwater. After precipitation, however, tree trunks are moist, which serve as suitable habitats for arthropods, resulting in their consequent upward movement to the arboreal habitats (Bowden et al., 1976; Bauer, 1979; Farrow and Greenslade, 1992). Subsequently, increased tree climbing by arthropods would increase the dispersal of arthropods by wind currents or would promote their falling from the arboreal habitats, thus leading to arthropod rain. In our study, the dispersal patterns of majority of the arthropod groups were observed to be influenced by temperature. Furthermore, temperature had a significant effect on detritivores, herbivores, and predators, indicating that predators responded directly to temperature and indirectly to an increased abundance of potential prey.

CONCLUSION

In conclusion, the abundances of fallen and moving arthropods differed among the vertical layers of a temperate conifer forest, and the arthropod rain was closely associated with weather conditions. The results that showed the upward and downward movements of arboreal arthropods among the vertical layers reveal the spillover effects of arboreal arthropods in the vertical structure of forests. Several studies have suggested that an arboreal-prey subsidy by abiotic physical conveyor (gravity) (i.e., arthropod rain) has important functional roles in trophic dynamics of the recipient system (ground surface) in forests (Pringle and Fox-Dobbs, 2008; Goncharov et al., 2016; Potapov et al., 2016; Rozanova et al., 2019). Thus, the findings of the study highlight the dependence of falling-prey subsidies into FF on the weather conditions and the importance of arthropod immigration from the ground to arboreal habitats. The arthropod movement among the vertical layers will be significantly affected by climate change. Climate warming leads to increased abundances of forest arthropods (Logan et al., 2003; Robinet and Roques, 2010). However, in the case that it causes drier conditions (i.e., low and infrequent precipitation), it should decrease the upward movement of wandering detritivores from the FF to the arboreal habitats, thus resulting in a decrease in arthropod rain within the forests.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

TY and NH formed and designed the field survey and wrote the manuscript. TY and YK performed the field survey. YF analyzed the data. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Japanese Society for the Promotion of Science under Grants 16K07775, 18H02223, and 19K06121.

ACKNOWLEDGMENTS

We thank Y. Imaizumi, N. Yamaguchi, and N. Takabe for providing assistance with the fieldwork.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.672601/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Forests and Global Change

MINI REVIEW
published: 12 August 2021
doi: 10.3389/ffgc.2021.710854

Climate Change Alters Temperate Forest Canopies and Indirectly Reshapes Arthropod Communities

Aurélien Sallé[1]*, Jérémy Cours[2], Elodie Le Souchu[1], Carlos Lopez-Vaamonde[3], Sylvain Pincebourde[4] and Christophe Bouget[2]

[1] Laboratoire de Biologie des Ligneux et des Grandes Cultures, INRAE, Université d'Orléans, Orléans, France, [2] INRAE, UR EFNO, Domaine des Barres, Nogent-sur-Vernisson, France, [3] INRAE, URZF, Orléans, France, [4] Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS – Université de Tours, Tours, France

Global change challenges the adaptive potential of forests. Large-scale alterations of forest canopies have been reported across Europe, and further modifications are expected in response to the predicted changes in drought and windstorm regimes. Since forest canopies are dynamic interfaces between atmosphere and land surface, communities of canopy-dwelling insects are at the forefront of major changes in response to both direct and indirect effects of climate change. First, we briefly introduce the factors shaping arthropod communities in the canopy of temperate forests. Second, we cover the significant impacts of a forest decline on canopy structure and functioning, and more specifically its contrasted effects on insect microhabitats, trophic resources and forest microclimates. Deleterious effects may be expected for several guilds of leaf-dwelling insects. Nonetheless, a forest decline could also lead to transient or long-lasting resource pulses for other canopy-dwelling guilds, especially saproxylic species depending on deadwood substrates and tree-related microhabitats. The novel microclimates may also become more favorable for some particular groups of insects. We pinpoint current knowledge gaps and the technological locks that should be undone to improve our understanding of the canopy biotope and biodiversity in temperate forests. We highlight the need for integrative approaches to reveal the mechanisms at play. We call for cross-scale studies and long-term collaborative research efforts, involving different disciplines such as community and disturbance ecology, plant and insect ecophysiology, and thermal ecology, to better anticipate ongoing functional and conservation issues in temperate forest ecosystems.

Keywords: biodiversity, forest decline, drought, insect, biological conservation

Edited by:
Akihiro Nakamura,
Xishuangbanna Tropical Botanical Garden (CAS), China

Reviewed by:
Carl Wardhaugh,
New Zealand Forest Research Institute Limited (Scion), New Zealand
Estefania Mico,
University of Alicante, Spain

****Correspondence:***
Aurélien Sallé
aurelien.salle@univ-orleans.fr

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *17 May 2021*
Accepted: *21 July 2021*
Published: *12 August 2021*

Citation:
Sallé A, Cours J, Le Souchu E, Lopez-Vaamonde C, Pincebourde S and Bouget C (2021) Climate Change Alters Temperate Forest Canopies and Indirectly Reshapes Arthropod Communities. Front. For. Glob. Change 4:710854. doi: 10.3389/ffgc.2021.710854

INTRODUCTION

Forest ecosystems worldwide are currently facing increasing levels of environmental stress, posing severe challenges to their adaptive capacity (Allen et al., 2010; Seidl et al., 2017). Biological invasions (Liebhold et al., 2017), land-use intensification (Seibold et al., 2019), and climate change (Seidl et al., 2017) lead to extended forest diebacks and declines worldwide (Allen et al., 2010). Drought and windstorms have been identified as major drivers of recent forest diebacks and declines in temperate Europe, either through their direct impacts on tree health and survival or by promoting large-scale outbreaks of opportunistic species (Carnicer et al., 2011; Sallé et al., 2014; Seidl et al., 2014, 2017; Biedermann et al., 2019; Senf et al., 2020). The frequency, intensity and spatial extent of both drought and windstorm have been increasing in Europe during the last decades, with a recent acceleration (Gregow et al., 2017; Senf et al., 2020). Damage from these disturbances are predicted to increase even further in temperate areas (Seidl et al., 2014; Samaniego et al., 2018; Spinoni et al., 2018). Consequently, temperate forest ecosystems are expected to undergo major changes in terms of structure and functioning in the near future (Senf et al., 2020).

Depending on disturbance regime, resistance, and resilience level of tree species and/or forest ecosystems, and interactions with other disturbances, the structure and functioning of trees, stands, and forests can be affected to varying degrees of severity (Brodribb et al., 2020). One of the fastest and most conspicuous modifications during forest declines and diebacks is the degradation of tree crown condition (**Figure 1**; Ishii et al., 2004). Droughts and windstorms can directly affect canopy structure by inducing leaf and twig shedding, and provoking branch and stem breakage (Bréda et al., 2006; Mitchell, 2013). They can also have long-lasting effects when they act as inciting factors of forest declines (Manion, 1981). Several long-term forest monitoring studies already highlight large-scale increase in defoliation in the Mediterranean area during the last decades (Carnicer et al., 2011; Senf et al., 2020), and unprecedented modifications of forest canopies will likely occur in the short-term in temperate Europe.

The canopy layer of temperate forests is a treasure trove of diverse plant resources, accumulating large amounts of foliage, wood, floral parts, fruits, cones, and seeds (**Figure 2**). At the interface with atmosphere, it is also the siege of peculiar microclimatic conditions (Leuzinger and Körner, 2007). In addition, the canopy conceals diverse but understudied arthropod communities (Floren and Schmidl, 2008; Ulyshen, 2011). Consequently, decline-driven extended change in canopy structure should dramatically alter the communities of canopy-dwelling arthropods and significantly affect forest biodiversity.

In this essay, we focus on the relationship between the climate-change-induced physical alterations of the canopy and arthropod communities. We briefly review (i) the factors affecting the composition and distribution of canopy-dwelling arthropods, and (ii) the consequences of forest decline on canopy-related microclimates and resources and how it affects arthropod biodiversity. We bring to the attention that a thorough understanding of the physical structure of altered canopies is needed to infer how climate change will reshape arthropod communities. Our aim is to highlight the gaps in our knowledge on the functional response of temperate forest ecosystems to global environmental change, and to stimulate cross-scale studies involving various disciplines to ameliorate predictions that should feed management plans.

FACTORS SHAPING ARTHROPOD COMMUNITIES IN THE CANOPY OF TEMPERATE FORESTS

The large amount of plant biomass in the canopy layer can support abundant arthropod populations and promote species richness according to the more-individuals hypothesis (i.e., higher energy availability promotes higher number of individuals, and higher species richness; Müller et al., 2018), or the resource-availability hypothesis (i.e., arthropod distribution reflects the availability of their resources; Wardhaugh, 2014). This large and diverse biomass may also enhance habitat heterogeneity, provide multiple trophic niches and allow the segregation of arthropod species according to the habitat heterogeneity hypothesis (i.e., species richness increases with increasing habitat heterogeneity due to greater niche dimensionality; Tanabe, 2002 but see Müller et al., 2018). The complex three-dimensional structure of tree crowns also provides ecological space of reduced predation for arthropods, known as an escape or enemy-free space (Lawton, 1983; Wardhaugh, 2014). In addition, the canopy provides a unique set of microhabitats and resources, with specific tree-related microhabitats such as mistletoe, suspended soils, epiphytes, perched dead branches, upper trunk cavities, and fruiting bodies of opportunistic fungi (Ulyshen, 2011; Larrieu et al., 2018), allowing the differentiation of dedicated habitat and/or trophic guilds of arthropods. Ultimately, this results in the establishment of complex food webs (Nakamura et al., 2017).

The temperate canopy layer is characterized by particular microclimates. For instance, the leaf surface of trees can be much warmer than local air temperature at the top of the canopy because they intercept a large amount of incoming radiation (Woods et al., 2018; Miller et al., 2021). Leaf temperature depends on the ecophysiological traits of the plant (Pincebourde and Woods, 2012), and is consequently species specific (Leuzinger and Körner, 2007). Canopy microclimates diverge largely from those of the understory. The shape and range of the vertical air temperature profile of temperate canopies depend on several variables including season, time of the day, canopy architecture, and tree species (De Frenne et al., 2021). This gradient contributes to the vertical distribution of arthropod species in the canopy, according to the physiological-efficiency hypothesis (i.e., arthropod distribution reflects their physiological tolerance to abiotic and biotic conditions, Wardhaugh, 2014). Consequently, resource availability, microhabitat richness, and abiotic conditions can be considered as critical factors affecting the abundance of arboreal arthropods (Mottl et al., 2020).

Forest stands also produce a microclimate in the understory that buffers against the atmospheric extremes for temperature and dryness (**Figure 2**). This buffer effect is present in all forests across latitudes and is relatively independent from tree species (De Frenne et al., 2019), suggesting that this biophysical effect relies almost entirely upon the canopy surface that absorbs or reflects incoming solar radiation. Thus, the amplitude of the buffering effect in the understory is lower when the forest canopy foliage is less dense (Zellweger et al., 2020).

Several studies underlined the vertical stratification of arthropod communities in temperate forests in response to the vertical gradient of resources, microhabitats, and microclimates (e.g., Tanabe, 2002; Bouget et al., 2011; Vodka and Cizek, 2013; Normann et al., 2016; Plewa et al., 2017; Seibold et al., 2018b; Weiss et al., 2019; Urban-Mead et al., 2021). This stratification applies to a diverse range of arthropod taxa and trophic or functional guilds (e.g., saproxylic species, leaf chewers, pollinators, and parasitoids), which results in particular species

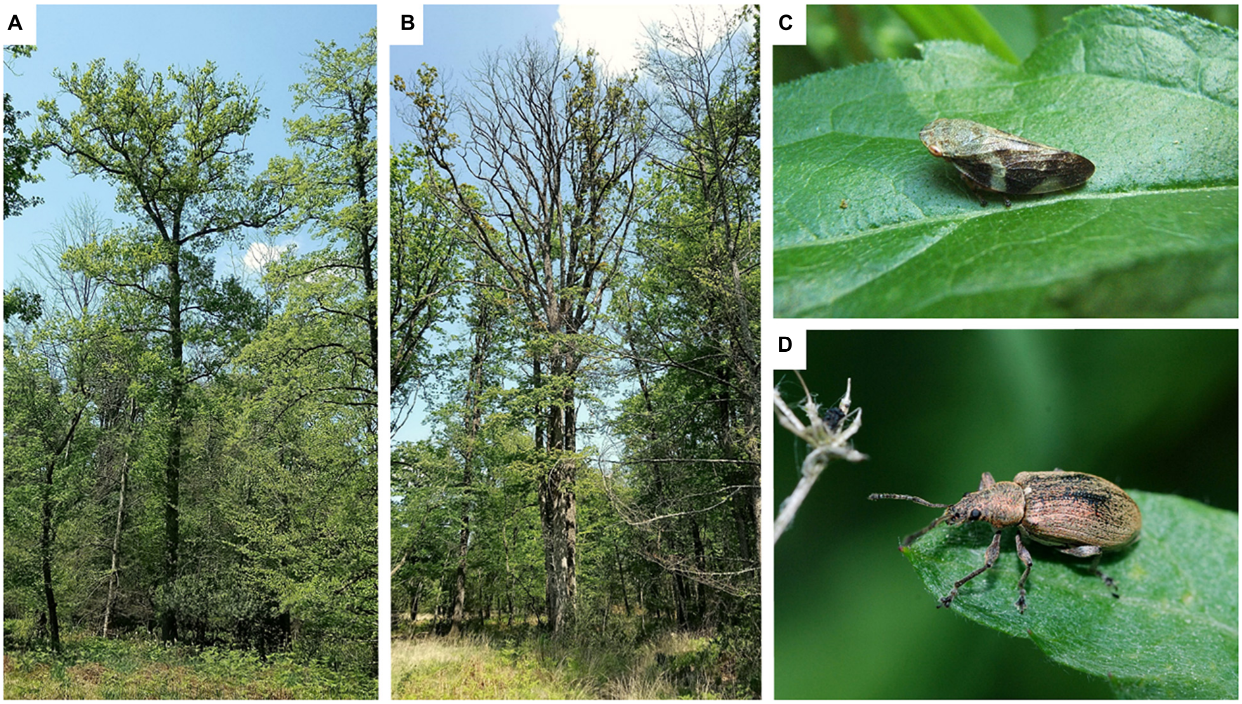

FIGURE 1 | Oaks (*Quercus petraea*) exhibiting moderate **(A)** and pronounced **(B)** symptoms of decline with a reduced foliage density and an accumulation of dead branches. Oak decline will have contrasted effects on canopy-dwelling insects: negative for the sap-feeding *Aphrophora alni* **(C)**, but positive for generalist leaf-feeder *Phyllobius pyri* **(D)**. Photo credits: Aurélien Sallé **(A,B)** and Sébastien Damoiseau **(C,D)**.

assemblages and community structure in the canopy layer (Maguire et al., 2014; Weiss et al., 2016; Šigut et al., 2018; Urban-Mead et al., 2021). Stratification patterns can markedly differ between temperate and tropical forests, but they share similar community structures (Weiss et al., 2019). Temperate forests probably shelter less insect taxa specific to the canopy than their tropical counterparts (Floren and Schmidl, 2008; Ulyshen, 2011), but up to 20–40% of saproxylic beetle species can be considered as exclusive to the canopy (Bouget et al., 2011).

In addition to canopy specialists, other species occasionally or temporarily use canopy microhabitats, as a hunting ground or to find mates for instance, and vertical migrations take place from a stratum to another, depending on the season, the stage of the life cycle, or even the time of the day (Ulyshen, 2011). As an example, several recent studies reported that even common species of scorpionflies (Mecoptera) and earwigs (Dermaptera) were quite abundant in the canopy layer while they were previously rather associated with lower forest strata (Vincent et al., 2020; Martínez-Pérez et al., 2021). Similarly, canopy top can be used by aquatic insects during the imago stage, suggesting cross-ecosystem connectivity involving the canopy layer (Le Naour et al., 2019). This underlines the fact that we still do not accurately evaluate the reliance of the overall forest arthropod community on canopy-related resources and processes.

We still have a limited knowledge of the taxonomic and functional diversity of arthropod communities dwelling in the canopy of temperate forests. Many studies have focused on saproxylic beetle communities, as key components of forest ecosystems (e.g., Bouget et al., 2011; Vodka and Cizek, 2013; Plewa et al., 2017; Seibold et al., 2018b). Other taxonomic groups and/or functional guilds have received comparatively little attention (but see Tanabe, 2002; Leksono et al., 2005; Larrivée and Buddle, 2009; Sobek et al., 2009; Maguire et al., 2014; Sallé et al., 2020; Vincent et al., 2020; Urban-Mead et al., 2021). The canopy of temperate forests may then shelter an underestimated pool of patrimonial species (Plewa et al., 2017; Tillier et al., 2020), and can be considered as a biotic frontier deserving special attention (Bouget et al., 2011).

DECLINE-DRIVEN MODIFICATIONS IN CANOPY HABITATS AND CASCADING EFFECTS ON ARTHROPOD COMMUNITIES

Forest declines and diebacks affect key microhabitats or trophic resources for arthropod communities, through reduced foliage density and flower or seed production (**Figures 1**, **2**; Ishii et al., 2004). For routine surveys of forest health, trees are considered in decline when more than half of their ramification is missing and/or more than half of their main branches are dead in the functional part of their canopy (**Figure 1**; Goudet and Nageleisen, 2019). The dynamic process of crown degradation implies the dieback of branches and twigs, progressing not only vertically from upper to lower levels but also horizontally from outer to

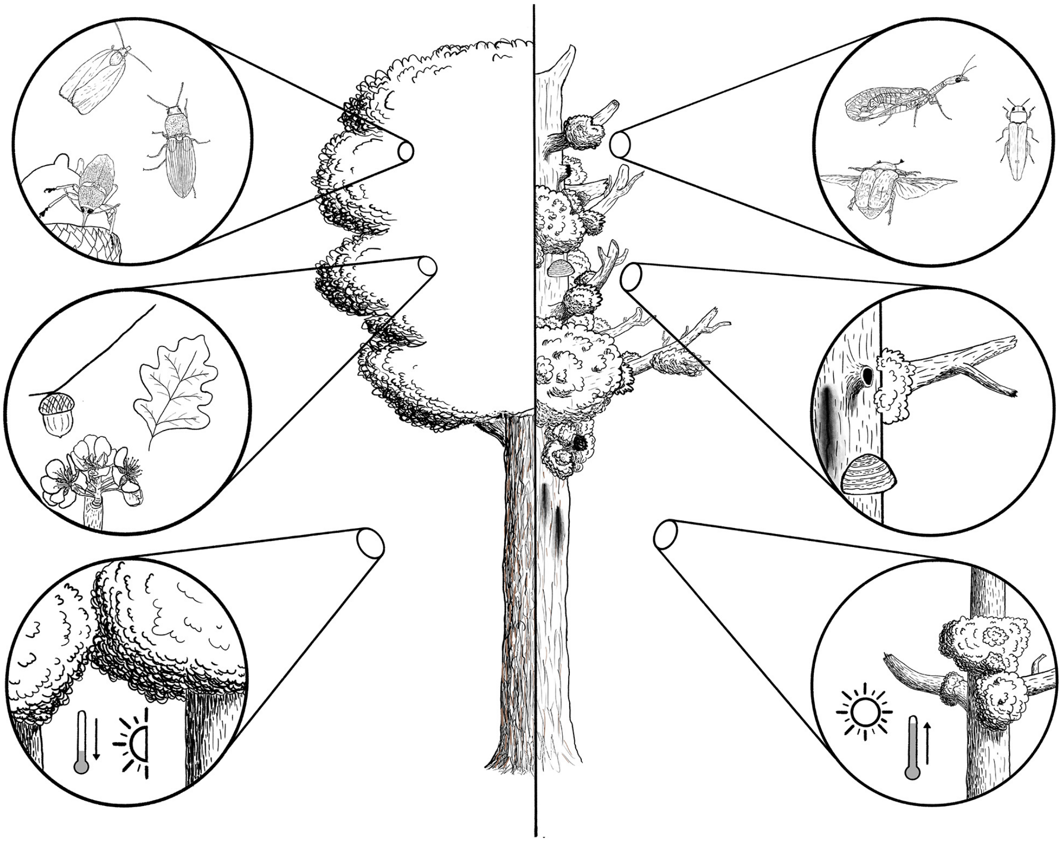

FIGURE 2 | Overview of decline-induced changes in arthropod communities (upper insets), resources and habitats (medium insets), and microclimates (lower insets) in forest canopies. Declining tree crowns (left part) should affect leaf-dwelling species but promote saproxylic species by reducing foliage, flower, and seed amounts but accumulating tree-related microhabitats like perched dead branches, fermented sap exudates, cavities, and fruiting bodies of pathogenic fungi. Reduced canopy cover will also reduce the thermal buffer on lower forest strata but increase the luminosity. Credits: Jérémy Cours.

inner parts of the tree crown (Drénou et al., 2015; **Figures 1**, **2**). This should affect the structure and composition of arthropod communities since experimental manipulations of branch density and structure can affect the abundance and trophic webs of arboreal arthropods (Halaj et al., 2000). Foliage quality, including biochemical and morphological leaf traits, can be also affected by the loss of tree vigor (Günthardt-Goerg et al., 2013; Hu et al., 2013). Likewise, the greater exposure of leaves in opened canopies can alter their phytochemical profile, and promote the accumulation of defensive metabolites (Yamasaki and Kikuzawa, 2003; Lämke and Unsicker, 2018).

Declines and diebacks can generate novel structures and favor the build-up of features uncommon on healthy trees. They promote tree-related microhabitats associated with reduced tree vigor or decaying trees (e.g., cavities initiated by break-offs of scaffold dead branches, perched dead branches and ascending dead branches emerging from the canopy, stress-induced broom-like mass of twigs, fruiting bodies of opportunistic fungi…) (**Figures 1**, **2**; Ojeda et al., 2007; Larrieu et al., 2018; Cours et al., 2021). Consequently, because forest declines and diebacks promote the accumulation of such biological legacies (Cours et al., 2021), they contribute to increase the structural complexity at multiple scales. The magnitude and sustainability of such changes will depend on disturbance regime on the one hand, and on stand characteristics on the other hand (e.g., Brodribb et al., 2020). In particular, tree characteristics such as resistance and tolerance to disturbance, propensity to accumulate microhabitats and decay rate of woody tissues should strongly influence the resources and habitat availability during the decline process. Foliage loss and accumulation of dead branches are frequently quantified during declines and diebacks as crown condition is generally used as proxy for tree health status (e.g., Goudet and Nageleisen, 2019). However, accurate description and quantification of tree-related microhabitats and foliage quality evolution during the entire process of tree decline is still missing.

The reduction of canopy cover negatively impacts the thermal buffering effect of the understory (**Figure 2**; Zellweger et al., 2020). Therefore, declines and diebacks would attenuate and even annihilate this buffer effect in the understory but we currently lack surveys to establish this link directly (Thom et al., 2020). In addition, the degradation of tree crown condition is expected to disturb considerably the vertical temperature profile from the ground to the upper canopy layer. The air temperature layers across the vertical gradient of forest canopies reflect the microclimate experienced by relatively large species. Nonetheless, the vast majority of arthropod species has a body size small enough to be influenced primarily by the microclimatic conditions at the plant surface where they live (Pincebourde and Woods, 2020; Pincebourde et al., 2021), and the influence of tree health status on leaf and bark microclimates is unknown.

The profound structural modifications affect habitat opportunities and trophic resources from micro- to macro-scales, with potentially marked cascading effects on communities of canopy-dwelling arthropods. Few studies have directly investigated how dieback or decline-induced change in canopy resources and microclimates can affect the diversity or functional structure of canopy-dwelling arthropod communities. Changes in foliage abundance and quality can markedly influence leaf-dwelling species, with contrasted effects according to their feeding guild. For instance, the abundance of sap-feeding Hemiptera and leaf-mining caterpillars or weevils can be negatively affected by degraded crown conditions (**Figure 1**; Martel and Mauffette, 1997; Stone et al., 2010; Sallé et al., 2020). However, studies conducted in different declining forests also showed that leaf-chewing beetles, like phyllophagous weevils, could be either promoted (in oak stands; Sallé et al., 2020) or unaffected by degraded crown conditions (in pinyon pine stands; Stone et al., 2010). Likewise leaf-chewing caterpillars were slightly promoted by maple decline (Martel and Mauffette, 1997). The accumulation of biological legacies, like perched deadwood or cavities, generally promotes saproxylic organisms following major disturbances (Beudert et al., 2015; Cours et al., 2021; Kozák et al., 2021). This stands true for canopy-dwelling arthropods as the abundance and species richness of saproxylic species is generally enhanced in the canopy of declining stands (**Figure 1**; Stone et al., 2010; Sallé et al., 2020; Vincent et al., 2020). These changes in sap-feeding, phyllophagous, saprophagous, and xylophagous species have in turn cascading effects on higher trophic levels and affect the community structure within the canopy (Trotter et al., 2008; Stone et al., 2010; Vincent et al., 2020).

The alteration of canopies can also alter their associated arthropod community through cascading effects on lower vegetation strata. The degree of canopy openness markedly influences the epiphyte communities of temperate forests (Nascimbene et al., 2013), and reduced epiphyte abundance have cascading effects on arboreal arthropod communities (e.g., Miller et al., 2007). Likewise, increased canopy openness markedly changes the understory and herbaceous layers (e.g., Dietz et al., 2020). This promotes floral resources and in turn affects some guilds of canopy-dwelling species, relying on below-canopy resources, such as pollinators (Davis et al., 2020; Mathis et al., 2021; but see Urban-Mead et al., 2021).

Declines and diebacks can consequently promote some particular microhabitat-, trophic-, or functional guilds of canopy-dwelling insects. In this regard, arthropod guilds can be arranged as "winners or losers," or "victims or perpetrators" of forest declines and diebacks (Sallé and Bouget, 2020; Sire et al., 2021). Depending on disturbance severity and stand characteristics, these biodiversity pulses could be either transient or long-lasting. Nevertheless, whether and how time scale of the decline process has consequences on decline-driven dynamics of canopy-dwelling communities is still poorly known – and we propose that the difficulty to monitor upper levels of canopy forests explains this gap. This will ultimately result in both biological conservation issues and opportunities that should be urgently assessed before forest declines and diebacks further increase in Europe and other parts of the world.

THE NEED FOR MULTIDISCIPLINARY INTEGRATIVE APPROACHES COMBINING CUTTING-EDGE TOOLS

Monitoring canopy microhabitats and biodiversity is challenging (Nakamura et al., 2017), but monitoring the canopy of declining trees adds further challenges related to safety issues (e.g., fall of tree limbs) compromising climbing. Their crowns are degrading more or less rapidly jeopardizing the installation of permanent monitoring structures and equipment.

To circumvent these issues, either ground-based or aerial surveys should be selected for canopy access. New processes combining drone imagery, airborne LiDAR, deep learning and modeling should be developed to better assess habitat diversity and abundance in the canopy (Müller et al., 2018; Frey et al., 2020; Santopuoli et al., 2020), and microclimates (Duffy et al., 2021). This would also allow to investigate the outcomes of crown diebacks at larger spatial scales, i.e., from tree to the landscape level, which could be more relevant for stakeholders and conservation managers, but also for the study of local biodiversity (Jackson and Fahrig, 2015; Percel et al., 2019). These approaches could be combined with high throughput metabarcoding that allows to characterize arthropod communities over large spatial scales (e.g., Cai et al., 2021; Sire et al., 2021).

We also need to scale-down the investigations at the plant surfaces and organ scale. Since microclimatic conditions are the most relevant for small-bodied organisms like arthropods (Pincebourde and Woods, 2020; Pincebourde et al., 2021), high resolution surveys of microclimate at plant surfaces (leaves and wood) and within plant matrices (e.g., leaves, wood, and galls) are required to better assess the outcomes of changing microclimates across canopy on forest arthropod communities.

Novel sampling and biomonitoring techniques would help to widen the range of arthropod taxa, and guilds, studied and improve their monitoring. This could be achieved by implementing long-lasting and self-contained ecoacoustic or visual monitoring techniques for canopy-dwelling communities (e.g., Rappaport et al., 2020). Approaches based on next generation sequencing and machine-learning could also be used to infer ecological networks and measure the effect of decline of tree canopies on species interactions (Bohan et al., 2017). Developing environmental DNA surveys in canopy-related microhabitats or matrices like cavities, suspended soils, or perched dead wood would also improve our knowledge of arthropod communities in the canopy.

We call for integrated whole-tree approaches, from forest floor to canopy top and from microhabitats to landscapes, to better understand the functional and biodiversity outcomes of canopy degradation on the whole forest ecosystem. This also claims for more integrated studies linking the physiological status of trees to the physical habitats, trophic resources, and microclimatic conditions they provide and ultimately to the biodiversity they shelter. For this, a better exploration of stress-induced horizontal gradient of inner to outer microhabitats in degraded tree crown, and fine characterization of arthropod community distribution within the canopy gradients, and in the above-tree stratum, would be necessary. Several studies have investigated the impacts of forest diebacks or declines on the diversity of specific taxonomic groups or functional, habitat, or trophic guilds (e.g., Martel and Mauffette, 1997; Trotter et al., 2008; Stone et al., 2010; Beudert et al., 2015; Sallé et al., 2020; Vincent et al., 2020; Cours et al., 2021; Sire et al., 2021). Nonetheless, because of the mixed impacts highlighted by several studies, a multi-taxa and multi-guild approach would be necessary to have a holistic view of the conservation and functional outcomes of ongoing climate changes on forest arthropod communities (Seibold et al., 2018a; Swart et al., 2020). Based on this comprehensive ecological assessment, forest management and biodiversity conservation strategies, including the retention of damaged or declining trees (e.g., Kašák and Foit, 2018), shall be designed in order to take advantage of decline patterns and processes for biodiversity promotion while facilitating the forest regeneration at the same time.

AUTHOR CONTRIBUTIONS

AS supervised the writing and wrote the first draft of the manuscript. JC and EL contributed to the writing and prepared the figures. CL-V, SP, and CB contributed to the writing. CB co-supervised the research theme with AS. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Région Centre-Val de Loire Project no. 2018-00124136 (CANOPEE) coordinated by AS.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Forests and Global Change

ORIGINAL RESEARCH
published: 22 September 2021
doi: 10.3389/ffgc.2021.675401

Response of Background Herbivory in Mature Birch Trees to Global Warming

Masahiro Nakamura[1,2], Moeko Minoshima[3], Chisato Terada[2], Kentaro Takagi[4], Kobayashi Makoto[4], Hideaki Shibata[5] and Tsutom Hiura[6]*

[1] Nakagawa Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Otoineppu, Japan, [2] Wakayama Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Kozagawa, Japan, [3] Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan, [4] Teshio Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Horonobe, Japan, [5] Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Japan, [6] Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo, Japan

OPEN ACCESS

Edited by:
Akihiro Nakamura,
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The University of Hong Kong, Hong Kong, SAR China

****Correspondence:***
Masahiro Nakamura
masahiro@fsc.hokudai.ac.jp

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *03 March 2021*
Accepted: *25 August 2021*
Published: *22 September 2021*

Citation:
Nakamura M, Minoshima M, Terada C, Takagi K, Makoto K, Shibata H and Hiura T (2021) Response of Background Herbivory in Mature Birch Trees to Global Warming. Front. For. Glob. Change 4:675401. doi: 10.3389/ffgc.2021.675401

Given the time scale based on the duration of exposure to global warming, natural climate-gradient studies and experimental manipulations have detected long-term (decades to centuries) and short-term (years to decades) ecological responses to global warming. Combination of these two complementary approaches within a single study may enable prediction of the likely responses of ecological processes to global warming. To understand how global warming affects plant–herbivore interactions within a canopy of Erman's birch, we combined an elevational gradient study and a warming experiment involving mature birch trees in which the soil and tree branches were warmed separately. In the elevational gradient study, herbivory by chewing insects and plant growth increased as elevation decreased, and the concentrations of condensed tannins and total phenolics in the leaves decreased. In the warming experiment, soil warming alone increased herbivory, and the addition of branch warming amplified the effect on herbivory. Soil warming alone decreased the tannin concentration, and the addition of branch warming led to a further reduction. The variation in herbivory was best explained by the tannin content of leaves. Our experimental results demonstrate that the decreased tannin content of leaves due to a combination of soil and branch warming was an important driver of increased herbivory in the canopy of the mature birch trees. The similar tendencies in the short- and long-term responses imply that global warming is likely to increase background herbivory in mature birch trees by decreasing the tannin content of leaves in the canopy.

Keywords: above-ground warming, below-ground warming, experimental manipulation, natural gradient, plant-mediated effect

INTRODUCTION

The impact of climate change on plant–herbivore interactions is an important topic in ecology (Bale et al., 2002; Robinet and Roques, 2010; Pureswaran et al., 2018). Within forest stands, insect species diversity is greatest in the high canopy, where associated biological activities are concentrated, rather than in the understory because of the greater plant productivity of mature trees (Basset et al., 2003). However, many field experiments that used warming to examine the effects of global warming have focused on saplings. Many of the physiological and morphological characteristics of woody plants vary during ontogenetic development (Bond, 2000). For example, plant defenses against herbivores often change dramatically from the seedling to the mature stage (Boege and Marquis, 2005; Barton and Koricheva, 2010). This ontogenetic difference, called age-dependent (size-dependent) plasticity (Fischer et al., 2014), has also been observed in plant responses to climate change (Nabeshima et al., 2010; Goodger and Woodrow, 2014). Nabeshima et al. (2010) demonstrated that responses of diameter growth of deciduous trees (*Acer mono var. glabrum, A. amoenum, Ostrya japonica, Carpinus cordata, Magnolia obovate,* and *Quercus crispula*) to annual changes in weather conditions (e.g., temperature, precipitation) depended on tree size. Thus, the experimental results obtained in sapling studies may not be entirely relevant for forest ecosystems.

Field-based approaches, most commonly involving natural climate gradients and experimental manipulation, have been used to detect the effects of global warming on natural ecosystems. Given the time scale based on the duration of exposure to global warming, the natural climate gradients provided by changes in elevation and latitude serve as space-for-time substitutions and are therefore useful for predicting long-term (decades to centuries) ecological responses (adaptation) to global warming (Shaver et al., 2000; Sundqvist et al., 2013). However, this approach has several limitations, as long-term adaptations to local conditions and co-varying factors may confound straightforward interpretations of natural gradients (Sundqvist et al., 2013). Conversely, experimental manipulations have fewer confounding factors and provide a mechanistic approach to test the effects of drivers of ecological processes directly (Shaver et al., 2000; Rustad et al., 2001). Therefore, experimental manipulation is useful for detecting short-term (years to decades) ecological responses to global warming (Dunne et al., 2004). However, because such experiments are unable to consider delayed responses, the occurrence of short-term changes may lead to inaccurate predictions of the effects of global warming on natural ecosystems (Wolkovich et al., 2012). Combination of these two complementary approaches within a single study might overcome some of their individual limitations (Anderson and Wadgymar, 2020) and allow predictions of global warming effects (Dunne et al., 2004).

Herbivory plays an important role as a driving force in ecosystem functions in forests (e.g., plant productivity and carbon [C], nutrient, and phosphorous cycles) (Wolf et al., 2008; Metcalfe et al., 2014). Although most studies have paid considerable attention to global warming as a very important factor that directly affects the abundance and species composition of insect herbivores by modulating their survival, development rates, and dispersal (Bale et al., 2002; Robinet and Roques, 2010; Heimonen et al., 2015), indirect effects have received less attention. However, a growing body of evidence shows indirect (plant-mediated) effects of global warming on insect herbivores (e.g., de Sassi et al., 2012; Nakamura et al., 2014; Rasmann et al., 2014; Hamann et al., 2021) and reflects the ability of plants to adjust to global warming via phenotypic plasticity (Nicotra et al., 2010). Indeed, some studies have emphasized that these indirect effects can be stronger than the direct effects (e.g., Barton et al., 2009; O'Connor, 2009).

Many of the effects of temperature on plant phenotypic plasticity, including leaf traits, may be complex (Weih and Karlsson, 2001). According to the C–nutrient balance hypothesis (Bryant et al., 1983), below-ground temperature elevation affects the primary (growth) and secondary (C-based defenses) metabolism of plants (Veteli et al., 2002; Zvereva and Kozlov, 2006). An increase in below-ground temperatures often accelerates soil nitrogen (N) mineralization (Rustad et al., 2001; Melillo et al., 2002), which is expected to lead to a decrease in the concentrations of C-based defensive compounds, as the C becomes allocated to plant growth rather than to defensive-compound synthesis (Veteli et al., 2002; Zvereva and Kozlov, 2006). Furthermore, increases in above-ground temperatures are expected to amplify the effects of increased below-ground temperatures by enhancing plant growth in boreal forests, where air (above-ground) temperatures are the most important limiting factor for plant growth (Ong and Baker, 1985; Weih and Karlsson, 2001). High air temperatures may accelerate C allocation to plant growth rather than to defensive-compound synthesis. Thus, in field experiments involving the canopy of mature trees, the growth of which is frequently limited by low temperatures, both above- and below-ground temperature increases must be considered, alone and in combination, to elucidate the mechanism by which increased temperature affects plant–herbivore interactions (Nakamura et al., 2014).

In this study, we combined an elevational gradient study and a warming experiment involving Erman's birch trees (*Betula ermanii* Cham.) growing on Hokkaido, a northern island of Japan, to predict the likely response of plant–herbivore interactions to global warming. Specifically, in the warming experiment, the canopy branches of mature birch trees 18–21 m in height and the soil surrounding their roots were warmed separately, and the results were compared with those of the elevational study. We predict that global warming would increase background herbivory in mature birches by reducing the concentration of leaf C-based defense compounds in the canopy. The background herbivory is defined as the damage to plants occurring when herbivore populations are at their normal densities (Kozlov and Zvereva, 2017). The following questions were addressed. (1) How do background herbivory, plant growth, and leaf traits of birch trees vary along an elevational gradient? (2) How do increased below-ground temperature (soil warming) alone and the combined effects of above- and below-ground (branch and soil) warming affect these same parameters of mature birch trees? (3) Which leaf traits explain the variation in the herbivory rate observed in the elevational gradient study and the warming experiment?

MATERIALS AND METHODS

Elevational Gradient Study

The focus of this study was Erman's birch (*Betula ermanii* Cham.), a common tree line and early successional species (pioneer) that is widely distributed from the plains to the mountains on the northern Japanese island of Hokkaido (Ito, 1987). The elevational gradient study was conducted on Mt. Youtei (42°49′N, 140°48′E, 1898 m a.s.l.), Hokkaido. The study area was a typical stratovolcano with a simple geography. The mean annual temperature at the foot of the mountain (176 m a.s.l.) is 5.7°C, and the mean annual precipitation is 1149 mm. Four sites (at 700, 1000, 1300, and 1600 m) were selected along the elevational gradient of Mt. Youtei in August 2012. The height of Erman's birch trees was 10–15 m at 700 m, 5–10 m at 1000 m, 3–5 m at 1300 m, and 2–3 m at 1600 m. Since the elevational difference was 900 m, the estimated temperature difference was approximately 5°C using a lapse rate of 0.6°C per 100 m. The vegetation turns into pure stands of Erman's birch toward higher elevations. At each site, eight Erman's birch trees growing along narrow forest roads (1–2 m in width) were randomly chosen, and two or three branches approximately 1 cm in diameter were sampled from the top of the canopy of each of these trees using a tree-height pole fitted with a sickle at the top. National and prefectural institutes reported no outbreaks of defoliating insects on Erman's birch on Mt. Youtei in 2012 (Sayama et al., 2014). Thus, we measured the rates of background herbivory on 50 current-year shoots, including 25 long and 25 short shoots from the canopy branches of each tree. The shoot development of Erman's birch can be divided into two types: long shoots continuously expand the leaves from early to late, while short shoots expand only the early leaves (Koike, 1995). Herbivory was caused mainly by leaf-chewing insects and late-instar larvae of leaf-mining insects, whereas sap-feeding and gall-making insects were not included. The percent area consumed was scored visually for each leaf, and the percentages were grouped into six ranked indices as follows: 0% = 0, 1–10% = 1, 11–25% = 2, 26–50% = 3, 51–75% = 4, and 76–100% = 5 (Nakamura et al., 2014). The median of each class was used for statistical analysis of herbivory (0 = 0, 1 = 5, 2 = 17.5, 3 = 37.5, 4 = 62.5, and 5 = 87.5%). To identify leaf traits potentially responsible for variation in herbivory rates, 10 leaves were sampled from the canopy of each tree. Ten leaf disks (6 mm radius) punched from each leaf were oven-dried at 40°C for at least 7 days. The mean leaf mass per area (LMA) of each leaf was then calculated. The N and C contents (percentage of dry mass) of the leaves were measured using an NC analyzer (NC-900, Sumika Chemical Analysis Service, Japan), and the C/N ratio was then calculated. The total N content per shoot was calculated as (N content (mg/g) × LMA (g/m^2) × leaf size (cm^2) × leaf number per shoot)/10,000. Five leaves from each tree were used to determine the concentrations of soluble condensed tannins and soluble total phenolics following the Butanol–HCl and Folin–Ciocalteu methods (Julkunen-Titto, 1985). Plant growth characteristics were assessed by measuring the numbers of leaves per shoot and the lengths of 50 current-year long shoots on each tree.

Warming Experiment

Our warming experiment was conducted in Hokkaido at a different site from the elevational gradient study on Mt. Youtei. The warming experiment, which was based on a factorial design of elevated temperature of soil surrounding the roots and canopy branches of mature birch trees, was conducted in the Nakagawa Experimental Forest of Hokkaido University (44°48′N, 142°14′E, 42 m a.s.l.), where the mean annual temperature is 5.4°C and the mean annual precipitation 1449 mm. In addition to the control condition (Control), the treatments comprised soil warming alone (SW) and soil warming together with canopy branch warming (SWBW). Direct observations were carried out from three steel scaffolds, each 36 m^2 (6 m by 6 m) and 20 m in height, with steps that were constructed specifically for this purpose between May and June 2010 around 10 tall *B. ermanii* trees 18–21 m in height (Nakamura et al., 2016). For the SW treatment, five mature birch trees whose canopies were accessible from two of the scaffolds were selected. In October 2010, we established two 36 m^2 (6 m by 6 m) plots at the base of these two scaffolds, one containing two mature birch trees, and one containing three. An electric heating cable made of copper resistance wire and 120 m in length was buried in the soil of each plot at a depth of 5–10 cm at 20 cm intervals, using a previously described method (Melillo et al., 2002; Nakamura et al., 2014). Five mature birch trees whose canopies were accessible from a scaffold were selected as the control. At the base of this scaffold, a 36 m^2 (6 m by 6 m) plot was similarly established, and the soil was dug to the same depth as in the warmed plots to ensure a similar level of disturbance to the control trees. Due to the difficulty of experimental manipulation, it was not possible to establish spatially replicated plots. We therefore treated individual tree as a replicate, but we recognize the limitation of not including spatial heterogeneity (Oksanen, 2001; Colegrave and Ruxton, 2018). Due to the early successional species (pioneer), there was plenty of space between the mature trees of *B. ermanii*. Therefore, these trees were not suppressed by surrounding trees. Soil temperature was set at approximately 5°C higher than the ambient temperature using thermal sensors (K cables) coupled with a controller to regulate the power supply. When the difference in temperature between the control and treatment dropped to <5°C, the controller relay switch opened, and power was supplied to the electric cable. When the difference was $\geq$ 5°C, the relay switch closed. The soil was warmed throughout the year. Soil warming increased the temperature of the soil by 5.10 ± 0.51°C (mean ± SD) compared with the ambient temperature.

For the SWBW treatment, we selected four of the five SW trees and warmed an area of approximately 0.5 m × 1.5 m in the canopy of each selected SW tree using infrared heat lamps (500 W, 200 V; Nippon Heater, Tokyo, Japan). The warmed canopy region of each selected SW tree was considered the SWBW treatment, and the rest of the canopy region was considered the SW treatment. The infrared lamps were fixed to the upper parts of the scaffolds (Nakamura et al., 2016). Warming of the canopy branches was started before leaf flush (late May) and continued until after leaf fall (mid-November) of 2012. At the onset of the experiment, the lamps were positioned approximately 30 cm above the tops of the branches. The lamps increased the temperature of the canopy branches by 1.37 ± 1.59°C (mean ± SD) above the ambient temperature (Nakamura et al., 2016).

Because phenotypic plasticity is a property of individual modules (leaves, shoots, and branches) triggered by local environmental conditions rather than a whole-plant response (de Kroon et al., 2005), a modular approach is particularly useful for examining the effects of temperature elevation on mature birch trees. Accordingly, we conducted shoot-level censuses to measure the responses of the mature birch trees to the treatments. Six branches per SW tree and control tree and four branches per SWBW tree were randomly selected for sampling and observation; as only a small part of the canopy was warmed, only four branches were available per tree for the SWBW treatment. Background herbivory rates were assessed using 10 randomly selected current-year shoots, which comprised 5 long and 5 short shoots from each branch using the same method described for the elevational gradient study on Mt. Youtei. Leaf traits including LMA, N and C contents, total N content per shoot, C/N ratio, soluble condensed tannins, and soluble total phenolics were assessed based on two leaves from each branch using the same method described for the elevational gradient study. To assess plant growth characteristics, the length and number of leaves per shoot of the 10 current-year shoots on each branch were measured. The measurements were conducted in September 2010 before the warming experiment started and in September 2012 during the warming experiment to accurately assess how individual mature trees responded to the warming treatments.

Statistical Analyses

In the elevational gradient study, data on herbivory rate, total N content per shoot, C/N ratio, LMA, the concentrations of condensed tannins and total phenolics, shoot length, and number of leaves per shoot were normally distributed (Shapiro–Wilk test, $P > 0.05$). As the data on N and C contents did not show a normal distribution (Shapiro–Wilk test, $P < 0.05$), they were arcsine (n)-transformed to satisfy the assumption of normal distribution. Correlation coefficients were used to analyze the strength of the relationships of elevation with herbivory rate, leaf traits, and plant growth characteristics. In all analyses in the elevational gradient study, Individual trees served as replicates ($n = 32$).

Before the warming experiment started (2010), the herbivory rate, condensed tannin concentration, and shoot length differed significantly among the Control, SW, and SWBW treatments ($P < 0.05$, **Supplementary Figures 1, 2**), but several leaf traits (N and C contents, total N content per shoot, LMA, C/N ratio, and total phenolic concentration) as well as number of leaves per shoot did not differ ($P > 0.05$). To remove the effect of heterogeneity from these variables before the warming experiment, effect sizes were used to compare the herbivory rate, leaf traits, and plant growth characteristics among treatments. We estimated the effect sizes using Cohen's *d* metric and 95% confidence intervals (95% CI) using the "meta" package version 4.18 in R 4.0.2 (Schwarzer et al., 2015). Cohen's *d* was calculated as the standardized mean difference in the value of each variable from before (2010) to after (2012) the warming experiment in each treatment. A positive effect size indicates that the value of a variable increased from 2010 to 2012 (from before to after the warming experiment), whereas a negative effect size indicates the inverse. Temporal variation between 2010 and 2012 was considered to have a statistically significant effect if the 95% confidence interval of the mean effect size did not include zero. Variation in the effect size of each variable among treatments was explored by calculating the heterogeneity index (Q_B). The index was tested against the chi-square distribution. All analyses were performed using random effect models. Individual trees served as replicates in the warming experiment analyses (control, $n = 5$; SW, $n = 5$; SWBW, $n = 4$).

To examine whether multiple leaf traits affected elevational and experimental variations in herbivory rates, generalized linear models (GLMs) were applied using a normal distribution with an identity link function; all leaf traits (C/N ratio, N and C contents, condensed tannin and total phenolic concentrations, and LMA) were set as the independent variables. We conducted separate analyses using data from the elevational gradient study and from both 2010 and 2012 to include variation before and after the warming experiment. There was no significant difference in the herbivory rates of the control trees between 2010 and 2012 (ANOVA, df = 9, $F = 0.9687$, $P = 0.354$), suggesting similar herbivory pressures during the 2-year period. Before the generalized linear models, we removed leaf traits with a variance inflation factor (VIF) value > 3 because a higher VIF indicates a greater influence of multicollinearity on the variance of an estimated regression coefficient. In both studies, to maintain a low VIF (<3), we removed the C/N ratio, which was strongly correlated with the N content. Model selections were performed based on an information theoretic approach using Akaike's information criterion (AIC; Burnham and Anderson, 2002). AIC values and Akaike weights were computed for all alternatives to select a best-fit model. According to the principle of parsimony, when alternative models had indistinguishable AIC values (ΔAIC < 2), the model with fewer parameters was selected (Burnham and Anderson, 2002).

RESULTS

Elevational Gradients in Herbivory, Leaf Traits, and Plant Growth

Herbivory rates increased significantly as elevation decreased ($F = 66.849$, $P < 0.001$, **Figure 1**). Most of the measured leaf traits

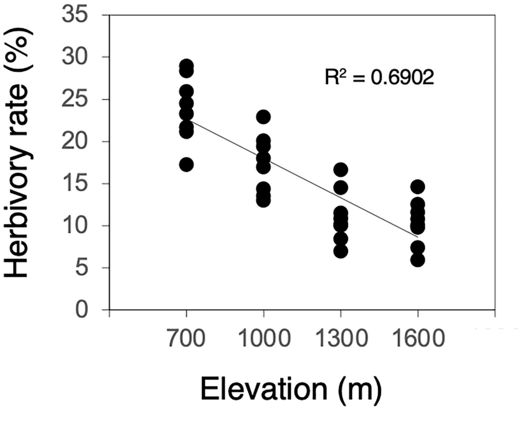

FIGURE 1 | Correlation between herbivory rate and elevation ($n = 32$). Solid line denotes significant correlation.

were significantly related to elevation. As elevation decreased, the N content, LMA, and concentrations of condensed tannins and total phenolics decreased significantly (N content, $F = 6.294$, $P = 0.018$, **Figure 2A**; LMA, $F = 39.356$, $P < 0.001$, **Figure 2C**; condensed tannins, $F = 51.324$, $P < 0.001$, **Figure 2F**; total phenolics, $F = 9.849$, $P = 0.004$, **Figure 2G**). In contrast, the total N content per shoot and C/N ratio increased significantly with decreasing elevation (total N, $F = 3.070$, $P = 0.090$, **Figure 2B**; C/N ratio, $F = 10.687$, $P = 0.003$, **Figure 2D**). Although the relationships were marginally significant, the C content also increased with decreasing elevation (C content, $F = 3.432$, $P = 0.074$, **Figure 2E**). Plant growth characteristics such as shoot length and the number of leaves per shoot increased significantly with decreasing elevation (shoot length, $F = 56.836$, $P < 0.001$, **Figure 2H**; leaf number, $F = 5.684$, $P = 0.024$, **Figure 2I**).

Response of Herbivory to Soil and Branch Warming

Significant differences in the effect size of temporal variation in herbivory were observed among the treatments ($Q_B = 13.62$, $P = 0.001$, **Figure 3**). The herbivory in control trees did not differ between 2010 and 2012 (before and after the warming experiment), whereas that in SW and SWBW trees increased (SW: 1.464, 95% CI: 0.068–2.860; SWBW: 4.601, 95% CI: 1.955–7.248). SWBW exhibited a stronger positive effect on herbivory than did SW alone.

Response of Leaf Traits to Soil and Branch Warming

Differences in the effect size of temporal variation in N and C contents, LMA, and C/N ratio among treatments were not significant (all $P > 0.05$, **Figures 4A,C–E**). Although difference in the effect size of temporal variation in total N per shoot was marginally significant ($Q_B = 5.34$, $P = 0.069$, **Figure 4B**), the effect sizes of temporal variation in condensed tannin and total phenolic concentrations differed significantly among the treatments (condensed tannins: $Q_B = 24.54$, $P < 0.001$, **Figure 4F**; total phenolics: $Q_B = 6.23$, $P = 0.044$, **Figure 4G**). These concentrations did not change during this period in control trees, whereas they decreased in SW (tannins: −4.228, 95% CI: −6.458

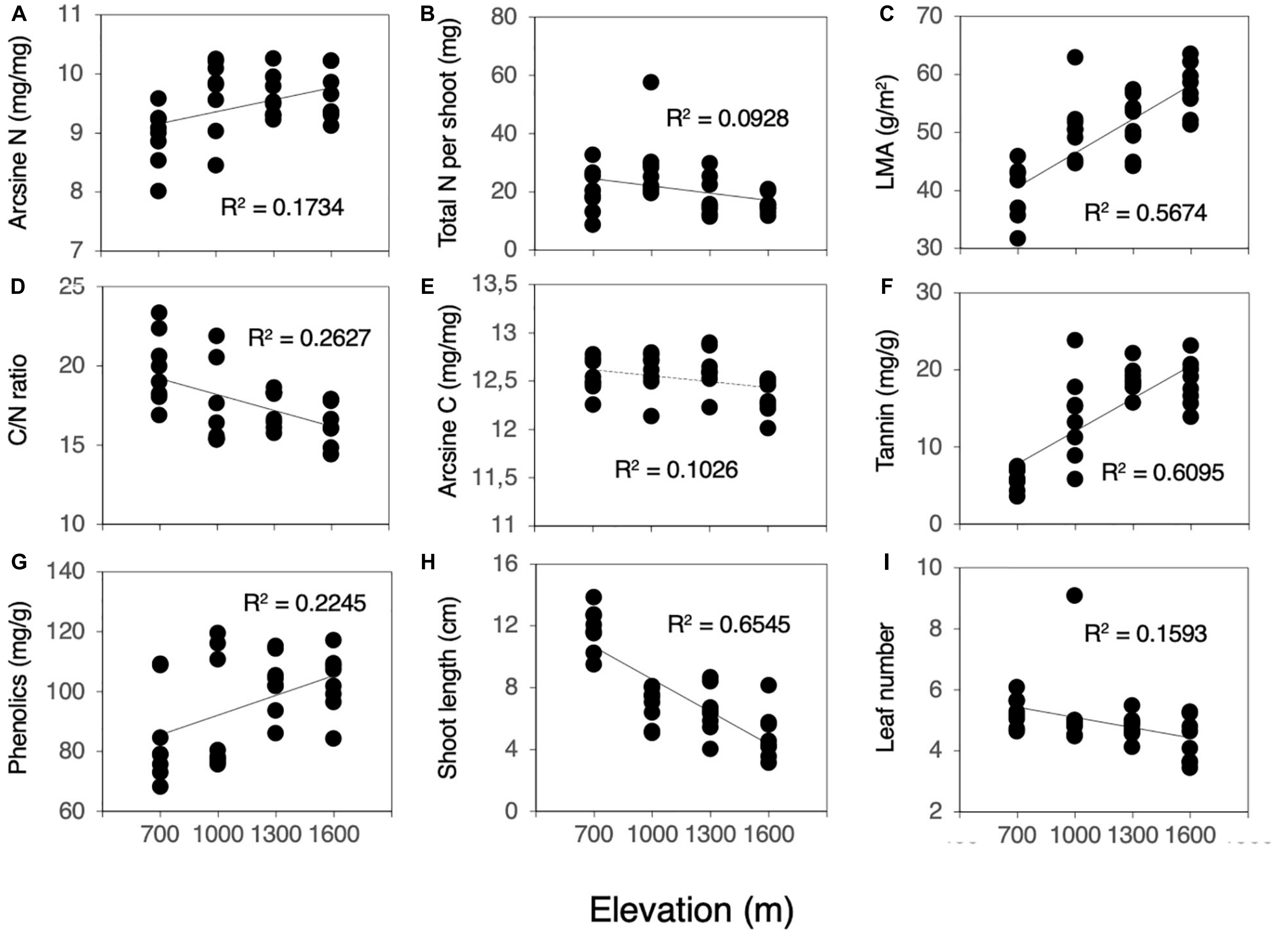

FIGURE 2 | Correlation between plant characteristics (leaf traits and plant growth) and elevation ($n = 32$). **(A)** N, **(B)** Total N per shoot, **(C)** LMA, **(D)** C/N ratio, **(E)** C, **(F)** Condensed tannin, **(G)** Total phenolics, **(H)** Shoot length, and **(I)** Number of leaves per shoot. The *y*-axis in **(A,E)** shows arcsine (n)-transformed N and C contents. Solid lines denote significant correlation, and dashed lines denote marginal significant correlation.

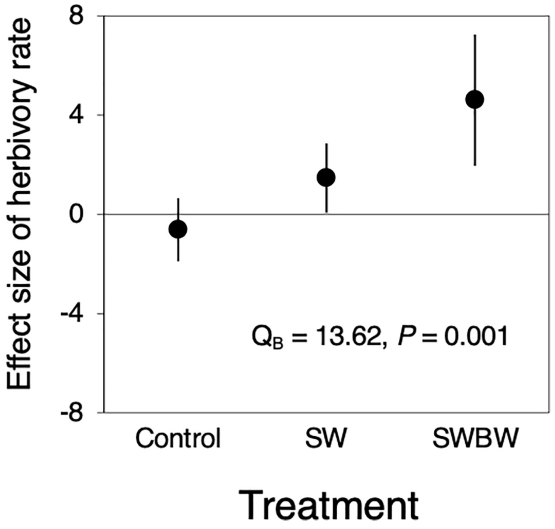

FIGURE 3 | Effect sizes (Cohen's *d*) of temporal variation (between 2010 and 2012) in herbivory rate in control trees (Control, *n* = 5), the soil-warmed trees (SW, *n* = 5), and soil and canopy branch-warmed trees (SWBW, *n* = 4). Positive effect size indicates that herbivory rate increased from 2010 to 2012 (before and after the warming experiment). Each dot indicates mean effect size. Each vertical line denotes 95% confidence interval (CI). The effect is significant if the 95% CI does not include zero. Significant Q_B value indicates between-treatment heterogeneity.

to −1.999; phenolics: −2.283, 95% CI: −3.876 to −0.690) and SWBW (tannins: −5.677, 95% CI: −8.784 to −2.2569; phenolics: −2.552, 95% CI: −4.419 to −0.685). Furthermore, SWBW showed a slightly stronger negative effect on the condensed tannin concentration than did SW.

Response of Plant Growth to Soil and Branch Warming

The effect sizes of temporal variation in shoot length and leaf number differed significantly among treatments (shoot length: Q_B = 20.56, *P* < 0.001, **Figure 4H**; leaf number: Q_B = 6.27, *P* = 0.044, **Figure 4I**). Shoot length and leaf number decreased during this period in control trees (shoot length: −1.895, 95% CI: −3.387 to −0.403; leaf number: −1.517, 95% CI: −2.923 to −0.110). However, shoot length increased in SW and SWBW trees during this period (SW: 1.823, 95% CI: 0.350–3.301; SWBW: 3.740, 95% CI: 1.443–6.038), but the difference in leaf number was not significant. These results suggest that SW and SWBW increased the shoot length and leaf number. Furthermore, SWBW yielded a slightly stronger positive effect on shoot length compared with SW.

Leaf Traits Explaining Herbivory Rates in the Elevational Gradient Study and Warming Experiment

According to the principle of parsimony, the model that included the condensed tannin concentration and N content was selected as the best-fit model for elevational variation in herbivory rates (**Supplementary Table 1**). The regression coefficient for the condensed tannin concentration was negative (−0.701, 95% CI: −0.954 to 0.104) (**Supplementary Table 2**). Although the slope was marginally significant, the regression coefficient for the N content was negative (−0.441, 95% CI: −0.947 to 0.352). To explain the experimental variation in herbivory rates, the model that included the condensed tannin concentration was selected as the best-fit model (**Supplementary Table 1**). The regression coefficient for the condensed tannin concentration was negative (−0.149, 95% CI: −0.251 to −0.047) (**Supplementary Table 2**).

DISCUSSION

Single and Combined Effects of Above- and Below-Ground Warming

A decrease in the defensive compounds of birch saplings during warming experiments has been reported in previous studies, predicting a critical influence of environmental changes during the juvenile stage (Kuokkanen et al., 2004; Zvereva and Kozlov, 2006), but to date, no detailed studies have considered the separate effects of above- and below-ground warming on mature birch trees (but see Weih and Karlsson, 2001). According to the C–nutrient balance hypothesis, in the presence of increased N availability for plants, C is used for plant growth rather than for defensive-compound synthesis (Bryant et al., 1983). Our warming experiment revealed that soil (below-ground) warming alone significantly increased shoot length and leaf number but decreased the concentrations of condensed tannins and total phenolics in the canopy leaves of mature birch trees. These findings imply that increased soil N availability likely caused more C to be allocated to plant growth than to defensive-compound synthesis, probably due to enhanced microbial N mineralization in the warmed soil (Rustad et al., 2001; Melillo et al., 2002). Contrasting soil (below-ground) and air (above-ground) temperatures are likely an important limiting factor in the growth of woody plants in boreal forest (Ong and Baker, 1985; Weih and Karlsson, 2001). Our warming experiment revealed that the addition of branch warming produced a stronger positive effect on shoot length than did soil warming alone but further decreased condensed tannin concentrations in canopy leaves. The greater C allocation to shoot length due to branch warming seemed to further lower condensed tannin concentrations. This implies that below- (soil) and above-ground (branch) warming may have different effects on the production of C-based defensive compounds and plant growth. However, the absence of branch warming alone in our warming experiment limits the interpretation of our experimental results. Thus, future studies examining the effects of branch warming alone are needed to understand the effects of branch warming on plant growth and defensive compounds in mature birch trees. Although an ontogenetic difference in plant responses (age-dependent plasticity) to climate change was expected (Nabeshima et al., 2010; Fischer et al., 2014; Goodger and Woodrow, 2014), similar to the sapling stage of birch trees, our warming experiment revealed that soil and branch warming significantly decreased defensive compounds even during the mature stage.

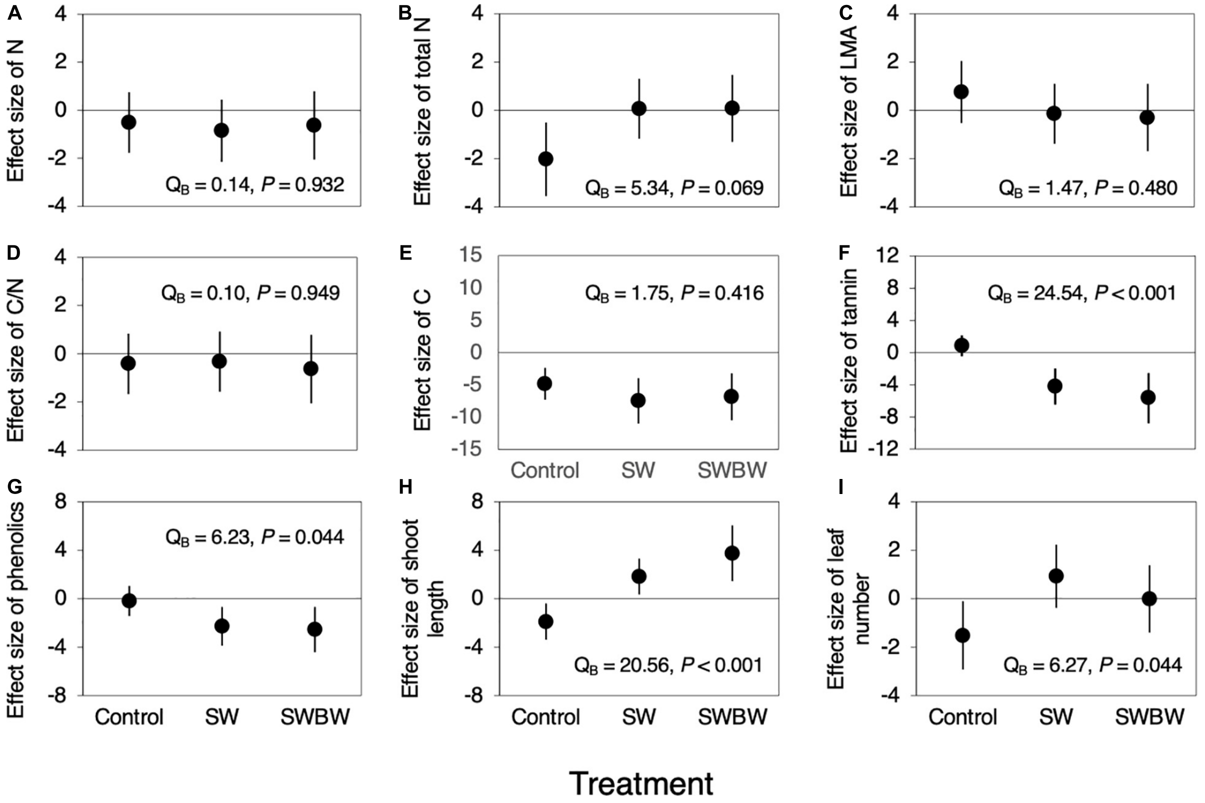

FIGURE 4 | Effect sizes (Cohen's *d*) of temporal variation (between 2010 and 2012) in leaf traits and plant growth characteristics in control trees (Control, $n = 5$), the soil-warmed trees (SW, $n = 5$), and soil and canopy branch-warmed trees (SWBW, $n = 4$). **(A)** N, **(B)** Total N per shoot, **(C)** LMA, **(D)** CN ratio, **(E)** C, **(F)** Condensed tannin, **(G)** Total phenolics, **(H)** Shoot growth, and **(I)** Number of leaves per shoot. Positive effect size indicates that leaf traits and plant growth characteristics increased from 2010 to 2012 (before and after the warming experiment). Each dot indicates mean effect size. Each vertical line denotes 95% confidence interval (CI). The effect is significant if the 95% CI does not include zero. Significant Q_B value indicates between-treatment heterogeneity.

Although an increase in the N content of canopy leaves in response to soil warming was expected, there was no effect on either the N content or the leaf C/N ratio, despite a tendency for the total N content per shoot to increase. This finding indicates that the increased plant growth due to soil warming may dilute leaf nutrient levels (Veteli et al., 2002).

The model selection revealed that experimental variation in herbivory rates was best explained by condensed tannin concentrations. Although soil warming alone reduced the condensed tannin concentrations of canopy leaves, this effect was amplified by the addition of branch warming. This combined effect was sufficient to drive a significant increase in background herbivory in the canopy of the mature birch trees. Our experimental results provide a mechanistic understanding of how, over the short term (years), the combination of soil and branch warming can induce positive indirect (plant-mediated) ecological effects in the form of increased background herbivory of mature birch trees. However, since individual trees were used as replicates in this study, spatial heterogeneity due to local conditions (e.g., soil types, precipitation, and temperature) may limit the interpretation of our experimental results. In the next step, plot replication and/or block design may provide more general results.

Elevational Gradient

The natural climate gradient is useful to predict long-term (decades to centuries) ecological responses to global warming (Shaver et al., 2000; Sundqvist et al., 2013). Our elevational gradient study with an estimated temperature difference of approximately 5°C (elevation difference of 900 m) revealed a significant increase in background herbivory in canopy leaves at relatively low elevations compared to the high elevations. Furthermore, condensed tannin and total phenolic concentrations decreased at relatively low elevations, while shoot length and leaf number increased. A meta-analysis of 1,027 plant species by Galman et al. (2017) showed that herbivory increased toward lower elevations for deciduous woody species. Similar to this elevational gradient, it is widely accepted that forests at low elevations suffer greater herbivory than those at high elevations (Kozlov, 2008; Kozlov et al., 2013; Lim et al., 2015; Wang et al., 2016; Zhang et al., 2016). These herbivory results supported the pattern detected in our elevational gradient study.

The model selection revealed that the elevational variation in herbivory rates was best explained by the condensed tannin concentrations and N content. This result was partly consistent with the model selection result of the warming experiment. Thus, elevational variation in herbivory likely occurs via a similar mechanism involving the positive indirect (plant-mediated) effects of warmer temperatures. However, the difference in the magnitude ([variable in 2012 – variable in 2010]/variable in 2010) of the herbivory rate from control to SWBW was 0.772, whereas the magnitude from 1600 to 700 m ([variable at 700 m – variable at 1600 m]/variable at 1600 m) was 1.312. Thus, the magnitude of the effect of elevational difference in the elevational gradient study on background herbivory was nearly twice as large as that of increased temperature in the warming experiment. There are at least three possible reasons for the smaller magnitude of the warming experiment effect. First, the magnitude of the effect of above-ground (branch) temperature elevation of only 1.4°C in the warming experiment may have been smaller than that of the above-ground temperature difference (∼5°C) estimated for an elevation difference of 900 m. Second, the period of temperature elevation achieved by branch warming was short, covering just one growing season (6 months). Third, since the soil was warmed in 36 m^2 plots, only a small section of the fine roots of each individual mature tree was warmed. These possible explanations imply that the actual magnitude of the effects of global warming on background herbivory may be closer to the result of the elevational gradient study than to that of the warming experiment. In the elevational gradient study, there were only eight individual trees (replications) at each site, but more individual trees may be needed to more accurately calculate the magnitude of the effect of elevational difference.

Prediction of Global Warming Effects

Combination of a natural climate gradient and an experimental manipulation into a single study can provide a research approach that more accurately predicts global warming effects (Dunne et al., 2004). Our results of these two complementary approaches revealed that the short-term responses (years) of plant–herbivore interactions and the growth in the canopy of mature birch trees detected in the warming experiment were consistent with the long-term responses (decades to centuries) detected in the elevational gradient study. Furthermore, a 38-year field monitoring study on forest dynamics in the Nakagawa Experimental Forest revealed an accelerated growth response to increased temperature in *B. ermanii* (Hiura et al., 2019). Together, these results yield insights into some of the potential effects of global warming. Specifically, we showed that global warming will increase background herbivory in mature birch trees by decreasing tannin concentrations in the canopy. In contrast to mature birch trees (*B. ermanii*), an early successional species (pioneer), mature oak trees (*Quercus crispula*), a late successional species (climax), showed decreased herbivory in response to soil warming (Nakamura et al., 2014) and toward lower elevations (Takafumi et al., 2021). These combined results suggest that global warming is likely to decrease background herbivory in mature oak trees. It is possible that the response of herbivory on mature trees to global warming may depend on tree species and their successional status, suggesting that the responses of herbivory to global warming in forests may be predicted from the species composition of woody plants. Several other aspects remain to be considered in future studies. First, triterpenoids on leaf surface exhibit defense properties against herbivores (Reichardt et al., 1984; Laitinen et al., 2004) and its content in silver birch leaves was reported to decrease toward southern latitudes (Makhnev et al., 2012). Since some of triterpenoids have been reported from Erman's birch (Yamaguchi et al., 2009), changes in the triterpenoid content due to global warming may affect background herbivory in mature birch trees. Second, both direct and indirect effects of global warming on insect herbivores should be considered in assessing the impact of global warming, because global warming directly affects the abundance and species composition of insect herbivores by modulating their survival, development rate, and dispersal (Bale et al., 2002; Robinet and Roques, 2010; Heimonen et al., 2015). Third, previous studies on birch saplings showed that tannin concentrations in birch leaves increased greatly in a CO_2-enriched atmosphere, which coincidently increase with increasing temperature (e.g., McDonald et al., 1999; Kuokkanen et al., 2003; Peltonen et al., 2005). Therefore, simultaneous increases in both temperature and CO_2 must be considered to better understand the responses of plant defenses and plant–herbivore interactions in the canopy of mature birch trees to actual global changes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

The idea for the study was conceived by MN and TH. The methodology was designed by MN, KT, and HS. Data was collected by MN and MM. Data analysis and interpretation was performed by MN, MM, CT, and KM. MN led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

FUNDING

This work was financially supported by Grants from Japan Society for the Promotion of Science (No. 26450188 to MN, No. 21248017 to TH), and from the Ministry of Environments (D-0909 to TH).

ACKNOWLEDGMENTS

We thank Ministry of the Environment and Agency for Cultural Affairs for allowing us to sample branches form Erman's birch trees at Mt. Youtei. Also thanks to staff members at Nakagawa Experimental Forest, Hokkaido University for their construction of steel pipe scaffolds and support during the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.675401/full#supplementary-material

Supplementary Figure 1 | Original values of herbivory rate before (2010) and after (2012) the warming experiment in control trees (Control: open bar, *n* = 5), the soil-warmed trees (SW: closed bar, *n* = 5), and soil and canopy branch-warmed trees (SWBW: closed bar, *n* = 4). Mean and SE are presented. One-way ANOVA was used to compare among treatments for each year. Tukey test was used for a multiple-comparison between all treatments. Different upper and lower case letters indicate significant differences ($P < 0.05$) after the multiple-comparison correction before (2010) and after (2012) the warming experiment, respectively.

Supplementary Figure 2 | Original values of leaf traits and plant growth characteristics before (2010) and after (2012) the warming experiment in control trees (Control: open bar, *n* = 5), the soil-warmed trees (SW: closed bar, *n* = 5), and soil and canopy branch-warmed trees (SWBW: closed bar, *n* = 4). **(A)** N, **(B)** Total N per shoot, **(C)** LMA, **(D)** CN ratio, **(E)** C, **(F)** Condensed tannin, **(G)** Total phenolics, **(H)** Shoot growth, and **(I)** Number of leaves per shoot. Mean and SE are presented. One-way ANOVA was used to compare among treatments for each year. Tukey test was used for a multiple-comparison between all treatments. Different upper and lower case letters indicate significant differences ($P < 0.05$) after the multiple-comparison correction before (2010) and after (2012) the warming experiment, respectively.

Supplementary Table 1 | Summary of the model selection procedure for the variation in herbivory rate in **(A)** the elevational-gradient study and **(B)** the warming experiment. The most likely multivariate models (ΔAIC < 2) are shown. The Log-likelihood value (LL), difference of the AIC between the current and the smallest model (ΔAIC), and Akaike weight (w_i) are shown.

Supplementary Table 2 | Summary of the best-fit models by GLMs explaining the variation in herbivory rate in **(A)** the elevational-gradient study and **(B)** the warming experiment.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 27 October 2021
doi: 10.3389/ffgc.2021.709403

Spatiotemporal Distribution of Herbivorous Insects Along Always-Green Mountaintop Forest Islands

Juliana Kuchenbecker[1]*, Luiz Eduardo Macedo-Reis[1,2], Marcílio Fagundes[3] and Frederico S. Neves[1,4]

[1] Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2] Maram Projetos Ambientais, Ipatinga, Brazil, [3] Departamento de Biologia Geral, Centro de Ciências Biológicas e da Saúde, Universidade Estadual de Montes Claros, Montes Claros, Brazil, [4] Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Insects make up the bulk of terrestrial diversity and about half of insect species are herbivores that have direct relationships with their host plants and are the basis of the entire food chain, on which wildlife and humanity depend. Some herbivorous insect traits, such as their spatio-temporal distribution, are especially relevant in the current scenario of global changes, which are more pronounced in high elevation areas, helping to improve the effectiveness of conservation actions. Here we evaluated the influence that different spatiotemporal scales have on three free-feeding herbivorous insect guilds (fluid-feeding, leaf-chewing, and xylophagous insects) in montane forest islands immersed in a grassland-dominated matrix (*campo rupestre*). We assessed whether species turnover or nestedness was the main component determining both spatial and temporal species composition variation (β-diversity) of the herbivorous insect community. We also checked the temporal effect on herbivorous insect guilds composition between vertical strata. We sampled herbivorous insects during two summers and two winters in 14 forest islands of different sizes and shapes in a natural mountainous fragment located in southeastern Brazil. A total of 6597 herbivorous insects representing 557 morphospecies were sampled, 290 of which were fluid-feeding, 147 leaf-chewing and 120 xylophagous insects. We found a main contribution of time scale in the organization of the herbivorous insect composition sampled in this study, mainly by turnover, with small differences among guilds. Additionally, we could see that climate determined the local variation of species, corroborating that we have a highly variable always-green system over space and time where the understory community varies less in comparison to the canopy community. Our findings suggest that long-term ecological research on herbivorous community structure in relation to climatic variation is a key element for future investigations, which can be decisive for the conservation

Edited by:
Louise A. Ashton,
The University of Hong Kong,
Hong Kong, SAR China

Reviewed by:
Antonio Rodriguez,
Swedish University of Agricultural Sciences, Sweden
Aurélien Sallé,
Université d'Orléans, France

****Correspondence:***
Juliana Kuchenbecker
jubelisario@gmail.com

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *13 May 2021*
Accepted: *07 October 2021*
Published: *27 October 2021*

Citation:
Kuchenbecker J, Macedo-Reis LE, Fagundes M and Neves FS (2021) Spatiotemporal Distribution of Herbivorous Insects Along Always-Green Mountaintop Forest Islands. Front. For. Glob. Change 4:709403. doi: 10.3389/ffgc.2021.709403

of herbivorous insect communities. We also suggest that the effects of anthropogenic pressures must be monitored in this system, since these forest islands may serve as warming refuges in a fragmented landscape holding an invaluable diversity of species that, without these old-growth forest reservoirs, would be doomed to disappear.

Keywords: Atlantic Forest, *campo rupestre*, chewing insects, fluid-feeding insects, xylophagous insects, β-diversity, Malaise trap

INTRODUCTION

Insects make up the bulk of terrestrial diversity (Sabrosky, 1953; Chapman, 2009; Stork, 2018), being considered the most important animals in terrestrial environments (Wilson, 1987). However, recent case studies have shown drastic declines in the total biomass of different insect groups (Schuch et al., 2012; Dirzo et al., 2014; Hallmann et al., 2017; Lister and Garcia, 2018), which can lead to the loss of multiple critical ecosystem services provided by them (e.g., pollination and decomposition) (Ashton et al., 2019). Out of their vast numbers, about half are represented by herbivorous insects that have direct relationships with their host plants (Stork, 2018) and are the basis of the entire food chain, on which wildlife and humanity depend (Cardoso et al., 2020). Many feeding guilds have evolved among herbivorous insects (e.g., fluid-feeding, leaf-chewing and xylophagous insect guilds), with high variability in spatio-temporal distribution (Neves et al., 2014; Kuchenbecker and Fagundes, 2018; Macedo-Reis et al., 2019). Several works report the distinction between some different guilds and the importance of working with each one (see Novais et al., 2018; Monteiro et al., 2020). Changes in habitat characteristics, such as vegetation structure and climatic variations, determine the organization of these guilds in different ways, and changes in the herbivore community have substantial effects on biological interactions throughout the food chain (Leal et al., 2016; Novais et al., 2016, 2019). In addition, some herbivorous insect traits, such as their spatio-temporal distribution (Lewinsohn et al., 2005), are especially relevant in the current scenario of global changes that are more pronounced in high elevation areas (Janzen and Hallwachs, 2019), helping to improve the effectiveness of conservation actions (Cooke et al., 2013; Callisto et al., 2019).

Interpreting the diversity distribution patterns of different herbivorous insect feeding guilds enables us to understand the influence of distinct ecological processes structuring herbivorous insect communities (see Novotny and Weiblen, 2005; Wardhaugh, 2014). Since tropical rainforests are notoriously heterogeneous environments (Basset et al., 2012; Kitching et al., 2013), one of the obvious spatial gradients of species diversity change is related to geographic distance. Insect diversity changes in this horizontal dimension may be driven by multiple factors, including the distance among habitat patches or even the distance of these patches to a continuous forest (e.g., da Silva et al., 2019). In addition, dispersal abilities of herbivorous insects differ among guilds, which may potentially influence them to move in different ways within and among habitats (Tews et al., 2004; Macedo-Reis et al., 2019). Seasonal variability is also common in tropical environments, especially with regard to species composition (Tylianakis et al., 2005), which is affected by resource availability (Shaw, 2004; Inclán et al., 2014) and can be guild-dependent. With a few notable exceptions (see Macedo-Reis et al., 2016, 2019), temporal patterns of insect diversity that reflect seasonal climatic conditions are frequently ignored in tropical rainforests (Didham and Springate, 2003). Particularly, in seasonal environments, both spatial and temporal patterns among herbivorous insects need more attention in order for us to understand these diversity patterns.

Naturally fragmented landscapes are exceptionally adequate for evaluating patterns and mechanisms that determine the distribution of species in environments without confounding effects of human-driven fragmentation and habitat loss (Fahrig, 2017; Haddad et al., 2017). Unlike oceanic islands, which are surrounded by a matrix that is a barrier to most terrestrial fauna, forest islands have more permeable matrices that harbor an important percentage of fauna (Cook et al., 2004; Driscoll, 2005; Yekwayo et al., 2016) and provide additional resources to species (Öckinger et al., 2012). In such systems, that occur immersed in grassland-dominated ecosystems on tropical mountaintops (Streher et al., 2017), vagile organisms like wasps, bees, dung beetles and fruit-feeding butterflies can seek different resources (da Silva et al., 2019; Perillo et al., 2020; Silva et al., 2020), where habitat diversity and configuration of the landscape may elicit different responses among them (Neves et al., 2021). Although the distribution of host plants at a given scale represents food resource availability and physical structure for herbivorous insects to find shelter and breeding sites (Lewinsohn and Roslin, 2008), evidence of this relationship in forests patches at landscape scales and its effects on herbivorous insect communities is still poorly documented (Rossetti et al., 2019).

There is a substantial change in habitat conditions between understory and canopy strata (Shaw, 2004; Basset et al., 2015), and this vertical stratification is often considered to be a key factor promoting the extreme diversity of tropical forests (Oliveira and Scheffers, 2019). This is mainly because vertical stratification is associated with major shifts in abiotic conditions, highlighting the great variety in resource availability that enables greater species coexistence at small scales (Brown, 1981; Scheffers et al., 2017). In fact, herbivorous insect diversity changes in this vertical dimension may be driven by factors such as resource quantity and quality and microclimate conditions (DeVries et al., 1999; Basset et al., 2001, 2003a,b; Brehm, 2007) and they usually show a clear stratification that decreases from the canopy to lower strata (Basset et al., 1992, 2003a,b; Neves et al., 2014). Nevertheless, few studies have investigated whether local patterns of species diversity are related to vertical stratification of different insect feeding guilds, which may be more informative regarding the functional role of arthropods in tropical forests (but see Grimbacher and Stork, 2007; Neves et al., 2014). Furthermore, along with the increasing interest in canopy ecosystems (Nadkarni et al., 2011; Silva J. O. et al., 2017; Swart et al., 2020), here we simultaneously measured herbivorous insect species diversity in both canopy and understory environments through time to contribute to the current knowledge on the subject (DeVries, 1988; Wolda, 1992; Skvarla et al., 2021).

The joint use of multiple scales (α-, β-, and γ-diversities; Whittaker, 1960) as well as a complimentary process-focused approach to decompose β-diversity into turnover (species substitution) and nestedness (a gradient of number of species) is an adequate framework to further advance our knowledge about how communities are structured in space and time, and the underlying mechanisms driving diversity patterns (Baselga, 2010). Indeed, species turnover has consistently been reported as the main component of total β-diversity for a wide range of taxa across different ecosystems (Soininen et al., 2018), and is a recurrent feature among insect communities (Leal et al., 2016; Silva L. F. et al., 2017; Macedo-Reis et al., 2019). However, such studies are still very necessary because distribution patterns vary with the scale of observation, which can range from centimeters to kilometers and from days to years (Allan and Castillo, 2007). Thus, it is important to assess both the horizontal and vertical spatio-temporal β-diversity in order to understand the mechanisms that determine the organization of insect communities, especially in megadiverse environments such as tropical forests.

Since global change would lead to multifaceted ecosystem modifications, including shifts in species distribution ranges and diversity (Chen et al., 2011) and other unpredictable cascading effects at different levels of ecological organization (Peñuelas et al., 2013), here we aimed to explore the spatiotemporal patterns of herbivorous insect diversity (i.e., richness, abundance, and composition) in forest islands in a mountain range in eastern South America (Coelho et al., 2016). Four spatiotemporal scales were considered: three spatial scales (within Malaise traps; between strata of vegetation within forest sites; and among forest islands within sampling dates) and one temporal scale (over 4 sampling dates). We then tested (i) whether different spatiotemporal scales contribute in a different way to the diversity of three herbivorous insect guilds (i.e., fluid-feeding, leaf-chewing, and xylophagous) in the forest islands; (ii) whether the variation of species composition over four seasons (spatial and temporal β-diversity) is determined by substitution (turnover), and (iii) whether the temporal variation of species composition (temporal β-diversity) varies between the vertical strata and among guilds. Specifically, we expect that:

i Spatial scale has the main contribution to the total diversity of herbivorous insects, since the forest islands are of different sizes, shapes and distances to the nearest island or to the continuous forest (Coelho et al., 2018) and the canopy faces higher levels of UV radiation, wind speed and variations in humidity and temperature than the understory (Basset et al., 2003b);

ii Species turnover among sites is more important than nestedness in determining community organization within a region, as nestedness patterns are considerably less common to herbivorous insects in fragmented habitats (Macedo-Reis et al., 2019);

iii Canopy presents the higher variation in species composition (temporal β-diversity) for all three herbivorous insect guilds, as canopy environmental conditions and resource availability vary more than in the understory (Basset et al., 2003b).

MATERIALS AND METHODS

Study Area

We conducted this study in a natural forest archipelago situated in Serra do Cipó (the southern portion of the Espinhaço Range), Minas Gerais state, southeastern Brazil (19°14′19″S, 43°31′35″W; **Figure 1**). The Espinhaço formation is an important mountainous formation in Brazil due to its high biodiversity and endemism (Silveira et al., 2016; Miola et al., 2020). It extends almost continuously for 1200 km from northeastern to southeastern Brazil, across two states (Bahia and Minas Gerais), and acting as a humidity barrier between two biomes, the Cerrado (Brazilian savannah) to the west and the Atlantic Forest to the east. Above 900 m. a.s.l. in this region a continuous grassland matrix called *campo rupestre* is predominant. In the *campo rupestre* matrix there are some forest islands that merge as natural patches of vegetation from mountaintops. These forest islands occur mainly at 1200 m a.s.l. and their maintenance is dependent on specific climate and soil parameters (see Coelho et al., 2016 for a review). They have a floristic composition similar to seasonal semi-deciduous forests associated with the Atlantic Forest domain (Coelho et al., 2016, 2018). The species composition includes families with wide geographical distributions, although many species are largely endemic and reveal the former isolation of this refuge, which can be called a cloud forest. Compared with lowland forests, these forest islands have a lower canopy and a greater abundance of epiphytes, with a significant representation of Araceae, Orchidaceae, Bromeliaceae, Pteridophyta, and Bryophyta. This elevated representativeness of epiphytes is a consequence of the high humidity in these formations (Whitmore, 1990; Van der Hammen, 1995; Webster, 1995). The plant families with the greatest species richness in these forest islands are Myrtaceae, Lauraceae, and Melastomataceae, followed by Fabaceae, Asteraceae, and Rubiaceae (Coelho et al., 2016). Parameters such as density, diameter, average height, richness and diversity of plant species vary conspicuously between the edge and the nuclear zones of the forest islands. The soil also differs spatially in its physicochemical characteristics and its conditions in nuclear zones are more favorable to the establishment of characteristic species of late stages of succession (Coelho et al., 2016). The climate is mesothermic (Cwb in the Köppen classification), with dry winters and rainy summers, a mean annual rainfall of 1500 mm and average annual temperature ranging from 17.4 to 19.8°C (Ferrari et al., 2016; Silveira et al., 2016).

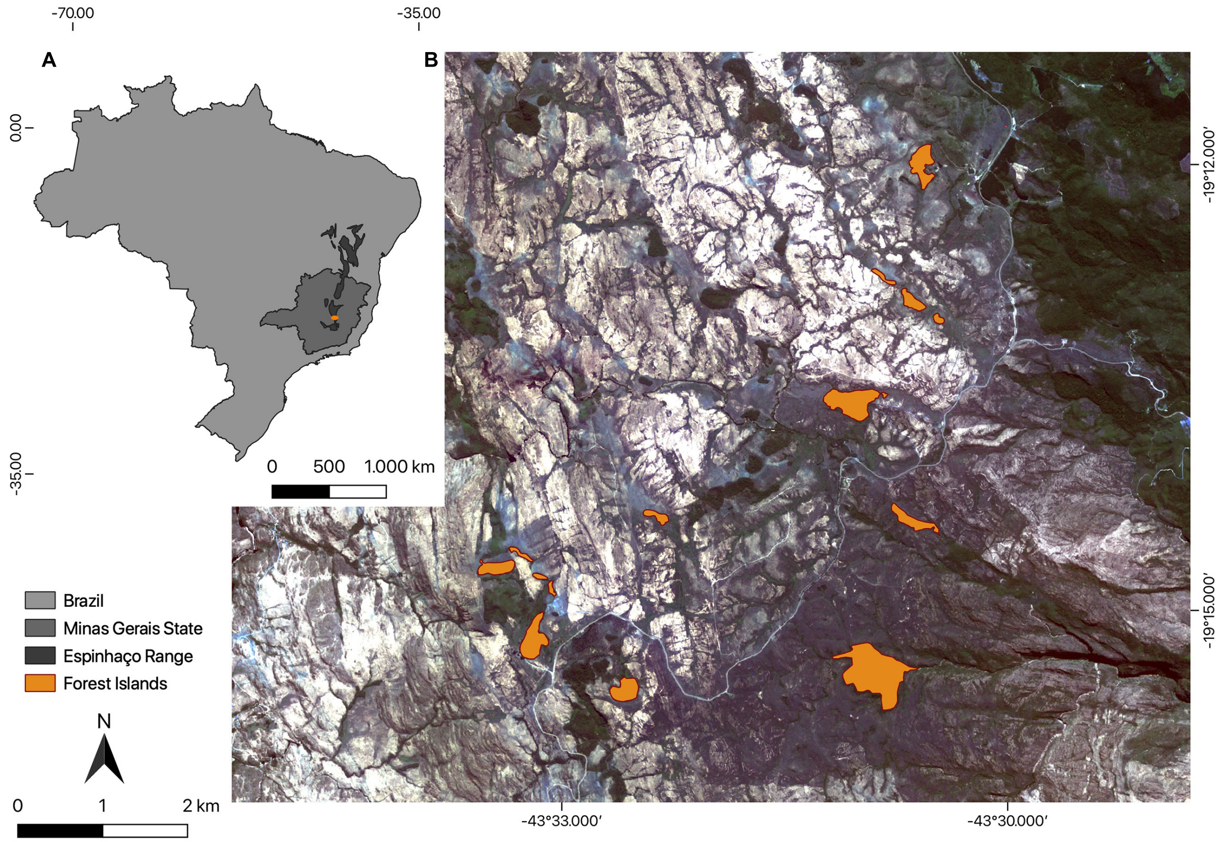

FIGURE 1 | Map showing **(A)** Espinhaço Range in Brazil and **(B)** the 14 naturally fragmented forest islands (orange shapes) where we sampled herbivorous insects in a *campo rupestre* matrix of the Serra do Cipó, southern portion of the Espinhaço Range Biosphere Reserve, Minas Gerais, Brazil.

Sampling Design

A total of 14 forest islands were selected. The chosen areas vary in size (1.21–39.89 ha, mean = 8.21 ha, SD = 10.24 ha) and shape (from circular to elongated limits), and are spaced at variable distances (0.1–1.5 km) (see **Supplementary Table 1** for more details). A 20 × 50 m plot was established in the center of each forest patch, where sampling was performed over two consecutive years (2014–2015) with two sampling events in the summer – rainy season (February 9–16, 2014; February 21–28, 2015) and two in the winter – dry season (August 16–23, 2014; August 22–29, 2015). In each sampling period we collected herbivorous insects using two Malaise traps (one soil Malaise trap and one canopy Malaise Window trap – mean height 4,5m ± SE 0,44 – see **Supplementary Table 1** to check the heights of each Malaise window traps) located in the center of each plot, which remained in the field for seven days (9408 trap-hours for each type) (Perillo et al., 2020). These types of traps are one of the most widely used non-attractant, static insect traps in surveys of insect diversity (Noyes, 1989; Muirhead-Thomson, 1991; Southwood and Henderson, 2000; also see Skvarla et al., 2021 for a review). Collected herbivorous insects were grouped into one of three guilds according to their feeding habits: fluid-feeding, leaf-chewing or xylophagous insects (wood-associated fauna) (Macedo-Reis et al., 2019). We considered as herbivorous insects every insect belonging to families that directly or indirectly depend on plant resources for survival (Moran and Southwood, 1982; Neves et al., 2014; Macedo-Reis et al., 2019). The insects were identified using taxonomic keys and separated into morphospecies based on external morphological features. All collected insects were stored in the entomological collection of the Laboratory of Insect Ecology at the Federal University of Minas Gerais (Universidade Federal de Minas Gerais – UFMG).

Herbivorous Insect Guilds

We chose to work with exophagous insects, which are free-living organisms that move within and between plant species in search of resources. They present different life-forms (Neves et al., 2010; Araújo, 2013) and can be classified into several trophic guilds according to their feeding habit (Novotny et al., 2010), such as fluid-feeding herbivores, with a modified oral apparatus or stylet to suck plant sap (Ribeiro et al., 1994); chewing herbivores, with well-developed jaws specialized in cutting and grinding plant tissue (Raupp, 1985); and xylophagous herbivores, that breed in inner bark (dead phloem tissue; Raffa et al., 2015) and some feed on symbiotic "ambrosia" fungi that grow in the nutrient poor xylem tissue of woody plants (Gohli et al., 2017). In the case of fluid-feeding herbivores, the stylet needs to overcome the physical protective barriers of plants, such as waxes and trichomes, to access plant tissues. Thus, they are mostly affected by sap nutrient content (Price, 1997; Huberty and Denno, 2006) and tend to specialize more than chewing and xylophagous herbivores by surviving for several generations on a single host plant (Denno and Perfect, 1994; Ødegaard, 2003; Novotny et al., 2010). Chewing herbivores generally have a broad diet of host plant species and are directly influenced by leaf mechanical properties and chemistry (Peeters, 2002; Read and Sanson, 2003). They are considered to comprise the most abundant and species-rich guild (Novotny et al., 2012). Xylophagous herbivores feed on several host families with generally low host specificity (Beaver, 1979; Macedo-Reis et al., 2016) and many of them are capable of quick dispersal and establishment in new habitats (Allen and Humble, 2002; Haack, 2006). The perception of landscape structure by all these insect guilds in different habitats depends on their dispersal ability and foraging range (Weibull et al., 2000; Toivonen et al., 2017; da Silva et al., 2019), which differ among guilds due to several factors, including habitat selection and mortality risks, potentially influencing them to move within forest environments (Tews et al., 2004; Macedo-Reis et al., 2019). Also, these herbivorous insect feeding guilds may respond differently to the same habitat conditions due to their level of specialization and life history constraints (Denno and Roderick, 1991; Vehviläinen et al., 2007; Sobek et al., 2009). Thus, different feeding guilds respond to environmental characteristics differently, especially if we consider different spatial scales (Neves et al., 2013; Lourenço et al., 2015).

Data Analysis

In order to show extrapolated values of species richness to a similar number of individuals we constructed rarefaction curves using the R package "iNEXT" (Chao et al., 2014; Hsieh et al., 2016), available at https://chao.shinyapps.io/iNEXTOnline/. We accounted for every individual collected in the two Malaise traps, in all different seasons, for the three guilds of herbivorous insects. Thus, we estimated the sample completeness for each individual sampled. For extrapolation curves, the number of individuals was up to twice the actual reference size (Chao et al., 2013). We then built rarefaction curves with different sampling intensities, these curves representing the gamma diversity of each stratum per herbivorous insect guild. The sample units (i.e., the 14 forest islands) were used as the independent variable and the richness of each herbivorous insect guild was the dependent variable. Thus, we were able to obtain information about the completeness of herbivorous insect species in relation to each sampling unit. We also assessed the alpha diversity in our smallest sampling unit for each herbivorous insect guild. For this, we used the hill number ^{0}D to show the observed accumulated species richness for our sampling units, with their respective confidence intervals. The non-overlapping of confidence intervals indicates that the insect richness found in the canopy is lower for all three guilds.

To test our hypothesis that spatial scale has a main contribution to the total diversity of herbivorous insects, we partitioned the γ-diversity (i.e., diversity in the 14 islands) into α-diversity (i.e., diversity within Malaise traps) and β-diversity (which was partitioned into $\beta 1$ – between strata within forest sites; $\beta 2$ – among forest islands within sampling dates and $\beta 3$ – over sampling dates). We performed these analyses with the "adipart" (additive diversity partitioning) function, which works with a statistic returned by a function that is assessed according to a nested hierarchical sampling design (our three dimensions), both from the R package "vegan" (Oksanen et al., 2018). We used this function only to describe the species richness per dimension.

We assessed the spatial and temporal variations of the herbivorous species composition (β-diversity) using the Sørensen dissimilarity index in a multiple-spatial and time approach (see Baselga, 2010; Neves et al., 2021). So, we decomposed total spatial and temporal β-diversity among the forest islands (using the Sørensen and Simpson index of similarity), which we partitioned into turnover and nestedness-related components using the "beta.multi" function in the "betapart" R package (Baselga and Orme, 2012). Data were aggregated by forest sites for the spatial partition of β-diversity among sites and aggregated by sampling dates for the partition of β-diversity over sampling dates. The function 'beta-multi' includes the functions to compute multiple-site dissimilarities β_{SOR}, β_{SIM} and β_{NES}, called beta.SOR(x), beta.SIM(x), and beta.NES(x), respectively, where x is a data frame in which sites are rows and species are columns. We used the results of this calculation to determine whether species substitution or species loss/gain influenced the general spatial pattern of temporal β-diversity (Baselga, 2010; Neves et al., 2021).

Linear mixed models (LMMs) were used to test the effect of the strata on each herbivore guild (fluid-feeding, leaf-chewing, and xylophagous) temporal β-diversity over seasonal samples, where temporal β-diversity was used as a dependent variable and the strata as an independent variable. The identity of each forest island (14 in total) was used as a random factor in LMMs, to account for spatial pseudoreplication (Bolker et al., 2009). The lme4 package and lmer function were used to perform LMM analyses (Bates et al., 2018). The function Anova of package car, which allows ANOVA in mixed models, was used to deem variables significant or not. All models were subjected to residual analyses to check for model fit and error structure suitability (Crawley, 2013). All statistical analyses were performed using R software v.4.0.

RESULTS

General Patterns

We recorded a total of 6597 herbivorous insects belonging to 41 families, two subfamilies and 557 morphospecies (**Table 1**). Among these morphospecies, 290 were fluid-feeding, 147 were leaf-chewing and 120 were xylophagous insects. Among fluid-feeding herbivorous insects, Cicadellidae and Psyllidae were the richest in species (28%) and most abundant (72.9%), with the Cicadelidae family alone representing 22.1% and 69.7% of the total richness and abundance, respectively. For leaf-chewing insects, Chrysomelidae and Curculionidae were the richest in species (23.5%) and most abundant (13.4%) while for xylophagous insects, Cerambycidae, and Scolytinae were the richest in species (9.7%) and Cerambycidae and Anobiidae were the most abundant (2.1%), together representing 61.2% of the total richness and 88.4% of the total individuals sampled. There

TABLE 1 | Herbivorous insects morphospecies richness and abundance sampled in Serra do Cipó, Minas Gerais, Brazil.

Guild	Taxa	Total richness	Total abundance	Strata			
				Understory		Canopy	
				Abundance	Richness	Abundance	Richness
Fluid-feeding	Achilidae	28	150	145	28	5	2
	Aetalionidae	2	2	2	2	0	0
	Aphididae	2	3	2	2	1	1
	Aphrophoridae	2	2	2	2	0	0
	Berytidae	1	4	4	1	0	0
	Cercopidae	7	10	9	7	1	1
	Cicadellidae	123	4601	4349	113	252	41
	Cixiidae	11	15	15	11	0	0
	Cydnidae	1	8	8	1	0	0
	Delphacidae	6	10	10	6	0	0
	Derbidae	3	99	98	3	1	1
	Flatidae	1	1	1	1	0	0
	Fulgoridae	1	3	3	1	0	0
	Issidae	2	5	5	2	0	0
	Kinaridae	1	1	1	1	0	0
	Largidae	1	1	1	1	0	0
	Lygaeidae	10	77	74	10	3	3
	Membracidae	16	33	32	15	1	1
	Miridae	21	81	70	20	11	3
	Pentatomidae	4	4	4	4	0	0
	Psyllidae	33	210	183	27	27	12
	Pyrrhocoridae	1	1	1	1	0	0
	Rhopalidae	3	4	3	3	1	1
	Thyreocoridae	1	1	1	1	0	0
	Tingidae	9	20	20	9	0	0
Leaf-chewing	Anostostomatidae	2	2	2	2	0	0
	Attelabidae	1	4	4	1	0	0
	Chrysomelidae	69	688	639	67	49	18
	Curculionidae	62	196	181	57	15	13
	Erotylidae	6	9	9	6	0	0
	Gryllidae	3	17	17	3	0	0
	Megalopodidae	1	1	1	1	0	0
	Meloidae	1	1	1	1	0	0
	Tettigoniidae	2	2	1	1	1	1
Xylophagous	Anobiidae	21	67	35	17	32	14
	Anthribidae	18	30	26	17	4	3
	Bostrichidae	5	19	3	3	16	2
	Brentidae	7	21	19	7	2	1
	Buprestidae	4	14	14	4	0	0
	Cerambycidae	27	72	58	23	14	8
	Cupedidae	4	4	0	0	4	4
	Platypodinae	7	42	30	4	12	7
	Scolytinae	27	62	12	8	50	22

was also a high number of rarely sampled species, comprising 244 singletons (43.8% of the total) and 84 doubletons (15.1% of the total). Of these, 120 singletons and 38 doubletons belonged to fluid-feeding insects guild, 58 singletons and 26 doubletons belonged to leaf-chewing insects guild and 65 singletons and 20 doubletons belonged to xylophagous insects guild. In addition, we had a greater number of unique morphospecies collected in the understory for all guilds. A total of 398 (71.5%) morphospecies were collected exclusively in the understory (224 fluid-feeding insects; 115 leaf-chewing insects; 59 xylophagous insects), while 63 (11.3%) were collected exclusively in the canopy (18 fluid-feeding insects; eight leaf-chewing insects; 37

xylophagous insects) and 96 (17.2%) in both strata (48 fluid-feeding insects; 24 leaf-chewing insects; 24 xylophagous insects).

The sampling effort was sufficient to characterize the insect community sampled, with the rarefaction-extrapolation accumulation curves presenting a greater sampling coverage for those carried out in the understory (**Supplementary Figure 1**), where the observed and extrapolated guilds richness and abundance was higher. The greatest sampling was found for fluid-feeding insects, followed by leaf-chewing insects and xylophagous insects. We observed that the fluid-feeding species richness ranged between 66 and 272 species and its total abundance ranged between 303 and 5043, which corresponds to 87% and 97% of the sampling coverage values, and 91% and 99% of the extrapolated sampling coverage abundance along the canopy and the understory, respectively (extrapolated sample coverage richness = 99 and 357 along the canopy and the understory, respectively). For the leaf-chewing insects, we observed species richness ranging between 32 and 139 species and its total abundance ranged between 65 and 855, which corresponds to 68% and 93% of the sampling coverage values, and 85% and 97% of the extrapolated sampling coverage abundance along the canopy and the understory, respectively (extrapolated sample coverage richness = 46 and 176 along the canopy and the understory, respectively). Finally, we observed that xylophagous species richness ranged between 61 and 83 species and its total abundance ranged between 134 and 197, which corresponds to 74% and 72% of the sampling coverage values, and 86% and 80% of the extrapolated sampling coverage abundance along the canopy and the understory, respectively (extrapolated sample coverage richness = 87 and 129 along the canopy and the understory, respectively). We also found that the diversity of the herbivorous insects sampled in this study was higher in the understory for all three guilds for both observed and extrapolated values (**Figure 2**).

Partition of Diversity

We observed a main contribution of temporal scale (β3) to the total diversity when partitioning the total insect species richness for each guild into its additive components [i.e., within Malaise traps (α), between strata within forest sites (β1), among forest islands within sampling dates (β2), and over sampling dates (β3)] (**Figure 3**). On the other hand, we found a smaller contribution of spatial scale between strata (β1) to the total diversity for each insect guild. The observed β3 over sampling dates was slightly greater for xylophagous insects (86.49%) than for fluid-feeding (79.90%) and leaf-chewing insects (83.43%). In contrast, the contribution of α, β1 and β2 was, respectively, only 7.45%, 5.17%, and 7.48% for fluid-feeding insects, 6.19%, 3.38%, and 7% for leaf-chewing insects and 3.95%, 3.25%, and 6.31% for xylophagous insects. When we decomposed spatial and temporal β-diversity into its turnover and nestedness components, we verified that it was mostly represented by the turnover (β_{SIM}) component in all cases. This component explained more than 75% of the total β-diversity for all guilds and strata (**Table 2**).

The species composition over time (Temporal β-diversity/β_{SOR}) varied between vegetation strata for fluid-feeding ($F = 54.12$; Df = 1; $p < 0.001$) and leaf-chewing insects ($F = 20.16$; Df = 1; $p < 0.001$), with higher values for the canopy (**Figure 4**). Temporal β-diversity of the xylophagous insects did not show variation between strata ($F = 1.27$; Df = 1; $p = 0.27$).

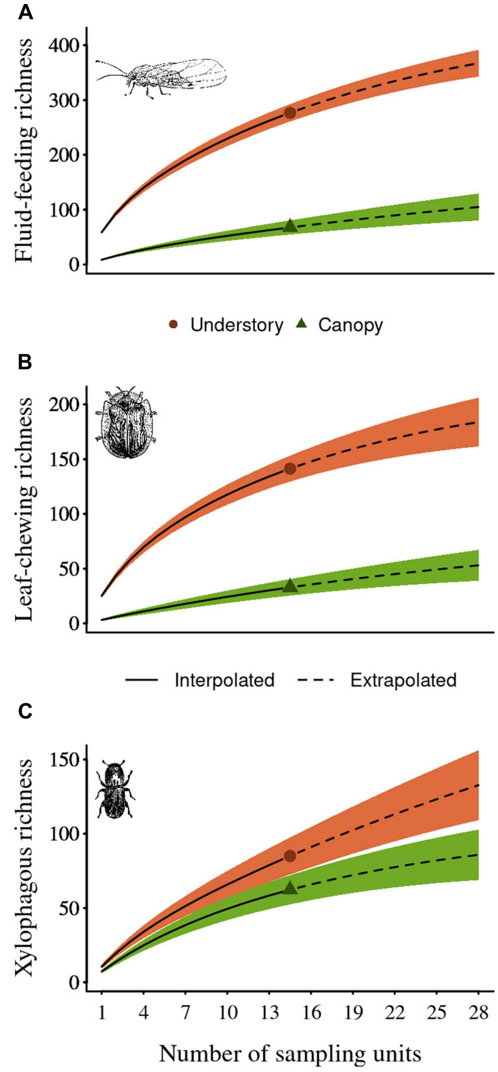

FIGURE 2 | Species diversity curves (^{0}D) for samples (solid lines) and extrapolated samples (dashed lines) as a function of sample size according to the count of incidences of **(A)** fluid feeding herbivores, **(B)** leaf chewing herbivores, and **(C)** xylophagous herbivores in each sampling unit (i.e., forest island). The bar error represents the 95 confidence interval. The red circles in the red line represent the observed samples for understory and the green triangle for the canopy, obtained by a bootstrap method based on 1000 iterations.

DISCUSSION

In this study, we evaluated the influence that different spatiotemporal scales have on three free-feeding herbivorous insect guilds (fluid-feeding, chewing and xylophagous insects) in montane forest islands immersed in a grassland-dominated matrix (*campo rupestre*). We highlight a main contribution

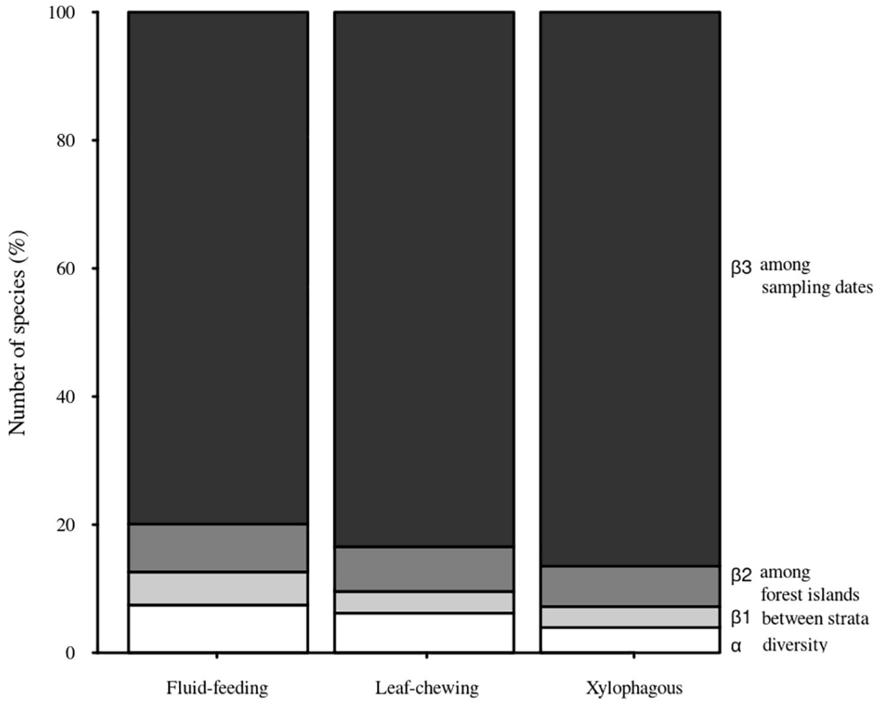

FIGURE 3 | Contribution of the diversity of Malaise traps (α), between strata within forest sites (β1), among forest islands within sampling dates (β2) and over sampling dates (β3) to the total diversity (γ) of herbivorous insects sampled in Serra do Cipó, Minas Gerais, Brazil.

TABLE 2 | β-Diversity using the Sørensen index for herbivorous insects collected in both strata and the importance (%) of turnover (β_{SIM}/β_{SOR}) for each guild at each stratum.

Stratum	Guild	Spatial β-diversity	Turnover (%)	Temporal β-diversity	Turnover (%)
Understory	Fluid-feeding	0.85	0.96	0.62	0.94
	Leaf-chewing	0.89	0.97	0.65	0.79
	Xylophagous	0.93	0.96	0.82	0.91
Canopy	Fluid-feeding	0.92	0.97	0.80	0.93
	Leaf-chewing	0.95	0.97	0.88	0.92
	Xylophagous	0.94	0.96	0.90	0.90

of time scale in the organization of the herbivorous insect community, mainly by turnover, with small differences among guilds. We could see the role of seasonality determining the local variation of species composition, corroborating that we have a highly variable always-green system over space and time, where the understory community varies less compared to the canopy community.

Among the several approaches that have been proposed to study patterns and processes that rule insect diversity across fragmented habitats, spatial metrics, such as patch (e.g., canopy cover, distance to continuous forest) and landscape attributes (e.g., percentage of forest in the landscape, number of patches, patch shape complexity) have been acknowledged to affect diversity patterns and distribution of several groups of insects in mountainous forest islands (da Silva et al., 2019; Perillo et al., 2020). Additionally, studies on temporal issues have drawn attention to help us understand and explain biodiversity dynamics in terrestrial fragmented habitats. Here, we found that the temporal scale exceeded the spatial scale in explaining herbivorous insect diversity, which is the opposite of what was expected for our first hypothesis. Most studies have found a higher predictive power of spatial scale than temporal ones over insect community structure in tropical ecosystems (da Silva et al., 2019; Castro et al., 2020). However, we found a higher importance of seasonal variation on the composition of all three insect guilds sampled in this study. These results indicate that, more than patch attributes, factors such as seasonal variation in temperature and humidity are drivers to temporal variation in resources that directly affect herbivorous insects that inhabit these forest islands. Thus, despite being an evergreen forest, the climatic seasonality of these forest islands is the most important driver for all herbivorous insect guilds sampled in this study and contribute more to their diversity and differences in species composition than spatial factors such as the distance among the forest islands

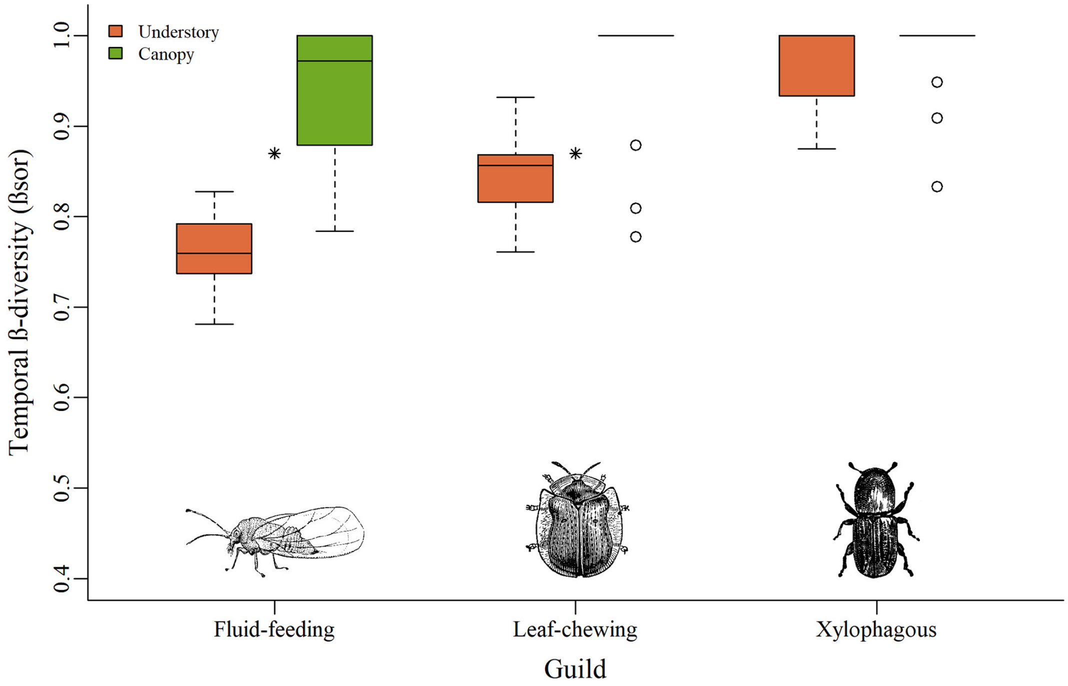

FIGURE 4 | Boxplots of temporal β-diversity relating each forest island to the sampled stratum, for all three guilds of herbivorous insects. Bars represent the interquartile range with the median value. Circles indicate outliers. Stars reflect temporal β-diversity of the two insect guilds that showed relation with the stratum. Dashed lines indicate minimum and maximum values.

or the vertical stratification (see Macedo-Reis et al., 2016; Novais et al., 2018).

We also found that the vertical stratum was an important factor driving the composition of fluid-feeding and leaf-chewing insect guilds, partially corroborating our third hypothesis. Similarly to our findings, wasps and bees (Perillo et al., 2020) and dung-beetles (da Silva et al., 2019) also have high temporal β-diversity (βsor) in this study system, indicating that the composition of insect communities varies greatly throughout seasons. In fact, all three herbivorous insect guilds, regardless of the strata, showed high temporal β-diversity, corroborating that we do have a highly variable system over space and time. However, fluid-feeding and leaf-chewing insects presented a greater temporal β-diversity for the canopy, which is the opposite of the pattern found for ants in a tropical dry-forest (Neves et al., 2021). The temporal β-diversity of both fluid-feeding and chewing insects being higher in the canopy than in the understory may be linked to the different effects of seasonality on specific microhabitats of these two strata. However, it may also be related to the insect diversity sampled in the understory that was much higher than that sampled in the canopy (which harbors more rare species and therefore has less redundancy) (see Skvarla et al., 2021). Besides, it is important to note that the insect guilds can respond differently to seasonal changes in resource availability between canopy and understory (see also Neves et al., 2014; Macedo-Reis et al., 2019).

Tropical forests have a vertical gradient of microclimatic conditions from the ground level to the canopy, with an increase of temperature and solar radiation, and a decrease in air humidity (Law et al., 2019). In this way, for herbivorous insects associated with forest islands system, it would be reasonable to think that the canopy has more unstable characteristics than the understory. For example, the increase in temperature and solar incidence is directly related to the survival of beetles from the Chrysomelidae and Curculionidae families, since it represents one of the main mortality factors of these insects (Lill and Marquis, 2007). In addition, the greater solar radiation can cause adult individuals of the Cicadellidae family to disperse less or to seek more humid and shaded areas (Ott and Carvalho, 2001; but also see Lessio and Alma, 2004). However, the canopy also has greater turnover of leaves and flowers (Basset et al., 2003b), resources that are especially important for fluid-feeding and chewing insects (Basset et al., 2003a). In this regard, it is known that the life cycle of individuals from the Cicadellidae and Psyllidae family probably follows the phenological pattern of leaf exchange in plants (Novais et al., 2018). Thus, we can expect that an environment with more changes in its conditions that presents, at the same time, more and better resource availability for its associated insects, will present greater temporal β-diversity for fluid-feeding and chewing insects. The same could not be observed for the guild of xylophagous insects, which is similar to the pattern reported among Scolytinae in Malaysian forests (Simon et al., 2003). This pattern could be explained by their close association with the range of suitable wood availability (Grove, 2002; Hulcr et al., 2008), and not leaves. Therefore, wood diameter and air moisture content seem to be better predictors of their diversity

(Macedo-Reis et al., 2016), since they feed and spend much of their lives inside the bark, with little interaction with the external environment (Wood, 1982; Zuo et al., 2016). Here, it is important to emphasize that although Lepidoptera like the Symphita larvae are very important representatives of chewing herbivorous insect guild, they were not considered in our results especially due to the limitations of sampling traps (see Schmidt, 2016; Rosa et al., 2019; Skvarla et al., 2021).

When we consider the β-diversity decomposition into turnover and nestedness, we found species turnover as the main driver of β-diversities for the three herbivorous insect guilds in all spatio-temporal scales. This high species replacement supported our second hypothesis and has been documented in similar environments as the most important component of dissimilarity in species over time (Oliver et al., 2016; Nunes et al., 2020; Neves et al., 2021). A greater species turnover implies in high substitution of species, which indicates that each forest island must have a unique community. From this perspective, forest islands can be considered transient environments with great conservation value for many species (Vieira et al., 2008), where their presence can be considered a sample of the dispersers coming from continuous forest and even from open surrounding areas, which, in part, can explain the high rates of rare species (43.8% singletons and 15.1% doubletons) sampled in this study. Similar rates of rare species have been commonly observed in tropical arthropod studies (Coddington et al., 2009), and especially in high-elevation tropical mountains (Perillo et al., 2017). Thus, we argue that species turnover is an important factor for maintaining high diversity in forest islands in tropical mountain areas. Besides, it is important to notice that this system must be linked to metacommunity structure. Preliminary data from a long-term research project (Long term ecological research from *campo rupestre* – CRSC) also points out to the existence of a dynamic of insect metacommunities among forest islands, where both local (size, shape) and landscape (degree of isolation) characteristics can jointly influence the spatiotemporal dynamics of the structure of metacommunities that inhabit this ecosystem. For example, recent studies carried out with different insect taxa, including ants (Brant et al., 2021), dung beetles (da Silva et al., 2019), fruit-feeding butterflies (Pereira et al., 2017), and wasps and bees (Perillo et al., 2020), have found high species turnover rates among forest islands, showing the importance of each forest island in maintaining the structure and conserving the communities associated with the entire archipelago. Despite the small distance among the forest islands, this provides a valid explanation of spatial and temporal dynamics, driven by habitat heterogeneity and environmental filters, with forest fragments connected by dispersal among habitats.

We presented a diverse array of spatiotemporal distributions among herbivorous insect guilds. Both spatial and temporal factors have mixed contributions in shaping the observed patterns, and the main differences among guilds reflect their use of resources. We showed that in altimontane forest systems there is a great change in the composition of herbivorous insects that are especially shaped by temporal variations in climatic characteristics. Besides, our findings suggest that there are differences between strata, where the understory of this studied system is more diverse and stable than the canopy. Since the montane forest islands are located at a higher elevation with a great propensity to be affected by global changes (Nunes et al., 2020), we suggest that long-term ecological research on herbivorous community structure in relation to climatic variation is a key element for future investigations, which can be decisive for the conservation of herbivorous insect communities. Beyond that, it can work as an advantageous strategy for the development of environmental conservation and monitoring programs, as long-term studies that include multiple sites within a regional species pool enable a thorough assessment of biodiversity change. Additionally, the effects of anthropogenic pressures, such as fire and the constant presence of cattle around this complex forest archipelago system, must be monitored (see Coelho et al., 2018) if we expect to manage and conserve biological diversity in a sustainable manner, since these forest islands may serve as warming refuges in a fragmented landscape holding an invaluable diversity of species that, without these old-growth forest reservoirs, would be doomed to disappear (see Janzen and Hallwachs, 2019). In a changing world, supporting and maintaining monitoring efforts in these systems, including other regions of the Espinhaço Range, will certainly bring an advance in knowledge and will be very useful for our current understanding of the effects of anthropogenic pressures and climate change on communities that are associated to naturally insular systems.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JK, LM-R, and FN: study design and data analyses. FN: project funding. JK: data collection. JK, LM-R, MF, and FN: writing the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the CNPq for funding the Long-Term Ecological Research "PELD Campos Rupestres da Serra do Cipó" (grant number 441515/2016-9) and FAPEMIG (grant number CRA-APQ-00311-15).

ACKNOWLEDGMENTS

We would like to highlight the invaluable help of Lucas Neves Perillo for assistance with field work and Marina Beirão for helping to draw up the map for this study. JK acknowledges a research scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) grant number (88887.288737/2018-00). FN thanks CNPq (306995/2019-0) for funding. Thank also go to Reserva Vellozia, GSG and Parque Nacional da Serra do Cipó for logistical support. This manuscript is an original research article submitted to journal Frontiers and Global Change in contribution to the research topic Understanding Patterns and Mechanisms of Forest Canopy Diversity and Ecosystem Functions in a Changing World.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.709403/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 27 October 2021
doi: 10.3389/ffgc.2021.727422

Persistent Effects of Historical Selective Logging on a Vascular Epiphyte Assemblage in the Forest Canopy of the Western Ghats, India

K. S. Seshadri[1]*, R. Ganesan[2] and Soubadra M. Devy[2]*

[1] Centre for Ecological Sciences, Indian Institute of Science, Bengaluru, India, [2] Ashoka Trust for Research in Ecology and the Environment, Bengaluru, India

Edited by:
Akihiro Nakamura,
Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (CAS), China

Reviewed by:
Romà Ogaya,
Ecological and Forestry Applications Research Centre (CREAF), Spain
Helena Einzmann,
Carl von Ossietzky Universität Oldenburg, Germany

***Correspondence:**
K. S. Seshadri
seshadriks@iisc.ac.in
Soubadra M. Devy
soubadra@atree.org

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: 18 June 2021
Accepted: 28 September 2021
Published: 27 October 2021

Citation:
Seshadri KS, Ganesan R and Devy SM (2021) Persistent Effects of Historical Selective Logging on a Vascular Epiphyte Assemblage in the Forest Canopy of the Western Ghats, India.
Front. For. Glob. Change 4:727422.
doi: 10.3389/ffgc.2021.727422

Forest canopies have been dubbed the last biological frontier and continue to remain underexplored. Vascular epiphytes form a rich assemblage of plants within the forest canopy and apart from sustaining diverse taxa, they also fulfill critical ecological functions. Vascular epiphytes are particularly sensitive to perturbations of microclimate and microhabitat within the canopy, especially from anthropogenic changes such as logging. The forests of the megadiverse Western Ghats in India harbor a rich assemblage of vascular epiphytes, but their ecology has not been examined systematically. We compared the diversity, abundance, and composition of a vascular epiphyte assemblage between an unlogged and a historically selectively logged forest in the southern Western Ghats, India, and identified factors affecting the epiphyte assemblage. Canopies of 100 trees each in selectively logged and unlogged forests were accessed using the single-rope technique. We found 20 species of vascular epiphytes with the assemblage dominated by members of Orchidaceae. The diversity and abundance of epiphytes were significantly greater in the selectively logged forest. One host tree, *Cullenia exarillata,* supported the greatest number of epiphytes in both forest stands. The niche widths of epiphyte species, computed with host tree species as a resource, were similar between the two stands but a greater number of species pairs overlapped in the selectively logged forest. Overall, epiphyte abundance was negatively associated with unlogged forests. Host tree species, tree height, and presence of moss on branches were positively associated with the abundance of epiphytes. Despite being ecologically important, no study has thus far examined the impact of selective logging on the epiphyte assemblage in the Western Ghats. Our findings contribute to the knowledge of vascular epiphytes from South and Southeast Asia and set the stage for future research and conservation.

Keywords: canopy science, community ecology, mixed-effects model, logging, single rope technique, vascular epiphyte, conservation, Western Ghats

INTRODUCTION

Forest canopies are defined as the aggregate of tree crowns in a stand of vegetation (Parker, 1995) and are a dynamic, functional interface between terrestrial biomass and the atmosphere (Ozanne et al., 2003; Nakamura et al., 2017). The canopies support diverse organisms ranging from insects to plants (Erwin, 1991; Nadkarni, 1994; Lowman and Schowalter, 2012). The presence of this rich diversity has been attributed to the structural complexity, availability of microhabitats, and resources within the canopy (reviewed in Ozanne et al., 2003). The loss or alteration to forests and the canopy is bound to profoundly influence aspects such as nutrient fluxes, carbon sequestration, plant water relations, and biodiversity support (Ozanne et al., 2003; Nadkarni et al., 2011; Lowman, 2020). Although globally acknowledged to be an important habitat, our knowledge and understanding of the canopies has only begun to gradually expand over the past four decades (Lowman, 2020).

Forest canopies serve as an important habitat for plants that germinate and root non-parasitically on other plants at all stages of life, termed epiphytes (Benzing, 2004; Zotz, 2016). Vascular epiphytes are diverse, comprising over 31,100 species distributed globally and accounting for nearly 10% of all extant plants (Benzing, 2004; Nakamura et al., 2017; Zotz et al., 2021). Although distributed globally, the diversity of vascular epiphytes peaks in the humid subtropical and tropical regions (Zotz and Hietz, 2001; Wolf and Flamenco-S, 2003; Zotz et al., 2021). Vascular epiphytes are vertically stratified on trees (Benzing, 1995) and occupy a three-dimensional space within the canopy, resulting in varying distribution patterns when observed at different ecological scales (Mendieta-Leiva and Zotz, 2015) such as individual trees (e.g., Freiberg, 1996), vertically on trees (e.g., Petter et al., 2015), across forest stands (e.g., van Leerdam et al., 1990; Nieder et al., 2000; van Dunné, 2002; Wolf and Flamenco-S, 2003; Alvarenga et al., 2009), and elevation gradients (e.g., Cardelús et al., 2005; Ding et al., 2016). Irrespective of their distribution, vascular epiphytes are vital to maintaining several canopy-atmosphere interactions (reviewed in Lowman and Schowalter, 2012) including nutrient flux regulation; temperature regimes (Scheffers et al., 2014); and plant-water relations (Van Stan and Pypker, 2015). They also enhance the structural and functional diversity of the canopy ecosystem by providing resources to a wide variety of organisms ranging from insects to mammals (Nadkarni and Matelson, 1989; Nadkarni and Longino, 1990; Nakamura et al., 2017).

Observed distribution patterns are attributed to the physiology of the epiphyte (Zotz and Hietz, 2001), microclimatic conditions (Krömer et al., 2006), and a varying degree of affinity to host tree characteristics (Wagner et al., 2015). Host trees are the fundamental unit of habitat for epiphytes, and it follows that tree size and architecture influence the diversity of epiphytes (Flores-Palacios and Garcia-Franco, 2006; Zotz and Schultz, 2008; Wolf et al., 2009; Zhao et al., 2015). Typically, large trees with greater diameter are older and have had greater time for epiphyte colonization events as well as a greater variation in light and humidity (Benzing, 2004; Burns, 2007). Furthermore, aspects of the host tree such as substrate characteristics (Kernan and Fowler, 1995), phenology (Einzmann et al., 2015), and branch throughfall (Winkler et al., 2005) may affect the patterns of epiphyte colonization and survival (Callaway et al., 2002). Disturbance from selective logging of host trees is predicted to negatively affect the vascular epiphyte assemblage (Petter et al., 2021) and indeed, studies have documented negative impacts of selective logging on epiphytes (e.g., Padmawathe et al., 2004; Wolf, 2005; Woods and DeWalt, 2013). However, there is increasing evidence indicating that selective logging where old-growth vegetation is retained can offset the negative impact and the epiphyte assemblages are similar to unlogged forests (Hietz, 2005; Hietz et al., 2006; Lõhmus and Lõhmus, 2010). As rapid deforestation continues in the tropics (Koh et al., 2004; Asner et al., 2005; Broadbent et al., 2008; Sodhi et al., 2009; Laurance, 2013; Struebig et al., 2015), it is imperative to document the impacts on diverse epiphyte assemblages.

The Western Ghats of India is one of the densely populated regions (Cincotta et al., 2000) and the natural landscapes are being threatened by increasing fragmentation and land-use change (Cincotta et al., 2000; Critical Ecosystem Partnership Fund, 2007). The Western Ghats of India and Sri Lanka are a global biodiversity hotspot (Myers et al., 2000) and while much of the landscape has been converted to different land uses, a large proportion of the natural landscape has been formally protected (Das et al., 2006). The vascular epiphyte assemblage in the Western Ghats has not been systematically studied and existing knowledge is from numerous regional checklists (e.g., Ganesan and Livingstone, 2000; Annaselvam and Parthasarathy, 2001; Mathew and George, 2015; Sebastian et al., 2021). The epiphyte diversity is likely to be underestimated because the forest canopy has largely been ignored. Furthermore, there is no systematic investigation of the impacts associated with activities such as logging of trees or forest stands, on vascular epiphyte assemblages, especially within the forest canopy of the Western Ghats.

We aimed to understand how disturbance to forests in the form of selective logging affects the vascular epiphyte assemblage. We achieved this by systematically documenting the epiphyte abundance and diversity in the forest canopy of wet-evergreen forests of the southern Western Ghats. A part of the wet-evergreen forests of the Kalakad Mundanthurai Tiger Reserve (KMTR), Tamil Nadu was selectively logged ∼40 years ago and this has altered the vegetation, impacting the regeneration process (Ganesan, 2000; Ganesan and Davidar, 2003; Nerlekar et al., 2019). Furthermore, long-term ecological research in KMTR has also documented the persisting effects of selective logging on taxonomic groups such as amphibians (Seshadri, 2014), birds (Ramachandran and Ganesh, 2012), butterflies (Devy and Davidar, 2001), lizards (Ishwar et al., 2003), and small carnivores (Mudappa et al., 2007). So far, 55 species of epiphytes have been documented from KMTR with a subset of them being restricted to the forest canopy (Ganesan and Livingstone, 2000).

Here, we (1) document the richness, diversity, and abundance of vascular epiphyte assemblages in unlogged and selectively logged forests. Given that selective logging of trees alters the substrate available for epiphytes, we hypothesized selectively logged forests to be lower in epiphyte species richness, diversity, and abundance; (2) compare the niche width and overlap of epiphytes, using the host tree as a resource, between the unlogged and selectively logged forests. During the selective logging activities, specific host tree species were logged, and we hypothesized that the loss of tree resources would result in narrower niches and greater extent of overlaps among epiphytes in the selectively logged forest; and (3) identify characteristics of the host tree as well as the microhabitat that are associated with the abundance of vascular epiphytes. We expected the abundance to be negatively associated with the selectively logged forest. Our novel findings serve as the baseline information to investigate the associations of epiphytes with their host trees and habitats in other parts of India. Our findings are at the interface of understanding forest canopy and disturbance ecology with broader implications for conserving vascular epiphytes in South and Southeast Asia.

MATERIALS AND METHODS

Study Area

We sampled trees in the wet-evergreen forests of Kalakad Mundanthurai Tiger Reserve (KMTR, 8°25′ to 8°53′ N and 77°10′ to 77°35′ E, 900 km^2) located within the Agasthyamalai Biosphere Reserve in the southern Western Ghats (**Figure 1**). The study area is located between 1,300 and 1,400 m asl and receives a mean annual rainfall of 3,000–3,500 mm, from the Northeast and the Southwest monsoon with a pronounced dry season between January to May (Ganesh et al., 1996). Trees in two contiguous sites, Kakachi (unlogged) and Upper Kodayar (selectively logged), were sampled in an area of ca. 4 km^2 each and are located within a north–south oriented saddle among hill ranges of KMTR. The unlogged forest comprises *Cullenia-Palaquium-Aglaia* vegetation type and the selectively logged forest comprises *Cullenia-Calophyllum-Aglaia* type (Ganesh et al., 1996; Ganesan, 2000). Trees such as *Cullenia exarillata*, *Palaquium ellipticum*, *Myristica dactyloides*, and *Calophyllum austroindicum* were logged in Upper Kodayar until the year 1988 for timber, supplied to the matchstick industry. The effects of selective logging on the tree vegetation were apparent even after 20 years after logging ceased (Ganesan and Davidar, 2003) and continue to linger on recruitment of trees even after 40 years (Nerlekar et al., 2019). The trees in unlogged and selectively logged forests grow between 30 and 35 m in height and have comparable average mean annual temperature (unlogged = 18.1°C vs. Selectively logged = 18.7°C) and relative humidity (unlogged = 71.7% vs. selectively logged = 76.7%). However, the light irradiance levels are nearly 15 times greater in the selectively logged forest (667 lux/m^2) than unlogged forest (44.5 lux/m^2; Ganesh and Tamizalagan, 2012).

Study Design

The vascular epiphyte assemblage was our study unit, the host tree, our sampling unit at the scale of selectively logged and unlogged forest (*sensu* Mendieta-Leiva and Zotz, 2015). We chose 100 trees each in the unlogged and selectively logged forests using a stratified random sampling approach. In a stratified search, trees were selected by two persons walking off from existing trails in the forest and scanning branches for epiphytes using binoculars (Bushnell® 8X40). Any tree >25 cm in diameter at breast height and having epiphytes on more than five branches was randomly chosen and termed the "focal" tree and the nearest four trees irrespective of epiphyte presence were chosen as "satellite" trees. Five such trees formed a cluster, resulting in 20 clusters in the unlogged and selectively logged forests, respectively. Clusters were 50–100 m apart and this design was used for analyzing neighborhood effects on the vascular epiphyte assemblage, as part of another study. To account for the habitat heterogeneity, 30 trees (6 clusters) were within 10 m of a stream and were considered to represent the riparian microhabitat type and the remaining 70 (14 clusters) trees were beyond 10 m and represented the non-riparian microhabitat type.

Trees were accessed using a modified single rope technique (Perry, 1978) by at least two persons and data collection was spread over 3 years from April 2008 to January 2010. Each spatially explicit epiphyte, including juveniles, was considered as an individual (Sanford, 1969). The number of epiphyte species, host tree species, microhabitat type (riparian or non-riparian), and geographic coordinates were recorded for each host tree. The following parameters were quantified for each sampling unit: tree height (m): from the base of the tree to the topmost part of the crown using a 50 m measuring tape; crown diameter (m): the extent of the tree crown, averaged from measurements of the distance from tree trunk to the edge of the crown in eight directions (N, NE, E, SE, S, SW, W, and NW); diameter of the tree: diameter of the trunk (DBH), measured 1.2 m above ground; association of moss with epiphyte occurrence: the presence or absence of moss or lichen on the tree branch at the site of epiphyte growth. Epiphyte species were ascertained in the field based on morphology using keys (Fischer, 1928; Sathish Kumar and Manilal, 1994).

Data Analysis

The number of epiphyte species was used as species richness. Shannon (H') index was used as a measure of diversity and followed up with a Diversity t-test (Hutcheson, 1970) to compare diversity between unlogged and selectively logged forests. Rank abundance curves of epiphytes were compared using a log-series distribution (Krebs, 1989) and followed up with a chi^2 goodness of fit test (Hammer et al., 2001). The evenness of the vascular epiphyte assemblage was computed using Simpson's index (Magurran, 2013). The relative abundance of epiphytes occurring thrice or more; abundance of epiphyte species on host trees; and host tree characteristics (tree height, average crown diameter, and average branch girth of the host tree) followed a non-normal distribution, and their ranks were compared using a Mann–Whitney U test. All the aforementioned analyses were performed in PAST® (Hammer et al., 2001). The occurrence and abundance of epiphytes on at least three host tree species was used to compute niche width using Levin's index and niche overlap was measured using Pianka's Index (Pianka, 1974) using the "spa" package (Zhang, 2016) in R ver. 4.0.5 (R Core Team, 2021). Levin's index is used to compute niche width for species

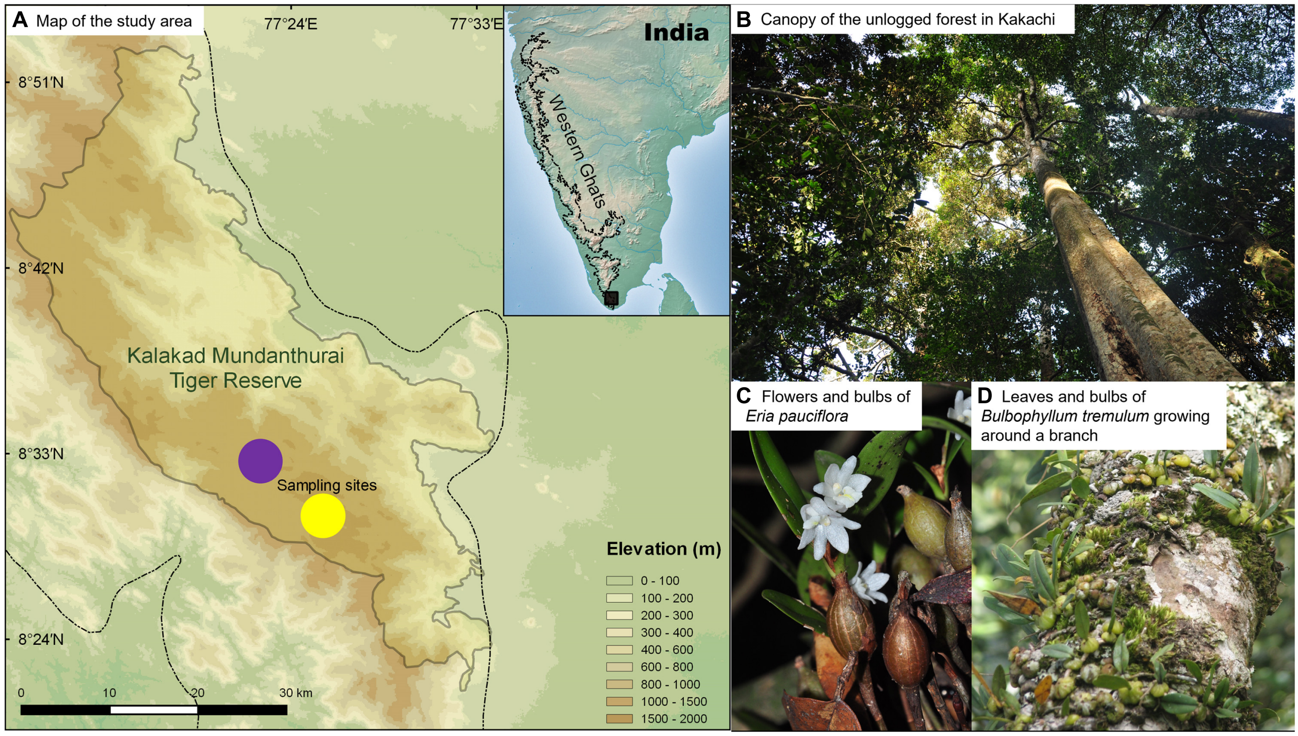

FIGURE 1 | (A) Map showing the study area. The dotted line indicates the Western Ghats. Circles represent the two sampling sites, Kakachi (purple, unlogged) and Kodayar (gold, selectively logged). **(B)** The forest canopy is seen from the ground in Kakachi. **(C)** Flowers and bulbs of *Eria pauciflora.* **(D)** Leaves and bulbs of *Bulbophyllum tremulum* growing around a branch of a *Cullenia exarillata* tree.

occurring in different resources (Feinsinger et al., 1981) and Pianka's index uses a resource utilization matrix to compute pairwise overlap values (Pianka, 1973, 1974). Levin's index values for those epiphyte species occurring more than thrice and common to both the unlogged as well as the selectively logged forest were compared using a Wilcoxon Rank Test (Hammer et al., 2001).

A generalized linear mixed model was used to examine the association of epiphyte abundance with host tree and habitat characteristics. This class of analysis was chosen because the inherent structure in our study design of 40 clusters and mixed models allow us to include a random effect (Bolker et al., 2009). The continuous variables were chosen based on variance-inflation factor (VIF) scores and variables scoring < 3 were retained (Zuur et al., 2009). Although DBH, crown diameter, and tree height scored < 3 VIF values, we found them to be correlated, and thus, only tree height was retained for the model. The abundance of epiphytes was a dependent variable in the generalized linear mixed-effects model. Fixed effects included the four categorical variables (habitat type: selectively logged vs. unlogged; association of moss with epiphyte occurrence: moss present vs. absent; microhabitat: riparian vs. non-riparian; host tree family: 16 families (Achariaceae; Anacardiaceae; Calophyllaceae; Clusiaceae; Ebenaceae; Elaeocarpaceae; Fabaceae; Lauraceae; Malvaceae; Meliaceae; Moraceae; Myristicaceae; Rubiaceae; Salicaceae; Sapotaceae; Unidentified) which were factorized and tree height was scaled before model fitting. The host tree family was used as a surrogate because the model failed to converge upon using the 22 host tree species as a fixed effect. Epiphytic abundance being a "count" variable (meaning it was never negative), a Poisson error function was used. The function "glmer" in the "lme4" package ver.1.1-27 (Bates et al., 2015) was used to perform the mixed-effects analysis in R ver. 4.0.5 (R Core Team, 2021).

RESULTS

Overview of the Vascular Epiphyte Assemblage

We found 2,129 individuals of vascular epiphytes (mean epiphyte abundance ± SD = 10.6 ± 17.4 indiv./tree; range = 0–115) belonging to 20 species, 13 genera, and 6 families (including two unidentified species, genera, and family) on 173 host trees of 22 species, 22 genera, and 17 families (including two unidentified species, genera, and family). Members of Orchidaceae comprised 85% of epiphyte abundance followed by Piperaceae (9.3%) and Melastomataceae (3.2%). Two other families, including one unidentified group, comprised the remaining 2.5% abundance (**Table 1**). The vascular epiphyte assemblage was dominated by *Eria pauciflora* followed by *Bulbophyllum* sp1 and *Oberonia brunoniana*. Among the 20 species, six are endemic to the Western Ghats, four have a pan-Asian distribution, three are endemic to the Western Ghats-Sri Lanka biodiversity hotspot,

TABLE 1 | Relative abundance of vascular epiphytes encountered in the unlogged and selectively logged forests of the Kalakad Mundanthurai Tiger Reserve (KMTR).

			Relative abundance (%)					
Family	**Genera**	**Species**	**Selectively logged**	**Unlogged**	**Total**	**Mann–Whitney *U***	***z***	**Distribution**
Apocynaceae	*Hoya*		0.4%	0.4%	0.4%			India–Sri Lanka
		Hoya pauciflora	0.4%	0.4%	0.4%	6	0.51	
Gentianaceae	*Fagraea*		2.9%	0.3%	2.0%			
		Fagraea ceilanica	2.9%	0.3%	2.0%			India, Indochina, and Malesia
Melastomataceae	*Medinilla*		4.7%	0.1%	3.2%			
		Medinilla beddomei	4.7%	0.1%	3.2%			Western Ghats
Orchidaceae			82.3%	90.2%	84.9%			
	Eria		44.7%	38.2%	42.6%			
		Eria pauciflora	42.5%	32.8%	39.3%	1005	3.7**	Western Ghats
		Eria pseudoclavicaulis	2.3%	5.4%	3.3%	18	0.37	Western Ghats
	Bulbophyllum		14.6%	45.1%	24.7%			
		Bulbophyllum sp1	9.1%	28.3%	15.5%	1089	0.53	
		Bulbophyllum fischeri	0.4%	1.7%	0.8%			Pan Asia
		Bulbophyllum tremulum	0.3%	0.0%	0.2%			Western Ghats
		Bulbophyllum sp2	4.9%	15.1%	8.3%	391	0.37	
	Cymbidium		0.0%	0.1%	0.0%			
		Cymbidium aloifolium	0.0%	0.1%	0.0%			Pan Asia
	Dendrobium		2.7%	4.0%	3.1%			
		Dendrobium heterocarpum	2.7%	4.0%	3.1%	105	0.62	Pan Asia
	Lipari		0.1%	0.0%	0.1%			
		Liparis elliptica	0.1%	0.0%	0.1%			Pan Asia
	Oberonia		16.2%	2.3%	11.6%			
		Oberonia sp	0.0%	0.1%	0.0%			
		Oberonia brunoniana	16.2%	2.1%	11.6%	102	1.11	Western Ghats
	Papilionanthe		2.0%	0.3%	1.4%			
		Papilionanthe subulata	2.0%	0.3%	1.4%			India–Sri Lanka
	Sirhookera		1.9%	0.3%	1.4%			
		Sirhookera latifolia	1.9%	0.3%	1.4%			India–Sri Lanka
Piperaceae	*Peperomia*		9.7%	8.6%	9.3%			
		Peperomia wightiana	7.5%	7.9%	7.6%	183.5	0.52	Western Ghats
		Peperomia sp 2	2.3%	0.7%	1.7%			
Unidentified	*Unidentified*		0.0%	0.3%	0.1%			
		Un id 1	0.0%	0.1%	0.0%			
		Un id 2	0.0%	0.1%	0.0%			

*Shown are the percentages for family, genera, and species. The difference in relative abundance was significant only for Eria pauciflora.** denotes $p < 0.01$.*

and one is found in India, Indochina, and Malesia. The remaining species were identified only to genera or as taxonomically distinct units and thus, their geographic distribution could not be determined. Epiphytes almost always co-occurred with moss and lichens in both unlogged and selectively logged forest (epiphyte individuals in unlogged: with moss present = 705, absent: 2; selectively logged: with moss present = 1,421, absent = 1). Additionally, we found 36 individuals of accidental epiphytes (trees and shrubs) and mistletoes, all greater in abundance and species richness in the selectively logged forest (5 species, 28 individuals) than unlogged forest (1 species and 8 individuals).

Species richness was similar between the selectively logged ($N = 16$) and the unlogged forests ($N = 18$). The composition of the vascular epiphyte assemblage was significantly more diverse in the selectively logged forest (Shannon $H' = 1.95$) compared to the unlogged forest (Shannon $H' = 1.8$; $t = 3.14$, df = 1559.7). The vascular epiphyte assemblage followed a log series distribution in both forests (selectively logged vs. unlogged; $\alpha = 2.5$ vs. 3.36; $x = 0.99$ vs. 0.99; $\text{chi}^2 = 339.6$ vs. 46.49; $p < 0.05$; **Figure 2**). The vascular epiphyte assemblage was more even in the selectively logged (evenness index = 0.44; range = 0.41–0.46) compared to the unlogged forests (evenness index = 0.33, range = 0.32–0.41) with 14 species being common to both. Two species were unique to the selectively logged and four were unique to the unlogged forest. Overall, the relative abundance of epiphytes was significantly lower in the unlogged forest (707 indiv.) compared to the selectively logged forest (1,422 indiv.; Mann–Whitney U: 2903, $z = 2.55$, $p = 0.009$). Among the epiphyte species encountered, *E. pauciflora* and *Bulbophyllum* sp1 were the two most abundant species in both the selectively logged and unlogged forest. While *O. brunoniana* was the third most abundant in selectively logged forest, *Bulbophyllum* sp2 was the

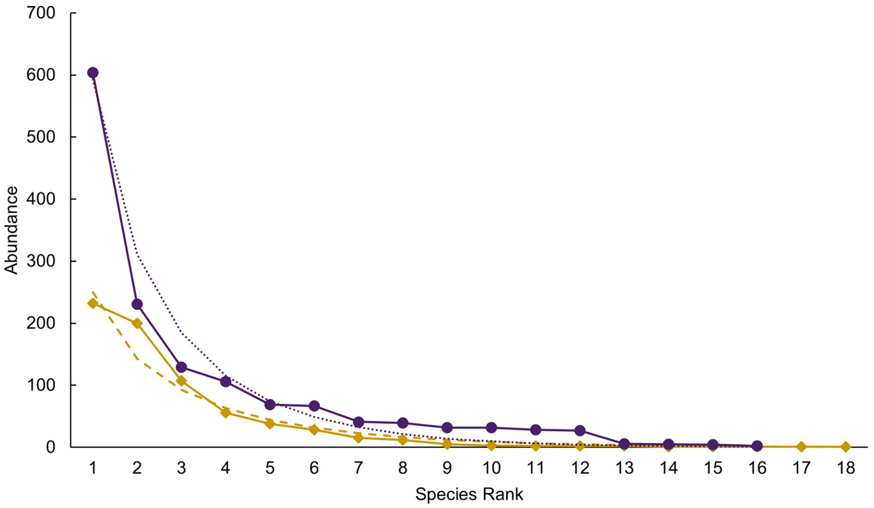

FIGURE 2 | The epiphyte rank abundance followed a log-series distribution in both the unlogged (dots and solid line in purple) and the selectively logged forest (diamond and solid line in gold). Species abundances are ranked with model estimates shown in purple dots (unlogged) and gold dashes (selectively logged).

third most abundant species in the unlogged forest (**Figure 3**). Only eight epiphyte species were encountered more than thrice each in selectively logged and unlogged forest and among them, the difference in abundance of *E. pauciflora* was statistically significant (**Table 1**).

Host Tree Composition

Epiphytes were encountered on 22 host tree species belonging to 22 genera, and 17 families including two unidentified tree species. Four tree species that hosted epiphytes were common to both the selectively logged and unlogged forest, 14 species were unique to the selectively logged, and four were unique to the unlogged forest. Overall, *C. exarillata* hosted the highest abundance of epiphytes in both selectively logged (n = 1096) and unlogged forest (n = 501) and the difference was statistically significant (**Figure 4**; Mann–Whitney U = 649.5, z = 3.55, $p < 0.01$). Among all the host tree species, *C. exarillata* was taller in the unlogged (26.5 ± 3.1 m, N = 49) compared to the selectively logged forests (23 ± 2.3 m, N = 46; Mann–Whitney U = 427.5, z = 5.2, $p < 0.01$) forest, but the crown diameter did not differ between unlogged (5.3 ± 2 m, N = 47) and selectively logged forest (5.3 ± 1.7 m, N = 45; Mann–Whitney U = 997.5, z = 0.5, $p > 0.5$). The differences in characteristics of other host tree species, common to both the unlogged and selectively logged forests, were not compared because of insufficient encounters (**Supplementary Table 1**).

Niche Width and Overlap on Host Trees

The niche widths of epiphytes occurring on three or more host tree species, and common to both selectively logged (Levin's Index, range = 1.1–4.8) and unlogged forest (Levin's Index, range = 1.1–2.0) were similar (Wilcoxon rank test, W = 15, z = 0.9, $p > 0.05$). In both the unlogged as well as the selectively logged forests, *O. brunoniana* had the widest niche whereas *Peperomia wightiana* had the narrowest niche in the unlogged forest and *Eria pseudoclavicaulis* had the narrowest niche in the selectively logged forest (**Table 2**). In the selectively logged forest, 28 species pairs overlapped by over 90% with 14 species pairs overlapping completely and the least extent of overlap was between *E. pseudoclavicaulis* and *Papilionanthe subulata* (0.40; **Supplementary Table 2**). In the unlogged forest, 15 species pairs overlapped by over 90%, only one species pair overlapped completely (*Bulbophyllum fischeri* and *Bulbophyllum* sp2), and the least extent of overlap was between *E. pseudoclavicaulis* and *Hoya pauciflora* (1.7; **Supplementary Table 2**). The greatest extent of niche overlap between species was similar between the unlogged and selectively logged forest but the lowest extent of overlap was smaller in the unlogged forest (**Table 3**).

Factors Determining the Vascular Epiphyte Abundance

The maximal additive model tested the influence of five variables as fixed effects and one random variable as a random effect on the abundance of epiphytes. Only four fixed effects had a statistically significant effect on epiphytic abundance (AIC = 2050.3, residual df = 179; cluster ID as random effect [variance ± SD]: 0.5 ± 0.7; **Table 4**). Epiphyte abundance was negatively associated with the unlogged forest. Epiphyte abundance was positively associated with tree height, the presence of moss, and four of the 16 host tree families (**Figure 5**). The association with riparian microhabitat was positive but the difference between non-riparian microhabitat was not statistically significant.

DISCUSSION

The ecology of vascular epiphytes growing in the forest canopies of tropical forests remains understudied. Access to forest canopies has been an impediment but since the 1980s methods such as the single rope technique have enabled easy access (reviewed in Lowman and Schowalter, 2012). Using

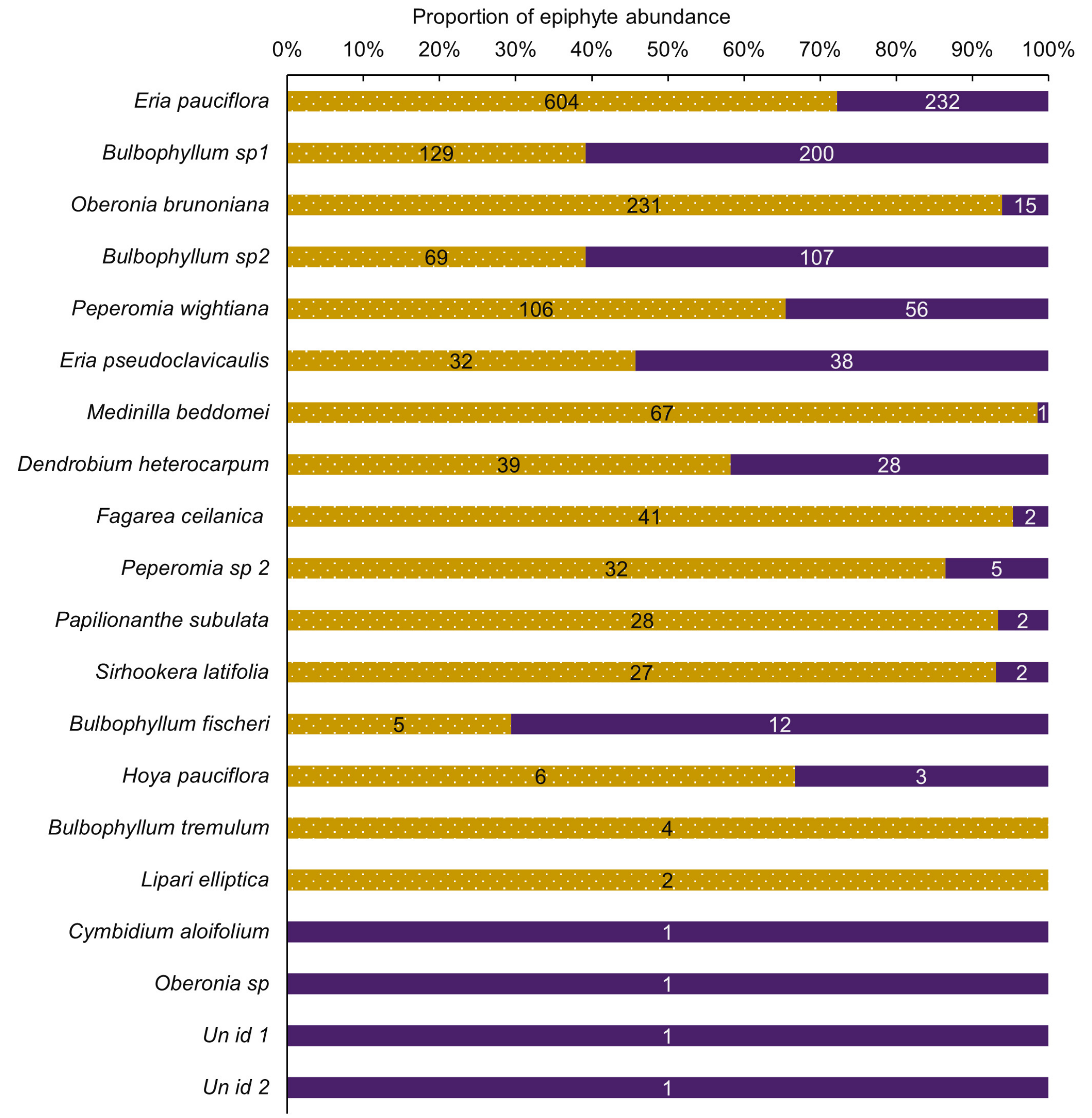

FIGURE 3 | Proportions of epiphyte species composition differed between unlogged and selective logged forests. Relative abundance of epiphytes in the unlogged (purple) and the selectively logged forest (dotted gold). The percentage of the total abundance is depicted. Actual relative abundance values are presented on bars.

the single-rope technique to access canopies, we documented the presence of a diverse assemblage of epiphytes (20 species, 2,129 individuals) growing in the forest canopy of the wet-evergreen forest in the southern Western Ghats of India. The vascular epiphyte assemblage was greater in diversity as well as relative abundance in the selectively logged forest. Epiphytes were encountered on more host tree species in the selectively logged forest but one host tree species, *C. exarillata,* hosted most of the epiphyte abundance in both selectively logged and unlogged forests. The niche breadth and overlap of epiphyte species, computed using the host tree as a resource, was wider and epiphyte species overlapped with each other to a greater extent in the selectively logged compared to the unlogged forest. Overall, epiphyte abundance was negatively associated with unlogged forest and positively associated with host tree height, presence of moss, and host trees belonging to four families. The location of the host tree in the riparian and non-riparian microhabitat did not have any statistically significant effect on epiphyte abundance. Taken together, our findings indicate that while the vascular epiphyte assemblage is influenced by the host tree characteristics, the effects of historical selective logging continue to persist even after 40 years since logging ceased. There are currently no other studies examining the ecological determinants of vascular epiphytes in the forest canopy in the Western Ghats, and our findings serve as a baseline for further research into the nature of epiphyte ecology in India as well as other parts of South and Southeast Asia.

The Vascular Epiphyte Assemblage

The epiphyte assemblage in both the unlogged and selectively logged forests was dominated by orchids, especially *E. pauciflora,*

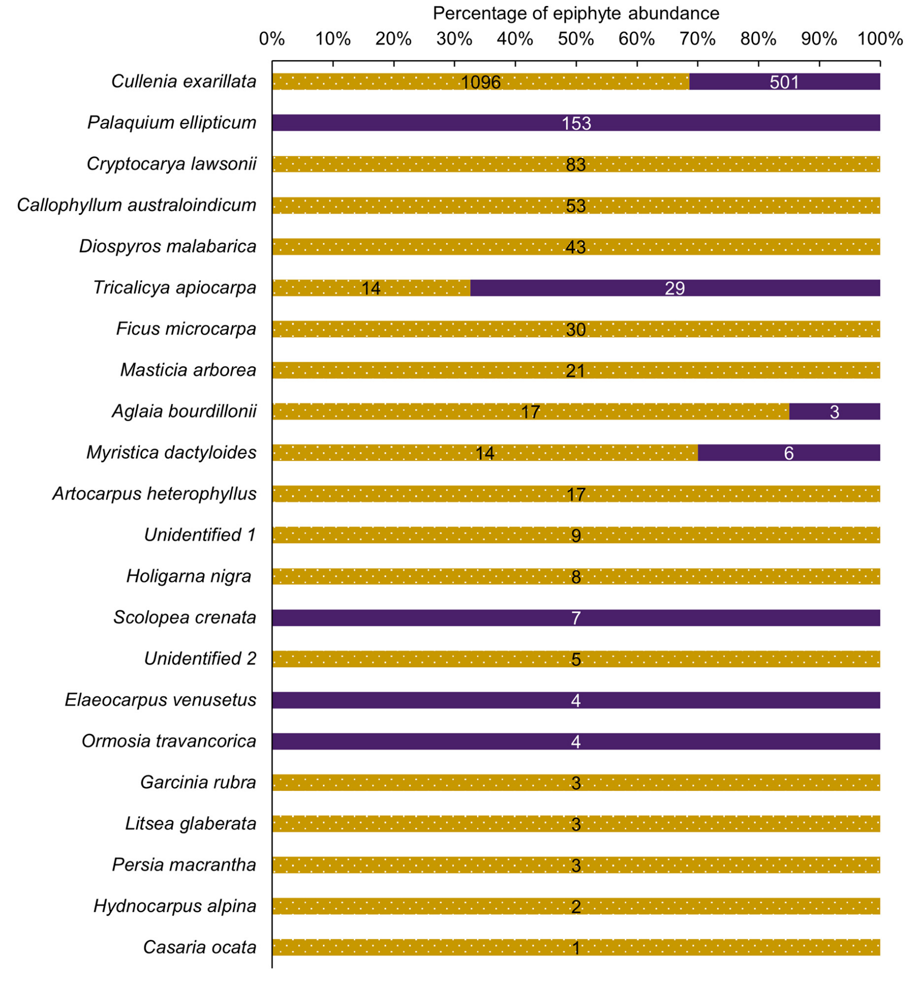

FIGURE 4 | Relative abundance of vascular epiphytes encountered on host tree species in the unlogged (purple) and the selectively logged forest (dotted gold) forest is shown as a percentage of the total abundance. Actual relative abundance values are shown on respective bars.

followed by *Bulbophyllum* spp. Both these species are drought-tolerant, and their increased abundance suggests that the epiphyte assemblage is responding to microclimate perturbations due to selective logging. Selective logging of trees is known to shift the epiphyte assemblage from a mesic to drought-tolerant species (e.g., Wolf, 2005) and epiphytes adapt to physical environmental gradients such as variation in atmospheric moisture and light availability (Benzing, 2004). Constraints of moisture availability are likely to bear a greater influence on epiphytes compared to light irradiance or nutrition (Zotz and Hietz, 2001). Although the study site receives >3,000 mm of rainfall annually, there is a pronounced dry season between January to May. Epiphytes such as *E. pauciflora* grow as a dense mat with bulbous, elongated stalks ending with a pair of leaves. We speculate that the dense mat likely traps canopy litter, thus accumulating canopy soil organic matter as opposed to *Bulbophyllum* spp. which creep along the branch, without forming a dense mat. Individuals of *Bulbophyllum* spp. also shed their leaves in subsequent years and only bare discoid bulbs from the plant body (Pers. Obs.). Other species such as *O. brunoniana* and *P. wightiana* do not have any vascular bulbs and yet were found in relatively high abundance in selectively logged forest. Both species appear to have moisture conserving strategies such as thick leathery strap-shaped leaves of *O. brunoniana* or possess thick succulent leaves in *P. wightiana*. The ability to tolerate drought could be critical for epiphytes to tide over xeric conditions in some periods of the year as they do not have access to soil moisture. Studying dispersal ability, the life span, and growth rates after colonization of these epiphytes would help us understand how quickly they were able to colonize the selectively logged habitat and proliferate. Such information would also help us identify epiphyte species that may be vulnerable to the impacts of climate change.

Niche Width, Overlap, and Host Bias

The dependence of epiphyte communities on a particular host has been considered as host preference or host specificity. However, Wagner et al. (2015) argue that determining specificity is a challenge given the diversity of trait associations of both the host tree as well as epiphytes. At best, any perceived association can only be termed as host bias. Host tree characteristics determine

TABLE 2 | Niche width of vascular epiphytes computed using the Levin's Index are presented for unlogged and selectively logged forests.

Species	Unlogged	Selectively logged
Bulbophyllum fischeri	1.4	–
Bulbophyllum sp1	1.9	1.2
Bulbophyllum sp2	1.9	2.8
Bulbophyllum tremulum	–	2.7
Dendrobium heterocarpum	–	1.1
Eria pauciflora	1.6	1.2
Eria pseudoclavicaulis	1.6	1.1
Fagraea ceilanica	–	1.2
Medinilla beddomei	–	1.4
Oberonia brunoniana	2.0	4.8
Papilionanthe subulata	–	5.0
Peperomia wightiana	1.1	2.0
Hoya pauciflora	3.0	–

Only epiphyte species occurring on three or more host tree species were used.

TABLE 3 | The minimal and maximal niche overlap values of 12 vascular epiphytes encountered in the unlogged and selectively logged forests are presented.

	Unlogged		Selectively logged	
Species	Min	Max	Min	Max
Bulbophyllum fischeri	0.23	0.99	–	–
Bulbophyllum sp1	0.21	1.00	0.41	1.00
Bulbophyllum sp2	0.21	0.98	0.61	0.95
Bulbophyllum tremulum	–	–	0.52	0.88
Dendrobium heterocarpum	–	–	0.42	1.00
Eria pauciflora	0.23	0.97	0.43	1.00
Eria pseudoclavicaulis	0.17	0.23	0.40	1.00
Fagraea ceilanica	–	–	0.41	1.00
Hoya pauciflora	0.54	0.59	–	–
Medinilla beddomei	–	–	0.41	0.98
Oberonia brunoniana	0.95	0.95	0.69	0.79
Papilionanthe subulata	–	–	0.44	0.44

Pianka's index of overlap was computed for epiphyte species occurring on three or more host tree species.

epiphyte colonization and growth (Benzing, 1995; Benzing, 2004). A decline in host tree densities will likely affect epiphytes that have specific requirements for establishment. In this study, the unlogged forest is dominated by the *Cullenia-Palaquium-Aglaia* series (Ganesh et al., 1996). Among the three, *C. exarillata* alone supported about 80% of the epiphyte abundance in both unlogged and selectively logged forests. One of the host tree species, *P. ellipticum* was naturally absent in the selectively logged sites and *C. exarillata* supported twice the number of epiphytes. The selective logging activities specifically targeted old-growth trees of large diameter and had affected the forest structure as well as regeneration of these species in the logged sites when measured 24 years after logging activities ceased (Ganesan and Davidar, 2003). A more recent examination of the forest stands indicates that the effects are still pervasive nearly 40 years after logging (Nerlekar et al., 2019). Despite the structural changes to the forest composition, the epiphyte assemblage did not appear to be drastically affected by the loss of host trees or change in host tree composition because the niche widths were statistically similar between unlogged and selectively logged forests.

The ability of *C. exarillata* to host a high abundance of epiphytes could be due to a combination of its relative abundance, large size, and bark features conducive to epiphyte colonization. Bark features of host trees are an important aspect that can affect the epiphyte distribution (e.g., Wyse and Burns, 2011) as is branch orientation (Mendieta-Leiva and Zotz, 2015; Wagner et al., 2015). The bark on *C. exarillata* tree is flaky and accumulates a large quantity of woody dust. This, along with the accumulation of canopy soil organic matter (Nadkarni, 1994), likely offers a rich nutrient source for the establishment of epiphyte communities (Zotz, 2016). Besides, the tree exhibits cauliflorous inflorescence which results in several woody knobs all along the branches. These branches after flowering/fruiting leave a rough surface which could serve as important micro-sites for deposition of the wind-dispersed epiphytic seeds. Several mammals visit the tree for feeding on the flowers (Ganesh and Devy, 2000) and they might be aiding as secondary dispersal agents of several epiphytic species as they move to other trees. In addition, the tree architecture may be conducive to intercepting wind-dispersed spores because the branches radiate horizontally from the trunk. Branches of different orientations and directions may provision diverse microclimatic gradients varying on the axes such as light, relative humidity, and the likelihood of intercepting wind-dispersed seeds. Future research will help us gain insights into the mechanisms behind the greater epiphyte abundance on *C. exarillata*.

We expected the epiphytes occurring in selectively logged forests to have a narrower niche width because fewer host trees would be available for colonization. We found that the niche widths of five epiphyte species common to the two forests were unaffected, meaning that the five epiphyte species used the same range of host trees or trees in both the unlogged and selectively logged forest. We found the niche among species to overlap to a greater extent in the selectively logged forest, indicating that fewer host trees had suitability for colonization and more than one species pair was sharing the host tree as a resource. Niches vary on multi-dimensional axes, and it remains to be studied if variability in other resources such as moisture or light availability resulted in the patterns we have observed. Future studies may also be able to quantify the microclimatic variability within host trees and evaluate colonization rates by quantifying seed or spore arrival on host trees in both forests. Taken together, the unaffected niche widths and greater overlaps in selectively logged forests suggest that the epiphyte assemblage shows a moderate degree of structural host bias (sensu Wagner et al., 2015).

Determinants of the Vascular Epiphyte Assemblage

Our generalized linear mixed model analysis indicated that the epiphyte abundance was positively associated with tree height, presence of moss on branches, and four host tree families; negatively associated with unlogged forest; and did not vary

TABLE 4 | The output of a generalized linear mixed-effects model built using the abundance of epiphytes on the individual tree as a dependent variable.

Number	Fixed effect	Estimate	Std. error	z	P	Significance
	(Intercept)	−1.85	0.94	−1.97	0.05	*
1	Forest type: unlogged	−1.76	0.24	−7.29	0	***
2	Host tree family: Anacardiaceae	−0.27	0.8	−0.34	0.73	
2	Host tree family: Calophyllaceae	0.07	0.73	0.1	0.92	
2	Host tree family: Clusiaceae	0.4	0.93	0.43	0.67	
2	Host tree family: Ebenaceae	3.87	0.79	4.92	0	***
2	Host tree family: Elaeocarpaceae	2.74	0.9	3.06	0	**
2	Host tree family: Fabaceae	0.73	0.89	0.82	0.41	
2	Host tree family: Lauraceae	1.06	0.73	1.45	0.15	
2	Host tree family: Malvaceae	1.6	0.72	2.23	0.03	*
2	Host tree family: Meliaceae	0.05	0.75	0.07	0.95	
2	Host tree family: Moraceae	0.91	0.73	1.25	0.21	
2	Host tree family: Myristicaceae	0.59	0.75	0.79	0.43	
2	Host tree family: Rubiaceae	1.91	0.74	2.6	0.01	**
2	Host tree family: Salicaceae	0.6	0.82	0.73	0.47	
2	Host tree family: Sapotaceae	1.31	0.72	1.8	0.07	.
2	Host tree family: unidentified	−0.44	0.77	−0.57	0.57	
3	Tree height	0.89	0.06	15.1	0	***
4	Moss: present	3.31	0.58	5.72	0	***
5	Microhabitat: Riparian	0.10	0.25	0.41	0.68	

*Fixed effects included forest type (logged vs. unlogged); host tree family; tree height (m); moss presence (vs. absent); and microhabitat (riparian vs. non-riparian). Cluster-ID, with five trees forming one cluster was used as a random effect (see Section "Data Analysis"). Numbers 1–5 refer to the fixed effects. All fixed effects except tree height (m) were categorical. Data from 200 trees and 40 clusters were used for model fitting. Significance P-values < 0 are represented by ***; <0.001 by **; <0.05 by *.*

between riparian on non-riparian zones. Host tree features are known to profoundly influence the abundance of epiphytes (Benzing, 2004). Several studies have found the diameter of the host tree (DBH) to be correlated with tree heights as well as epiphyte abundance (e.g., Zotz and Vollrath, 2003) and more generally, tree size is correlated with epiphyte abundance (Hietz-Seifert et al., 1996; Hietz, 2005; Wolf et al., 2009; Dislich and Mantovani, 2015). Tree height or DBH is used as a proxy for tree size and we find the tree height to be similar between both unlogged and selectively logged forests and overall, older and thus larger, trees were likely to support a greater abundance of epiphytes. A similar trend has been observed in another study from Asia by Zhao et al. (2015). The only other study from India, by Padmawathe et al. (2004), found the richness and abundance of epiphytes were significantly correlated with crown diameter but not with tree height. However, they treat crown diameter and tree height as two separate variables while the two may be correlated. While we also measured the average crown diameter and DBH, we used only tree height because the two were strongly correlated.

The presence of moss also emerged as a key driver for epiphyte abundance from our models. Mosses and lichens comprise the non-vascular epiphyte community and vary within the tree based on microclimate and substrate characteristics (e.g., Holz et al., 2002). In the temperate forests, the bryophytic vegetation is early colonizers and these poikilohydric (ability to increase or decrease moisture holding capacity) flora create thick mats of dead and decaying vegetation which enables colonization by other vascular epiphyte assemblages (Nadkarni, 2000). Studies suggest that the mats of lichens and mosses may provide a substrate for colonization of vascular epiphyte either by trapping seeds or spores or buffer the moisture requirements or all three (reviewed in Zotz, 2016). The mechanisms of seed arrival and establishment of vascular epiphytes as well as associations with other plants such as mistletoe or lianas continues to be understudied, especially in the tropics. Our finding that the vascular epiphytes were almost always found with non-vascular epiphytes indicates similarities in the community ecology of vascular epiphytes in both tropical and temperate forests. We expected the epiphyte abundance to be associated with riparian habitats because moisture availability is a known physiological constraint (Zotz and Hietz, 2001) for epiphyte colonization and survival. On the contrary, we found that the presence of the host tree in the riparian zone did not support a greater number of epiphytes compared to those in the non-riparian zones, irrespective of the logging status. The strong positive association with the presence of moss and their poikilohydric nature is likely to have buffered epiphyte assemblages to a similar extent in both selectively logged and unlogged sites. However, future evaluation of the performance of epiphyte assemblage as measured by colonization or growth rates in riparian and non-riparian zones is necessary because climate change effects will likely affect the two zones differently (Seavy et al., 2009).

The tree height and presence of moss on host trees could be attributed to the host tree characteristic itself. We were unable to account for the host tree as a fixed effect in the model because of model convergence issues and therefore, we used the host tree families as a proxy, assuming that the characteristics influencing the epiphyte assemblage would be similar among species within the family. Among the 16 families used to build the model, a positive association was observed in only four families viz., Ebenaceae (1 host tree species), Elaeocarpaceae (1 host tree

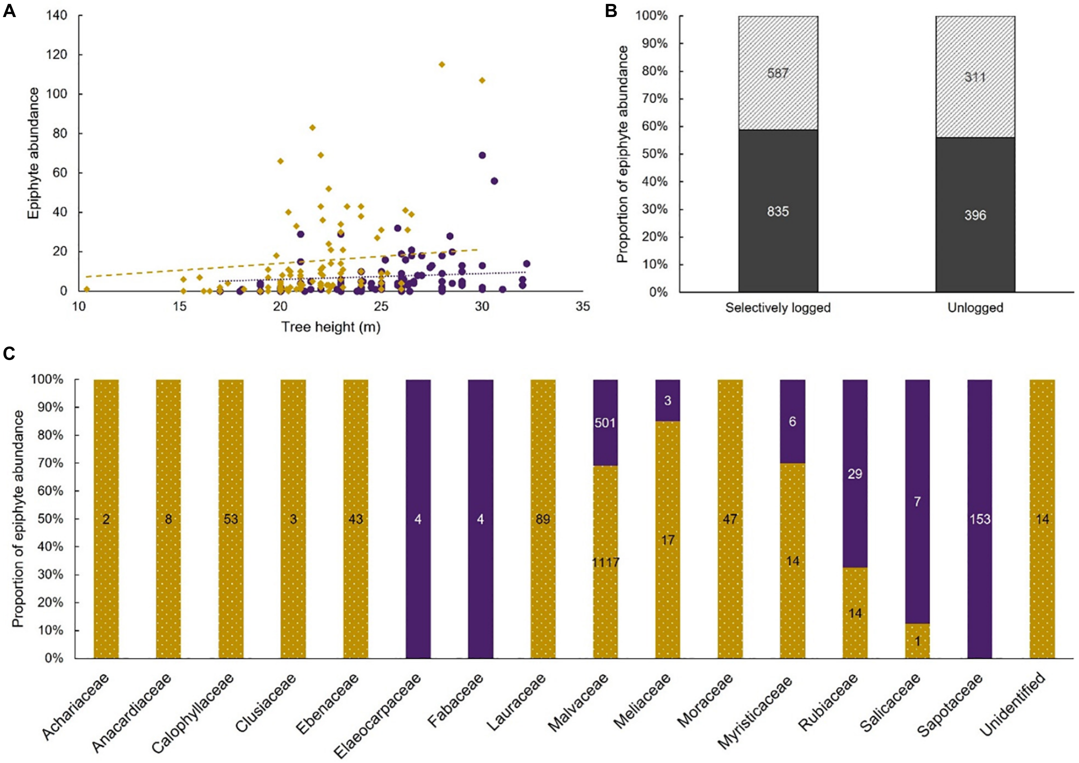

FIGURE 5 | Epiphyte abundance was positively associated with increasing tree height **(A)**. Associations within the unlogged forest (purple dots) and the selectively logged forest (gold diamond) are shown along with trendlines for unlogged (purple dotted) and selectively logged (gold dashed); similar proportions of epiphytes were found in the riparian (diagonal stripes) and non-riparian (solid gray) microhabitats of both the unlogged and the selectively logged forest **(B)**. Abundance values are depicted on respective bars, and epiphyte abundance was positively associated with host tree species in the family Ebenaceae, Elaeocarpaceae, Malvaceae, and Rubiaceae. The proportion of epiphyte abundance for each host tree family in the unlogged (solid purple) and the selectively logged forest (dotted gold) are depicted **(C)**.

species), Malvaceae (2 host tree species), and Rubiaceae (1 host tree species). Examining the five host tree species belonging to the four families (**Supplementary Table 1**) suggests that the rough or fissured bark texture may be aiding the epiphyte abundance. Further studies could artificially modify the substrate and determine colonization rates on these host tree species to determine if bark features are indeed an important driver for the rich abundance of epiphytes.

The status of the forest, whether selectively logged or unlogged, was statistically significant in the model and unlogged forests were negatively associated with epiphyte abundance. This was an unexpected result, and we speculate that the old-growth vegetation in the unlogged forest, the dense, unbroken canopy, and the slower recruitment of host trees resulted in a saturated habitat for epiphyte colonization. Tree falls would create gaps in the forest canopy, facilitating the recruitment of young trees, but such gaps were uncommon (Pers. Obs.). The dense and continuous canopy could also affect the wind flow and thus, seed and spore dispersal in the unlogged forest compared to the more open, selectively logged forest. Long-term studies of air temperature, relative humidity, and light irradiation in both selectively logged and unlogged sites show a marked increase in light irradiation in the selectively logged forest (Ganesh and Tamizalagan, 2012). All these factors, in combination, may have enabled the epiphyte abundance to increase in selectively logged sites, resulting in a positive association.

Conserving Epiphyte Assemblages in the Western Ghats of India

Commercial logging of trees is a major driver of land-use change and has profound consequences on the structure and function of the ecosystem (Brown and Gurevitch, 2004; Fredericksen and Fredericksen, 2004; Padmawathe et al., 2004; Asner, 2005; Ernst et al., 2006; Peres et al., 2006; Villela et al., 2006; Broadbent et al., 2008; Burivalova et al., 2014). Such land-use modifications invariably impact vascular epiphytes as they depend on existing flora for survival. In our study site, the forests were logged about four decades ago and information about its impacts on floral diversity is limited to three reports on trees and other vegetation but not on epiphytes (Ganesan, 2000; Ganesan and Davidar, 2003; Nerlekar et al., 2019). Although few studies exist in Asia, several studies have examined the impacts of logging on the epiphyte community in the neotropics and suggest that the impacts of selective logging are fewer compared to clear felling (e.g., Wolf, 2005; Wolf et al., 2009). Studies also highlight the importance of retaining old-growth trees within selectively logged sites as an effective management strategy to conserve biodiversity (Rosenvald and Lõhmus, 2008). Parts of the Western Ghats are well protected, but a diverse range of habitats lay outside the protected areas and need to be conserved (Das et al., 2006). Wild harvest of orchids for medicine and trade (Hinsley et al., 2018) may also be an emerging threat to epiphytic orchids in India and it is necessary to document its impacts.

CONCLUSION

Our findings indicate the presence of distinct distribution patterns of epiphytes at the scale of forest type as well as between host trees. It appears that after 40 years since logging activities ceased, the vascular epiphyte assemblage does not match that of the unlogged forest. Although there was higher epiphytic abundance in the selectively logged forest, we caution against using our findings as evidence to support indiscriminate or selective logging because we only present a snapshot of the selectively logged forest during its succession. Despite 40 years since logging, the impacts appear to persist on vascular epiphytes as a greater abundance of drought-resistant species were found in the selectively logged forest. Our study has caveats such as the unavailability of data on moisture, light irradiance, and tree architecture for all the trees sampled. The lack of a baseline before the forest was logged or from elsewhere within the Western Ghats prevents us from making unified inferences. Nevertheless, the findings open an avenue for further research about the physiology of epiphytes, the changes to faunal diversity supported by epiphytes, and highlights the need to document patterns of epiphyte colonization on trees differing in characteristics such as architecture or bark texture. Such studies would provide novel insights, expand our understanding of the forest canopy, and help conserve them in the rapidly changing forests of South and Southeast Asia.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

SD and RG designed the study and secured funding. KS collected field data and analyzed data. RG identified species. KS wrote the manuscript with inputs from SD and RG. All authors have approved the submission.

FUNDING

This study was financially supported by the Department of Science and Technology, Government of India, by a grant (SR/S0/AS-01/2004) to SD and RG. KS was supported by the DST-INSPIRE faculty fellowship (DST/INSPIRE/04/2019/001782) by the Department of Science and Technology during manuscript preparation.

ACKNOWLEDGMENTS

C. K. Sreedharan, PCCF and Chief Wildlife Warden, Tamil Nadu Forest department provided the necessary research permits. A. Ramkumar, Conservator of Forests and Field Director, KMTR, and The Deputy Directors and Range Forest Officers, Kodayar provided their valuable suggestions and support. Tamizalagan, Johnson, Murthy, Esaki-Muttu, Mythri, Aditya, and Mathivannan provided field support. The accommodation was provided by Tamil Nadu Electricity Board. Ganesh T., Jan Wolf, and Gerhard Zotz provided critical comments on early drafts of this manuscript. Vidisha M. K. and the two reviewers provided useful suggestions to the manuscript. We thank them all.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.727422/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Forests and Global Change

ORIGINAL RESEARCH
published: 08 November 2021
doi: 10.3389/ffgc.2021.712165

Extending Our Scientific Reach in Arboreal Ecosystems for Research and Management

Charles H. Cannon[1]*, Colby Borchetta[1], David L. Anderson[2], Gabriel Arellano[3], Martin Barker[4], Guillaume Charron[5], Jalene M. LaMontagne[6], Jeannine H. Richards[7], Ethan Abercrombie[8], Lindsay F. Banin[9], Ximena Tagle Casapia[10,11], Xi Chen[12], Polina Degtjarenko[13], Jane E. Dell[14], David Durden[15], Juan Ernesto Guevara Andino[16], Rebeca Hernández-Gutiérrez[17], Andrew D. Hirons[18], Chai-Shian Kua[1], Hughes La Vigne[5], Maurice Leponce[19], Jun Ying Lim[20], Margaret Lowman[21], Andrew J. Marshall[22], Sean T. Michaletz[23], Benjamin B. Normark[24], Darin S. Penneys[25], Gerald F. Schneider[26], Joeri S. Strijk[27], Bashir B. Tiamiyu[28], Tara L. E. Trammell[29], Yalma L. Vargas-Rodriguez[30], Samantha R. Weintraub-Leff[15], Alexis Lussier Desbiens[5] and Matthew Spenko[31]

[1] Center for Tree Science, The Morton Arboretum, Lisle, IL, United States, [2] Canopy Watch International, Boise, ID, United States, [3] Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, United States, [4] School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom, [5] Department of Mechanical Engineering, Université de Sherbrooke, Sherbrooke, QC, Canada, [6] Department of Biological Sciences, DePaul University, Chicago, IL, United States, [7] Department of Biology, University of Louisville, Louisville, KY, United States, [8] Department of Biology, Washington University in Saint Louis, Saint Louis, MO, United States, [9] UK Centre for Ecology & Hydrology, Penicuik, United Kingdom, [10] Instituto de Investigaciones de la Amazonia Peruana (IIAP), Iquitos, Peru, [11] Laboratory of Geo-Information Science and Remote Sensing, Wageningen University and Research, Wageningen, Netherlands, [12] School of Ecology and Environmental Science, Yunnan University, Kunming, China, [13] Department of Botany, University of Tartu, Tartu, Estonia, [14] Geosciences and Natural Resources Department, Western Carolina University, Cullowhee, NC, United States, [15] National Ecological Observatory Network, Battelle, Boulder, CO, United States, [16] Grupo de Investigación en Biodiversidad, Medio Ambiente y Salud-BIOMAS- Universidad de las Américas, Quito, Ecuador, [17] Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico, [18] Myerscough College, Preston, United Kingdom, [19] Biodiversity Monitoring and Assessment, Royal Belgian Institute of Natural Sciences, and Evolutionary Biology, and Ecology and Université Libre de Bruxelles, Brussels, Belgium, [20] Department of Biological Sciences, National University of Singapore, Singapore, Singapore, [21] TREE Foundation, Sarasota, FL, United States, [22] Department of Anthropology, Department of Ecology and Evolutionary Biology, Program in the Environment, and School for Environment and Sustainability, University of Michigan, Ann Arbor, MI, United States, [23] Department of Botany and Biodiversity Research Centre, The University of British Columbia, Vancouver, BC, Canada, [24] Department of Biology, University of Massachusetts Amherst, Amherst, MA, United States, [25] Department of Biology and Marine Biology, University of North Carolina Wilmington, Wilmington, NC, United States, [26] Department of Biology, Utah State University, Logan, UT, United States, [27] Institute for Biodiversity and Environmental Research, Universiti Brunei Darussalam, Jalan Tungku Link, Brunei, [28] Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, [29] Plant and Soil Sciences Department, University of Delaware, Newark, DE, United States, [30] Universidad de Guadalajara, Centro Universitario de los Valles, Ameca, Mexico, [31] Mechanical, Materials, and Aerospace Engineering, Illinois Tech, Chicago, IL United States

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Edited by:
Brett Scheffers,
University of Florida, United States

Reviewed by:
Romà Ogaya,
Ecological and Forestry Applications Research Center (CREAF), Spain
Lily Leahy,
James Cook University, Australia

***Correspondence:**
Charles H. Cannon
ccannon@mortonarb.org

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *20 May 2021*
Accepted: *04 October 2021*
Published: *08 November 2021*

Citation:
Cannon CH, Borchetta C, Anderson DL, Arellano G, Barker M, Charron G, LaMontagne JM, Richards JH, Abercrombie E, Banin LF, Tagle Casapia X, Chen X, Degtjarenko P, Dell JE, Durden D, Guevara Andino JE, Hernández-Gutiérrez R, Hirons AD, Kua C-S, La Vigne H, Leponce M, Lim JY, Lowman M, Marshall AJ, Michaletz ST, Normark BB, Penneys DS, Schneider GF, Strijk JS, Tiamiyu BB, Trammell TLE, Vargas-Rodriguez YL, Weintraub-Leff SR, Lussier Desbiens A and Spenko M (2021) Extending Our Scientific Reach in Arboreal Ecosystems for Research and Management. Front. For. Glob. Change 4:712165. doi: 10.3389/ffgc.2021.712165

The arboreal ecosystem is vitally important to global and local biogeochemical processes, the maintenance of biodiversity in natural systems, and human health in urban environments. The ability to collect samples, observations, and data to conduct meaningful scientific research is similarly vital. The primary methods and modes of access remain limited and difficult. In an online survey, canopy researchers (*n* = 219) reported a range of challenges in obtaining adequate samples, including ∼10% who found it impossible to procure what they needed. Currently, these samples are collected using a combination of four primary methods: (1) sampling from the ground; (2) tree climbing; (3) constructing fixed infrastructure; and (4) using mobile aerial platforms, primarily rotorcraft drones. An important distinction between instantaneous and continuous sampling was identified, allowing more targeted engineering and development strategies. The combination of methods for sampling the arboreal ecosystem provides a range of possibilities and opportunities, particularly in the context of the rapid development of robotics and other engineering advances. In this study, we aim to identify the strategies that would provide the benefits to a broad range of scientists, arborists, and professional climbers and facilitate basic discovery and applied management. Priorities for advancing these efforts are (1) to expand participation, both geographically and professionally; (2) to define 2–3 common needs across the community; (3) to form and motivate focal teams of biologists, tree professionals, and engineers in the development of solutions to these needs; and (4) to establish multidisciplinary communication platforms to share information about innovations and opportunities for studying arboreal ecosystems.

Keywords: canopy crane, tree climbing, robotics, epiphytic, tree canopy, canopy access, canopy biology, drones (UAV)

INTRODUCTION

The arboreal ecosystem is a critical terrestrial environment, fundamental to global carbon, water, and nutrient cycles (Barker and Becker, 1995; Bassow and Bazzaz, 1997; Santiago et al., 2004) and biodiversity maintenance (Ozanne et al., 2003; Nitta et al., 2020). Large trees store a substantial proportion of the world's terrestrial biomass (e.g., Slik et al., 2013) and forested ecosystems contain nearly half of all global above-ground biomass, primarily in the form of wood in large trunks and limbs (Chen et al., 2014). Remarkably, more water flows through the stems of plants and the pores of leaves than through the mouths of rivers, in the world's water cycle (Schlesinger and Jasechko, 2014), enough to generate local weather patterns (Makarieva et al., 2014). While the living tissue (cambium, phloem, parenchyma in the sapwood, leaves, buds, and reproductive parts) contributes a small proportion of overall tree biomass, these highly reactive organs represent an immense physiological interface with both the abiotic and biotic environment. Arboreal processes, therefore, have consequences for global biogeochemical, hydrological, and ecological cycles.

Terry Erwin and Janice Scott coined the term "arboreal ecosystem" to capture the vast complexity and importance of the poorly explored above-ground terrestrial environment created by trees (Erwin and Scott, 1980; Erwin, 1982). They identified it as a last frontier for terrestrial biodiversity research. Arboreal ecosystems span the planet and scale in density and structure, from complex multi-layered tropical rainforest to lone urban street trees. The structural complexity of the canopy is a useful proxy for habitat richness and correlates with the biodiversity of dependent taxa (e.g., Gouveia et al., 2014). Structural complexity, in turn, is correlated with canopy height and environmental factors, with the highest complexity found in evergreen tropical rainforests (Ehbrecht et al., 2021) where trees commonly exceed 70 m (Banin et al., 2012; Shenkin et al., 2019).

Arboreal structure influences and is influenced by microclimate, which includes irradiance, temperature, humidity and carbon dioxide concentrations, and is highly dynamic across space and time (Davies-Colley et al., 2000; Newmark, 2001). In temperate zones, bud burst and the initiation of growth after months of winter dormancy occurs over several days, coordinated with local weather patterns. In forests around the world, large synchronous pulses of seed production by plant populations, known as mast events, occur at irregular intervals across years (Kelly and Sork, 2002; Pearse et al., 2020). The many important plant biotic interactions in the arboreal environments include herbivory (Lowman, 2001), pollination (van Dulmen, 2001; Kettle et al., 2011), seed dispersal (Seidler and Plotkin, 2006; Jordano et al., 2007), and seed predation (Janzen, 1971). These interactions can occur on short time scales and early detection can be important for management decisions. Detailed observation and sampling are important for understanding mature tree biology and for identifying critical canopy traits and functions that determine trees' vulnerabilities to environmentally driven decline (McDowell et al., 2018).

Our ability to understand canopy-dwelling organisms also depends on how we access the arboreal ecosystem, because spatial distribution of canopy-dwelling organisms is inherently three-dimensional. Sedentary and mobile organisms partition themselves vertically according to microclimatic and biotic gradients from the forest floor to the canopy (Petter et al., 2016; Oliveira and Scheffers, 2019), as well as laterally within the canopy from within the trunk to the surface of peripheral branches (Volf et al., 2019). Fine-scale environmental conditions are especially important for sedentary organisms such as epiphytes (Zotz, 2007; Petter et al., 2016) and lichens as well as for organisms with specific niche requirements (Dial et al., 2006). An ability to collect samples *in situ* and to monitor growth and behavior would greatly contribute to our understanding of the evolution, maintenance, and management of terrestrial biodiversity.

Trees are also immensely important to humans living in cities, as a major component of green infrastructure in the built environment. Trees improve human health (Kardan et al., 2015), help us recover from surgery (Ulrich, 1984), clean the air we breathe (Nowak et al., 2014), and provide numerous other benefits (Turner-Skoff and Cavender, 2019). However, trees in the built environment face several novel challenges. Tree care professionals must monitor tree health and vigor to diagnose pests and diseases and assess the risk of structural failure. The built environment is also capable of harboring a surprising amount of biodiversity and biotic interactions (Trammell et al., 2020) and basic research can help translate the rules of life into cities (Langen et al., 2021).

Little is known about how adult trees balance investments in secondary metabolites against herbivores and resource acquisition. Most study of plant functional traits is limited to seedlings and saplings, which often do not scale to the mature tree (Falster et al., 2018). For example, the abundance and diversity of secondary metabolites appears to be greatest during early developmental phases in plants (Coley and Barone, 1996; Endara et al., 2017; Sedio, 2017). A serious gap exists in our knowledge of how resource acquisition and defensive traits against herbivores and pathogens change across space and time (Barton and Boege, 2017). Collecting leaves for trait mapping, molecular and DNA analyses, species identification and new species discovery remains a challenge, especially in hyperdiverse tropical forest ecosystems. Difficulties of collecting any biotic material from the canopy in such systems delays the advance of our knowledge and development of conservation action to preserve them, as well as the systems they inhabit.

To better understand these arboreal processes, we need more fine-grained data. Canopy research is an active field that supports an international community of researchers (Nadkarni et al., 1996, 2011) but our ability to easily and precisely access the many different dimensions of the arboreal ecosystem remains limited. Even with advanced training and equipment, obtaining the necessary observations and samples with the desired speed and precision presents many practical difficulties. These logistical challenges range from accessing terminal branches and twigs with precision and control to working in remote settings far away from roads, networks, and power sources. To better understand this key habitat, we need new methods that can adequately capture rapid changes in canopy structure, vegetative, and reproductive traits, microhabitat, and microclimate through repeated measurements, across seasons and years. In this paper, we bring together a multidisciplinary, international perspective that includes biologists, tree-care professionals, and engineers to (1) collate existing and proposed access methods for studying the arboreal ecosystem; (2) highlight newly emerging engineering approaches to overcome physical and logistical barriers to accessing tree canopies; and (3) point a way forward to create better modes of access for research and monitoring of the arboreal ecosystem.

THE MULTIDISCIPLINARY COMMUNITY SEEKING ACCESS TO THE TREE CANOPY

To assess the current status of methods for accessing tree canopies, we conducted two online surveys from Oct 23, 2020 to Dec 4, 2020. The first survey, with a total of 27 questions, collected basic information about each respondent's current ability and future aspirations for accessing the tree canopy (see **Supplementary Material 1**). Invitations to take the survey were shared through social media platforms, including Twitter, Facebook, and various listservs (e.g., Ecolog, EvolDir, and UrbanForests), and directly with specific groups of interested colleagues, including the now-closed International Canopy Network, staff and collaborators of the Morton Arboretum, and direct messages to specific individuals. In total, 219 people responded, with Ecolog (54), EvolDir (37) and Twitter (26) providing the most completed surveys. Completion of the survey took 7 min on average.

The respondents to the first survey were asked whether they were willing to participate in a subsequent survey and in a continued discussion of how to overcome challenges in tree canopy research. Over three-quarters of the respondents to the first survey (171 or 78%) indicated their interest in participating in the discussion. The second survey was sent only to this smaller group of continuing participants. It contained six open-ended questions designed to gather more detailed information about the specific needs and objectives of the respondents in their tree canopy work (see **Supplementary Material 2**). We received 51 responses (28% of the short list), and average time to completion was 15 min. Additionally, we held two virtual workshops with the respondents eager to continue their participation in a community discussion. This manuscript and plans for further community development are a result of the virtual discussions, both synchronous and asynchronous, that emerged from the workshops.

Most of the responses (**Figure 1**) were from researchers who either study trees directly (*n* = 130 of 216 total; 60%) or study biotic or abiotic elements of the canopy environment (*n* = 40; 19%), while a smaller fraction manage or care for trees (*n* = 13; 6%). Respondents with an engineering background were in the minority (*n* = 7; 5%) with the distribution of the survey biased toward biologists and arborists. Most respondents worked at universities (**Figure 2**; *n* = 121; 56%), while non-profits, government agencies, and self-employment were all roughly equally represented (*n* = 32, 26, 24, respectively). A smaller proportion worked for private companies (*n* = 13; 6%). Additionally, most respondents were affiliated with institutions in the United States (*n* = 134 of 209 total; 64%) followed by Germany, Mexico, and France (*n* = 11, 8, 8, respectively). The trees of interest to the community are found across most land use types (**Figure 3**); although the greatest fraction is found in natural forests and parks and preserves, a substantial proportion

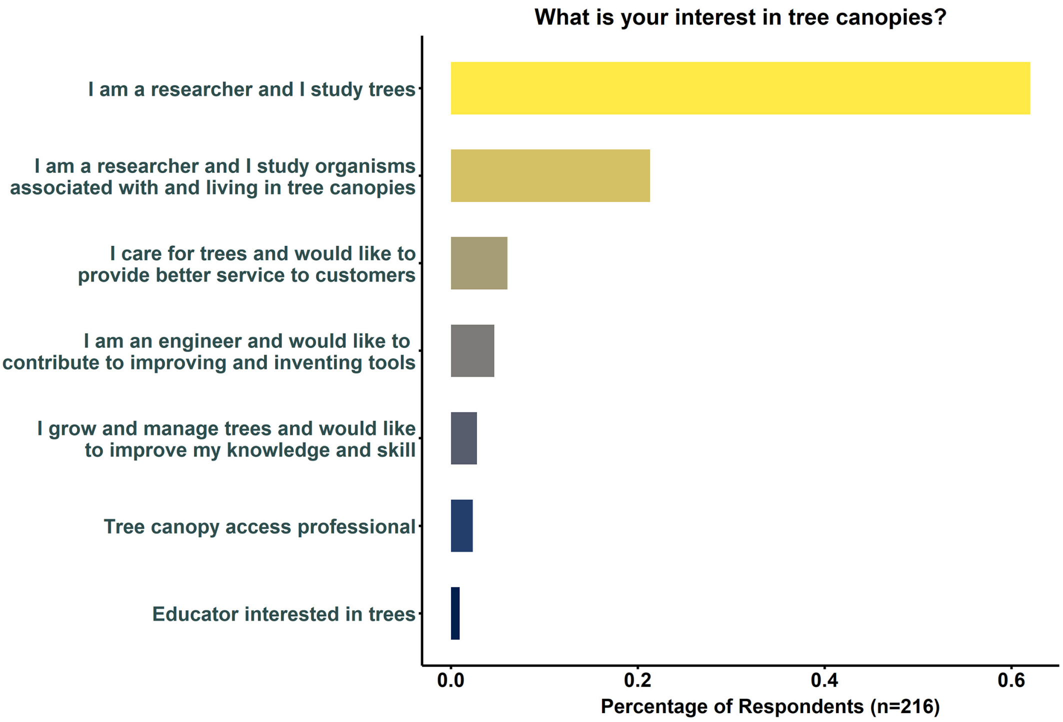

FIGURE 1 | Motivations for gaining access to tree canopies of respondents to an online survey about conducting research and management in the arboreal ecosystem (*n* = 216). Each respondent could only choose one option. An "other" option was included, which generated the bottom two answers.

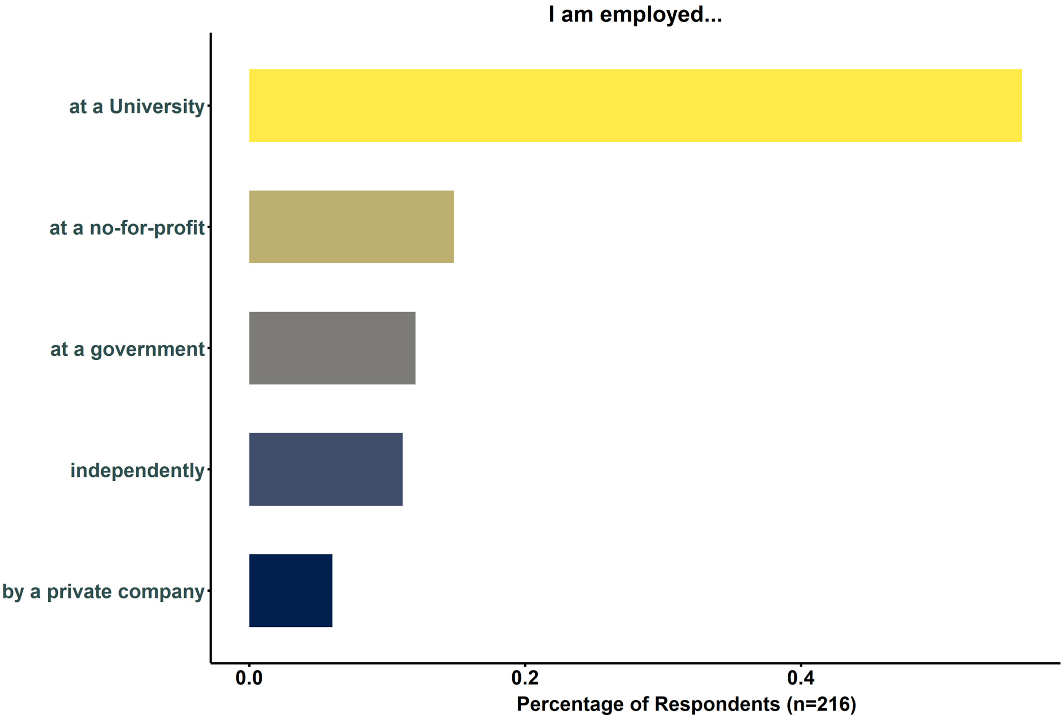

FIGURE 2 | Place of employment of respondents to an online survey about conducting research and management in the arboreal ecosystem (*n* = 216). Each respondent could only choose one option.

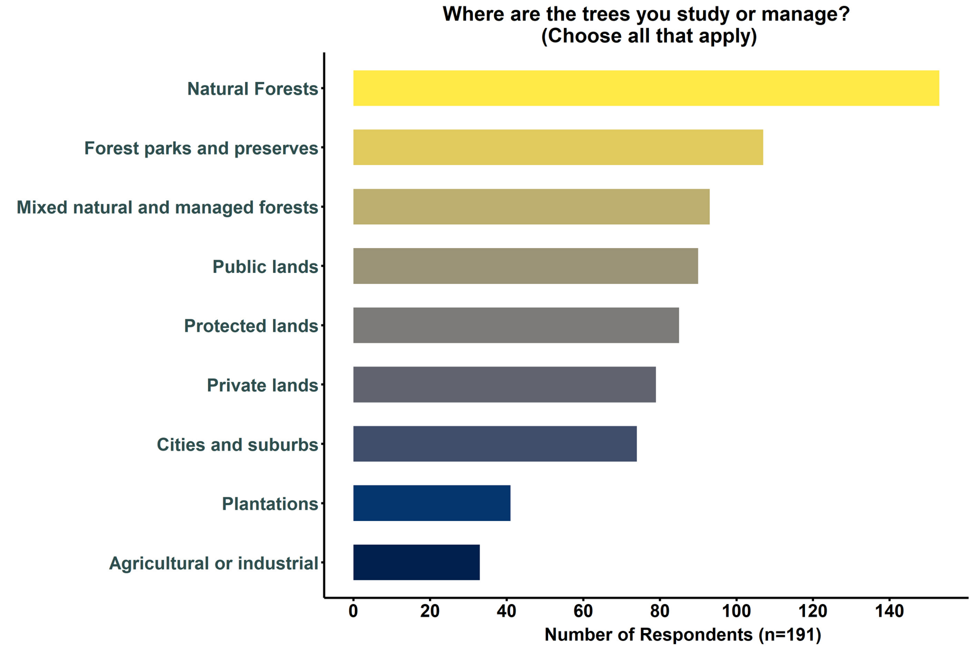

FIGURE 3 | Location of the trees of interest, by land use type, for respondents to an online survey about conducting research and management in the arboreal ecosystem (*n* = 191). Each respondent could choose all applicable options.

occur on private land, in urban areas, and as part of agroforestry production systems. The trees of interest also span a broad range of vegetation types and seasonal precipitation, with increasing trends toward broadleaved wet forests from temperate to tropical regions (**Figure 4**).

Overall, the respondents indicated that their access to the tree canopy is constrained (**Figure 5**). When asked directly whether they felt their access was limited, the median score was 64 out of 100, with higher scores reflecting greater agreement (**Figure 5A**). Some respondents indicated that they were completely unable to get the samples needed to conduct their research (**Figure 5B**) and the median was 49 out of 100. Additionally, the tool used to collect the sample often has an impact on which sample was selected (**Figure 5C**—median of 58 out of 100), further limiting the power of the possible research conducted in the canopy. Finally, there was overwhelming agreement that accessing the canopy took too much time (**Figure 5D**—median of 66 out of 100) and that time is an important consideration (**Figure 5E**). The strongest indicator that the researcher requires better access to the tree canopy is apparent when a comparison is made between how often respondents currently access the canopy and how often they would, given an effective and useful tool were available (**Figure 6**). Clearly, we need better tools to study the tree canopy.

HISTORICAL AND CURRENT APPROACHES TO CANOPY ACCESS

The earliest examples of accessing the canopy for research involve the use of local climbers (Wallace, 1869), felling trees (Beccari, 1904), and the construction of elaborate hoist systems (Perry, 1978; Perry and Williams, 1981; Mitchell, 1986). E.J.H. Corner famously trained pig-tailed macaques to collect samples in Malaya (Taylor, 2019) but the approach was impractical and was abandoned (Corner, 2013). Rope-based climbing methods have been a mainstay for canopy access because the equipment is relatively inexpensive and portable (Barker, 2002; Anderson et al., 2015, 2020; Falen Horna and Honorio Coronado, 2018). Cranes, towers, walkways, temporary platforms (Kettle et al., 2011), and even dirigibles have been used to create a stable infrastructure from which people can access the canopy. Researchers gather arboreal samples from the ground by using shotguns, slingshots, throwlines, and fogging entire trees. More recently, Unmanned Aerial Vehicles (UAVs) have created a new avenue to gain a close look and even collect samples from trees. Some of these achievements have been reviewed and celebrated (Mitchell, 1986; Moffett, 1993; Parker et al., 1995; Mitchell et al., 2002; Lowman et al., 2012), stimulating important research initiatives focused on the arboreal ecosystem and the continued improvement of our ability to access the canopy.

In our study, we asked respondents in two online surveys to indicate how they gathered samples from the arboreal environment (in the first survey as multiple choices from an itemized list, including an "other" text option; in the second survey as a free text response). To simplify further discussion, the word "sample" is used interchangeably for the remainder of the article for any type of physical sample, digital image, or measurement data that could be collected. After compiling the results, we categorized these methods for access (**Figure 7**)

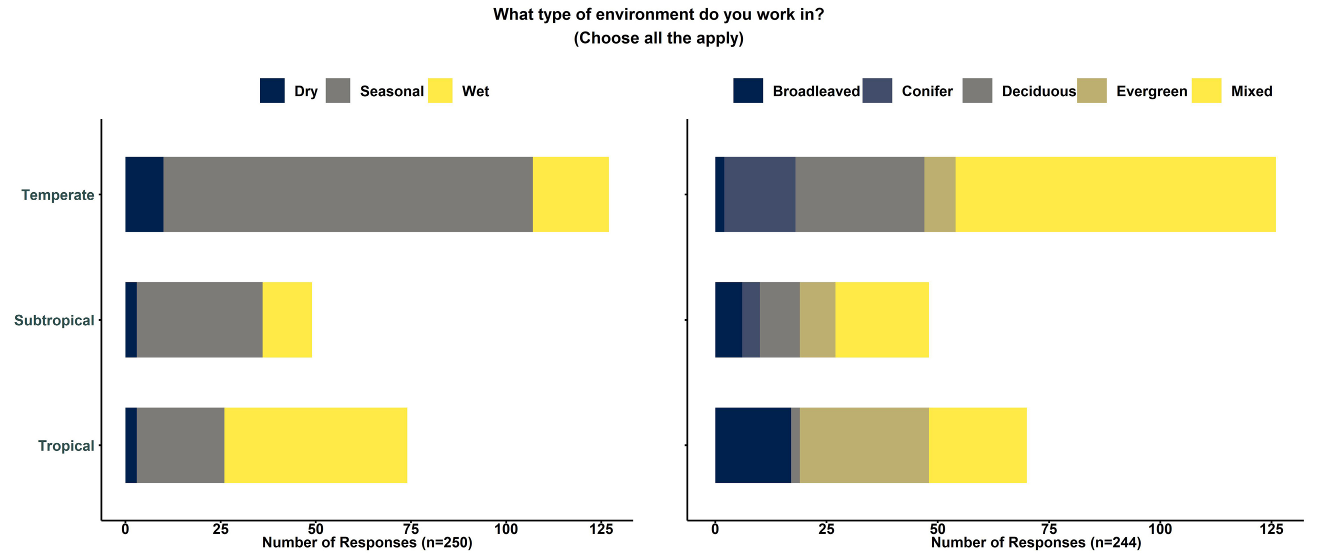

FIGURE 4 | Climate and type of forest where respondents work. Each respondent could choose all applicable options in an online survey about conducting research and management in the arboreal ecosystem.

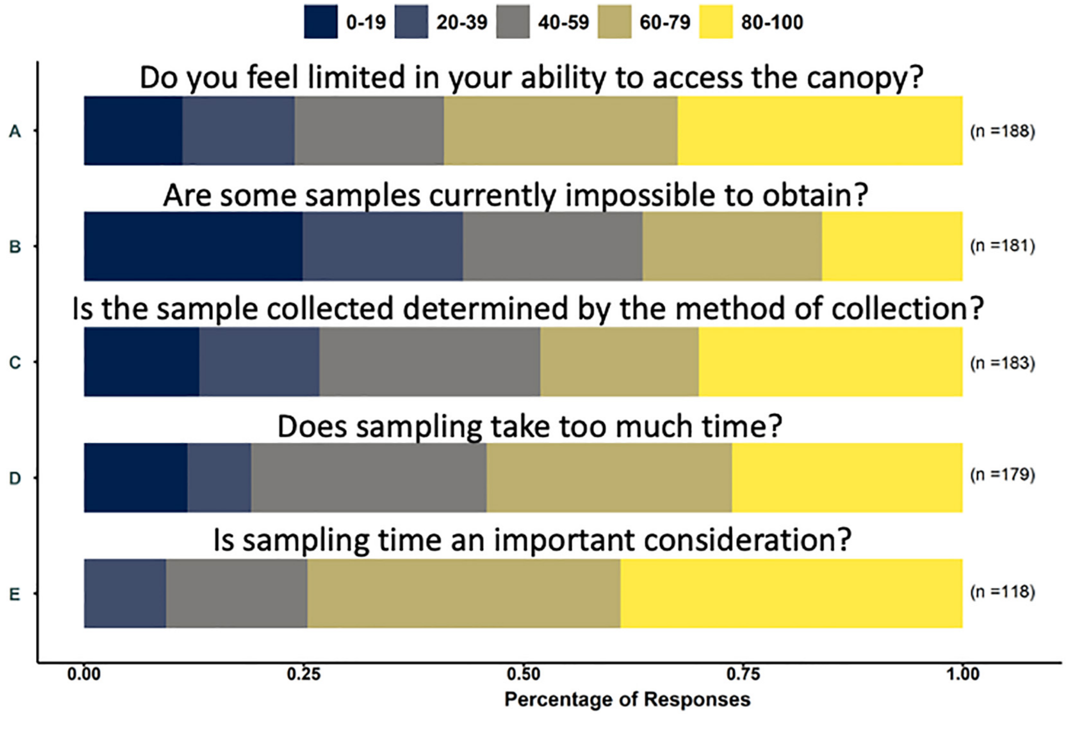

FIGURE 5 | Responses to questions about current ability to access tree canopies from respondents in an online survey about conducting research and management in the arboreal ecosystem. Questions were: **(A)** Do you feel limited in your ability to access the canopy? **(B)** Are some samples currently impossible to obtain? **(C)** Is the sample collected determined by the method of collection? **(D)** Does sampling take too much time? **(E)** Is sampling time an important consideration? Respondents expressed their agreement with the question by choosing a number between zero and 100, with 100 being total agreement.

based on "how?" and "when?". How the sample was obtained included: (1) from the ground, (2) climbing the tree, (3) using infrastructure, or (4) piloting an UAV. Sampling frequency included instantly in a single sampling event or continuously over some period of time. Here, we summarize the results of these surveys according to this classification system.

Our respondents mostly gained canopy access from the ground, with a third (128 out of 384 total answers) indicating

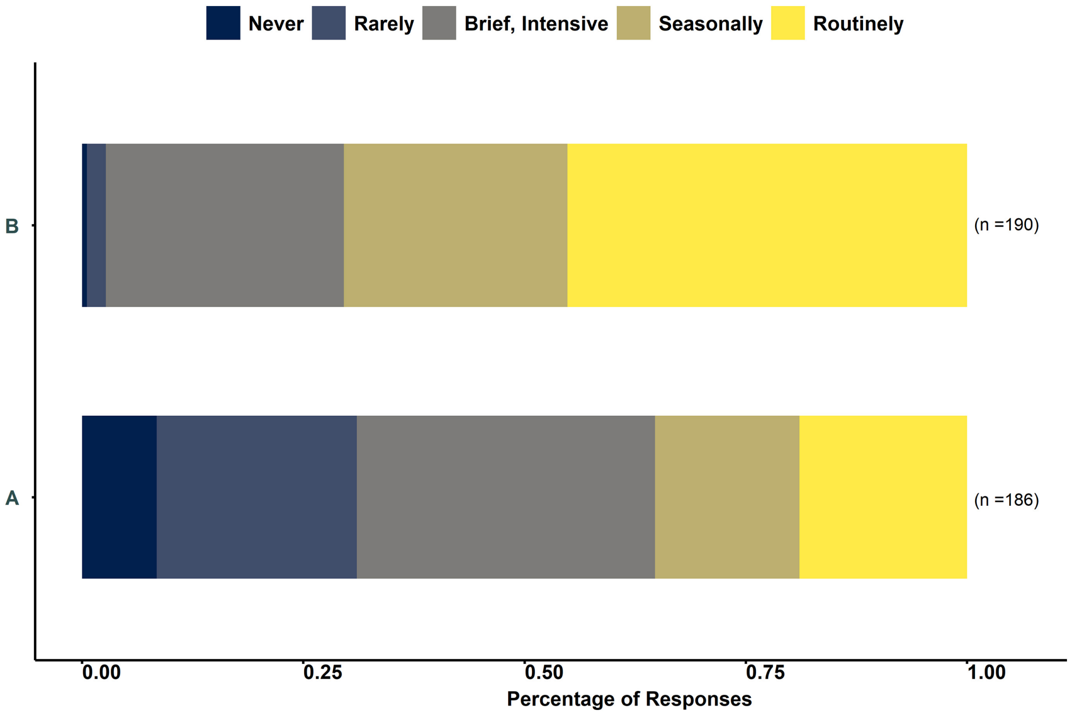

FIGURE 6 | Frequency of tree canopy access reported by respondents given **(A)** their current methods of access and **(B)** their desired level of access, if sampling were quick and effective.

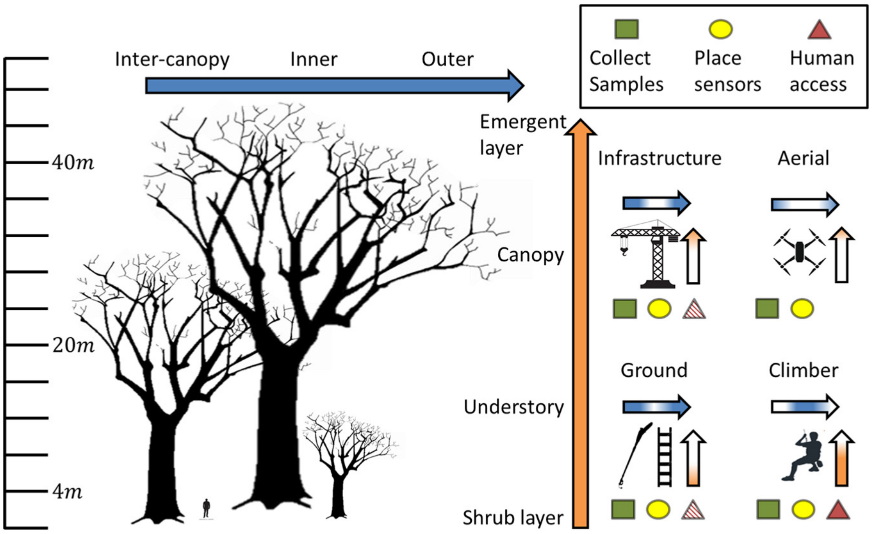

FIGURE 7 | The relative ability of each of the predominant methods of tree canopy access to obtain a sample, given the sample's position in the tree canopy. The blue shading indicates the horizontal accessibility, from the spaces between tree crowns, the inner crown and the outer crown, left to right. The orange shading indicates the vertical accessibility, from the ground to the emergent portion of the canopy. Shading on horizontal and vertical arrows indicates current access, given each method: darker indicated better access. The modes of sample collection are also shown: instantaneous (collecting a sample) by a square; continuous (place a sensor) by an ellipse; and direct human action by a triangle. Darker shading indicates the overall accessibility by that particular mode.

that they either used some type of slingshot or shotgun to knock down a sample or they simply searched and picked up samples directly from the ground, which are often dropped by animals as they forage. While this method is fast and easy, it greatly limits the researcher's ability to objectively choose samples. Generally, imprecision and inability to select the exact location of a sample is a major limitation and this difficulty increases with greater heights and forest structural complexity. Methods requiring a long pole to hoist the sampling device into the tree are limited to relatively low heights in the canopy and necessitate transport of long poles through a forest environment, which is difficult. If upper "sunlit" canopy samples are required, the challenge is even more daunting, and sampling is slow and possibly destructive to the canopy matrix. The use of firearms to shoot down samples also involves a certain amount of personal risk, requires adequate safety training, and is not legal or desirable in all locations, especially in the built environment. The use of ropes to pull down branches can cause significant damage to the canopy and unintended injuries to the tree. Hybrid methods exist by which a line is placed in the canopy (possible using climbing or aerial access) so that sampling equipment can be raised and lowered from the ground. These types of access can also cause unintended structural damage to the tree canopy, which may be problematic for long-term studies.

Climbing was the second most frequently used method (105 out of 384 answers). Rope-based access methods ["rope access"—(Jepson, 2000)] have experienced several innovations over the past two decades. New tools and devices (e.g., hybrid climbing systems that employ both mechanical devices and specialized friction knots) have improved climber efficiency and safety and requiring less physical effort and time. Ropes and specialized equipment for climbing trees are widely available commercially, and relatively affordable and portable, allowing sampling even in remote settings. These methods also involve using slingshots and line launchers to secure the initial ropes and lines. Importantly, with adequate training and expertise, samples can often be obtained from all parts of the tree, including delicate branch tips, epiphytes, and even the open spaces between trees (Anderson et al., 2020), although risk and difficulty increase substantially with taller trees with more expansive, complex crowns.

A main limitation of rope-based methods is that specialized training and experience is needed to execute more sophisticated movements and some trees cannot be safely climbed, for example dead snags growing in isolation from living trees that could permit peripheral access. Many tree-climbing activities are also regulated in most countries with respect to safety in the workplace, as this activity exposes operators to the risk of falling from height (Longo et al., 2013). Climbing can also be time consuming and tiring, so ultimately the number of trees climbed, and number of samples gathered within a time frame is constrained. Sampling can also only be performed on structurally sound trees and repeated rope access must be carefully planned to minimize damage (Chavana-Bryant et al., 2019). Finally, rope access can be combined with other methods, allowing vertical and horizontal movement away from cranes, walkways, and towers (see below). When sampling is performed by a human, rope access offers a flexible and effective means for unbiased and replicable sampling in tree canopies.

Construction cranes (Nakamura et al., 2017) and canopy walkways or towers (Lowman and Bouricius, 1995) require the greatest initial investment, but once installed require no further training or specialized equipment other than the assistance of a crane operator. Therefore, they provide access across the widest range of people, considering age and physical ability. Further, they allow ready access to the open spaces between trees. The physical infrastructure of cranes and walkways cannot reach the interior tree crown, however, limiting access to the outer crown and branch tips without some type of augmentation or sampling device. They also only provide access to a fixed limited number of trees and environments in the immediate vicinity of the crane or walkway. Additionally, sampling from this population of trees must be carefully managed to avoid disturbing or changing the environment, which can affect long-term study results. because moving platforms through the canopy can cause damage if not done carefully. Other types of infrastructure include bucket trucks and mechanical means of lifting a person into the tree canopy. These tools provide excellent access to the outer canopy but require substantial capital investment and are only applicable when the tree is accessible by road and grows in the open.

Several of the respondents (50 out of 384 answers) use aerial techniques to gain access to the canopy. A small proportion reported use of mobile infrastructure, such as flying balloons or rafts (Hallé et al., 2000; Mitchell et al., 2002; Dorrington, 2005) but these remain expensive, logistically difficult to transport, and unavailable to most scientists. Remote sensing with drones was the most common aerial method used to sample the canopy. Over the past few years, Unmanned Aerial Vehicles (UAVs) have evolved rapidly and been adopted by scientists in a multitude of applications. Declining acquisition costs, increasing reliability, and ease of use have driven demand, while airspace regulators have enacted rules to facilitate low-risk UAV operations. Collecting physical samples with a UAV has also become possible over the past few years. The first devices used cutting blades or knives attached firmly to the UAV through a pole.[1,2] This, however, can create vibrations and destabilizes the UAV when it meets a branch. A critical development was the suspension of the sampling device beneath the drone. This configuration allows stable operations and a greater sampling reach while keeping the UAV at a safe distance from natural obstacles.

New aerial sampling tools were developed by various labs with multiple actuators and a vision system to assist the operator during sample collection (Finžgar et al., 2016; Käslin et al., 2018; Kutia, 2019; Charron et al., 2020). Among the solutions available on the market, the DeLeaves canopy sampling tool (Charron et al., 2020) has now been used by scientists in a number of field experiments (NEON, 2019; Schweiger et al., 2020; Arseneau et al., 2021). This tool allows tree canopy samples to be collected in about 6 min (i.e., from UAV takeoff to return with the sample) and has proven to be effective in a variety of environments and for more than 30 tree species. The approach also removes a substantial amount of direct human risk involved in other methods, when people are climbing, shooting, or being suspended to collect samples. Recent work used the DeLeaves canopy sampling tool on a custom UAV to assist the pilot during sampling. In that system, an RGB-D camera was used to detect

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[1] "How Mythbuster Jamie Hyneman Hacked a Drone to Trim His Trees." *Popular Mechanics*, Popular Mechanics, 14 Nov. 2017, www.popularmechanics.com/flight/drones/a26102/jamie-hyneman-drone-plants/.

[2] "Sampler Drones for Forestry Research." *Rausser College of Natural Resources, UC Berkeley*, nature.berkeley.edu/garbelottowp/?p=1801.

and locate suitable branches for sampling before an on-board computer guided the UAV to autonomously collect the selected branches (La Vigne et al., 2021). This assistance significantly reduces the pilot workload and accelerates the sampling process by more than six times.

UAV sampling still has limitations. For example, it is necessary to maintain a safe distance from the top of the tree to avoid any contact with the propellers. This limits sampling to the upper third and the outer edge of a tree. Finally, while battery life has improved, flight time and payload remain limited (e.g., the DJI M300 has a 9 kg max takeoff weight, a 2.7 kg max payload, a 55 min max flight time and a max speed of 23 m/s). However, in practice, the flight time when fully loaded with sensors and sampling equipment is closer to 15 min. For missions of long duration, multiple sets of batteries need to be carried and recharged in the field when possible (e.g., the DJI M300 has a 2.7 kg battery pack of 548 Wh). The noise associated with UAVs may also disrupt organisms inhabiting natural ecosystems. While there are instances of UAVs having been attacked by birds (Yaacoub et al., 2020; Chaari and Al-Maadeed, 2021), which can disrupt research progress and harm birds, best practices have been developed to fly UAVs around birds (Junda et al., 2015). Noise or activity produced may also create other types of unsafe situations for researchers. For instance, the UAV noise may disturb unseen nests of bees or wasps, which in one instance has resulted in the death of a researcher due to bee stings (pers. obs.).

The respondents also pointed out that the site context can also present significant challenges for gaining canopy access. In remote locations, beyond the simple fact that newer technologies require electrical power, complicating their heavy implementation, their noisy operation and high visibility can call attention to researchers working in areas where illegal activities or military conflict are occurring. Developed and populated landscapes, such as cities and towns, present different challenges associated with tree canopy access. While line launching techniques (e.g., slingshot) are used in natural forested areas within cities, they pose considerably more risk in urban forest settings used for recreation, endangering bystanders and property. UAV-based techniques pose additional challenges associated with permission and security concerns in populated landscapes. Finally, sensors or other types of equipment placed within urban tree and forest canopies for continuous or repeated measurements can be stolen or damaged.

MODES OF ACCESS AND EXPERIMENTAL DESIGN

Beyond the practical details of how the sample is collected, when and where the sample is collected is potentially of even greater importance. Previously, canopy sampling has tended to be arbitrary, non-random, unrepresentative, and opportunistic. Also, low replication or pseudoreplication (*sensu* Hurlbert, 1984) are potential weaknesses. Yet few canopy-based studies report sampling issues (Kapos et al., 1993; Baldocchi and Collineau, 1994; Koch et al., 1994), although logistics and experimental design in arboreal ecosystem research remain undeniably challenging. But the focus among researchers has shifted from simple access to experimental design and sampling issues (Barker and Pinard, 2001; Bongers, 2001; Nakamura et al., 2017) centered on hypothesis driven quantitative approaches (Rinker and Jarzen, 2004; Volf et al., 2019). Ideally, modes of access and experimental design of arboreal ecosystem research need to be equally rigorous as other terrestrial ecosystem research (Barker and Pinard, 2001; Mitchell, 2001; Sutton, 2001). The quantity and quality of data and samples collected in the canopy should not be compromised based upon how and when the sample can be collected. For example, researchers are not always sufficiently mobile in the upper canopy, so to avoid under- or over-sampling or pseudo-replication, independent replicates can be achieved spatially, by simultaneous sampling by researchers, sensors or traps (e.g., Bassow and Bazzaz, 1997; Bar-Ness et al., 2012) or temporally across days or weeks (e.g., Perez-Salicrup et al., 2001). Devising the best strategies for achieving these comprehensive approaches requires collaboration and coordination across the community of arboreal researchers.

Modes of access and experimental design can vary considerably depending on the aspect of the arboreal ecosystem that is being sampled. For example, the epiphyte community requires capturing the plants living attached to the limbs and branches of a tree. The limited access to the canopy has been calculated to miss, for example, more than 50% of lichen diversity (Boch et al., 2013), much of which is endemic to the upper canopy (Fritz, 2008; Ellis, 2012; Marmor et al., 2013). To study epiphyte communities and obtain complete species lists during inventories, various complementary methods are used, including surveying fallen twigs and branches (Sarmento Cabral et al., 2015; Gasparyan et al., 2018), using tree pruners (Degtjarenko et al., 2016, 2020), and employing rope-based methods (Fanning et al., 2007; Boch et al., 2013; Kiebacher et al., 2016). Canopy crane access has permitted detailed characterization of epiphyte assemblages across forest strata, but these have been limited to only a few locations (Zotz, 2007; Petter et al., 2016) and gondolas can damage the upper or outer canopy. Trees or branches felled for other purposes or that naturally fall also permit comprehensive sampling of epiphyte diversity (Marmor et al., 2013; Kaufmann et al., 2019). The latter methods are not always applicable in regular surveys or permissible in highly protected forests.

The collection of arthropods and other invertebrates from tree crowns relies on a multitude of sampling methods, including fogging from the ground and insect traps (e.g., Malaise, flight interception, pitfall, sticky traps) that can be lifted with ropes into trees. The integrative IBISCA (Investigating the Biodiversity of Soil and Canopy Arthropods) approach is a good example of the combination of these methods with a wide variety of canopy access tools (e.g., cranes, balloons, rafts, climbers) leading to the development of new sampling protocols (Leponce et al., 2012, 2021). Recent large-scale, multi-taxa projects to access the tree canopy have relied on tree felling, canopy cranes, cherry pickers (Nakamura et al., 2017; Volf et al., 2019; McCaig et al., 2020; Mottl et al., 2020), or ground-level fogging using insecticide (Swart et al., 2020). Canopy-dwelling vertebrates including birds, frogs, lizards, and mammals are commonly studied observationally either using binoculars, climbing into the canopy, or using remotely sensed images to identify nests (Milne et al., 2021). For more sentient taxa (birds and mammals) and for behavioral studies in particular, there is risk of unintended disturbance or demonic intrusion (Hurlbert, 1984) when researchers introduce themselves into the arboreal environment, thus changing the naturally occurring parameters in the experiment (Barker and Pinard, 2001). Censusing from the canopy accurately captures richness patterns (Anderson, 2009) and can elucidate patterns of vertical stratification (Scheffers et al., 2013). Traps to capture small mammals have been implemented in the canopy and can be installed directly or from the ground (McClearn et al., 1994; Kays, 1999; Lambert et al., 2005).

While methods of access are generally the focus of experimental design, an important distinction about the timing of access was identified in remote discussions among the co-authors, during and after the virtual workshops. Specifically, samples are either collected (1) instantaneously or (2) continuously. Instantaneous samples serve as a snapshot in time, typically involving a physical sample, a measurement, or an image or data taken by cameras or sensors, that is collected a single time without expectations of returning to the site. Continuous samples, on the other hand, can involve either placing a sensor or trap in the tree or repeated measurements of the same part of the tree. These two approaches require fundamentally different techniques and impose different functional constraints on the experimental design. Most methods used by canopy researchers focus on instantaneous sampling, where a physical specimen is removed from the tree. Continuous monitoring and repeated measurements are undergoing a revolution because of advances in sensor technology and camera traps. Sensors have been placed in tree canopies using UAVs (Farinha et al., 2020) and improving upon these methods is one major avenue for innovation. Additionally, repeated sampling of the same tree, preferably even the same part of the canopy, can be necessary during experimental trials or long-term monitoring programs. These repeated measurements often involve the construction of canopy infrastructure but UAVs again might provide a new means of achieving this research. Controlling the structural and physiological impact and logistical burden of these repeated sampling techniques is important aspect to consider (Zandt, 1994).

Remote sensing provides information about forest canopies through a sensor signal resulting from the interaction of electromagnetic energy with the canopy components (Weishampel et al., 1996; Baret and Buis, 2008). The level of detail and type of information obtained from the canopy mostly depends on the type of platform (satellite, airborne, UAV) and sensor (multi/hyperspectral, radar, laser) used. Traditional remote sensing uses satellite imagery and it has improved in terms of the type of sensors, spatial and temporal resolution (Chavana-Bryant et al., 2019; Lechner et al., 2020). UAVs provide relevant canopy information at flexible times, with a higher spatial resolution and a relatively cheaper price when compared to satellite data (Lechner et al., 2020; Dainelli et al., 2021). Arboreal camera traps are effective at capturing photos of canopy vertebrates if foliage is removed from the immediate vicinity of the camera to prevent false triggering (Di Cerbo and Biancardi, 2013; Gregory et al., 2014; Whitworth et al., 2016; Bowler et al., 2017; Nazir and Kaleem, 2021). Fixed cameras in the canopy as Phenocams or camera traps also provide valuable temporal information to study growth, phenology, harvest traits (Aasen et al., 2020). Ecoacoustic monitoring is an emerging field (Ducrettet et al., 2020; Pérez-Granados and Traba, 2021) that could be adapted to canopy research similarly as cameras.

The combination of machine learning techniques with remote sensing data allows several canopy studies ranging from semi and automatic identification and quantification of canopy species using conventional RGB cameras (Tagle Casapia et al., 2019; Ferreira et al., 2020; Wang et al., 2021), multispectral cameras (Gini et al., 2014; Wagner et al., 2020) or hyperspectral sensors (Dalponte et al., 2014), to assess health status (Dainelli et al., 2021), phenology (Feng et al., 2021), above-ground biomass estimation/quantification using RGB, radar or Lidar (Marks et al., 2014; Brede et al., 2019; Dainelli et al., 2021), canopy traits (Thomson et al., 2018; Ganivet and Bloomberg, 2019), or to detect fauna with thermal sensors (Spaan et al., 2019; Zhang et al., 2020). In all the cases, to be most effective, calibration against carefully chosen samples at the top of the canopy is required to provide accurate results (Käslin et al., 2018; Chavana-Bryant et al., 2019; Schweiger et al., 2020). In addition, while we are aware that some high-tech methodologies such as airborne laser-guided imaging spectroscopy could help to solve these issues especially in tropical forests, these methodologies are expensive and not accessible for all researchers (Baraloto et al., 2010; Asner et al., 2017). Thus, setting a workflow that allows drone-based leaf sampling and drone-guided imaging spectroscopy would be valuable in terms of cost reduction and canopy data accessibility.

ENGINEERING AND ROBOTIC POSSIBILITIES

Given the need for more precise and versatile ways to access the arboreal ecosystem, both for instantaneous sampling and for continuous monitoring, recent progress in robotics can enable new capabilities. Historically, robotics has been used to perform tasks known as "the 3Ds" (typically, dull, dangerous, or dirty, but also sometimes difficult, demeaning, and demanding), particularly repetitive tasks in manufacturing and warehouse fulfillment (Bogue, 2016). Sensors, sometimes located on mobile robots to create deployable and reconfigurable sensor arrays, are also used to monitor certain areas for extended periods of time and in a variety of environmental conditions. Given these examples, one can presume that teleoperated robots could be deployed in the exploration of a tree canopy during an expedition, to address many of the sampling issues discussed above. Automating data collection can also provide solutions to creating large, replicable datasets and collecting repeated measurements. To inspire new ways to perform canopy research, we provide a short review of recent relevant robotics progress and the challenges that remain.

Robots are typically classified either as manipulator arms or mobile robots. Manipulator arms are common in factory settings, moving objects or performing tasks such as painting or welding. While manipulators may employ different joints and linkages, they are generally similar to each other and could best be deployed along with canopy infrastructure, where a fixed anchor point would be available. For example, when placed on a canopy crane, a manipulator arm could extend the reach and precision of sample collection. Mobile robots have a much more varied form and are typically classified according to their mode of locomotion. These include wheeled, tracked, and legged (or combinations thereof) robots for terrestrial locomotion, and fixed-wing, rotorcraft, or ornithopters (flapping wing) systems for aerial flight.

Many of the challenges of mobile robot mechanical design lie in understanding how to move in challenging environments, including deformable terrain such as sand or mud, overcoming specific obstacles (like stairs), or climbing vertical surfaces. Tree-climbing robots have been developed, using wheels (Megalingam et al., 2021), arrays of small toes with sharp spines that engage with asperities in the climbing substrate, also known as microspines (Megalingam et al., 2021), "legs" that grasp the trunk (Lam and Xu, 2011), or trunk jamming mechanisms.[3] These platforms are much slower than human climbers and none have demonstrated the ability to reliably and repeatedly navigate trunks with branches. Other robots can deploy observation masts up to 12 m high using three interlocked steel tapes[4] or grow like a vine by everting thin plastic tubes using compress air (Haggerty et al., 2021). These could be deployed from the ground, from within the tree canopy, or from aerial platforms; however, they have many logistical issues that limit their successful deployment for research.

Aerial platforms have the advantage of being able to rapidly fly over an area to easily access the topmost branches of the tree canopy or for reconnaissance to identify and target sampling sites. In general, above-canopy flight is a simple task because of the presence of Global Navigation Satellite System (GNSS) signals, such as GPS, which allow the robot to accurately localize itself in the environment. However, below-canopy flight is much more difficult because the canopy effectively blocks all GNSS signals. Thus, aerial platforms that navigate below the canopy must be teleoperated or rely on sophisticated sensors such as vison or lidars coupled with advanced control and path planning algorithms for the robot to adequately localize itself. When operating above the canopy, suspended payloads could be transported by these platforms to collect samples or retrieve sensors near any structure in the canopy. UAVs have also been flown with nets to capture insects (Löcken et al., 2020) or various devices to capture spores (Crazzolara et al., 2019).

Although direct contact with arboreal elements (leaves, twigs, fine vines) should generally be avoided, some UAV platforms are protected against intermittent contact (e.g., through physical guards or duct around propellers) or are even designed to take advantage of direct contact with their environment (e.g., using cages around the drone (Klaptocz et al., 2013; Kalantari and Spenko, 2014). This allows UAVs to fly within forests and inspect enclosed spaces, crevasses within glaciers, tunnels, or underground mines. Some aerial platforms can also land on high power electrical lines for in-contact inspection (Mirallès et al., 2018) or perch on surfaces/branches of various orientations for extended monitoring (Roderick et al., 2017; Hang et al., 2019; Mehanovic et al., 2019; Nguyen et al., 2019). Many new commercial UAVs now also have sensors to autonomously avoid obstacles when navigating around hiking trails, construction sites, or underground mines (e.g., Skydio, Emesent).

Mobile robots are commonly used to carry sensors to measure values of interest. The sensors (e.g., RGB cameras, range sensors such as lidars or radars, Infrared (IR) sensors, and hyperspectral cameras, among others) transported by the robot could be used to reconstruct the 3D environment through photogrammetry or point clouds (Scher et al., 2019). Many robots are also equipped with manipulator arms including end-effectors to interact with the environment (Zhang et al., 2020) through grasping or specialized samplers (e.g., branch cutter, aspirating devices, pumps, drills) to collect branches, flowers, bark, water, or insects. Robots could also be used to precisely install ropes or as a platform to deploy instrumented darts (Farinha et al., 2020) or other projectiles. Many industrial fields are investing heavily to advance robotics technology. These include aerial manipulation for structural inspection and maintenance, agricultural robotics, mining, manufacturing, fulfillment centers, medical robotics, autonomous driving, among others. Many of the technologies in these fields could be re-used to provide better access to arboreal ecosystems for research and management.

One of the main challenges with robotics is the required resources, expertise, cost and time needed to develop, use, and maintain these systems. To successfully develop tools that will be used in the field, coordinated efforts between scientists and engineers are required. Priority should be established by identifying the most pressing and common needs from scientists, but also the needs for which viable technological solutions can be developed by the engineering team. Equipment sharing should be favored, as well as concerted sampling efforts (i.e., collecting samples that can provide data for multiple studies). Technological solutions that can be used in multiple contexts can also be beneficial. For example, the creation of robotic augmentation to extend and improve the human ability to sample specific parts of the tree in structurally challenging regions of the canopy could be useful either for someone working from the ground or for someone who has climbed up the trunk of the tree. Likewise, devices developed to allow a hovering UAV to place a sensor precisely in the canopy could also be used by a person working from a canopy crane or walkway.

FUTURE GOALS AND DIRECTIONS

Tree canopy research has grown substantially over the last three decades (Moffett and Lowman, 1995; Barker, 2015; Lowman, 2021), with an acceleration in innovative techniques and possibilities led by technological developments in sensors,

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[3] https://www.youtube.com/watch?v=2qKDixMGaqY&ab_channel=KAZ-corpLTD

[4] https://www.faulhaber.com/en/markets/factory-automation-robotics/zippermast/

robotics, and UAVs. Here, we hope to facilitate this acceleration and focus innovation on the tools and technologies that could have the broadest impact and encompass the entire arboreal ecosystem. Experimental design, scientific questions, and management practices should not be determined by the methods and modes of accessing tree canopies. The community of scientists who study the biology of tree canopies originally had to overcome tremendous obstacles and recent developments are both a cause and a consequence of creative methods for accessing tree canopies (Barker and Sutton, 1997). Even today, most people working with trees state that their canopy access is limited and if they had effective and accurate equipment, they would routinely collect samples from the arboreal ecosystem.

This study represents a first step toward building a multidisciplinary community of academic scientists, tree professionals, and research engineers to address the limitations we face in accessing the arboreal ecosystem. The results provide important information from diverse perspectives about current abilities, limitations, and aspirations. Our virtual discussions, both synchronous and asynchronous, have created an effective framework for characterizing the methods and modes of accessing the canopy. To find common ground among these many interests and to maximize the impact for global efforts to study the arboreal ecosystem, we have identified four main priorities to advance these efforts:

1) broaden participation geographically and professionally;
2) identify the most common needs;
3) create teams of those who need access, e.g., biologists, with those who can help provide access, e.g., engineers and climbers, organized around common needs; and
4) establish a communication platform for sharing information.

Broadening participation, both geographically and professionally, will allow an effective interchange of advances, an efficient communication channel about different end user's values, and create opportunities for novel insights from under-represented perspectives. During the initial stages of our discussions, the responses to our survey were dominated by those working at institutions and universities in the United States and the United Kingdom. Subsequent discussions, during the virtual workshops and asynchronous collaborations on this manuscript, were more diverse, as demonstrated by the authors here. A more focused effort to include participants from tropical countries, is needed (Botanic Gardens Conservation International [BGCI], 2021). In some of these countries, the resources and infrastructure for research can be limited (e.g., Jarrín-V et al., 2021) or the value and emphasis of different types of research can be substantially different (Ciocca and Delgado, 2017).

Tree managers and tree care professionals, both municipal and private, work with trees every day and have a great deal of experience and motivation to access tree canopies. The value of trees in our built environment, both for human health and pleasure, has become increasingly apparent to the general public and city planners (Matsler et al., 2021). Combating pests, treating diseases, pruning, and assessing structural integrity of trees would all be aided by easy, precise, and low-cost access to the tree canopy. Cheap and enjoyable means of obtaining arboreal samples could also allow community scientists to meaningfully participate and contribute to research related to community health, environmental justice, and important scientific questions about urban ecology and evolution (Langen et al., 2021). This connection between the public and the arboreal ecosystem can also be a powerful way to educate and inform students and tourists about its importance and value, even generating forest conservation and sustainable alternative incomes for indigenous people. To engage participants from these two different segments of global society, we will contact relevant societies and associations, international and national, like the International Society for Arboriculture, to learn how to make their members aware of our activities and objectives.

By identifying the common needs, we will form working groups that connect the scientists and professionals who need routine and efficient access with the engineers and roboticists with the appropriate expertise and interest to improve and invent the needed tools. Combining technical expertise from these two scientific fields may provide solutions through the application of existing technologies to the challenges faced in gaining canopy access (Cannon, 2012). By starting with the "lowest hanging fruit," we can have the broadest impact and understand the functional requirements and engineering approaches that span several modes of access. The intersection of the means of access (e.g., canopy infrastructure, climbing, UAVs) with the needs of access (e.g., physical leaf sample, multispectral imagery, arthropod samples) will determine the best strategy for designing and improving these tools and how we might best incorporate robotic and engineering solutions.

Effective communication is important to any distributed and multidisciplinary effort. Given the rapid pace of innovation and change in global circumstances, we seek to establish an effective platform, built on existing social media networks and digital sharing technologies. This communication would not only coordinate activities and discussions within the network but would act as an information hub for technological advances, training opportunities, community events, and even expeditions. Informing the community about canopy samples being collected for one purpose can potentially have significant and simultaneous value for multiple research programs, especially for studies of sedentary plant associates (e.g., fungi, lichens, bacteria, mites, scale insects, whiteflies) and for samples collected in poorly known and remote tropical forests and other biodiversity hotspots. Researchers would benefit from standardized protocols for sample and data collection and distribution to allow for comparative studies across taxa and projects. The organization of workshops at large scientific and professional conferences, targeted events with municipalities and non-profits, and continued online virtual engagement will be an important part of growing the community and increasing inter-disciplinary and cross-agency collaborations and networking.

Finally, we recognize that expense and training will remain a barrier to entry for many people whose professional or personal needs do not require regular tree canopy access. Efforts to remove these barriers, to maximize benefits and share the load as much as possible, will be sought. For example, the creation of regional hubs of expertise and equipment to facilitate the use or even provide service of canopy sampling tools or personnel to occasional or one-time users in the scientific and public communities, potentially even on a contract basis. This would help the community combine efforts and maximize benefit obtained from existing tools and expertise and innovate on new ones. Moreover, an awareness of the types of specimens sought by other researchers could potentially provide an easy solution for obtaining samples simultaneously for several purposes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Illinois Institute of Technology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CC, CB, and MS conceived of the project and conducted the surveys. CC, CB, MS, and AD organized and led the workshops. CC and CB analyzed the data. CC, CB, DA, GA, MB, GC, and JR made substantial creative contributions to the manuscript. All authors contributed to the writing, editing, and revising of the manuscript.

FUNDING

The research presented in this article was supported by the Center for Tree Science at The Morton Arboretum.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.712165/full#supplementary-material

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Conflict of Interest: Some of the authors provide professional services, like tree climbing or sell UAV sample collection devices, that are discussed in the paper. The lead author and most of the other authors have no conflict of interest and have paid careful attention to any possible advocacy or promotion of a particular service caused by these select few potential conflicts.

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ORIGINAL RESEARCH
published: 23 November 2021
doi: 10.3389/ffgc.2021.716278

Structuring Interaction Networks Between Epiphytic Bryophytes and Their Hosts in Yunnan, SW China

Hai-Xia Hu[1,2,3†], Ting Shen[4†], Dong-Li Quan[5†], Akihiro Nakamura[1,3] and Liang Song[1,3,6*]

[1] CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, China, [2] University of Chinese Academy of Sciences, Beijing, China, [3] Core Botanical Gardens, Chinese Academy of Sciences, Mengla, China, [4] Institute of Botany, University of Liège, Liège, Belgium, [5] Center for Integrative Conservation, Southeast Asia Biodiversity Research Institute, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, China, [6] Ailaoshan Station for Subtropical Forest Ecosystem Studies, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Jingdong, China

Edited by:
Brett Scheffers,
University of Florida, United States

Reviewed by:
Julian Donald,
University of Exeter, United Kingdom
Wen Xing Long,
Hainan University, China

****Correspondence:***
Liang Song
songliang@xtbg.ac.cn

† These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *28 May 2021*
Accepted: *01 November 2021*
Published: *23 November 2021*

Citation:
Hu H-X, Shen T, Quan D-L, Nakamura A and Song L (2021) Structuring Interaction Networks Between Epiphytic Bryophytes and Their Hosts in Yunnan, SW China. Front. For. Glob. Change 4:716278. doi: 10.3389/ffgc.2021.716278

Ecological networks are commonly applied to depict general patterns of biotic interactions, which provide tools to understand the mechanism of community assembly. Commensal interactions between epiphytes and their hosts are a major component of species interactions in forest canopies; however, few studies have investigated species assemblage patterns and network structures of epiphyte–host interactions, particularly non-vascular epiphytes in different types of forest. To analyze the characteristics of network structures between epiphytes and their hosts, composition and distribution of epiphytic bryophytes were investigated from 138 host individuals using canopy cranes in a tropical lowland seasonal rain forest (TRF) and a subtropical montane moist evergreen broad-leaved forest (STF), in Southwest China. We structured binary networks between epiphytic bryophytes and their hosts in these two forests, which presented 329 interactions in the TRF and 545 interactions in the STF. Compared to TRF, the bryophyte–host plant networks were more nested but less modular in the STF. However, both forests generally exhibited a significantly nested structure with low levels of specialization and modularity. The relatively high nestedness may stabilize the ecological networks between epiphytic bryophytes and their hosts. Nevertheless, the low modularity in epiphyte–host networks could be attributed to the lack of co-evolutionary processes, and the low degree of specialization suggests that epiphytes are less likely to colonize specific host species. Vertical distribution of the bryophyte species showed structured modules in the tree basal and crown zones, probably attributing to the adaptation to microclimates within a host individual. This study highlights the nested structure of commensal interaction between epiphytic bryophytes and host trees, and provides a scientific basis to identify key host tree species for conservation and management of biodiversity in forest ecosystems.

Keywords: commensal interaction, epiphyte, modularity, nestedness, specialization

INTRODUCTION

Human disturbance and climate change induce a severe biodiversity crisis, which not only directly leads to species extinction, but also indirectly causes community collapse as species co-extinction through interspecific interactions. Hence, biotic interactions remain a core theme to understand the general mechanisms of community assembly and disassembly in order to scientifically guide biodiversity conservation and restoration (Taylor et al., 2016; Naranjo et al., 2019).

Ecological networks provide a powerful tool to elucidate complex interspecific interactions in different types of communities (Borrett et al., 2014; Landi et al., 2018). There is a long tradition of describing ecological networks in bipartite models which generate simple binary interaction matrices (Blick and Burns, 2009; Gruber et al., 2009; Arthur, 2020). In addition to depicting the interactions among species, quantitative descriptions of network properties are possible to explore the process of colonization during successions (Fontaine et al., 2011; Peralta, 2016) and to predict community stability under disturbance in the future (Vizentin-Bugoni et al., 2019; Baumgartner, 2020). Many empirical studies have uncovered different network patterns, such as resource–consumer networks (Welti and Joern, 2015; Costa et al., 2021), plant–pollinator networks, plant–seed disperser networks (Veron et al., 2018; Stein et al., 2021), as well as commensal networks in the last decade (Sayago et al., 2013; Francisco et al., 2018). However, the ecological processes and underlying causes that drive the network structure are still under debate (Bluethgen, 2010; Calatayud et al., 2017; Landi et al., 2018; Pinheiro et al., 2019). Much effort is required to understand general structures of interaction networks and to clarify the underlying mechanisms that mediate ecological networks.

Epiphytes constitute a large part of the global plant biodiversity and provide a unique component in forest ecosystems (Nieder et al., 2001; Song et al., 2015b; Toivonen et al., 2017). Since the survival and growth of epiphytes mainly depend on their host plants, studies on the patterns and mechanisms of commensal interactions between epiphytes and host trees are essential to understand the diversity of epiphytic communities. So far, some patterns of epiphyte–host interaction networks have been reported in different forest ecosystems such as a nested structure, low connectance, low degree of specialization, and low modularity (Francisco et al., 2019; Naranjo et al., 2019; Fontúrbel et al., 2021). The mechanisms behind network patterns of epiphyte species and their host plants are poorly understood, although non-random processes seem to be recognized in shaping the structure of epiphyte networks (Fontúrbel et al., 2021). Furthermore, epiphyte–host network studies are often restricted to communities in a single forest type (e.g., boreal forest, temperate forest, subtropical or tropical forest) and/or in specific vascular epiphyte groups (e.g., orchids) (Zhao et al., 2015; Ceballos et al., 2016; Francisco et al., 2018, 2019). Although the database EpIG-DB has integrated existing data on epiphyte assemblages at the continental scale (Mendieta-Leiva et al., 2020), we are still in the process of establishing general knowledge about the epiphyte–host networks among all epiphyte groups in different forest types.

Epiphytes attached to host trees are more sensitive to changes in humidity, light and temperature in different habitat environments (Coyle, 2017). On the one hand, climate differences at a regional scale act as environmental filters to constraint epiphyte community assembly. For example, the typical elevational changes in epiphyte species composition were found to be explained by humidity gradients, as species richness showed mid-elevational peaks which corresponded to humidity gradients (Song et al., 2015a; Quiel and Zotz, 2021). A study from the temperate oceanic climate zone of Western Europe revealed that annual rainfall and average temperature gradients are responsible for the frequency and abundance of epiphytes (Klinghardt and Zotz, 2021). On the other hand, host attributes such as bark texture (Zarate-Garcia et al., 2020), tree size (Francisco et al., 2018) and species richness (Calatayud et al., 2017) are the important factors which may affect the biodiversity of epiphyte community in forest stands.

Additionally, microclimatic gradients on individual host trees provide a range of different conditions for epiphyte assembly from the tree base to upper canopy (de Oliveira and de Oliveira, 2016). It has been reported that more bryophyte species occurred in the tree crowns than the trunks and base zones in subtropical forests and tropical forests (Shen et al., 2018). Humidity, temperature and light incidence are the main microclimate factors determining growth and survival of epiphytes at different vertical zones that contribute to the variation in species composition and development of epiphyte species in forests (Padilha et al., 2017). Although numerous studies suggested that the epiphyte species distribute along vertical microclimatic gradients on host individuals (Zotz, 2007; Li et al., 2015), epiphyte–host network structures along different vertical zones have rarely been studied (but see Francisco et al., 2019).

Here, we investigated epiphytic bryophytes and their hosts using the canopy cranes in the tropical and the subtropical forests in Yunnan, Southwest China. The preceding study by Shen et al. (2018) investigated the vertical composition and distributions of epiphytic bryophytes in the same study sites, but our study re-analyzed the data by constructing commensal networks of bryophyte–host interactions and quantified network properties using six network metrics. In this study, we aim to (a) characterize the structures of the epiphytic bryophyte–host networks, (b) assess the roles of forest types, host traits, and vertical zonation in structuring the bryophyte–host networks. Based on previous knowledge, we hypothesize that networks present a nested structure with low level of modularity, and bryophyte species attached on the base and crown zones are grouped into different modules. We also hypothesize that occurrence of higher bryophyte diversity is more likely in the humid subtropical forest than in the monsoonal tropical forest; and the host size and abundance are two main drivers of epiphytic community structure.

MATERIALS AND METHODS

Study Area

This study was conducted at the two canopy crane sites located in the southwest of China. While these two sites are only 330 km apart, the climate and the forest canopy composition are different (Shen et al., 2018). One site is situated in the tropical lowland seasonal rain forest (hereafter referred to as TRF) of Bubeng within Xishuangbanna National Nature Reserve (101°35′E, 21°37′N), at an elevation of around 700 m a.s.l. Mean annual temperature and mean annual precipitation of this region are 21.0°C and 1,532 mm, respectively. The vegetation in the TRF is dominated by *Parashorea chinensis* (Lan et al., 2012). The other site is the subtropical montane moist evergreen broad-leaved forest (hereafter referred to as STF) of Xujiaba within Ailao Mountain National Nature Reserve (101°01′E, 24°32′N), at an elevation of around 2,420 m a.s.l. Mean annual temperature is 11.6°C, and mean annual precipitation is 1,859 mm (Song et al., 2015b). The vegetation in the STF is dominated by *Castanopsis rufescens* (Shen et al., 2018). In both sites, rainy season occurs from May to October, and dry season between December and April (Li et al., 2011).

Data Collection

At each site, the canopy crane is established in the primary forest, covering an area of approximately 1 ha. Within this area, we conducted fieldwork during the dry season from October 2016 to April 2017. Both forests can be vertically divided into three tree layers: a canopy layer of trees (> 30 m in the TRF; > 25 m in the STF), a subcanopy layer (16–30 m in the TRF; 16–25 m in the STF) and an understory layer (6–16 m in both TRF and STF). A total of 69 target trees, with a diameter at breast height (DBH) greater than 3 cm, were selected randomly in each forest until we obtain a total of 14 tree species. We recorded host tree characteristics such as species identity, height, and DBH (**Supplementary Tables 1, 2**). Considering micro-climatic variations along the vertical gradient on the host trees, epiphytic bryophytes were sampled from several vertical zones set for each tree according to its height: we subdivided the height of the trees into six vertical zones (Z1-6) for canopy trees and four vertical zones (Z1-4) for sub-canopy and understory trees. Samples were taken using frame quadrats (20 cm × 20 cm for large branches, 80 cm × 5 cm for small branches) and taking an estimated total of 400 cm^2 of twigs in each vertical zone of the host tree by a ladder and canopy sampling techniques such as a telescopic tool and the canopy crane. Owing to the differences in tree size, the number of quadrat repeats varied from 4 to 6; hence, the total area sampled per vertical segment of each host tree ranged from 1,600 to 2,400 cm^2. Detailed investigation methods are described in Shen et al. (2018).

Network Metrics

As most epiphytic bryophytes are spore plants with asexual reproduction that restricted us to count the number of individuals, we recorded the presence and absence of epiphytic bryophyte species in binary matrices. To describe the interaction between epiphytic bryophytes (in rows) and host trees (in columns), we constructed the bipartite matrices, in which the cell with the number 0 or 1, respectively, represents absence or presence of epiphytic bryophyte species as observed on each host tree species. Based on the matrix data, we developed a bipartite graph whose nodes represent bryophyte species (top) and tree species (bottom) (Guimera et al., 2007). Links represent observed interactions when at least one individual of an epiphyte species was found on at least one individual of a host tree species (Gruber et al., 2009; Burns and Zotz, 2010). Similarly, to test the modularity structure across the vertical zones, the frequency-weighted matrices were constructed using the occurrence of bryophyte species (in rows) across vertical zones (in columns).

Network statistics were used to describe the structure of the epiphytic bryophyte–host networks. The following metrics were chosen: vulnerability, generality, connectance (C), nestedness (*NODF*), and modularity (Q). Vulnerability and generality represent the average number of species that forms links with another group. Connectance (C) measures network complexity (Heleno et al., 2012), corresponding to the number of actual links in relation to the number of theoretically possible links with the value varying from 0 (without interaction) to 1 (all species linked to each other) (Dunne et al., 2002). Nestedness was examined by NODF (the nestedness metric based on overlap and decreasing fill), which varies from 0 (when there is no nesting) to 100 (indicating maximum nesting) (Almeida-Neto et al., 2008). Modularity was analyzed with the Q index, ranging from 0, where the community has no more links within modules than expected by chance, to a maximum value of 1 (Barber, 2007; Arthur, 2020). Modularity was further analyzed using standardized, within-module connectivity (z) and among-module connectivity (c) coefficients proposed by Guimera et al. (2005). These values are calculated for each species to represent how well the species is connected to others within (z) and outside (c) its modular networks. According to the threshold values of z (2.5) and c (0.62) for binary networks, species were divided into four roles: peripheral species ($z \leq 2.5$ and $c \leq 0.62$); connectors ($z \leq 2.5$ and $c > 0.62$); module hubs ($z > 2.5$ and $c < 0.62$); and network hubs ($z > 2.5$ and $c > 0.62$) (Olesen et al., 2007). Peripherals tend to form a few interactions within a module and rarely any to other modules, in contrast, connectors play an important role in network coherence by connecting modules together. Module hubs play the role of hubs with many links, most of which are in their own modules, whereas network hubs play the roles of connectors and module hubs simultaneously (Dormann and Strauss, 2014; Ines Borthagaray et al., 2018).

Data Analysis

Species accumulation curves were plotted by Shen et al. (2018) to evaluate sampling completeness, showing that curves approached their asymptotes. We generated histograms that showed the proportion of epiphytic bryophyte species attached to the given number of host tree species.

Using simple linear models, we checked whether the increase in the number of individuals of each host species was positively related to the bryophyte species richness. We then analyzed whether the bryophyte species richness on individual trees vary with tree size, as we predicted that the species richness would increase with tree height and DBH.

The statistical significance of observed network metrics was tested using the null model that generated 1,000 random networks in which the number of nodes and interactions are given by the observed networks (Barber, 2007). Data analyses were performed using software R version 4.0.2. All network statistics were calculated and visualized with the "bipartite" package (Gruber et al., 2009) except for the modularity metrics which were analyzed by the "igraph" package (Csardi and Nepisz, 2006).

RESULTS

In total, we recorded 85 and 100 epiphytic bryophyte species in the TRF and STF, respectively (**Supplementary Tables 3, 4**). The number of interaction links was lower in the TRF (329 links) than STF (545) (**Figure 1**). The largest number of bryophyte species was recorded on *Parashorea chinensis* (61 species) in the TRF, and *Castanopsis rufescens* (70) in the STF. *Plagiochila parvifolia* was the only bryophyte species recorded on all host species in the TRF, whereas five species, *Plagiochila assamica*, *Porella oblongifolia*, *Wijkia deflexifoia*, *Sinskea phaea*, and *Herbertus aduncus*, were attached to all host tree species in the STF. Similar proportions of bryophyte species (25% in the TRF and 21% in the STF) were recorded on only one tree species in both forests (**Figure 2**).

Species interaction networks in both TRF and STF showed low levels of connectance and low levels of specialization (**Table 1**). In the TRF, epiphytic bryophytes interacted, on average, with 6.70 host tree species (representing the degree of generality), and host tree species interacted with 29.10 epiphyte species (vulnerability). In the STF, the value of generality and vulnerability were 9.07 and 43.21, respectively. The networks in the TRF and STF displayed significantly nested structure (**Table 1**) and presented low modularity with six modules in the TRF ($Q = 0.030$, $P < 0.001$, **Table 1**) and also six modules in the STF ($Q = 0.003$, $P < 0.001$) (**Figure 3**). Surprisingly, the values of all other metrics, except for modularity (Q), were higher in the STF than in the TRF (**Table 1**). Across the vertical zones of host trees, epiphytic bryophytes formed four modules in the TRF and three modules

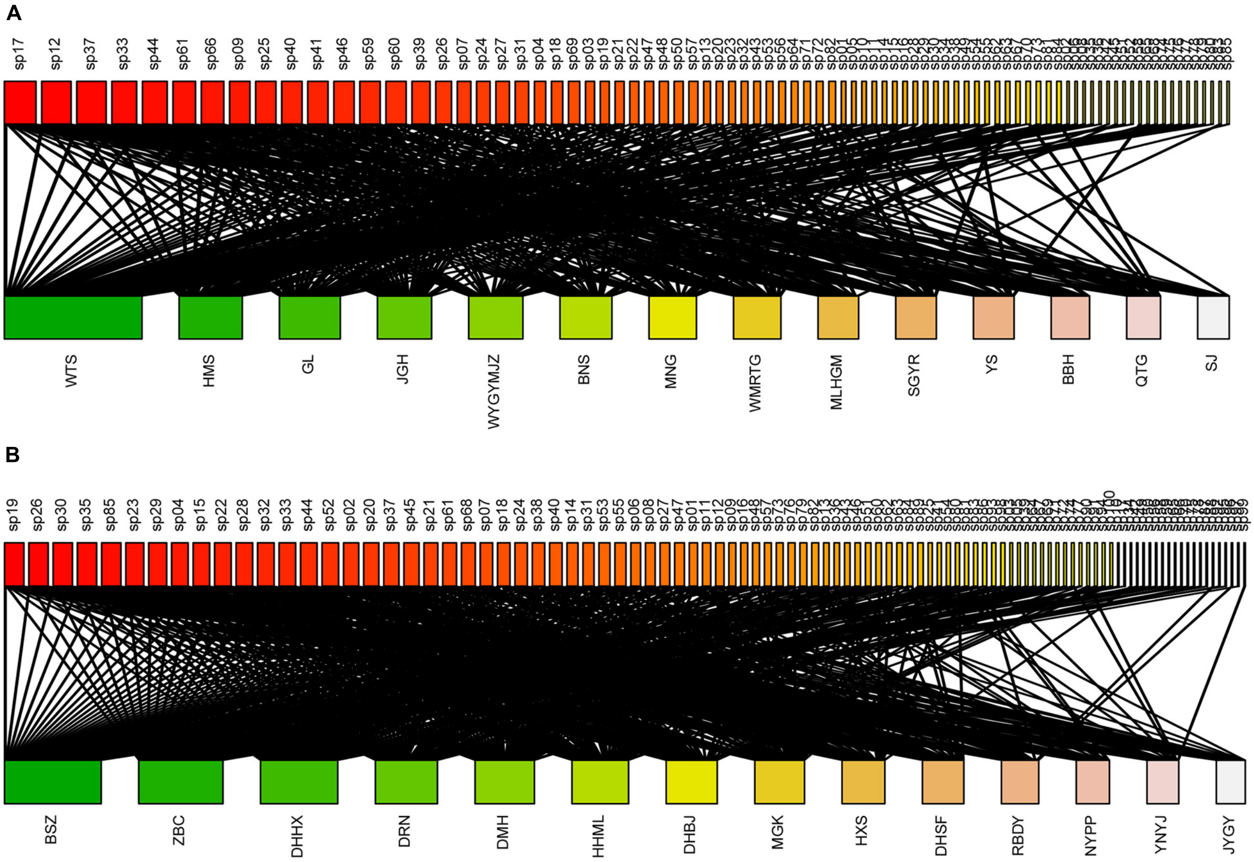

FIGURE 1 | The bipartite epiphytic bryophyte–host tree networks of the **(A)** tropical lowland seasonal rain forest (TRF) and **(B)** subtropical montane moist evergreen broad-leaved forest (STF). For each network, epiphytic bryophytes are displayed as rectangles at the top, and the host trees are shown as rectangles at the bottom of each bipartite graph. The width of the rectangles represents the number of links for each species. Black links indicate observed interactions between epiphytic bryophytes and hosts. The label added to each rectangle refers to the species codes of epiphytic bryophytes and trees. The names of species and corresponding species codes are provided in **Supplementary Tables 1–4**.

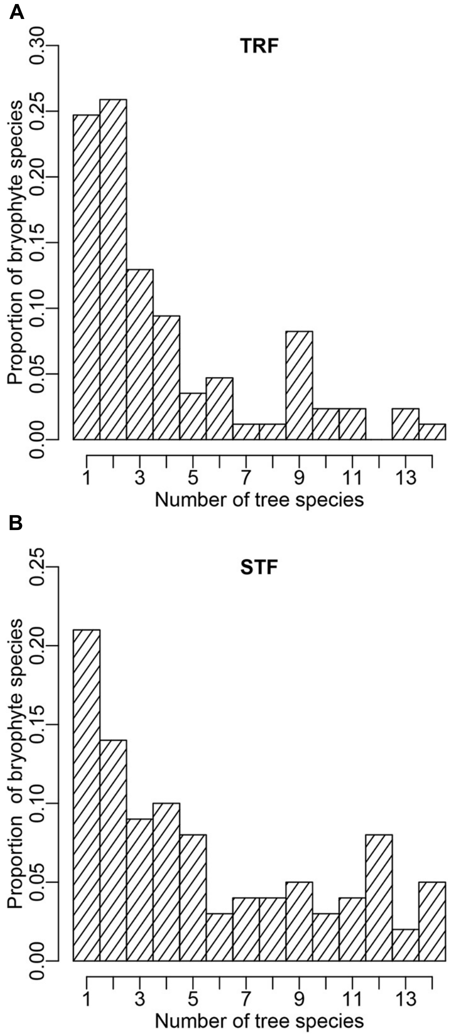

FIGURE 2 | Probability distribution structure of epiphytic bryophyte species attached to tree species in the **(A)** tropical lowland seasonal rain forest (TRF) and **(B)** subtropical montane moist evergreen broad-leaved forest (STF). The X-axis presents the number of tree species to which a bryophyte species attaches; the Y-axis presents the proportion of bryophyte species that can attach to given number of tree species.

TABLE 1 | Species-level and network-level indices in the subtropical montane moist evergreen broad-leaved forest (STF) and tropical lowland seasonal rain forest (TRF).

Index	TRF	STF
Species level		
Tree species	14	14
Tree individual	69	69
Bryophyte species	85	100
Link	329	545
Network level		
Connectance (*C*)	0.277 ($P < 0.001$)	0.389 ($P < 0.001$)
Generality (Ave. host species per bryophyte species)	6.720 ($P < 0.001$)	8.692 ($P < 0.001$)
Vulnerability (Ave. bryophyte species per host species)	28.812 ($P < 0.001$)	44.244 ($P < 0.001$)
Nestedness (*NODF*)	49.874 ($P < 0.001$)	65.171 ($P < 0.001$)
Modularity (*Q*)	0.0303 ($P < 0.001$)	0.0028 ($P < 0.001$)
Number of modules	6	6

Values in brackets are the P-values derived from the null model approach.

in the STF (**Figure 4**). In both forests, bryophyte species typically attached on the base and crown of hosts were grouped into different modules.

In the TRF, *c*-values ranged between 0 and 0.71 (with 13 of 85 bryophytes and 2 of 14 tree species exceeding the threshold of 0.62), and *z*-values range between −1.67 and 2.12 (no species exceeding the value of 2.5). In the STF, *c*-values range between 0 and 0.78 (with 41 of 100 bryophytes and 9 of 14 tree species exceeding the threshold of 0.62), and *z*-values range between −2.33 and 1.61 (no species exceeding the value of 2.5). Both networks in this study represented the lack of species representing module and network hubs ($z > 2.5$). Five tree species (*Elaeocarpus japonicus*, *Vaccinium duclouxii*, *Castanopsis rufescens*, *Machilus bombycine,* and *Illicium macranthum*) in the STF can be considered as peripheral species, while *Pseuduvaria indochinensis* and *Drypetes hoaensis* are the connector species in the TRF (**Figure 5**).

The number of interacting bryophyte species significantly increased with increasing number of recorded individuals per host tree species. The slope of the fitted linear model was higher in the STF than TRF (4.489 in the STF and 2.212 in the TRF) (**Figure 6**). Similarly, the number of epiphyte species increased significantly with tree height and DBH with higher slopes in the STF than TRF (**Figure 7**).

DISCUSSION

Common Characteristics of Bryophyte-Host Networks

Both bryophyte–host interaction networks in two forests exhibited nested structures with low modularity. Although the observed structures are a common pattern for commensal interaction (Naranjo et al., 2019), we acknowledge that relatively small sample sizes collected within the reach of canopy cranes may cause sampling bias and phylogenetic effects on estimates of host specificity, possibly representing incomplete forest community structures (Novotny et al., 2002).

Our study showed that more bryophyte species interacted with two or more tree species (generalists), while the smaller proportions of bryophyte species (25% in the TRF and 21% in the STF) interacted with a specific tree species (specialists). A possible explanation is that epiphytic bryophytes are generally pioneer species representing wide niche and great adaptability, colonizing the tree surface at early stages of tree growth to provide suitable substrates (Burns, 2007), while a few specialists subsequently colonize and thrive under specific conditions. Specificity is expected to be weaker in commensal

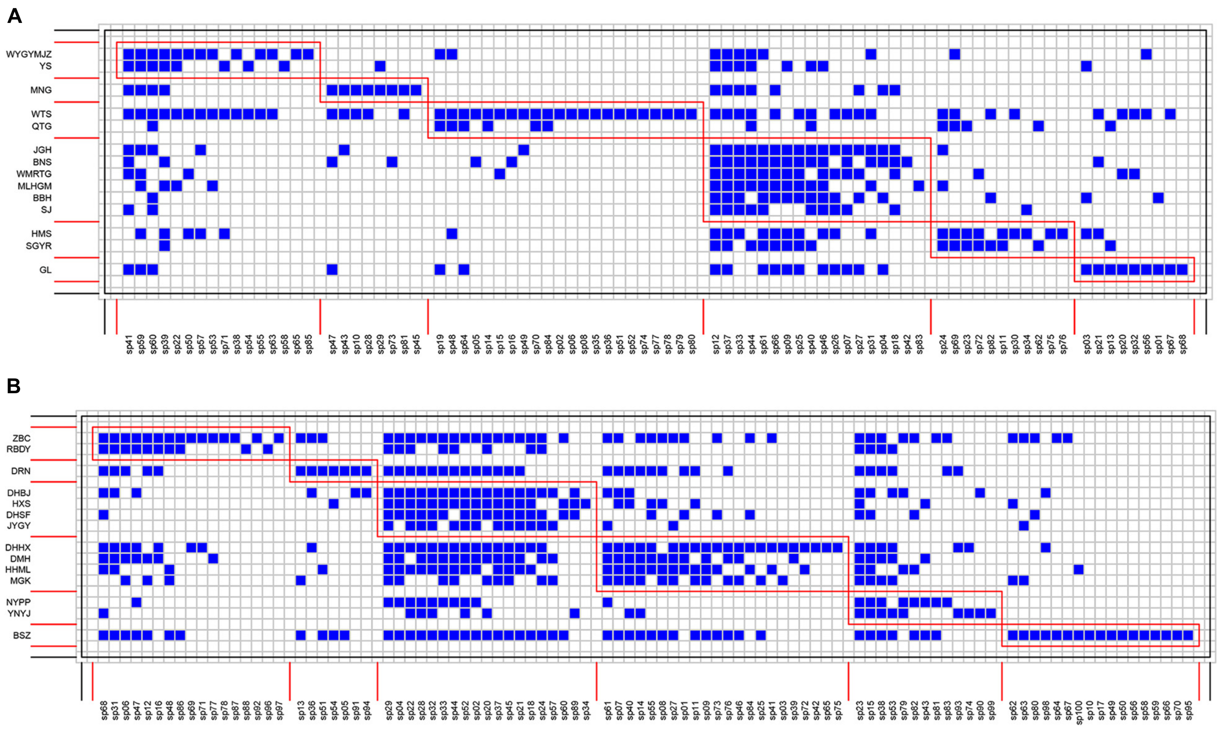

FIGURE 3 | Interaction matrices featuring modules (red boxes) of bryophyte–host tree networks in the **(A)** tropical lowland seasonal rain forest (TRF) and **(B)** subtropical montane moist evergreen broad-leaved forest (STF). Species are sorted according to their modular affinity, showing tree species as rows and bryophyte species as columns. Each blue square indicates the presence of an interaction between bryophyte and their host species. Red boxes delineate the six modules in TRF **(A)** and in STF **(B)**.

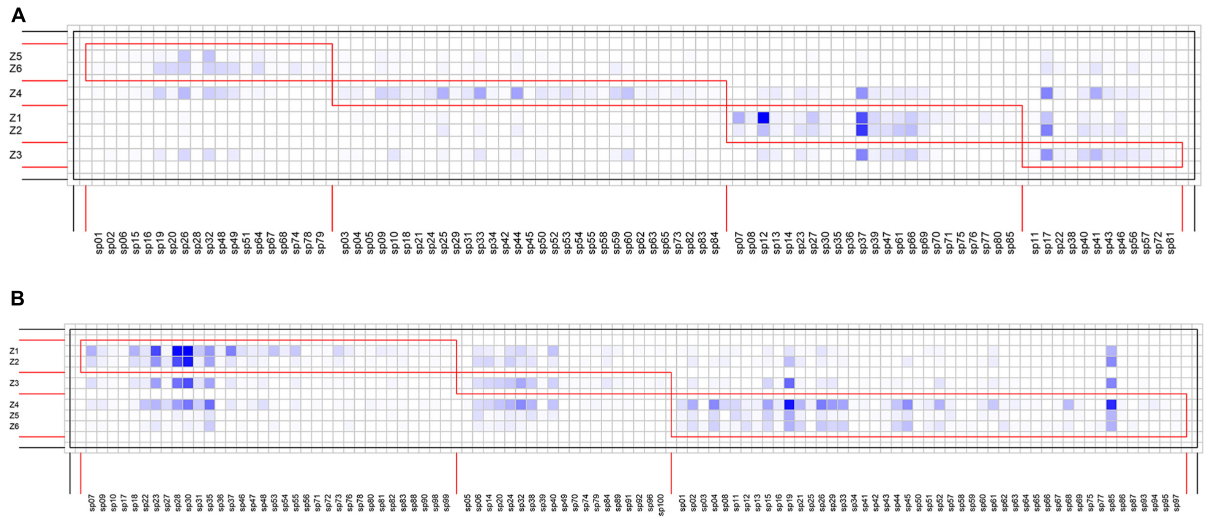

FIGURE 4 | Interaction matrices featuring modules (red boxes) of epiphytic bryophytes according to affinity with the vertical zones of the host trees. Tree vertical zones are shown as rows and bryophyte species as columns. Square cells in the darker blue color indicate more frequent interactions. **(A)** Four modules are detected in the tropical lowland seasonal rain forest (TRF), whereas **(B)** three modules are detected in the subtropical montane moist evergreen broad-leaved forest (STF).

interactions than other types of interactions (Bluthgen et al., 2006). The low level of specialization in epiphytic network structure may be related to the weak selection effects on epiphyte colonization by the host species (Silva et al., 2010) and low coevolution intensities in epiphyte bryophyte–host interactions (Fontaine et al., 2011). However, networks between epiphytes and hosts are not a simple random distribution of individuals, and epiphytes generally present their preferences

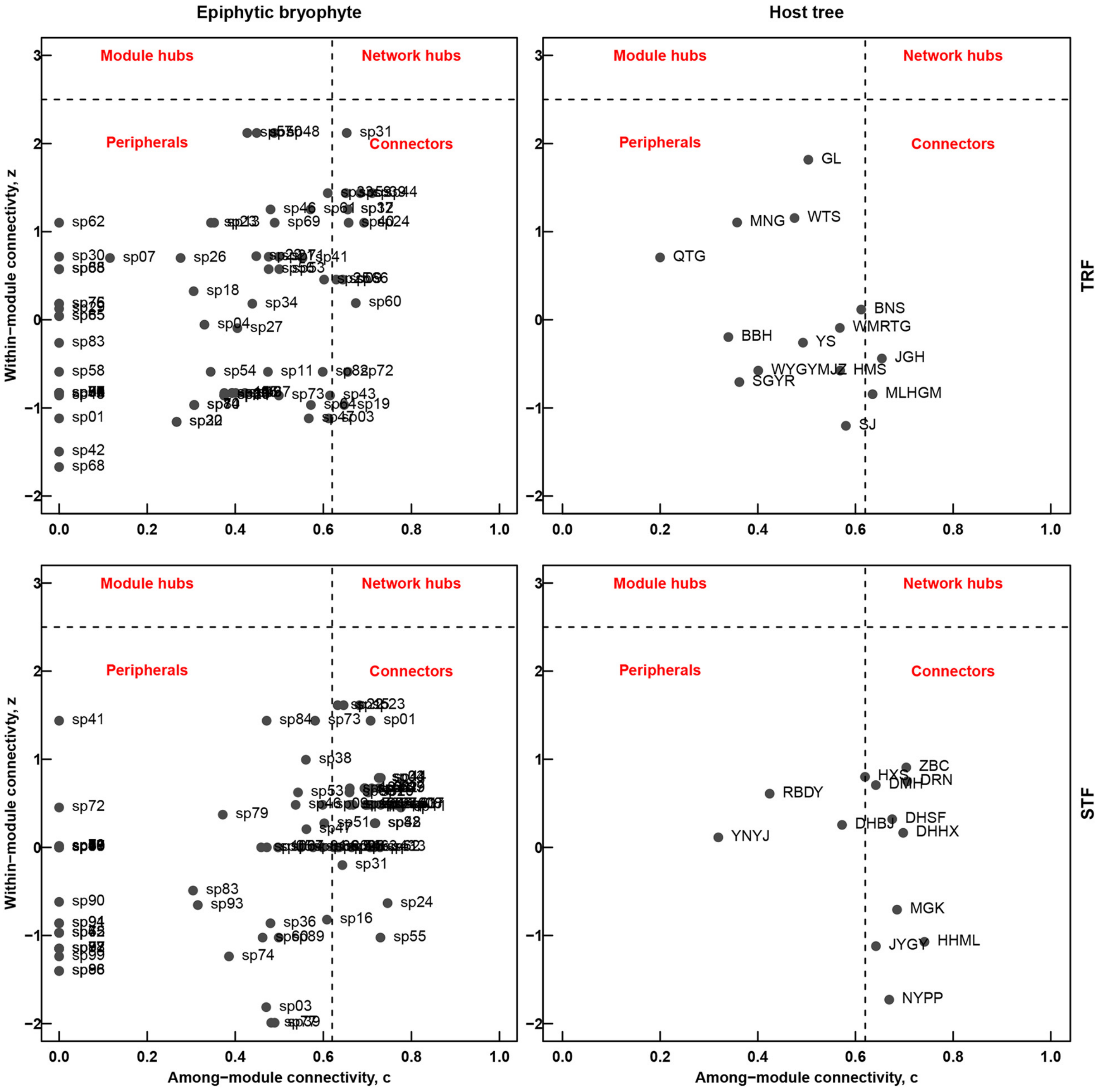

FIGURE 5 | Roles of the epiphytic bryophyte species (left) and host tree species (right) in the modular and the entire network structures in the tropical lowland seasonal rain forest (TRF) (top) and subtropical montane moist evergreen broad-leaved forest (STF) (bottom). The coefficients z and c refer to among-module connectivity and within-module degree, respectively. Dashed lines represent threshold values for c (0.62) and z (2.5), indicating the topographical space of network hubs (top right-hand rectangle, high *z*- and *c*-values), module hubs (top left-hand rectangle, high *z*- and low *c*-values), connectors (bottom right-hand rectangle, low *z*- and high *c*-values) and peripheral species (bottom left-hand rectangle, low *z*- and *c*-values).

for particular host traits and microclimatic conditions rather than for host species identifies *per se* (Wagner et al., 2015; Taylor et al., 2016).

Our epiphytic bryophyte–host networks showed a nested pattern, which is in line with the majority of other epiphyte–host networks (Naranjo et al., 2019; Olivia Cortes-Anzures et al., 2020; Fontúrbel et al., 2021). For ecological networks, nested structure indicates that specialists tend to interact with an appropriate subset of species that interact with more generalists. High nestedness may also play an important role in stabilizing the ecological networks between epiphytic bryophytes and their hosts (Piazzon et al., 2011; Song et al., 2017); however, this mechanism is still speculative and needs to be further investigated. One possible hypothesis is that the abundance of species may be one of the factors contributing to the formation of nested structure. The dominant tree species with high abundance were the generalists, which support the most epiphytic bryophyte species, including generalists and specialists. Under the nested structure, the large-scale species loss of epiphytic community requires the reduction of dominant tree species, while the abundance of

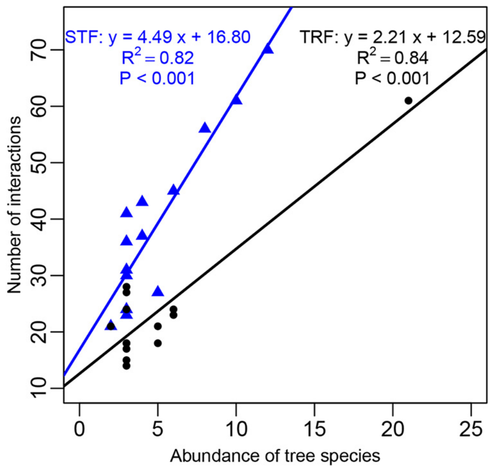

FIGURE 6 | Abundance of the 14 host plant species plotted against the number of interactions with epiphytic bryophyte species. Black solid circles represent those in the tropical lowland seasonal rain forest (TRF) and blue solid triangles represent the tree species in the subtropical montane moist evergreen broad-leaved forest (STF). The solid line is the best fit linear regression: the black line in TRF (slope = 2.21, R^2 = 0.84, $P < 0.001$); and the blue line in STF (slope = 4.49, R^2 = 0.82, $P < 0.001$).

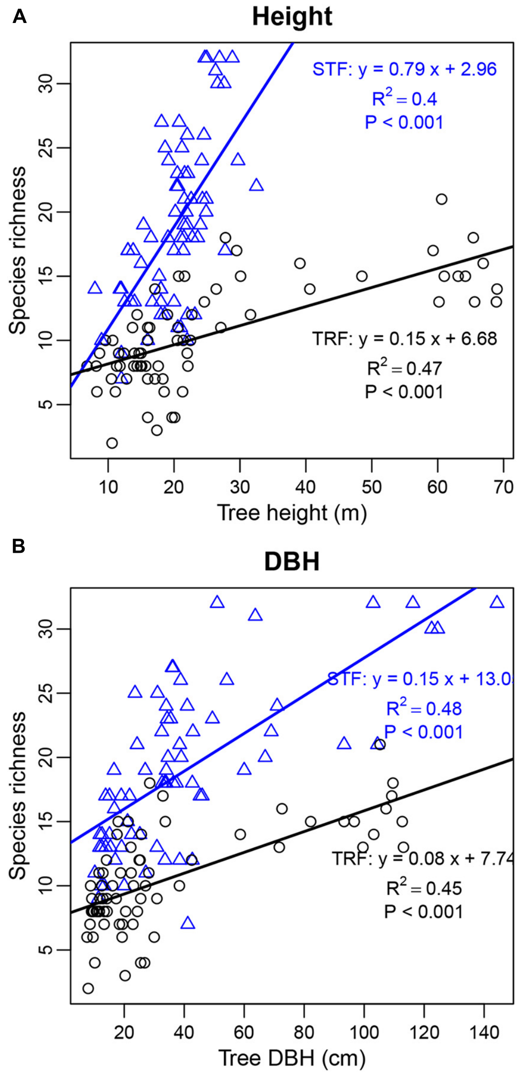

FIGURE 7 | Linear relationship of tree height **(A)** and DBH **(B)** with the number of bryophyte species found on individual host trees. Black circles and blue triangles are from the tropical lowland seasonal rain forest (TRF) and subtropical montane moist evergreen broad-leaved forest (STF), respectively. The solid lines indicate the overall trends of linear regression: black lines in TRF [**(A)** slope = 0.15, R^2 = 0.47, $P < 0.001$; **(B)** slope = 0.08, R^2 = 0.45, $P < 0.001$] and blue lines in STF [**(A)** slope = 0.79, R^2 = 0.40, $P < 0.001$; **(B)** slope = 0.14, R^2 = 0.48, $P < 0.001$].

dominant species is quite stable, which ensures the stability of epiphytic community.

Unlike other ecological networks, there are low levels of modularity (*Q*) in both TRF and STF, which may reflect the existence of lower niche partitioning caused by low host or microhabitat preferences by epiphytes. Networks were divided into modules as species within modules interact more frequently with each other (Guimera et al., 2007; Francisco et al., 2019). Some studies argued that species within modules may have adapted to the same habitat conditions and have close phylogenetic relationships (Olesen et al., 2007; Watts et al., 2016). At species level, both TRF and STF lacked network hubs: we found 9 host tree connectors in the STF, while only *Pseuduvaria indochinensis* and *Drypetes hoaensis* were the connector species in the TRF. Similar results have been reported on pollination networks and seed dispersal networks that most species act as peripherals and few species act as module and network hubs (Olesen et al., 2007; Vizentin-Bugoni et al., 2021). Our study suggests that, although highly nested interactions provide more stable networks, the loss of highly connected species (i.e., connectors in this study) may cause altered network structures.

Factors Structuring the Bryophyte–Host Networks

We recorded more bryophyte species and links in the STF than in the TRF, despite having collected the same number of host species and individuals in both forests. Furthermore, the metrics of network properties in the STF were higher than those in the TRF, except for modularity. High level of connectivity, vulnerability and generality indicate the complexity of network structure, while nested structure with low level of modularity is correlated with the stability of network. These results may reflect more complex and stable structure of epiphytic bryophyte community in the STF. Relatively high epiphyte richness and network complexity in the STF may be attributable to the hump-shaped pattern of tree bole bryophyte species richness with increasing altitude in the same area, where our STF site is located in the middle of the elevational transect (Song et al., 2015a). Climatic differences between two sites may be strong drivers of epiphyte community assembly (Saiz et al., 2020). Despite higher annual temperature in the tropical forest than in subtropical forest, relatively lower rainfall in the TRF may have acted as an indispensable factor limiting epiphytic bryophyte abundance.

On the other hand, the difference in the structure of epiphyte communities might be explained, at least partially, by the host tree identify (and the substrate and microclimate conditions they provide) at different sites (Patino and Gonzalez-Mancebo, 2011). According to previous study, even in the same type of forests (but with different dominant tree species), network properties varied widely among study sites (Taylor et al., 2016). In our study, the dominant species in the TRF (*Parashorea chinensis*) and STF (*Castanopsis rufescens*) belong to different taxonomic groups, which may have resulted in the difference in microhabitats they provide for epiphytes.

It has been repeatedly confirmed that epiphyte–host interactions correlate well with host size, because large tree size provides more surface areas, various microhabitats, and longer exposure time for the colonization of epiphytes (Agglael Vergara-Torres et al., 2010; Sayago et al., 2013; Zotarelli et al., 2019). For example, in a tropical montane forest, DBH alone explained 6% of the epiphyte community variation (Zhao et al., 2015), and a study conducted in two tropical dry forests found a positive relationship between epiphyte richness and host tree size (i.e., height and DBH) (Siaz-Torres et al., 2021). Some researchers treated the host tree individuals as isolated islands, and the epiphyte diversity is predicted by the theory of island biogeography which depicts that larger hosts are able to support a larger number of epiphytic species (Spruch et al., 2019). In addition to size, host abundance could influence the plant–host network through the neutral allocation of species interactions, as proposed by Calatayud et al. (2017). In our study, *Parashorea chinensis* and *Castanopsis rufescens* were the most common species among sampled trees. These tree species may provide greater opportunities for colonization by epiphytic bryophyte, which may have resulted in the majority of interactions in the networks. A study of a Mexican oak forest, in contrast, revealed that interaction intensities were not determined by their host abundance (Olivia Cortes-Anzures et al., 2020). The inconsistent result may suggest that the interaction patterns are attributable to the combination of neutral and niche processes.

We observed similar module patterns in the vertical distributions of bryophyte–host interactions in the two forests. Bryophyte species attached on the base zones (Z1, Z2) were grouped into one module, while those recorded on crown zones (Z4, Z5, Z6) were grouped into another. Our results support the findings of Francisco et al. (2019), who subdivided the epiphyte–host networks in a tropical cloud forest into three modules among the hosts' vertical zones. The clumped epiphyte distributions along vertical zones may be related to the adaptation to microclimates within a host with increasing light intensity, wind speed, and air temperature and declining air humidity along the entire vertical gradient from the tree base to canopy (Krömer et al., 2007; Shen et al., 2018). We found that epiphytic bryophyte species such as *Taxiphyllum taxirameum* in the TRF and *Thuidium cymbifolium* in the STF commonly occurred on the base of host individuals, and these species may prefer shade and high humidity habitat. In contrast, *Frullania fuscovirens* in the TRF and *Frullania chenii* in the STF attached on the crown zones are adapted to intense radiation and drought stress in the forest canopy. These findings carry conservation implications for the potential impacts of climate change on epiphytic bryophyte distributions, as the changes in microclimatic conditions due to anthropogenic warming may results in the shift in their vertical distributions or in local extinctions (Pardow and Lakatos, 2013; Zanatta et al., 2020).

CONCLUSION

Our study shows that the STF supports a higher diversity of epiphytic bryophytes and more complex structures of bryophyte–host networks in comparison to the TRF in southwest China. Despite that the TRF and STF represent different biomes, both sites showed some common patterns in epiphyte–host networks. In both sites, size and abundance are two attributes affecting the structure of epiphytic communities and tree vertical zones were closely related to their network modules. In light of our study, forest management and conservation in these areas should focus on *Castanopsis rufescens, Pseuduvaria indochinensis,* and *Drypetes hoaensis* in the STF and *Parashorea chinensis* in the TRF that contribute greatly to epiphytic bryophyte diversity and community stability as network connectors. Once these key species are lost, the network structure may break apart or cause a sharp decline of the biodiversity in the forest canopies. Some bryophyte species (such as *Taxiphyllum taxirameum and Frullania fuscovirens* in the TRF; *Thuidium cymbifolium and Frullania chenii* in the STF) were only recorded on specific vertical zones, and these species may be sensitive to environment changes and used as bioindicators.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

LS, TS, D-LQ, and H-XH conceived the research idea and were involved in statistical analyses and interpretations. TS and LS led the data collection. H-XH and D-LQ draft the manuscript. All authors contributed to the final manuscripts.

FUNDING

This study was supported by the National Natural Science Foundation of China (Nos. 32171529 and 31670452), the Natural Science Foundation of Yunnan Province (No. 202101AT070059), the CAS "Light of West China" Program, the Lancang-Mekong Cooperation (LMC) Special Fund (Biodiversity Monitoring and Network Construction along Lancang-Mekong River Basin Project), the CAS 135 Program (Nos. 2017XTBG-F03 and 2017XTBG-F01), the candidates of the Young and Middle-Aged Academic Leaders of Yunnan Province (No. 2019HB040), and the Yunnan Ten Thousand Plan Young and Elite Talents Project (YNWR-QNBJ-2020-066).

ACKNOWLEDGMENTS

We thank Ailaoshan Station for Subtropical Forest Ecosystem Studies and the Xishuangbanna Station for Tropical Forest Ecosystem Studies for their supports in field survey.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.716278/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Forests and Global Change

ORIGINAL RESEARCH
published: 14 January 2022
doi: 10.3389/ffgc.2021.816570

Vertical Stratification of Insect Species Developing in Water-Filled Tree Holes

***Martin M. Gossner*[1,2]* and *Jana S. Petermann*[3]**

[1] *Forest Entomology, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland,*
[2] *Department of Environmental Systems Science, Institute of Terrestrial Ecosystems, ETH Zürich, Zurich, Switzerland,*
[3] *Department of Biosciences, University of Salzburg, Salzburg, Austria*

Forest ecosystems have a distinct vertical dimension, but the structuring of communities in this three-dimensional space is not well understood. Water-filled tree holes are natural microcosms structured in metacommunities. Here, we used these microcosms as model systems to analyze how insect communities and the occurrence and abundance of individual species are influenced by biotic and abiotic microhabitat characteristics, the vertical position of the tree hole, and stand-scale habitat availability. We found that both the characteristics of water-filled tree holes and their insect communities differ significantly between canopy and ground level. Individual insect species showed contrasting responses to the vertical position of the tree holes when important environmental factors at the stand and the tree-hole scale were considered. While some species, such as the mosquito *Aedes geniculatus* and the beetle *Prionocyphon serricornis*, decreased in abundance with increasing tree-hole height, the biting midge *Dasyhelea* sp., the non-biting midge *Metriocnemus cavicola* and the hoverfly *Myiatropa florea* increased in abundance. Our results suggest that vertical stratification in forests is most likely driven not only by variation in tree-hole microhabitat properties, i.e., niche separation, but also by individual species traits, such as adult dispersal propensity, food preferences and mating behavior of adult stages, and interspecific competition of larval stages. Therefore, communities of insect species developing in tree holes are likely structured by competition–colonization trade-offs predicted by metacommunity theory.

Keywords: dendrotelmata, tree hole, canopy, vertical stratification, niche separation, metacommunity, dispersal, microcosm

Edited by:
Brett Scheffers,
University of Florida, United States

Reviewed by:
Aurélien Sallé,
Université d'Orléans, France
Alan Feest,
University of Bristol, United Kingdom

****Correspondence:***
Martin M. Gossner
martin.gossner@wsl.ch

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *16 November 2021*
Accepted: *21 December 2021*
Published: *14 January 2022*

Citation:
Gossner MM and Petermann JS (2022) Vertical Stratification of Insect Species Developing in Water-Filled Tree Holes. Front. For. Glob. Change 4:816570. doi: 10.3389/ffgc.2021.816570

INTRODUCTION

Forest ecosystems have a distinct vertical dimension, covering a height of up to >100 m and forming a complex and heterogeneous structure (Parker, 1995). In tropical forests in particular, the canopy contributes significantly to overall diversity (Basset et al., 2015). Although they were identified as one of the last biotic frontiers by Terry Erwin (1983) almost 40 years ago, canopy communities and the drivers that structure these communities are still not well understood. In temperate forests, several studies have demonstrated that canopy assemblages are not just nested subsets of ground assemblages (Bouget et al., 2011) and might show contrasting responses to forest management (Leidinger et al., 2019) and forest health (Sallé et al., 2021). This stratification is likely driven by differences in species resource requirements related to microhabitat and food, but also to microclimate preferences, species interactions, dispersal and mating behavior (Bouget et al., 2011; Ulyshen, 2011).

Tree-related microhabitats (TreM) have been shown to be crucial for promoting biodiversity in forests and have therefore become an important measure for forest conservation and management (Asbeck et al., 2021). One type of TreM that occurs throughout the vertical extent of trees is the water-filled tree hole or dendrotelma (Kitching, 2000; Larrieu et al., 2018). Gossner et al. (2016) showed that water-filled tree holes can occur in large numbers in the canopy but also near the ground. Thus, these microhabitats are ideal study systems to investigate the vertical stratification of species occurrence.

Spatial niche separation due to different microhabitat preferences of species could be important for structuring local communities across scales (species sorting perspective; Leibold et al., 2004). In general, microclimate shows a gradient along the vertical extent of trees (Parker, 1995), and this might affect the occurrence, development and phenology of species in these microhabitats (Gossner, 2018). Tree-hole properties such as size, amount of detritus (the basic resource in these microhabitats), and water chemistry are likely additionally important niche parameters (Gossner et al., 2016). Independent of the vertical position, stand-scale variables, such as the density of suitable microhabitats, might also affect the communities in water-filled tree holes (Gossner et al., 2016; Petermann et al., 2020). A likely reason for this is that communities in these microhabitats are organized as metacommunities, with local communities forming as a result of limited dispersal (Leibold et al., 2004). However, besides environmental and spatial filters, the outcome of biotic interactions also needs to be considered (Kraft et al., 2015; Cadotte and Tucker, 2017). According to the patch dynamics paradigm, there might be a competition–colonization trade-off in species, as some species are likely superior competitors and others superior colonists (Leibold et al., 2004). This might lead to a higher abundance of superior competitors in situations of high microhabitat density, while superior colonists might dominate under low microhabitat density. The relative importance of these different factors in driving the occurrence and abundance of single species is, however, still poorly understood.

In this study, we used data from two large-scale projects on the insect communities of natural tree holes occurring across vertical strata in differently managed forests. Previous publications based on these data focused on the consequences of forest management for communities in water-filled tree holes (Gossner et al., 2016; Petermann et al., 2020). Here, we address the importance of the vertical position of tree holes, as a proxy for microclimatic conditions, relative to other environmental drivers at the tree-hole scale (e.g., tree-hole size and chemistry, which likely also change with height) and stand scale (tree-hole density) in structuring insect communities developing in tree holes. In particular, we focus on the abundance and occurrence of the most frequently observed species. We expect that species have preferences regarding the vertical stratum when accounting for differences in microhabitat properties with height, with some species preferring tree holes at a lower and other species at a higher vertical position on the tree. This is expected because of differences in microclimatic preference, availability of resources for adults (e.g., flowers, vertebrate blood), and mating behavior (e.g., mating swarms of dipterans above tree tops; Downes, 1969; Ulyshen, 2011). In addition, we expect that niche separation, due to variation in height-independent tree-hole properties, and competition–colonization trade-offs, due to dispersal limitations, drive the abundance and occurrence of species, independent of the vertical position of the tree holes.

MATERIALS AND METHODS

Study Sites

The study was conducted within the Biodiversity Exploratories project[1] (Fischer et al., 2010) in three different regions of Germany: the Biosphere Reserve Schwäbische Alb (ALB) in the southwest (48°34′–48°53′N; 9°18′–9°60′E; 460–860 m a.s.l.), the National Park Hainich and the surrounding Hainich-Dün region (HAI) in the center (50°94′–51°38′N; 10°17′–10°78′E; 285–550 m a.s.l.), and the Biosphere Reserve Schorfheide-Chorin (SCH) in the northeast (52°51′–53°11′N, 13°36′–14°01′E; 3–140 m). The distance between ALB and HAI and between HAI and SCH is approx. 300 km and between ALB and SCH 600 km. ALB is located in a low mountain range with a mean annual precipitation of 700–1000 mm and a mean temperature of 6–7°C. The forest landscape is fragmented by agriculture and rural settlements. HAI is a forested hill chain and comprises the largest coherent area of deciduous trees in Germany. It has a mean annual precipitation of 500–800 mm and a mean annual temperature of 6.5–8°C. SCH is a post-glacial, flat landscape and one of the driest regions in Germany, with a mean annual precipitation of 500–600 mm and a mean annual temperature of 8–8.5°C. Forests in all regions would naturally be dominated by European beech, *Fagus sylvatica*, but differ greatly in their past and present management intensity.

Study Plots

This study is based on a compiled dataset from two projects, one focusing on ALB (29 plots) and HAI (24) in 2009 and 2011, respectively (Gossner et al., 2016), and one conducted in ALB (25), HAI (25) and SCH (25) in 2014 (Petermann et al., 2020). The experimental plots were 100 m × 100 m (1 ha) in size and covered the specific range of forest management intensities found in the region, from unmanaged beech forests to managed beech and conifer forests [spruce (*Picea abies*) in ALB und HAI; pine (*Pinus sylvestris*) in SCH]. Unmanaged forests were set aside 20–70 years ago, but all forests were influenced by humans at some point.

Sampling and Assessment of Plot and Tree-Hole Parameters

All water-filled tree holes were mapped in each 1-ha plot in spring and early summer after rain filled up the tree holes in 2009, 2011, and 2014. The number of tree holes per 1 ha (TH density) was used as a predictor of species abundance or occurrence. In both

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[1] www.biodiversity-exploratories.de

projects pan holes (maintaining an unbroken bark lining) and rot holes (penetrating through to the wood of the tree) (Kitching R. L., 1971) were considered, but sampling followed a slightly different approach. Tree holes at ≥2 m height were defined as "canopy holes" and those at <2 m height as "ground holes", following the classification of Kitching R. L. (1971) and Yanoviak and Fincke (2005). On each plot, either all available pan tree holes with a volume ≥50 ml plus all available rot holes were sampled (Gossner et al., 2016), or two canopy and two ground holes with a volume of 200–1000 ml were sampled, including both pan and rot holes (Petermann et al., 2020). Canopy tree holes were reached by using the single-rope climbing technique (Perry, 1978). In total, 318 tree holes were sampled.

For each tree hole, we measured the height above ground, opening area (Area, cm^2), potential volume (Vmax, ml) and amount of detritus (mass in g). The proportion of total detritus relative to the maximal water volume (PropDetritustot; g/ml) was used as an independent predictor variable.

Among water properties, dissolved oxygen (O_2 mg/l) and pH were measured in the field with mobile electrodes (Oxi 330, pH 330, WTW GmbH; Gossner et al., 2016) or multiprobes (Hach lange HQ40D.99.101301, field meter; Petermann et al., 2020). Nitrate (NO_3^- in mg/L), ammonium (NH_4^+ in mg/l) and phosphate (PO_3^{4-} in mg/l) concentrations were measured using photometric determination (Gossner et al., 2016; Petermann et al., 2020).

In the laboratory, all insect eggs, larvae and pupae, and all other invertebrates in each sample were transferred to 70% ethanol. Subsequently, insect larvae and pupae were identified using a stereo microscope and insect larval identification keys. We identified all specimens to either the species or morphospecies level. For details on sampling and measurements, see Gossner et al. (2016) and Petermann et al. (2020).

Statistical Analyses

All analyses were performed in R version 4.0.2 (R Core Team, 2020). In a first step, we tested whether insect communities and environmental parameters differed between ground and canopy holes. To visualize differences in community composition, we performed non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities using the "metaMDS" function in the *vegan* package (Oksanen et al., 2020) with 1000 permutations. Twenty-eight tree holes were excluded because they did not contain insect larvae, and another five were excluded because they contained only exclusive species and NMDS did not converge when these tree holes were included, resulting in 285 tree holes. We tested for differences in communities between regions, years and strata (ground vs. canopy) based on the same 285 tree holes using the "adonis" function in *vegan*.

To test for differences in total insect abundance, species richness, and plot- and tree-hole-scale parameters between ground and canopy holes, all 318 tree holes were included. We used generalized mixed effects models to analyze abundance (Poisson family, "glmer" function) and linear mixed effects models for all other response variables ("lmer" function), with stratum (ground vs. canopy) as a predictor and plot nested within region as the random effects structure, using the *glmmTMB* package (Brooks et al., 2017). Results are shown as histograms, separated into ground and canopy holes, created using the *ggplot2* package (Wickham, 2016).

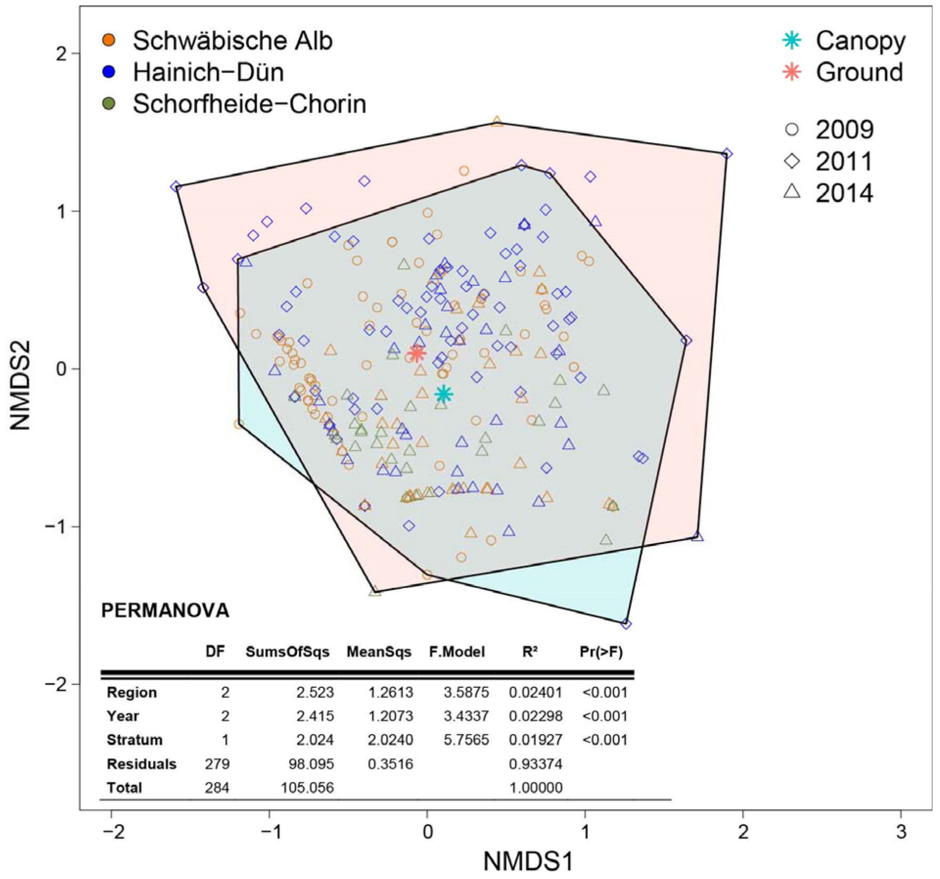
In a second step, we tested the importance of different plot- and tree-hole-scale predictors, including tree-hole height as a continuous predictor variable, for the abundance or occurrence of particular insect species. We again used generalized linear mixed effects models implemented in *glmmTMB*, with a Poisson (abundance) or binomial (presence/absence) distribution, with all environmental variables as predictors and, as above, plot nested within region as the random effects structure. All environmental variables were standardized to zero mean and unit variance using the "decostand" function in the *vegan* package (Oksanen et al., 2020) to allow comparisons of effect sizes. We performed two models for all abundance-based models, one with tree-hole density based on all tree holes and one using canopy tree-hole density for canopy tree holes and ground hole density for ground holes. We did that, because we expected different communities in canopy and ground holes and thus different importance of canopy and ground hole densities for some species. For occurrence-based models we were facing problems with model convergence and thus only used pooled tree holes density as predictor. Residual diagnostics were calculated using the *DHARMa* package (Hartig, 2020) and a test for multicollinearity was completed using the "check_collinearity" function in the *performance* package (Lüdecke et al., 2021). Neither significant zero inflation nor problems with dispersion or multicollinearity were observed. We then plotted the standardized estimates using the "plot_model" function in the *sjPlot* package (Lüdecke, 2021). Significant effects of tree-hole height above ground on either the abundance or occurrence of particular species were plotted using the "predictorEffect" function in the *effects* package (Fox, 2003; Fox and Weisberg, 2018, 2019).

RESULTS

In the 318 tree holes studied (ground: 194, canopy: 124), we found 43 (morpho)species with a total of 13,456 individuals. The most abundant species were *Metriocnemus cavicola* (Diptera: Chironomidae; 5909 individuals, 194 tree holes), *Myiatropa florea* (Diptera: Syrphidae; 747, 139), *Prionocyphon serricornis* (Coleoptera: Scirtidae; 3099, 131), *Dasyhelea* spec. (Diptera: Ceratopogonidae; 1646, 109), *Aedes geniculatus* (Diptera: Culicidae; 1027, 88), *Chironomidae* spec. (Diptera: Chironomidae; 114, 32), Muscidae spec. (Diptera: Muscidae; 509, 26), Culicidae spec. (Diptera: Culicidae; 42, 15), Dolichopodidae spec. (Diptera: Dolichopodidae; 22, 15), and *Pericoma* spec. (Diptera: Psychodidae; 29, 14).

Community composition differed significantly between regions ($F_{2,279}$ = 3.588, $P < 0.001$), years ($F_{2,279}$ = 3.434, $P < 0.001$), and height strata ($F_{1,279}$ = 5.757, $P < 0.001$), but the explained variance (R^2) was low (region: 0.024; year: 0.023; stratum: 0.019; **Figure 1**).

Tree holes were observed at heights of up to 34 m in the canopy, although median height was 0.58 m and mean height was 4.13 m, indicating that most of the sampled canopy holes



PERMANOVA

	DF	SumsOfSqs	MeanSqs	F.Model	R²	Pr(>F)
Region	2	2.523	1.2613	3.5875	0.02401	<0.001
Year	2	2.415	1.2073	3.4337	0.02298	<0.001
Stratum	1	2.024	2.0240	5.7565	0.01927	<0.001
Residuals	279	98.095	0.3516		0.93374	
Total	284	105.056			1.00000	

FIGURE 1 | Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances of insect communities in water-filled tree holes sampled in the canopy and near the ground in three regions of Germany between 2009 and 2014 (stress = 0.142). Colored hulls indicate the space covered by the communities in the canopy (at ≥2 m height; blue) and near the ground (at <2 m; red). The table shows the results of a PERMANOVA, testing for differences in community composition between region, year and height stratum. Please note that the R^2 value for stratum shows the effect strength after controlling for differences between individual regions and between individual years (Type I sum of squares, sequential).

occurred at rather low heights (**Figure 2A**). Tree holes in the canopy (≥2 m) were smaller overall, but contained more detritus relative to their size and had a higher water pH and ammonium concentration than ground holes (**Figure 2**). Insect abundance and species richness, did not differ between ground and canopy holes, nor did hole opening area or dissolved oxygen, nitrate and phosphate concentration at the tree-hole scale (**Figure 2**). At the plot scale, tree-hole density tended to be higher at ground level ($P = 0.051$; **Figure 2**).

The abundances of the most frequent species, *Metriocnemus cavicola*, *Myiatropa florea*, *P. serricornis*, *Dasyhelea* sp. and *A. geniculatus*, were significantly affected by tree-hole height above ground, with *P. serricornis* and *A. geniculatus* negatively and *Metriocnemus cavicola, Myiatropa florea* and *Dasyhelea* sp. positively affected (**Figures 3**, **4**; for separation between canopy and ground holes, see **Supplementary Figure 2** in the **Supplementary Material**). In addition, *Metriocnemus cavicola* was positively affected by overall tree-hole density at the plot scale (**Figure 3**). When tree-hole density was separated into canopy and ground holes, *P. serricornis* also responded positively to tree-hole density (**Supplementary Figure 1** in the **Supplementary Material**). This was also the only model in which the marginal R^2 slightly increased by separating between canopy and ground holes. All other models showed higher marginal R^2 when using overall tree-hole density. Water chemistry parameters had contrasting responses in the most frequently found species (**Figure 3**). While the abundance of *A. geniculatus* was positively affected by dissolved oxygen concentration, *Dasyhelea* sp. and *P. serricornis* showed negative responses. Higher water pH affected *Metriocnemus cavicola* and *Dasyhelea* sp. positively, but *Myiatropa florea* and *P. serricornis* negatively. Ammonium and nitrate concentration affected *A. geniculatus* (only nitrate) and *Dasyhelea* sp. negatively, whereas *Myiatropa florea* (only nitrate) and *P. serricornis* showed a positive response. Only *Metriocnemus cavicola* showed a response to phosphate concentration and this was positive. Regarding the size of the tree holes, *A. geniculatus* preferred holes with large and *Myiatropa florea* with small opening areas (**Figure 3**). While *Dasyhelea* sp. was more abundant in small holes (low Vmax), *Myiatropa florea* and *P. serricornis* were more abundant in large holes.

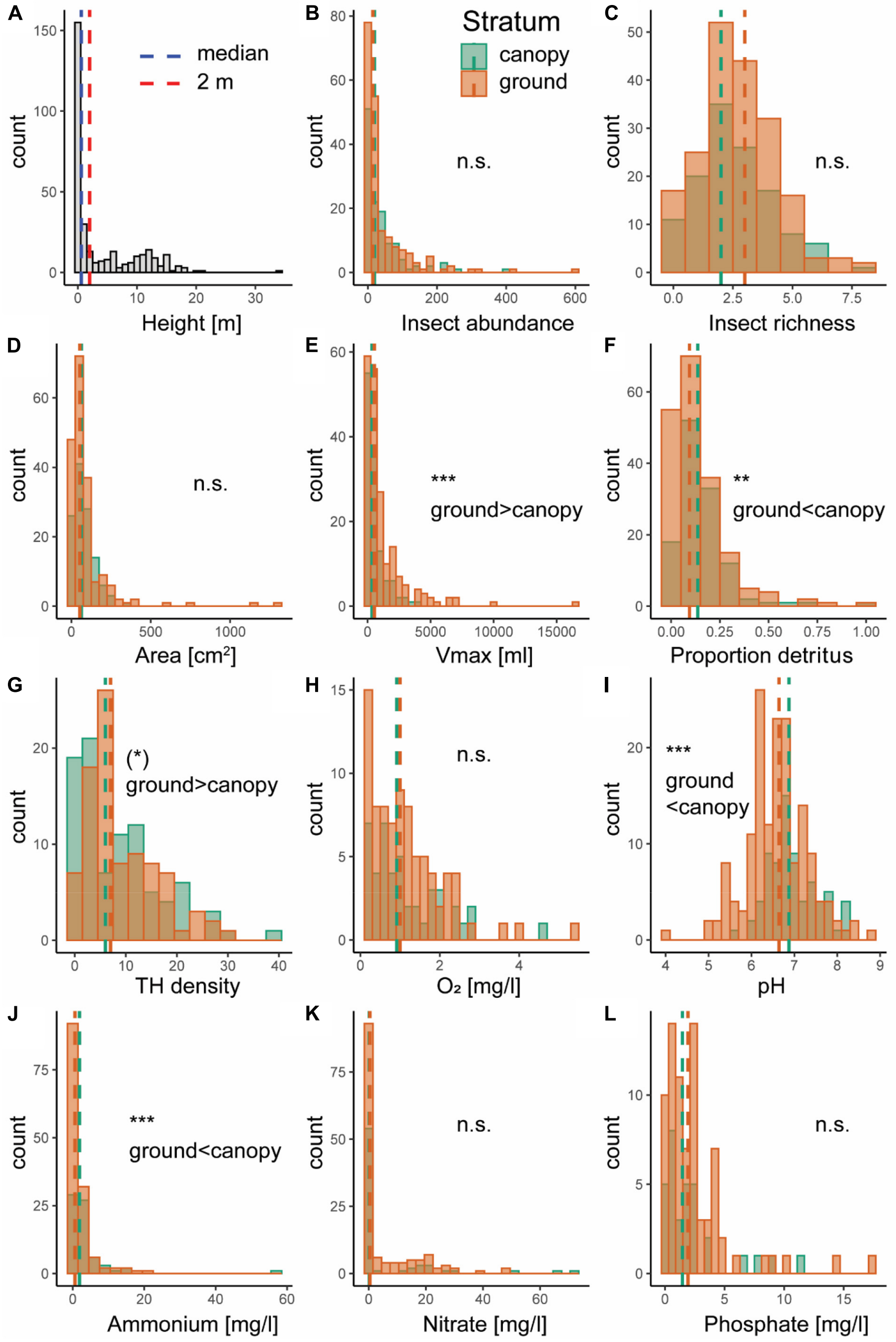


FIGURE 2 | Histograms of tree-hole height **(A)**, insect abundance **(B)**, insect species richness **(C)**, tree-hole opening area **(D)**, tree-hole maximum volume (Vmax; **E**), total amount of detritus relative to tree-hole Vmax (proportion detritus; **F**), plot-level tree-hole density **(G)**, and tree-hole water chemistry: dissolved oxygen concentration (O_2) **(H)**, pH **(I)**, ammonium concentration **(J)**, nitrate concentration **(K)**, and phosphate concentration **(L)**. Values for ground holes (<2 m height; orange) and canopy holes (>2 m height; green) in panels **(B–L)** are indicated by different colors, with median values shown as dashed lines. Asterisks indicate significance based on generalized (for insect abundance; Poisson distribution) or linear (all other variables) mixed effects models [(*) $P = 0.051$, ** $P < 0.01$, *** $P < 0.001$, n.s. not significant].

The total amount of detritus relative to tree-hole size affected the abundance of all analyzed species, with positive effects for *Metriocnemus cavicola*, *Dasyhelea* sp. and *Myiatropa florea*, and negative effects for *A. geniculatus* and *P. serricornis*.

The occurrence of the five next most frequent species showed only three significant responses to our predictor variables. The occurrence of a Culicidae species that could not be identified to the genus or species level showed a positive response to tree-hole

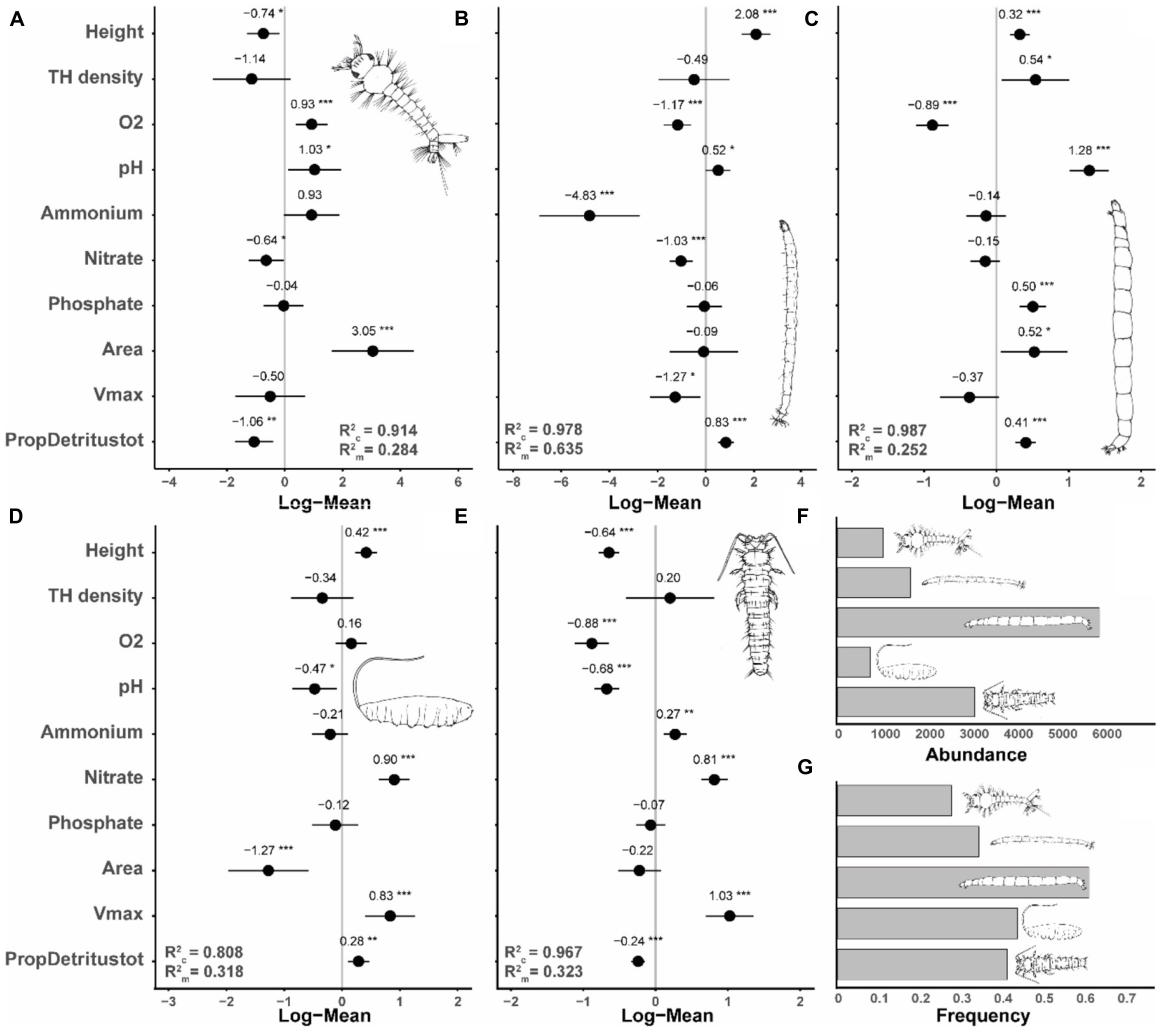


FIGURE 3 | Standardized model coefficients (including conditional and marginal R^2-values) of generalized linear mixed effects models with a Poisson distribution, showing the importance of tree-hole height above ground, tree-hole density (canopy and ground holes combined), tree-hole opening area, tree-hole maximum water volume (Vmax), water chemistry [dissolved oxygen concentration (O_2), pH, and ammonium, nitrate and phosphate concentrations], and total amount of detritus relative to Vmax (PropDetritustot) in determining the abundance of the five most frequently observed insect species. **(A)** Diptera, Culicidae: *Aedes geniculatus*, occurrence in $n = 88$ tree holes; **(B)** Diptera, Ceratopogonidae: *Dasyhelea* sp., $n = 109$; **(C)** Diptera, Chironomidae: *Metriocnemus cavicola*, $n = 194$; **(D)** Diptera, Syrphidae: *Myiatropa florea*, $n = 139$; **(E)** Coleoptera, Scirtidae: *Prionocyphon serricornis*, $n = 131$. **(F,G)** show the total abundance and the frequencies of occurrence of the five species, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

opening area, Chironomidae species occurred more frequently with higher nitrate concentration, and Pericoma sp. at lower pH (**Figure 5**).

DISCUSSION

Our study provides novel insight into the importance of the vertical position of microhabitats, relative to other abiotic and biotic drivers, in determining insect community structure and abundance of individual species. By using water-filled tree holes as a model system, we showed that canopy communities differ significantly from those near ground level. Our analyses of individual species revealed that this pattern is due to contrasting responses of individual species to the vertical position of the tree holes, when controlling for important environmental factors at the plot and tree-hole scale. Our results suggest that vertical stratification in forests is most likely driven not only by changes in tree-hole microhabitat properties (niche separation) with height above ground, but also by other factors such as adult species traits (e.g., dispersal propensity, food, mating behavior) and interspecific competition. Therefore, communities of insect species developing in tree holes are likely structured by competition–colonization trade-offs predicted by metacommunity theory.

We found significant differences in the composition of insect communities developing in water-filled tree holes. This confirms findings from previous studies that height above

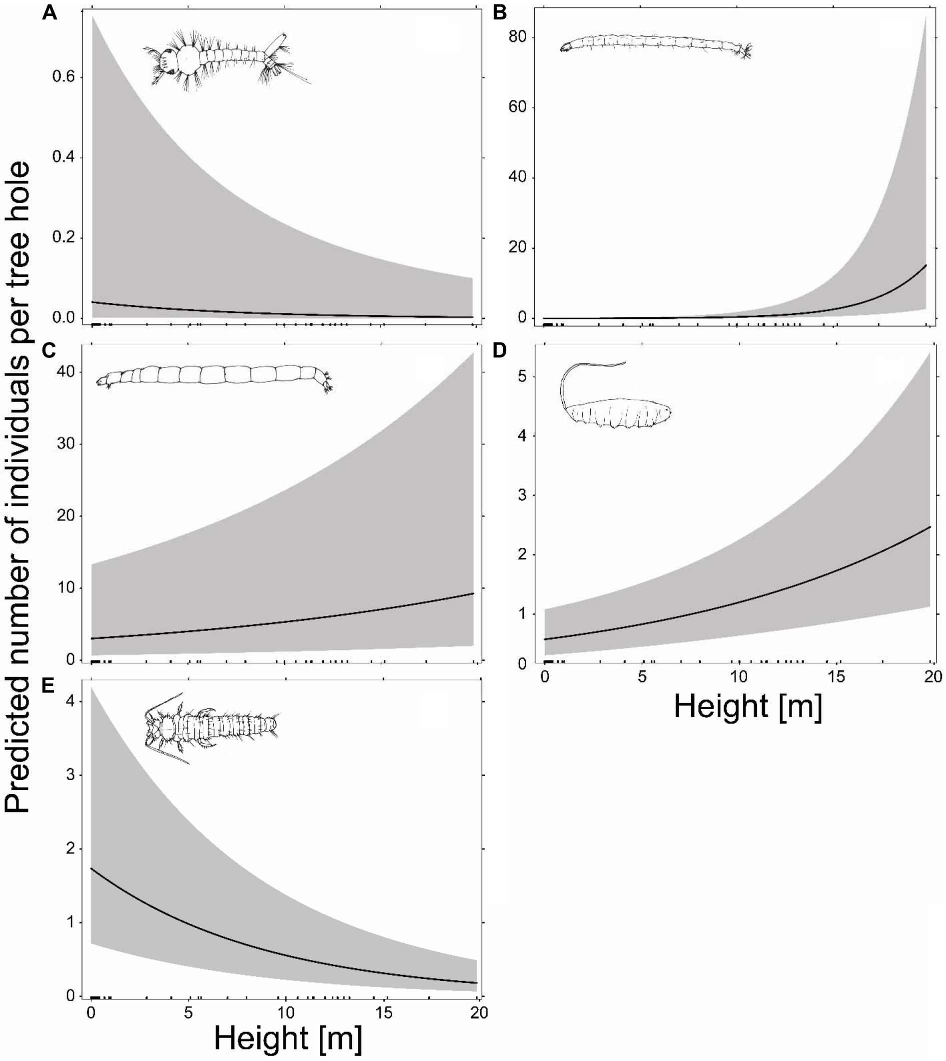


FIGURE 4 | Prediction of the abundance of the most abundant species in our study as a function of tree-hole height above ground, based on generalized linear mixed effects models with a Poisson distribution, including tree-hole-scale and plot-scale variables (for tree-hole density canopy and ground holes were combined) as additional predictors. The gray bands show 95% confidence intervals. The bars on the x-axis indicate the tree holes sampled along the vertical gradient. **(A)** Diptera, Culicidae: *Aedes geniculatus* occurrence in *n* = 88 tree holes; **(B)** Diptera, Ceratopogonidae: *Dasyhelea* sp., *n* = 109; **(C)** Diptera, Chironomidae: *Metriocnemus cavicola*, *n* = 194; **(D)** Diptera, Syrphidae: *Myiatropa florea*, *n* = 139; **(E)** Coleoptera, Scirtidae: *Prionocyphon serricornis*, *n* = 131.

ground is an important factor structuring insect communities of these microhabitats in temperate (Blakely and Didham, 2010; Gossner et al., 2016; Petermann et al., 2020) and tropical forests (Yanoviak, 1999). However, the underlying mechanisms might be manifold.

Several properties differed between the ground and canopy holes studied here and could have caused niche separation of species. While the volume of tree holes was on average larger near the ground than in the canopy (**Figure 2E**), the proportion of detritus and thus the resource concentration was higher in the canopy (**Figure 2F**). Because of the branching pattern of beech canopies, the main tree species on which we found tree holes in our study (see also, von Brandt, 1934; Gossner et al., 2016), the size of tree holes decreases with height. In addition, stem holes, which can be quite large, become less abundant in the canopy. However, leaf biomass is greatest in the upper canopy of closed forests, causing high leaf input into canopy holes and thus a higher resource concentration in the canopy (**Figure 6A**). Moreover, the pH and ammonium concentration of the water in tree holes were significantly higher in the canopy than near the ground, which might be caused by greater drought disturbance in the canopy (Blakely and Didham, 2010). These changes with greater tree height are generally accompanied by increases in temperature and conductivity (both not measured), as well as larger temporal fluctuations in environmental parameters (Yanoviak, 1999; Blakely and Didham, 2010; Gossner, 2018). These changes are most likely due to windier conditions and increased exposure to sunlight the

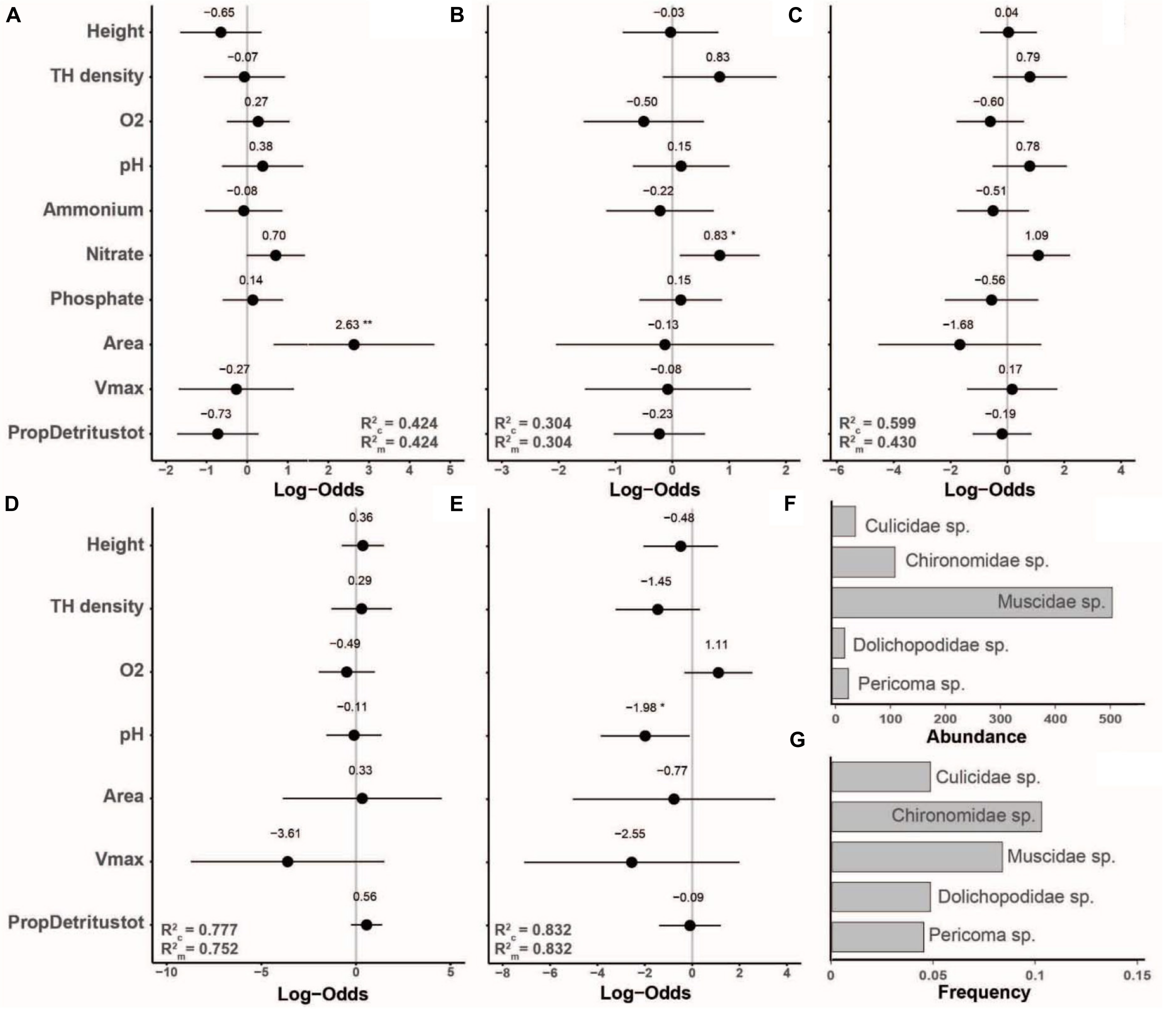


FIGURE 5 | Standardized model coefficients (including conditional and marginal R^2-values) of generalized linear mixed effects models with binomial distribution based on occurrence data, showing the importance of tree-hole height above ground, tree-hole density, tree-hole opening area, tree-hole maximum water volume (Vmax), water chemistry [dissolved oxygen concentration (O_2), pH, and ammonium, nitrate and phosphate concentrations] and total amount of detritus relative to Vmax (PropDetritustot) in determining the occurrence of less frequently observed insect species that were observed in >10 but <88 tree holes. **(A)** Diptera, Culicidae sp., occurrence in $n = 15$ tree holes; **(B)** Diptera, Chironomidae sp., $n = 32$; **(C)** Diptera, Muscidae sp., $n = 26$; **(D)** Diptera, Dolichopodidae sp., $n = 15$; **(E)** Diptera, Psychodidae, *Pericoma* sp., $n = 14$. Please note that in **(D,E)** a reduced model was used because there were many missing values. **(F,G)** show the total abundance and the frequencies of occurrence of the five species, respectively. $^*p < 0.05$, $^{**}p < 0.01$.

closer the tree holes are to the upper boundary of the canopy (Parker, 1995).

Various microhabitat properties significantly affected the insect communities developing in the water-filled tree holes in our three study regions, confirming results from previous studies (Blakely and Didham, 2010; Gossner et al., 2016). Although we found no significant differences in overall insect abundance and species richness between our coarse categories canopy vs. ground, either a decrease (e.g., in Nothofagus forest of New Zealand and tropical forests in Panama; Yanoviak, 1999; Blakely et al., 2008) or an increase (e.g., in Fagus forests of Germany; Gossner et al., 2016; Petermann et al., 2020) in species richness with height has been observed in previous studies. These differences might be due to different adaptations of the species to drought in the different ecosystems. It has been shown that environmental filters such as drought disturbance can eliminate many species that survive in wetter environments (Chase, 2007). The combination of effective drought resistance strategies and faster development under high temperatures in the canopy might have positive effects on the species richness of insect communities in water-filled tree holes in the canopies of Central European forests.

We observed clear contrasting effects of individual species to the microhabitat properties, which likely relates to species-specific traits. The general importance of species traits in determining the responses of communities in water-filled tree holes to environmental drivers has already been shown by Petermann et al. (2020). While the mosquito *Aedes geniculatus* and another non-identified mosquito species preferred holes

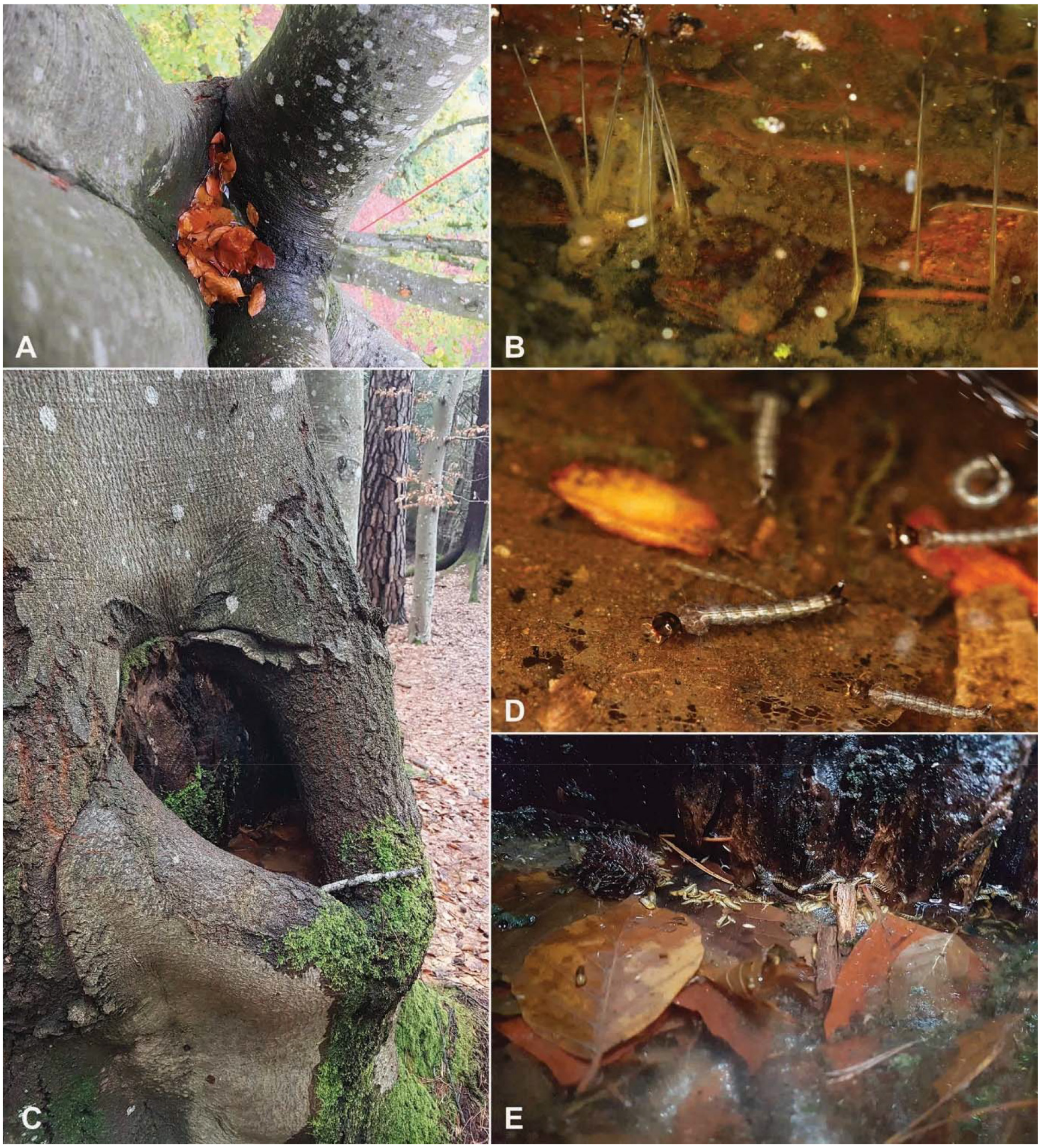


FIGURE 6 | (A) Water-filled tree hole in a branch fork of a beech tree at 20 m height. At this height these microcosms receive considerable leaf litter input, resulting in a large amount of detritus relative to the tree-hole volume. **(B)** The abundance of the hoverfly *Myiatropa florea* (Diptera: Syrphidae) increases with tree-hole height. These rat-tailed maggots are characterized by a long, tube-like, telescoping breathing siphon that acts like a snorkel, allowing the larvae to breathe air while submerged. **(C)** Near ground level, very large water-filled tree holes with a volume >10 l are sometimes found. **(D)** The larvae of the mosquito *Aedes geniculatus* (Diptera: Culicidae) prefers holes with wide openings near ground level. Larvae breathe atmospheric air through a siphon and filter algae, bacteria, and other microbes from the surface microlayer. **(E)** The beetle *Prionocyphon serricornis* (Coleoptera: Scirtidae) depends on large holes that do not dry out frequently. They survive freezing of the water in tree holes (most of the water column in the photo is frozen) by staying in bark crevices at the upper water level and become active as soon as the top layer melts.

with a larger opening area (**Figures 6C,D**), independent of tree-hole volume, the hoverfly *Myiatropa florea* (**Figure 6B**) and the beetle *Prionocyphon serricornis* (**Figure 6E**) preferred large holes with a rather small opening area. In contrast, the biting midge *Dasyhelea* sp. was more abundant in smaller holes. This suggests that mosquitoes prefer large opening areas for oviposition (Bentley and Day, 1989; Clements, 1999), whereas hoverflies and beetles, which have longer developmental times, need a larger water volume and more stable water levels so that tree holes do not dry out during their development. Small biting midges such as *Dasyhelea* sp. might have a competitive advantage in small holes due to their fast development.

Species such as the non-biting midge *Metriocnemus cavicola* and the beetle *P. serricornis* seem to do well under low dissolved oxygen concentrations, while mosquitoes depend on high concentrations. As mosquito larvae are air breathers (**Figure 6D**), they do not directly depend on oxygen in the water. Thus, the positive relationship with dissolved oxygen concentration might be indirect *via* a higher abundance of microorganismic food species such as bacteria and ciliates (Schmidl et al., 2008). In contrast, *P. serricornis*, which lives saprophagously by shredding leaves and detritus, can store air in its tracheal system (Klausnitzer, 1984) and thus tolerates relatively anoxic conditions (Schmidl et al., 2008). The negative relationship with oxygen supply that we observed for saprophagous *Metriocnemus cavicola* is in contrast to results from previous studies (von Brandt, 1934; Schmidl et al., 2008) and might be caused by the fact that the canopy stratum was not considered in the previous investigations.

Moreover, biting (*Dasyhelea* sp.) and non-biting (*Metriocnemus cavicola*) midges, as well as hoverflies (*Myiatropa florea*), seem to reach higher densities under high resource concentrations (see also, Schmidl et al., 2008), whereas high resource concentrations might reduce densities of mosquitoes and *P. serricornis* due to inhibition by leachates (Blakely, 2008; Schmidl et al., 2008). Water chemistry, including nutrient concentrations, might also be important cues for oviposition (Bentley and Day, 1989; Clements, 1999), which could explain the observed responses of all abundant species to water chemistry.

The strong response of *Myiatropa florea* to microhabitat properties is surprising, as this species is known to be a habitat generalist, also occurring in other water bodies, and showed no clear preferred physicochemical conditions in a previous study on water-filled tree holes (Schmidl et al., 2008). Because of its relatively large size, however, it might depend on properties, e.g., tree-hole volume, that support complete development in tree holes, given the rather ephemeral character of this microhabitat in terms of water availability.

There was still a significant effect of height after accounting for changes in microhabitat properties with height, which might be related to shifts in unmeasured variables such as temperature. In addition, many insects have flight height preferences (Basset et al., 2003), which likely influences where they feed, mate and oviposit (Scholl and Defoliart, 1977; Sinsko and Craig, 1979; Copeland and Craig, 1990). Moreover, the availability of food might play a role in height effects. Mosquitoes, for instance, need vertebrate blood for egg maturation and thus prefer lower forest strata where vertebrates are overall more abundant in temperate forests (Shaw, 2004).

Dispersal limitation could be an additional driver of species occurrence and abundance. Competition–colonization trade-offs most likely help to structure insect communities of water-filled tree holes, as predicted by the metacommunity patch-dynamics paradigm (Leibold et al., 2004). Good colonizers might be poor competitors and thus might be pushed to less suitable marginal habitats. In our study, positive responses to tree-hole density might characterize species that are limited by a low colonization ability, requiring high densities of suitable habitats to be able to colonize from source habitats, but that have a high competitive ability, giving them an advantage under high tree-hole density. The non-biting midges (*Metriocnemus cavicola*) and the marsh beetle (*Prionocyphon serricornis*) might be examples of this dispersal guild. In contrast, the syrphid *Myiatropa florea* and the mosquito *Aedes geniculatus* seem to be good colonizers (strong negative effect of overall tree-hole density although not significant) but poor competitors, so they can reach high densities even when habitat availability is low. This is supported by the observation that non-biting midges are weak, slow flyers, whereas the syrphid *Myiatropa florea* and the mosquito *Aedes geniculatus* are strong, fast flyers leading to differences in colonization potential (Kitching R., 1971; Verdonschot and Besse-Lototskaya, 2014). The midges seem to compensate for the lower dispersal success by higher fecundity, laying large clutches of up to 100 eggs (Kitching R., 1971; Kitching, 1972). This high fecundity combined with high drought and/or freezing tolerance may make midges (drought and freezing tolerance) and marsh beetles (freezing tolerance) (see **Figure 6**; Gossner, 2018) superior competitors under the harsh conditions that prevail in tree holes.

CONCLUSION

Water-filled tree holes are important tree-related microhabitats promoting forest biodiversity. Our study clearly shows that communities in these microhabitats are structured by complex and potentially interacting mechanisms proposed by metacommunity theory, such as niche separation, competition and dispersal limitations. Besides larval requirements, traits of adult life stages, such as food preferences and behavioral traits related to mating and oviposition, likely also shape these communities. Our study therefore clearly supports the conclusion of Srivastava et al. (2004) that these microcosms serve as a useful model system in ecology. These microhabitats host complex interactions involving different trophic levels, yet they are discrete, small enough to measure abiotic properties and collect all insect species present, and easy to manipulate. Water-filled tree holes thus open multiple possibilities to study responses of whole communities to environmental perturbation.

DATA AVAILABILITY STATEMENT

The datasets are publicly available in the Biodiversity Exploratories Information System: http://doi.org/10.17616/R32P9Q (Gossner and Weisser, 2017; Petermann et al., 2018a,b), https://www.bexis.uni-jena.de/ddm/data/Showdata/19086, https://www.bexis.uni-jena.de/ddm/data/Showdata/24146, and https://www.bexis.uni-jena.de/ddm/data/Showdata/24148.

AUTHOR CONTRIBUTIONS

MG conceived the ideas for this study, analyzed the data, and wrote the first draft of the manuscript. MG and JP led the two underlying projects. JP commented on all versions of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work has been funded by the DFG Priority Program 1374 "Biodiversity-Exploratories" (DFG-WE 3081/21-1, DFG21234_P).

ACKNOWLEDGMENTS

We thank the managers of the three Exploratories, K. Wells, S. Renner, K. Reichel-Jung, I. Steitz, S. Weithmann, S. Gockel, K. Wiesner, K. Lorenzen, J. Vogt, A. Hemp, M. Gorke and M. Teuscher, for their work in maintaining the plot and project infrastructures; S. Pfeifer, M. Gleisberg, C. Fischer and J. Mangels for giving support through the central office, J. Nieschulze, M. Owonibi and A. Ostrowski for managing the central database, and M. Fischer, E. Linsenmair, D. Hessenmöller, D. Prati, I. Schöning, F. Buscot, E.-D. Schulze, W. W. Weisser and the late E. Kalko for their roles in setting up the Biodiversity Exploratories project. We thank the administration of the Hainich National Park, the UNESCO Biosphere Reserve Swabian Alb and the UNESCO Biosphere Reserve Schorfheide-Chorin, as well as all landowners, for this excellent collaboration. Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen and Brandenburg. We are grateful for fieldwork and lab support from P. Lade, A. Rohland, N. Sichardt and A. L. Roberts and thank M. Dawes for English editing and A. Sallé and A. Feest for the valuable suggestions they provided through their reviews.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.816570/full#supplementary-material

Supplementary Figure 1 | Standardized model coefficients (including conditional and marginal R^2-values) of generalized linear mixed effects models with a Poisson distribution, showing the importance of tree-hole height above ground, plot-level tree-hole density (separated by canopy and ground), tree-hole opening area, tree-hole maximum water volume (Vmax), water chemistry (dissolved oxygen concentration (O_2), pH, and ammonium, nitrate and phosphate concentrations), and total amount of detritus relative to Vmax (PropDetritustot) in determining the abundance of the five most frequently observed insect species. **(A)** Diptera, Culicidae: *Aedes geniculatus*, occurrence in $n = 88$ tree holes; **(B)** Diptera, Ceratopogonidae: *Dasyhelea* sp., $n = 109$; **(C)** Diptera, Chironomidae: *Metriocnemus cavicola*, $n = 194$; **(D)** Diptera, Syrphidae: *Myiatropa florea*, $n = 139$; **(E)** Coleoptera, Scirtidae: *Prionocyphon serricornis*, $n = 131$. **(F,G)** show the total abundance and the frequencies of occurrence of the five species, respectively. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Supplementary Figure 2 | Prediction of the abundance of the most abundant species in our study as a function of tree-hole height above ground, based on generalized linear mixed effects models with a Poisson distribution, including tree-hole-scale and plot-scale variables (tree-hole density was assessed separately for canopy and ground holes) as additional predictors. The gray bands show 95% confidence intervals. The bars on the x-axis indicate the tree holes sampled along the vertical gradient. **(A)** Diptera, Culicidae: *Aedes geniculatus* occurrence in $n = 88$ tree holes; **(B)** Diptera, Ceratopogonidae: *Dasyhelea* sp., $n = 109$; **(C)** Diptera, Chironomidae: *Metriocnemus cavicola*, $n = 194$; **(D)** Diptera, Syrphidae: *Myiatropa florea*, $n = 139$; **(E)** Coleoptera, Scirtidae: *Prionocyphon serricornis*, $n = 131$.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Forests and Global Change

ORIGINAL RESEARCH
published: 11 February 2022
doi: 10.3389/ffgc.2022.743039

Distribution of a Foliage Disease Fungus Within Canopies of Mature Douglas-Fir in Western Oregon

Yung-Hsiang Lan[1]*, David C. Shaw[1], E. Henry Lee[2] and Peter A. Beedlow[2]

[1] Department of Forest Engineering, Resources, and Management, Oregon State University, Corvallis, OR, United States,
[2] U.S. Environmental Protection Agency, Pacific Ecological Systems Division, Corvallis, OR, United States

Edited by:
Akihiro Nakamura,
Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (CAS), China

Reviewed by:
Romà Ogaya,
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Government of British Columbia, Canada
Christopher Lee,
California Department of Forestry and Fire Protection (CAL FIRE), United States

***Correspondence:**
Yung-Hsiang Lan
skylan12@gmail.com

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: 17 July 2021
Accepted: 14 January 2022
Published: 11 February 2022

Citation:
Lan Y-H, Shaw DC, Lee EH and Beedlow PA (2022) Distribution of a Foliage Disease Fungus Within Canopies of Mature Douglas-Fir in Western Oregon.
Front. For. Glob. Change 5:743039.
doi: 10.3389/ffgc.2022.743039

Nothophaeocryptopus gaeumannii is a common native, endophytic fungus of Douglas-fir foliage, which causes Swiss needle cast, an important foliage disease that is considered a threat to Douglas-fir plantations in Oregon. Disease expression is influenced by fungal fruiting bodies (pseudothecia), which plug the stomata and inhibit gas exchange. Trees are impacted when pseudothecia plug stomates on 1-year-old and older needles resulting in early needle abscission. Mature (100 years+) trees appear to be less impacted from disease, and we hypothesize this is due to the greater emergence of pseudothecia on older than younger needles, which allows for more needle retention. We measured the density of pseudothecia occluding stomates across 2- to 5-year-old needles from upper, middle, and lower canopy positions of mature trees at three sites in the Oregon Coast Range and two sites in the western Oregon Cascade Mountains. Binomial generalized linear mixed model (GLMM) was used to test for the effects of canopy position (upper, middle, and lower), sites, needle age (2–5 years old), and years (2016 and 2017), and their interactions on the pseudothecia density. Pseudothecia density varied annually depending on sites, needle age and canopy positions. Pseudothecia density peaked on 3-, and 4-year-old needles, however, needles emerging from the same year, like 2-year-old needles in 2016 and 3-year-old needles in 2017 both emerged in 2014, had consistently similar patterns of pseudothecia density for both years, across site and canopy positions. Canopy position was important for 3-, and 4-year-old needles, showing less pseudothecia in the lower canopy. This research confirms that *N. gaeumannii* pseudothecia density is greatest in 3- and 4-year old needles in mature trees in contrast to plantations where pseudothecia density usually peaks on 2-year-old needles, and that pseudothecia density (disease severity) is generally lower in mature trees. Something about mature forest canopies and foliage appears to increase the time it takes for pseudothecia to emerge from the needles, in contrast to younger plantations, thus allowing the mature trees to have greater needle retention.

Keywords: Douglas-fir, fungal disease, foliar pathogen, *Nothophaeocryptopus gaeumannii*, Swiss needle cast, tree canopy

INTRODUCTION

Nothophaeocryptopus gaeumannii is a common native, endophytic fungus which occurs only in Douglas-fir (*Pseudotsuga menziesii*) foliage (Hansen et al., 2000). The fungus can cause a foliage disease known as Swiss needle cast, which is currently defoliating and decreasing growth of Douglas-fir along the Pacific Coast in Oregon, Washington, and SW British Columbia (Ritóková et al., 2016; Shaw et al., 2021). Disease is caused when the fruiting bodies of the fungus, known as pseudothecia (**Figure 1**), emerge from and plug the stomates which causes reduced gas exchange and carbon starvation (Manter et al., 2000). This fungus may be unusual for a pathogen in that newly emerged needles are the predominant substrate for new infection by ascospores (Rohde, 1937; Chastagner and Byther, 1983) and colonization within needles is exclusively intercellular and non-lethal to cells (Stone et al., 2008a). Swiss needle cast induced reductions in tree growth of coastal Douglas-fir stands vary in space and time depending upon elevation, aspect, proximity to the coast, and site conditions and primary environmental factors influencing the degree of pathogen dynamics (Rosso and Hansen, 2003; Coop and Stone, 2007). Evidence from dendrochronological studies indicate that Swiss needle cast impacts in Oregon were least severe in the first half of the 20th century and increased in frequency, severity and range after ∼1980 due to increasing winter temperature associated with climate change (Black et al., 2010; Lee et al., 2013, 2017). The Swiss needle cast epidemic in the most recent decades is a primary economic and ecological concern to the timber industry in the Pacific Northwest because disease severity is greater in young coastal Douglas-fir plantations than in mature stands but reasons for this remains elusive (Lan et al., 2019; Mildrexler et al., 2019). Coastal young Douglas-fir trees typically retain up to 4 years of needles but may have only current and 1-year-old foliage in severely infected plantations (Hansen et al., 2000; Maguire et al., 2002; Zhao et al., 2011) due to early needle abscission when between 25 and 50% of the stomates are occluded (Hansen et al., 2000; Manter et al., 2003; Stone et al., 2008a). Anecdotal evidence from epidemiological studies suggest needle retention appears to drive the growth impacts on the tree, with foliage retention of only 2 years causing a reduction in growth of about 30% (Maguire et al., 2002, 2011; Shaw et al., 2021). Needle retention and fungal fruiting body presence and abundance on 1- and 2-year-old foliage have routinely been used as indices of disease severity in Douglas-fir plantations (Hood, 1982; Michaels and Chastagner, 1984; Hansen et al., 2000). However, disease severity on 2-year-old needles may be misleading for mature Douglas-fir trees which typically have greater needle retention of more than 5 years (Lan et al., 2019) and lesser frequency and magnitude of growth losses (Lee et al., 2017).

Recent epidemiological evidence suggests that disease severity, as measured by incidence times the percentage of pseudothecia occluding stomates (Mulvey et al., 2013), is greatest in 2-year-old needles for young trees, and 3–5 year-old needles for older trees (Lan et al., 2019). The reason for this difference remains elusive, as foliar nitrogen and leaf wetness did not explain the difference. The reason mature trees have greater foliage retention, and are healthier, may be influenced by the timing of pseudothecia emergence. While there is considerable epidemiological evidence of disease severity for young Douglas-fir plantations in coastal Oregon and Washington (Ritóková et al., 2016, 2021), the data and knowledge gaps of disease severity for mature Douglas-fir are large due primarily to comparably few epidemiological studies on mature Douglas-fir and scaling issues from sampling only 2-year-old needles for measuring incidence.

This manuscript extends our previous epidemiological study (Lan et al., 2019) to examine the differences in disease severity between needle age classes and canopy position of mature Douglas-fir trees based on within-leaf measurements of the density of pseudothecia occluding stomates. There is no published data on pseudothecia density of multiple needle age classes in mature trees. Lan et al. (2019) found a greater percentage of 3–5 year-old needles of mature Douglas-fir were infected by the presence of pseudothecia than younger needles, but the amount of stomata occluded by pseudothecia on infected needles (i.e., pseudothecia density) was not measured. Consequently, the pathogenicity of *N. gaeumannii* and epidemiology of mature trees are still not very clear. Because the physiological effects of SNC (impaired CO_2 uptake and photosynthesis) are associated with the physical blockage of stomata, the density of pseudothecia occluding stomates is a relevant response variable for assessing disease severity across needle age classes and canopy positions of mature trees. We hypothesized that disease severity is less in mature trees because the time since initial infection to the emergence of pseudothecia is longer for mature than young trees. Within the mature tree crowns, we also explored whether pseudothecia density varied with needle age, canopy position, and site. We believe that this analysis provides important insight into spatial and temporal dynamics of Swiss needle cast disease progression in mature tree canopies. Also, filling a data gap of the differences in mature and young trees can lead to a better understanding of the ecology and epidemiology of this important foliage disease.

MATERIALS AND METHODS

Study Sites

We sampled the same mature trees from five of the seven sites in western Oregon that were included in the study by Lan et al. (2019); three sites were in the long-term ecological monitoring plot (LTEM) system established by the United States Environmental Protection Agency (hereafter EPA; Lee et al., 2007; Beedlow et al., 2013) and two sites were not part of the LTEM system. Moose Mountain and Falls Creek are located on the west slope of the Cascade Range in the Willamette National Forest, and Cascade Head, Woods Creek, and Klickitat Mountain are in the Siuslaw National Forest in the Coast Range (**Figure 2**). The forests were unmanaged mature stands of Douglas-fir that were 120–150 years old and ranged in height from 55 to 70 m. Elevation of sites varied from 140 to 670 m. Annual precipitation varied from 1700–2500 mm. Associated tree species included western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*), in Cascade Head Sitka spruce (*Picea sitchensis*)

FIGURE 1 | Photo of pseudothecia blocking stomates on the underside of a Douglas-fir needle.

is also associated. Monthly precipitation and mean temperature data from 2010 to 2017 at all sites were downloaded from PRISM at Oregon State University[1] by providing GPS coordinates. We use average winter mean temperature (December, January, and February) and average summer precipitation (May, June and July), the climatological variables that are associated with Swiss needle cast severity (Manter et al., 2005), to compare the weather conditions across the sites (**Figure 3**).

Field Sampling

Branch samples were collected on the south side of each tree in late May through early June in 2016 and 2017, after bud-break and before new branchlets were elongated. The May–June period is also optimal for sampling because *N. gaeumannii* asci mature during April–June in western Oregon and sporulation occurs mid-May to July (Rohde, 1937; Chastagner and Byther, 1983; Michaels and Chastagner, 1984). We collected 1–3 branches from three canopy positions (lower, middle, and upper crown) in each of three trees at each site (total number of trees = 15). At least one branch > 1 m in length was selected to ensure sufficient needle material for measurements. Several shorter branches were chosen if there were no branches greater than 1 m in length. Branches were transported to the lab and stored in a 5°C cold room. Three young trees (20 – 30 years old) next to the mature trees at each site were sampled at the same time by Lan et al. (2019) and data are used here for comparison to older stands.

Lab Analysis

For each canopy position of 15 mature trees, 50 individual needles were randomly selected from the cohort of each foliar age class and needles were taped on an index card and stored at −20°C. All needle age classes from 2 to 6 years old were examined for *N. gaeumannii* pseudothecia incidence and density. The pseudothecia incidence is defined as the percentage of the 50 needles with pseudothecia present. Pseudothecia density was determined by selecting the first 10 needles with pseudothecia present beginning at the top of the card and working down,

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[1] http://www.prism.oregonstate.edu/explorer/, accessed 11 April 2021.

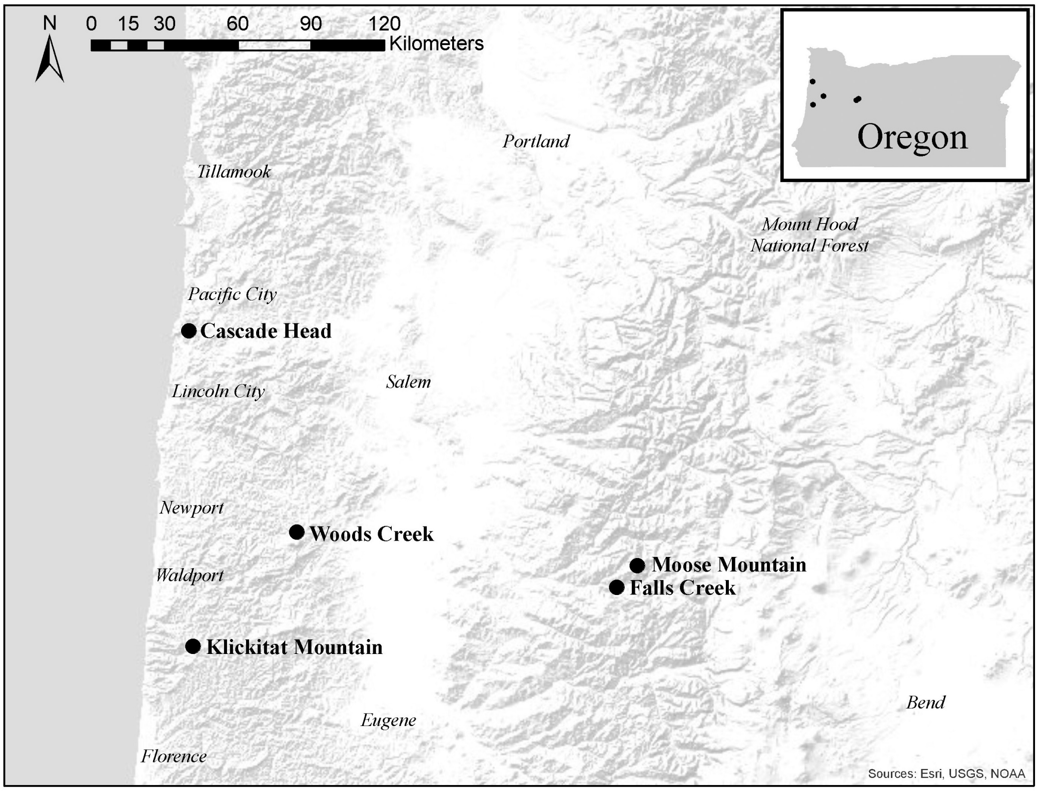

FIGURE 2 | Map of study sites in the Oregon Coast Range and western Cascade Mountains of western Oregon, United States. Cascade Head, Falls Creek, and Moose Mountain are in the long-term ecological monitoring plot (LTEM) system, and Klickitat Mountain and Woods Creek are not part of the LTEM system. Cascade Head, Klickitat Mountain, and Woods Creek are in the Siuslaw National Forest. Falls Creek and Moose Mountain are in the Willamette National Forest.

and then counting the percent of stomates occluded in three regions (base, middle, and tip) of the needle (Mulvey et al., 2013). The entire length of the needle was evenly divided into three regions, for each region we picked a starting point using a random number table, and examined 100 contiguous stomates from the starting point to determine the number that were occluded by pseudothecia. Pseudothecial occlusion in the three regions was then averaged for each needle and then averaged for 10 needles per canopy position per tree.

Disease severity is typically determined by multiplying the incidence times the density of stomates blocked by pseudothecia for 2-year-old needles (Mulvey et al., 2013; Ritóková et al., 2021; Shaw et al., 2021). Disease severity is an important metric used to compare disease importance in forest stands (Shaw et al., 2021), while density alone is most important in casting of individual needles (Hansen et al., 2000; Manter et al., 2003; Stone et al., 2008a). The 2-year-old foliage is the key age class in coastal forests but foliage retention of severely diseased trees can be less than 2 years (Ritóková et al., 2016). Where disease severity is lower, pseudothecia can take as long as 4 years to mature when environmental conditions are less favorable for fungal growth (Stone et al., 2008a). In this study, we measured density of stomates plugged with pseudothecia for needles aged 2–5 years and included incidence data from Lan et al. (2019). We used the pseudothecia occlusion density rather than incidence as the primary measure of disease expression because there were many incidence values at or near zero.

Statistical Analysis

Binomial generalized linear mixed model (GLMM) was used to test for effects of canopy positions (upper, middle, and lower), sites, needle ages (2–5 years old), years (2016 and 2017), and their interactions on the pseudothecia density at the 0.05 level of significance. In the GLMM, canopy position, site, and needle age were treated as fixed effects with interaction terms in the GLMM whereas year was treated as an additive fixed effect, the individual trees on the same site were treated as random effects. We first ran the GLMM by using only 2-year-old needles of mature and young trees, to confirm the statistical differences of pseudothecia incidence and density between mature and young trees. We analyzed pseudothecia incidence and density ratios (0–1) using binomial GLMM assuming a logit-linear model with four fixed factors and one random factor for individual trees.

To understand more deeply about pseudothecia density on mature trees, we first ran the GLMM by using the complete dataset. In the preliminary results, there were interactions involving canopy positions, sites, and needle age, so we also ran the GLMM by year and by needle age, to test for differences in pseudothecia density on canopy position and site separately. We conducted a Bonferroni mean separation test to

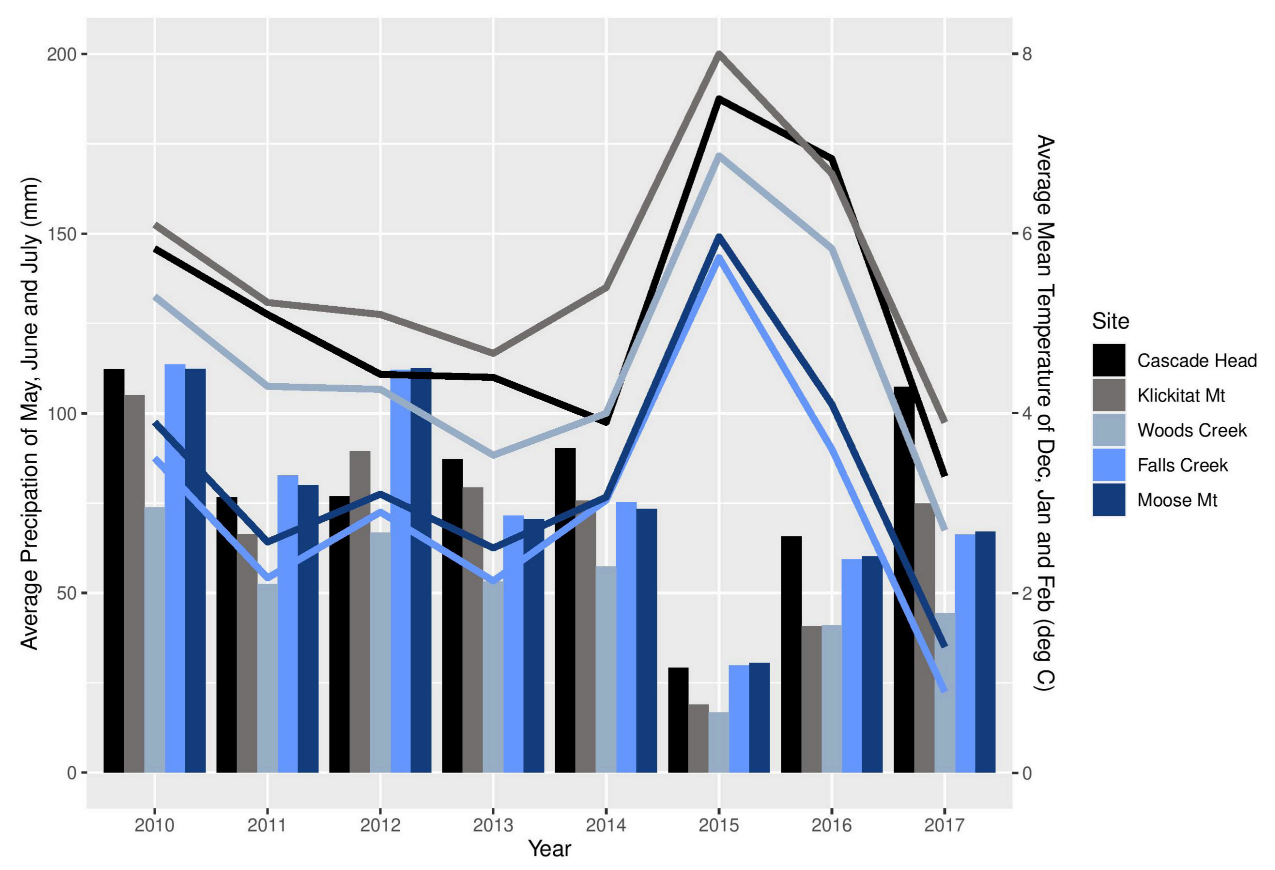

FIGURE 3 | Average winter (December, January, and February) mean temperature (lines) and average summer (May, June, and July) precipitation (bars) of each study site from 2010 to 2017.

infer which treatment means were different. GLMM tests were performed using R (v. 4.0.2, R Core Team, 2020) and package dplyr (Wickham et al., 2017), emmeans (Lenth, 2018), ggplot2 (Wickham, 2009), nlme (Pinheiro et al., 2017), glmmTMB (Brooks et al., 2017), and multcomp (Hothorn et al., 2008).

RESULTS

Mature Douglas-fir trees were less severely infected by *N. gaeumannii* than nearby young Douglas-fir trees (10–20 years old) based on examination of pseudothecia incidence and density for 2-year-old needles (**Table 1**). The mean percentage of 2-year-old needles with pseudothecia present, or incidence, ranged from 1.3 to 98% across sites and years for mature Douglas-fir trees, compared with 44 to 100% for the young plantations (**Table 2**). Tree age differences (i.e., mature vs. young trees) in pseudothecia incidence and density for 2-year-old needles were highly significant at the 0.05 level of significance ($p = 0.02$ and $p < 0.001$ respectively, **Table 1**). To a lesser extent, site and canopy position effects and their interactions on incidence and density for 2-year-old needles were also significant at the 0.05 level of significance based on the binomial GLMM, however, year effect was significant only in pseudothecia incidence but not density (**Table 1**).

Pseudothecia incidence was uniformly high for all needle classes of mature trees at Cascade Head where disease severity was greatest. For less severely diseased stands of mature trees, incidence varied by needle age class and peaked for 3- and 4-year old needles rather than for 2-year-old needles. The mean percentage of occluded stomates on 2-year-old needles with pseudothecia present, or pseudothecia density, ranged from 0.1 to 6.5% for mature trees in comparison to a range of 2–21% for young plantations. The highest pseudothecia density recorded for mature trees were: Cascade Head 9.3% for 6-year-old needles, Klickitat Mountain 4.0% for 5-year, Woods Creek 8.4% for 4-year, Moose Mountain 8.8% for 4-year, and Falls Creek 8.6% for 3-year old needles (**Table 2**). Because of the limited sample size in this study, the variation of pseudothecia density and incidence between tree to tree could be large (**Table 2**).

Disease severity (incidence times density) was consistently greater for 2-year-old needles of young plantation trees than for 2–5 years old needles of mature trees (**Figure 4**). Disease severity was greatest for 3- and 4-year old needles of mature trees, although 5-year-old needles were similar to 4-year old needles at Cascade Head and Moose Mountain.

TABLE 1 | Summary of GLMM of pseudothecia incidence and density, across five study sites (15 trees) in western Oregon, tree age (mature or young trees), year, canopy positions and their interactions.

	Density					Incidence				
	Estimates	**SE**	**z value**	**p value**		**Estimates**	**SE**	**z value**	**p value**	
(Intercept)	−2.49	0.24	−10.56	<0.001	***	2.18	0.48	4.51	<0.001	***
treeageYoung	1.02	0.29	3.49	<0.001	***	2.22	0.95	2.33	0.020	*
canopyMiddle	−0.67	0.36	−1.86	0.063	.	1.87	0.64	2.91	0.004	**
canopyLower	−1.59	0.49	−3.20	0.001	**	0.00	0.37	0.00	1.000	
siteKlickitat Mt	−0.80	0.40	−2.00	0.046	*	−2.95	0.66	−4.50	<0.001	***
siteWoods Creek	−1.00	0.42	−2.37	0.018	*	−6.55	0.95	−6.89	<0.001	***
siteFalls Creek	−2.48	0.74	−3.37	0.001	***	−5.47	0.76	−7.22	<0.001	***
siteMoose Mt	−1.89	0.58	−3.28	0.001	**	−3.88	0.67	−5.81	<0.001	***
year2017	0.19	0.29	0.64	0.523		1.15	0.49	2.32	0.021	*
treeageYoung:canopyMiddle	0.25	0.43	0.58	0.559		−1.57	1.26	−1.25	0.213	
treeageYoung:canopyLower	0.34	0.57	0.60	0.550		0.29	1.15	0.25	0.800	
treeageYoung:siteKlickitat Mt	0.04	0.49	0.08	0.939		1.99	1.21	1.64	0.101	
treeageYoung:siteWoods Creek	0.87	0.49	1.76	0.078	.	4.01	1.34	2.99	0.003	**
treeageYoung:siteFalls Creek	1.50	0.79	1.90	0.057	.	4.90	1.31	3.75	<0.001	***
treeageYoung:siteMoose Mt	0.85	0.65	1.32	0.186		2.58	1.18	2.18	0.029	*
canopyMiddle:siteKlickitat Mt	−0.53	0.72	−0.73	0.465		−2.44	0.70	−3.49	<0.001	***
canopyLower:siteKlickitat Mt	−0.05	0.89	−0.06	0.952		−0.88	0.47	−1.87	0.061	.
canopyMiddle:siteWoods Creek	−2.65	1.80	−1.47	0.141		−1.45	1.12	−1.29	0.196	
canopyLower:siteWoods Creek	1.62	0.69	2.37	0.018	*	1.29	0.89	1.45	0.148	
canopyMiddle:siteFalls Creek	0.36	1.12	0.32	0.749		−1.32	0.83	−1.59	0.113	
canopyLower:siteFalls Creek	1.85	1.04	1.77	0.077	.	1.27	0.61	2.07	0.038	*
canopyMiddle:siteMoose Mt	0.25	0.90	0.28	0.778		−1.35	0.71	−1.91	0.057	.
canopyLower:siteMoose Mt	0.49	1.14	0.43	0.665		0.48	0.48	1.01	0.312	
treeageYoung:year2017	0.33	0.35	0.93	0.351		−0.85	1.20	−0.71	0.475	
canopyMiddle:year2017	0.25	0.48	0.53	0.599		−0.73	1.05	−0.70	0.485	
canopyLower:year2017	0.68	0.61	1.11	0.267		0.19	0.72	0.27	0.791	
siteKlickitat Mt:year2017	−0.45	0.61	−0.74	0.457		−1.92	0.62	−3.08	0.002	**
siteWoods Creek:year2017	−3.51	1.79	−1.96	0.050	*	−1.15	1.12	−1.02	0.308	
siteFalls Creek:year2017	−0.02	0.98	−0.02	0.986		−1.71	0.84	−2.02	0.044	*
siteMoose Mt:year2017	−0.57	0.86	−0.66	0.507		−2.92	0.70	−4.18	<0.001	***
treeageYoung:canopyMiddle:siteKlickitat Mt	0.43	0.82	0.52	0.601		1.89	1.42	1.33	0.183	
treeageYoung:canopyLower:siteKlickitat Mt	0.71	0.99	0.72	0.472		0.41	1.33	0.31	0.757	
treeageYoung:canopyMiddle:siteWoods Creek	3.54	1.83	1.94	0.053	.	3.76	1.73	2.17	0.030	*
treeageYoung:canopyLower:siteWoods Creek	−0.09	0.77	−0.12	0.907		1.31	1.65	0.80	0.425	
treeageYoung:canopyMiddle:siteFalls Creek	0.16	1.18	0.14	0.891		1.89	1.69	1.12	0.262	
treeageYoung:canopyLower:siteFalls Creek	−0.80	1.13	−0.71	0.478		−1.40	1.48	−0.95	0.345	
treeageYoung:canopyMiddle:siteMoose Mt	0.44	0.97	0.46	0.647		2.52	1.46	1.73	0.084	.
treeageYoung:canopyLower:siteMoose Mt	0.84	1.21	0.70	0.486		1.11	1.42	0.78	0.434	
treeageYoung:canopyMiddle:year2017	−0.66	0.57	−1.16	0.246		0.44	1.91	0.23	0.818	
treeageYoung:canopyLower:year2017	−0.58	0.71	−0.82	0.414		−0.48	1.75	−0.28	0.782	
treeageYoung:siteKlickitat Mt:year2017	−1.37	0.75	−1.83	0.067	.	0.70	1.36	0.51	0.609	
treeageYoung:siteWoods Creek:year2017	1.74	1.83	0.95	0.340		−1.92	1.59	−1.21	0.227	
treeageYoung:siteFalls Creek:year2017	−0.37	1.04	−0.35	0.723		1.15	1.56	0.74	0.458	
treeageYoung:siteMoose Mt:year2017	−0.93	0.97	−0.96	0.338		0.09	1.35	0.07	0.947	
canopyMiddle:siteKlickitat Mt:year2017	−0.15	1.15	−0.13	0.899		1.97	1.16	1.70	0.089	.
canopyLower:siteKlickitat Mt:year2017	−1.49	1.76	−0.85	0.397		−0.82	1.03	−0.80	0.421	
canopyMiddle:siteWoods Creek:year2017	−12.37	3909.49	0.00	0.997		0.73	1.67	0.44	0.662	
canopyLower:siteWoods Creek:year2017	−0.72	2.57	−0.28	0.781		−0.91	1.41	−0.65	0.517	
canopyMiddle:siteFalls Creek:year2017	−2.65	2.79	−0.95	0.341		0.65	1.37	0.48	0.632	
canopyLower:siteFalls Creek:year2017	−2.56	1.90	−1.34	0.179		−1.46	1.16	−1.26	0.209	
canopyMiddle:siteMoose Mt:year2017	1.08	1.20	0.90	0.371		−0.83	1.37	−0.61	0.544	

(Continued)

TABLE 1 | (Continued)

	Density				Incidence			
	Estimates	**SE**	**z value**	**p value**	**Estimates**	**SE**	**z value**	**p value**
canopyLower:siteMoose Mt:year2017	0.31	1.50	0.21	0.835	−1.30	1.07	−1.22	0.224
treeageYoung:canopyMiddle:siteKlickitat Mt:year2017	0.04	1.40	0.03	0.977	−2.04	2.09	−0.98	0.329
treeageYoung:canopyLower:siteKlickitat Mt:year2017	1.74	1.90	0.92	0.360	0.30	2.02	0.15	0.880
treeageYoung:canopyMiddle:siteWoods Creek:year2017	11.94	3909.49	0.00	0.998	−2.08	2.44	−0.85	0.393
treeageYoung:canopyLower:siteWoods Creek:year2017	0.16	2.63	0.06	0.951	−0.65	2.30	−0.28	0.779
treeageYoung:canopyMiddle:siteFalls Creek:year2017	2.52	2.84	0.89	0.374	−0.40	2.47	−0.16	0.873
treeageYoung:canopyLower:siteFalls Creek:year2017	1.54	2.00	0.77	0.440	1.06	2.21	0.48	0.633
treeageYoung:canopyMiddle:siteMoose Mt:year2017	−0.81	1.36	−0.60	0.550	0.07	2.22	0.03	0.975
treeageYoung:canopyLower:siteMoose Mt:year2017	−0.47	1.64	−0.29	0.776	0.86	2.09	0.41	0.681

Only 2-year-old needles were used. Asterisks indicate significance at the 0.05 (*), 0.01 (**), and 0.001 (***) levels. Estimates are based on a logit transformation.

Within Crown and Site Patterns of Mature Trees

Pseudothecia density varied by canopy position, site, and year depending upon needle age class (**Figures 5**, **6** and **Table 3**). Site differences in pseudothecia density of 2-year-old needles were statistically significant at the 0.05 level, with greater pseudothecia density at the Cascade Head site than the inland sites (**Figures 4**, **5**). Canopy position differences in pseudothecia density of 3- and 4-year-old needles were statistically significant at the 0.05 level, with greater pseudothecia density in the upper canopy than in the lower canopy (**Figure 6**).

Site differences in pseudothecia density were statistically significant at the 0.05 level of significance (**Table 3**), indicating that temporal and spatial variability might exist in response to local weather variability. Only current year foliage is infected by *N. gaeumannii,* and variability of weather during the year foliage emerges can influence infection success (Stone et al., 2008a). Year-to-year variation in temperature in winter and rainfall in summer is common (**Figure 3**). Pseudothecia density of 2-year-old needles was greatest at Cascade Head, notably in 2017, whereas that of 4-year-old needles in 2016 was greatest at Woods Creek (**Figure 5** and **Table 2**). Pseudothecia density peaked in either 2- or 3-year-old needles at Cascade Head and 3- or 4-year-old needles at the inland sites depending upon year (**Figure 5**).

Canopy position differences were statistically significant at the 0.05 level (**Table 3**). Needles from upper canopy position had significantly higher pseudothecia density than the needles from middle ($p = 0.024$, **Table 3**) and lower canopies ($p < 0.001$, **Table 3**). In addition, these trends in canopy position also persisted over time for needles that emerged from the same year. For example, the canopy position trends in 2-year-old needles in 2016 was similar to the canopy position trends in 3-year-old needles in 2017, which they both emerged from 2014. The canopy position trends are particular for the 2- and 3-year old needles in 2016. In contrast, the canopy position trends in 3-year-old needles in 2016 did not persist in 4-year-old needles in 2017 due, in part, to a combination of pseudothecia density in the upper canopy peaking in 2016 followed by greater needle abscission in 2017 (**Figure 6**). The needle cohorts that emerged from the same year had similar trends among sites, implying a weather effect.

DISCUSSION

Swiss needle cast is an important disease of plantation Douglas-fir along the coast, yet mature stands are apparently not currently severely infected except in limited areas such as Tillamook, Oregon (Black et al., 2010). Older trees have lower disease severity on 2-year-old needles (Lan et al., 2019) and older stands rarely show up in Swiss needle cast aerial detection surveys (Mildrexler et al., 2019). In this study, pseudothecia density, as well as disease severity, peaks on 3-, and 4-year-old needle age classes in mature Douglas-fir trees (**Figure 4**). This is consistent with previous findings that pseudothecia incidence peaks on 3-year or older needles for mature Douglas-fir (Lan et al., 2019). Pseudothecia density is also very low across all our mature tree samples compared to young tree 2-year-old needles, with density never exceeding 10% for any needle age class (**Table 2**). Manter et al. (2000) states that carbon assimilation decreases proportionally with increasing stomate occlusion, while Hansen et al. (2000) suggest leaves are cast above 50% occlusion and Manter et al. (2003) suggest casting with as low as 25% stomate occlusion. Therefore, it is likely that the levels of stomate occlusion found in older trees do not influence needle function as strongly as in younger trees, although we did not test this.

The combination of lower pseudothecia density and the emergence of pseudothecia on 3- or 4-year-old needles, rather than 2-year-old needle age class, is potentially the key difference between Swiss needle cast disease expression in young and mature Douglas-fir. This supports our hypothesis, that the reason mature stands do not typically suffer from disease caused by *N. gaeumannii* is because needle retention is typically above 3 years (Lan et al., 2019). The lower pseudothecia density on mature trees also makes needle casting due to disease less likely in mature needles.

Why Do Pseudothecia Emerge on Older Needles in Mature Forests?

The factors that control timing of pseudothecia emergence from needles are not well understood, yet they may be critical to explaining disease epidemiology because the *N. gaeumannii* is an endophyte that appears to only impact leaves when

TABLE 2 | Disease incidence (%) and pseudothecia density (%) of 2-year old needles of young plantations (data used to determine disease severity in Lan et al., 2019) and 2–6 year age classes on mature trees (n = 15).

Sites	Young plantation 2 year		Mature 2 year		Mature 3 year		Mature 4 year		Mature 5 year		Mature 6 year	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Incidence												
Cascade Head	99.78 ± 0.22	100 ± 0	91.78 ± 2.32	97.78 ± 1.31	86.4 ± 9.27	93.11 ± 5.43	77.96 ± 3.79	90.01 ± 7.26	81.57 ± 7.97	97.22 ± 2.78	84.51 ± 1.35	85.71 ± 14.29
Klickitat Mountain	96.22 ± 1.61	85.78 ± 4.06	26.44 ± 7.39	10 ± 5.36	69.78 ± 7.03	41.12 ± 9.3	39.18 ± 6.96	50.19 ± 10.15	26.59 ± 12.55	34.65 ± 8.82	15.53 ± 7.99	28.04 ± 11.67
Woods Creek	94.89 ± 3.27	44.44 ± 8.07	2.22 ± 0.91	1.33 ± 0.58	30.22 ± 7.68	8.44 ± 2.84	33.78 ± 5.97	22.44 ± 4.71	8.12 ± 1.64	27.78 ± 4.2	9.33 ± 3.25	6.29 ± 2.29
Moose Mountain	96.89 ± 1.57	68.67 ± 9.88	22.44 ± 6.97	1.78 ± 0.91	54.89 ± 13.96	34.44 ± 10.94	52.51 ± 12.41	40 ± 12.41	51.59 ± 14.25	34.79 ± 11.06	39.5 ± 19.19	33.5 ± 12.82
Falls Creek	98.89 ± 0.59	97.33 ± 1.15	7.78 ± 2.3	2.44 ± 0.87	62.67 ± 10.39	38 ± 7.26	51.95 ± 6.97	65.79 ± 10.72	56.57 ± 8.21	51.66 ± 9.08	56.16 ± 12.14	53.64 ± 11.65
Density												
Cascade Head	12.82 ± 1.84	17.89 ± 3.08	4.56 ± 1.03	6.51 ± 1.34	7.52 ± 0.91	6.28 ± 0.7	6.63 ± 1.09	6.4 ± 0.88	7.36 ± 1.08	4.12 ± 0.57	9.33 ± 2.48	6.1 ± 0.7
Klickitat Mountain	6.22 ± 0.73	2.07 ± 0.38	1.84 ± 0.5	1.16 ± 0.52	2.04 ± 0.68	2.02 ± 0.47	2.68 ± 0.68	3.06 ± 1.18	2.36 ± 0.46	4.05 ± 1.96	1.42 ± 0.57	2.44 ± 1.42
Woods Creek	20.82 ± 2.07	4.65 ± 1.09	2.07 ± 1.14	0.07 ± 0.05	7.78 ± 2.41	1.59 ± 0.81	8.41 ± 1.78	6.94 ± 0.87	6.91 ± 1.6	5.7 ± 0.9	4.44 ± 1.24	2.72 ± 1.22
Moose Mountain	8.63 ± 1.18	3.18 ± 0.44	0.84 ± 0.15	1.25 ± 0.73	3.9 ± 0.7	4.51 ± 1.49	3.2 ± 0.73	8.82 ± 1.49	3.27 ± 1.07	6.63 ± 2.17	2.14 ± 0.51	4.07 ± 1.07
Falls Creek	7.93 ± 0.97	6.15 ± 1.75	0.71 ± 0.13	0.35 ± 0.27	8.55 ± 2.08	2.69 ± 0.58	5.54 ± 1.69	6.89 ± 1.36	3.98 ± 0.48	3.46 ± 1.05	3.95 ± 0.79	3.51 ± 0.89

pseudothecia emerge and block gas exchange (Manter et al., 2000, 2005). Manter et al. (2005) has shown that winter temperature may explain timing of pseudothecial development, while Lan et al. (2019) showed that leaf wetness and leaf nitrogen do not explain differences in Swiss needle cast disease severity using 2-year-old needles of young and mature trees. Evidence from dendrochronological studies suggest winter temperature is strongly associated with disease impact at wetter, cooler sites while summer conditions are more important at less humid, warmer sites (Lee et al., 2013). In 2015, the PNW experienced the warmest winter on record with winter temperatures 3.4°C above historical average (Mote et al., 2019). The anomalously warm winter of 2015 likely contributed to the high disease severity at the coast site while below-average spring and summer precipitation and above-average summer temperatures in 2015 likely decreased pseudothecia incidence and density at inland sites outside of the coastal fog zone (Lee et al., 2013, 2017).

What Factors Could Control Timing of Pseudothecia Emergence?

Although we did not specifically test why pseudothecia emerge on older needles of mature trees, canopy architecture is distinct between young plantations and mature tree crowns. The mature trees are taller, with longer vertical foliage distribution and more complex microclimatic variation (Parker et al., 2002). Leaf temperature in winter may be reduced in mature trees compared to younger stands due to age differences in canopy height and architecture, and this could slow the development of pseudothecia production (Manter et al., 2005). Spore dispersal could also be different within older forest stands with complex crowns compared to even-structured young stands where canopy connectivity and homogeneity may allow for better colonization of needles. Other factors that might influence pseudothecia development time could be related to differences in leaf structure or chemistry between young and mature trees (Lan et al., 2019).

Patterns Within Mature Tree Crowns

We found that *N. gaeumannii* pseudothecia density varies year to year depending on needle age and canopy position in mature Douglas-fir of western Oregon. Needles emerging from the same year, for example, 2-year-old needles in 2016 and 3-year-old needles in 2017 both emerged in 2014, have similar patterns of pseudothecia density across needle ages which likely relates to differences in weather during the year that needles are infected (**Figure 5**). *Nothophaeocryptopus gaeumannii* only infects the current-year needles from May–August when pseudothecia disperse spores (Michaels and Chastagner, 1984) and leaf wetness is important for fungal colonization (Manter et al., 2005). The weather during these months may cause the similar pseudothecia occlusion for the same-year needle cohorts due to similarities in weather-driven fungal colonization. The site effect was important for 2-year-old needles in our study, with highest pseudothecia densities at the Cascade Head site, but site was also important for 3- and 4-year-old needles. Distance from coast is important to Swiss needle cast disease severity and needle retention in young stands, with Swiss needle cast impacts greater

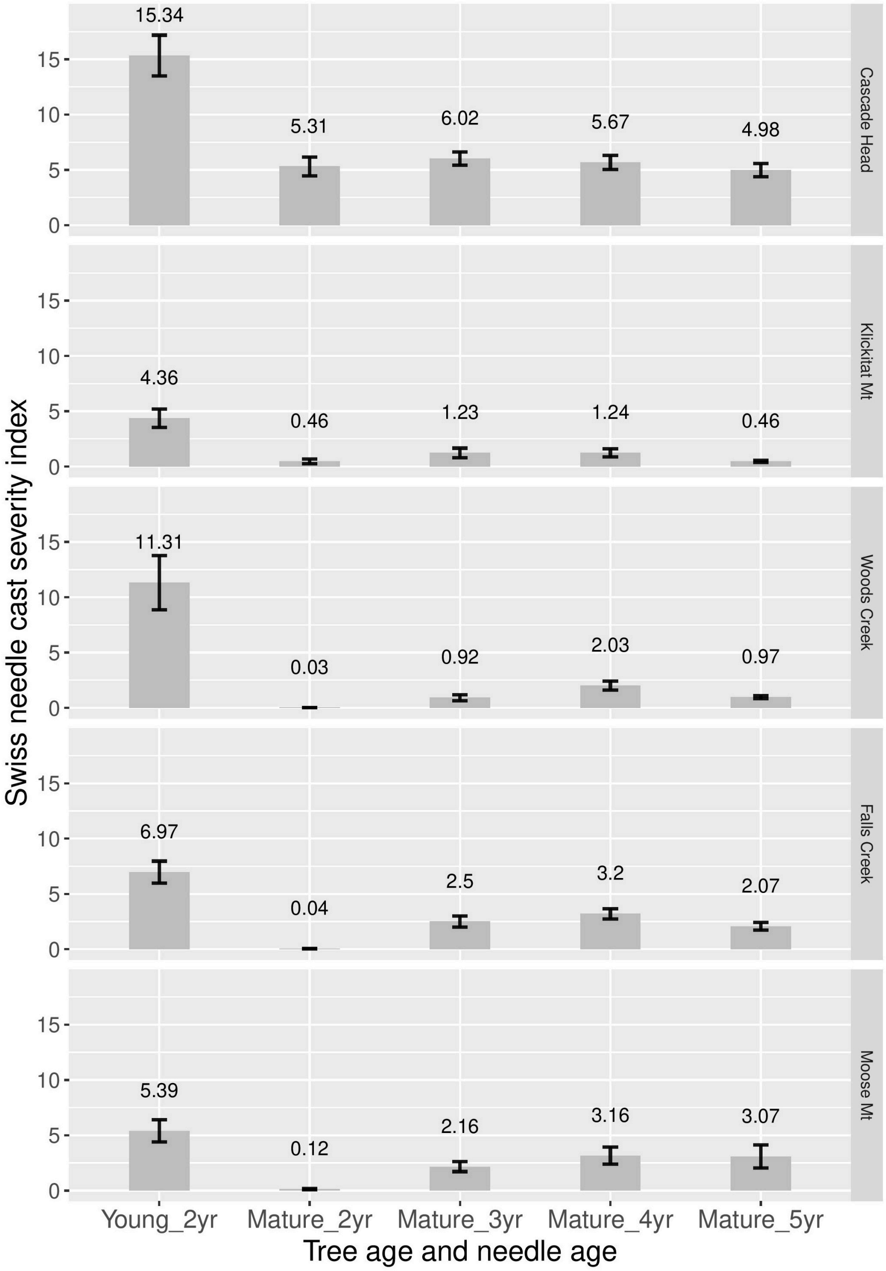

FIGURE 4 | Swiss needle cast average disease severity (incidence × density) for 2-year-old needles in young nearby plantations, and for 2, 3, 4, and 5-year old needles in mature stands. Two years and three canopy positions were averaged at each site.

at lower elevation areas and closer to coast (Hansen et al., 2000; Rosso and Hansen, 2003; Lee et al., 2013; Shaw et al., 2014, 2021; Ritóková et al., 2021).

Disease severity and pseudothecial density measured on 2-year-old needles is highest in the upper crowns of plantation-grown Douglas-fir compared to middle and lower crown positions (Hansen et al., 2000; Manter et al., 2005; Shaw et al., 2014; Lan et al., 2019; Ritóková et al., 2021). Our pseudothecia density data across older needles is generally consistent with Douglas-fir crown vertical patterns for 2-year-old needles in plantations. The lower canopy position had consistently lowest density of pseudothecia for all needle age classes (**Figure 6** and **Table 3**), while the upper canopy had the highest density, except the mid canopy of 4-year old needles in 2017.

Foliage diseases are generally thought to be most impactful in the highest humidity regions of a tree crown, which is typically the lower and inner crown (Tattar, 1989; Scharpf, 1993). In Christmas tree plantation settings in Pennsylvania and western Washington, *N. gaeumannii* was most severe in the lower and inner portion of the tree crown (Merrill and Longenecker, 1973;

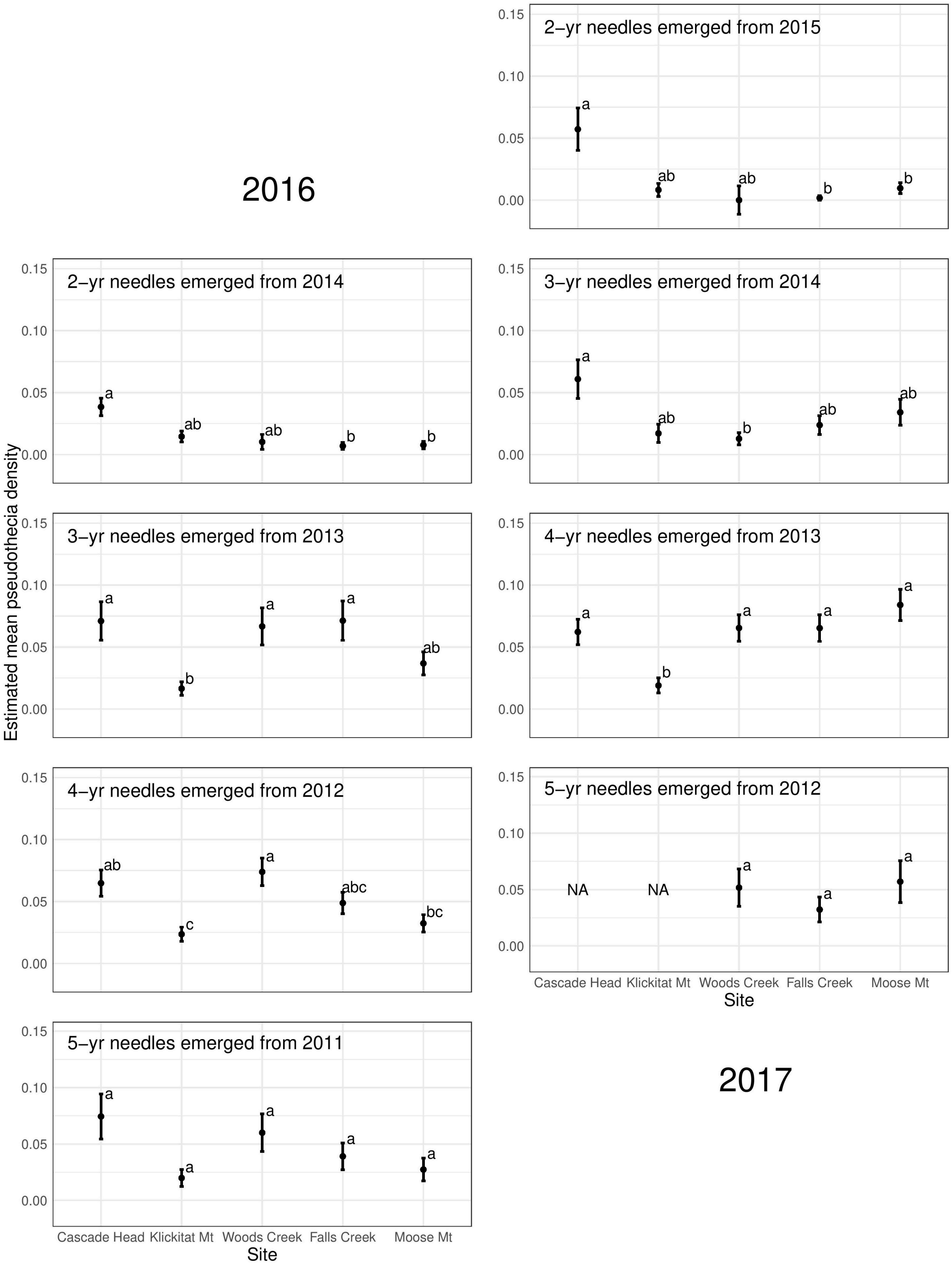

FIGURE 5 | Pseudothecia density by site for 2- to 5-year old needles in 2016 and 2017 from mature Douglas-fir trees in western Oregon. Sites listed on the *X*-axis are from coast (left) to inland (right). Different letters represent that the two groups are statistically different.

Chastagner and Byther, 1983). However, in western Oregon, disease severity of *N. gaeumannii* is consistently greatest in the upper crown for plantation trees (Hansen et al., 2000; Shaw et al., 2014; Ritóková et al., 2021), while the mid- and upper crown of mature trees show greater pseudothecia density in our study. This implies that environmental conditions for the fungus are more favorable in the mid and upper crown of mature trees, consistent with younger trees in western Oregon forests. Dye et al. (2020) analyzed the 22-year mean May through September low cloudiness (i.e., stratus, stratocumulus, and fog) and found

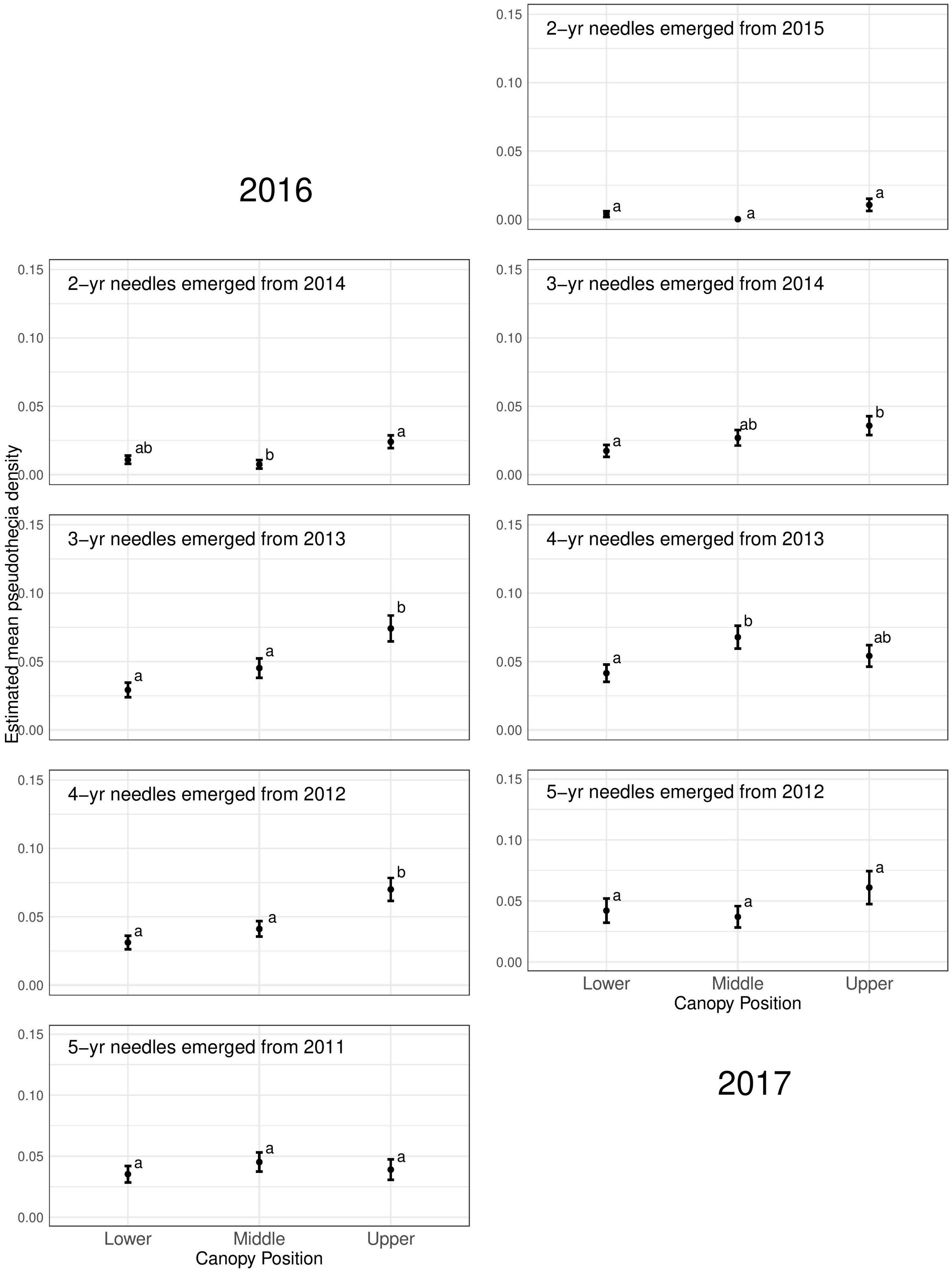

FIGURE 6 | Pseudothecia density by canopy position for 2- to 5-year old needles in 2016 and 2017 from mature Douglas-fir trees in western Oregon. Different letters represent that the two groups are statistically different.

a strong decline of low clouds in May and June, and a peak in July in the terrestrial highlands mode (Moose Mt. and Falls Creek). Seasonally, coastal fog frequency increases to a peak in August and is highest from June to September (Johnstone and Dawson, 2010). The coastal summer fog maintains needle wetness during the period of low summer precipitation and high summer temperatures. The coastal mode (Cascade Head, Klickitat Mt. and Woods Creek) had consistent low cloudiness that also peaked in late summer. The fog may wet the upper and mid crowns of co-dominant and dominant trees more

TABLE 3 | Summary of GLMM of pseudothecia density across five study sites (15 trees) in western Oregon, needle age (2–5-year-old), year, canopy position and their interactions.

	Estimate	**SE**	***z* value**	***p* value**	
(Intercept)	−2.32	0.26	−8.88	<0.001	***
canopyMiddle	−0.53	0.23	−2.25	0.024	*
canopyLower	−1.16	0.29	−4.07	<0.001	***
needleage3 year	−0.06	0.21	−0.31	0.759	
needleage4 year	−0.43	0.24	−1.78	0.075	.
needleage5 year	−0.20	0.28	−0.72	0.473	
siteKlickitat Mt	−0.99	0.42	−2.37	0.018	*
siteWoods Creek	−1.79	0.47	−3.80	<0.001	***
siteFalls Creek	−2.50	0.58	−4.33	<0.001	***
siteMoose Mt	−2.35	0.52	−4.48	<0.001	***
year2017	−0.14	0.06	−2.62	0.009	**
canopyMiddle:needleage3 year	0.45	0.32	1.41	0.159	
canopyLower:needleage3 year	0.69	0.37	1.84	0.065	.
canopyMiddle:needleage4 year	0.92	0.34	2.71	0.007	**
canopyLower:needleage4 year	1.14	0.39	2.92	0.004	**
canopyMiddle:needleage5 year	0.41	0.37	1.11	0.265	
canopyLower:needleage5 year	0.73	0.42	1.73	0.084	.
canopyMiddle:siteKlickitat Mt	−0.63	0.55	−1.14	0.253	
canopyLower:siteKlickitat Mt	−0.68	0.72	−0.95	0.341	
canopyMiddle:siteWoods Creek	−2.82	1.78	−1.59	0.113	
canopyLower:siteWoods Creek	1.20	0.54	2.21	0.027	*
canopyMiddle:siteFalls Creek	−0.47	0.93	−0.50	0.615	
canopyLower:siteFalls Creek	0.81	0.78	1.04	0.301	
canopyMiddle:siteMoose Mt	0.86	0.57	1.50	0.133	
canopyLower:siteMoose Mt	0.59	0.72	0.82	0.412	
needleage3 year:siteKlickitat Mt	0.18	0.40	0.44	0.663	
needleage4 year:siteKlickitat Mt	0.42	0.43	0.97	0.332	
needleage5 year:siteKlickitat Mt	−0.46	0.62	−0.73	0.465	
needleage3 year:siteWoods Creek	1.56	0.42	3.67	<0.001	***
needleage4 year:siteWoods Creek	2.49	0.43	5.79	<0.001	***
needleage5 year:siteWoods Creek	1.93	0.46	4.23	<0.001	***
needleage3 year:siteFalls Creek	2.67	0.53	5.02	<0.001	***
needleage4 year:siteFalls Creek	3.02	0.55	5.52	<0.001	***
needleage5 year:siteFalls Creek	2.03	0.59	3.46	<0.001	***
needleage3 year:siteMoose Mt	1.81	0.48	3.74	<0.001	***
needleage4 year:siteMoose Mt	2.61	0.50	5.22	<0.001	***
needleage5 year:siteMoose Mt	1.92	0.55	3.48	<0.001	***
canopyMiddle:needleage3 year:siteKlickitat Mt	−0.30	0.75	−0.40	0.692	
canopyLower:needleage3 year:siteKlickitat Mt	−0.13	0.91	−0.15	0.883	
canopyMiddle:needleage4 year:siteKlickitat Mt	−0.07	0.72	−0.10	0.918	
canopyLower:needleage4 year:siteKlickitat Mt	−0.09	0.89	−0.11	0.916	
canopyMiddle:needleage5 year:siteKlickitat Mt	1.12	0.85	1.32	0.186	
canopyLower:needleage5 year:siteKlickitat Mt	1.22	0.99	1.23	0.220	
canopyMiddle:needleage3 year:siteWoods Creek	2.63	1.81	1.45	0.147	
canopyLower:needleage3 year:siteWoods Creek	−1.76	0.67	−2.62	0.009	**
canopyMiddle:needleage4 year:siteWoods Creek	2.03	1.81	1.12	0.262	
canopyLower:needleage4 year:siteWoods Creek	−2.16	0.65	−3.31	<0.001	***
canopyMiddle:needleage5 year:siteWoods Creek	2.69	1.82	1.48	0.138	
canopyLower:needleage5 year:siteWoods Creek	−1.46	0.68	−2.15	0.031	*
canopyMiddle:needleage3 year:siteFalls Creek	−0.27	0.98	−0.27	0.785	
canopyLower:needleage3 year:siteFalls Creek	−1.55	0.86	−1.80	0.072	.
canopyMiddle:needleage4 year:siteFalls Creek	−0.49	0.99	−0.50	0.620	
canopyLower:needleage4 year:siteFalls Creek	−1.71	0.86	−1.98	0.048	*

(Continued)

TABLE 3 | (Continued)

	Estimate	SE	*z* value	*p* value	
canopyMiddle:needleage5 year:siteFalls Creek	0.40	1.02	0.40	0.693	
canopyLower:needleage5 year:siteFalls Creek	−0.82	0.90	−0.90	0.366	
canopyMiddle:needleage3 year:siteMoose Mt	−1.15	0.67	−1.72	0.086	.
canopyLower:needleage3 year:siteMoose Mt	−0.85	0.82	−1.04	0.299	
canopyMiddle:needleage4 year:siteMoose Mt	−1.51	0.67	−2.26	0.024	*
canopyLower:needleage4 year:siteMoose Mt	−1.21	0.81	−1.49	0.137	
canopyMiddle:needleage5 year:siteMoose Mt	−1.14	0.74	−1.54	0.123	
canopyLower:needleage5 year:siteMoose Mt	0.10	0.84	0.12	0.904	

Asterisks indicate significance at the 0.05 (*), 0.01 (**), and 0.001 (***) levels. Estimates are based on a logit transformation.

than the lower canopy, and help explain canopy differences of *N. gaeumannii* density, although leaf wetness was not consistently higher on upper canopy foliage (Lan et al., 2019).

In western Oregon, winter temperature influences disease severity at a range of spatial and temporal scales (Manter et al., 2005; Stone et al., 2008a,b; Zhao et al., 2012; Lee et al., 2013, 2017; Wilhelmi et al., 2017). Leaf canopy temperature may be a key predictor of variations in *N. gaeumannii* density within the canopy of mature Douglas-fir. A wide range of biological responses from leaf photosynthesis (Jordan and Ogren, 1984) to net ecosystem exchange (Kim et al., 2016) are better predicted by leaf temperature than air temperature. Several thermal imaging and radiation absorption modeling studies show leaf temperature is higher in the upper crown than in the lower crown and understory (Still et al., 2019; Miller et al., 2021) due to more direct daytime solar irradiation at the treetop than at the bottom of the canopy (Sinoquet et al., 2001). The gradients in leaf canopy temperature are most likely an important factor for fungal development, notably in the cool winter months when temperatures are most limiting to the formation of pseudothecia. In coastal Oregon, pseudothecial primordia form in epistomatal chambers October to April following initial infection in the summer months (Stone et al., 2008a). Pseudothecia density was generally low ($< 10\%$) for all needle age classes in mature trees, implying less colonization of the needles or slower growth within the needles. Winton et al. (2003) have demonstrated that pseudothecia density is significantly correlated with quantitative PCR, or the abundance of fungi within the needle. Therefore, other factors may be influencing colonization success of needles in mature trees. Although more recently, Montwé et al. (2021) contend that there is not a clear relationship between pseudothecia density, fungal DNA load and needle retention. They propose that there is a more complex unknown pathology involved in needle loss.

CONCLUSION

Nothophaeocryptopus gaeumannii causes disease in plantation and forest trees when over 25% of the stomates of young (1 and 2-year-old) foliage are occluded, causing early needle casting. Our hypothesis that disease severity is lower in mature trees because pseudothecia emerge later on older needles is supported. Within the mature tree crowns, we also found that pseudothecia density varied with needle age, canopy position, and site. In older and mature trees, pseudothecia *of N. gaeumannii* were most abundant on 3- and 4-year-old needles and density of pseudothecia on all needles was very low ($< 10\%$). Something about mature forest canopies and foliage appears to decrease the success of needle colonization or increase the time it takes for pseudothecia to emerge from the needles, in contrast to younger plantations (Lan et al., 2019), thus allowing the mature trees to have greater needle retention. Tree crown and canopy architectural differences may help explain these results, because mature trees have more complexities in microenvironmental patterns with deeper crowns, more shade, and less crown connectivity between trees, but this needs more studies in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Y-HL contributed to the study design, field sampling, data process and analysis, and writing. DS contributed to the study design and writing and especially for the discussion. EL contributed to the data analysis cooperation and stats interpretation. PB contributed to the study design, field sampling, especially organizing the climbing crew, and discussion. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We acknowledge the Swiss Needle Cast Cooperative at Oregon State University for providing funding and technical support during the research. We thank the EPA long term ecological monitoring network team for tree climbing and needle sampling supports. We really appreciate the tree climbing and ground support of Mike Bollman, Ron Waschmann, Steve Cline, Gabi Ritóková, Rong Fang, Rebecca Hsu, Brian Chiu, Eric Forsman, Dave Woodruff, Jimmy Swingle, Chia-Yun Hsu, Mark Ko, and Hans Song. We also thank Alexis Danley, Shannon Burton, and Lori Lewis for lab work.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer CL declared a past collaboration with one of the authors DS to the handling editor.

frontiers | Frontiers in Forests and Global Change

ORIGINAL RESEARCH
published: 13 April 2022
doi: 10.3389/ffgc.2022.734448

The Impact of Anthropogenic Disturbance to the Canopy Microclimate of Tropical Forests in the Southern Western Ghats, India

Rubin Sagar* and M. Soubadra Devy

Ashoka Trust for Research in Ecology and the Environment (ATREE), Bengaluru, India

Anthropogenic disturbances are a pressing driver of forest degradation and are known to affect the microclimate within forests. Most organisms experience the microclimate and hence, associated changes may drive species communities in rainforests. However, such knowledge remains limited in the case of forest canopies, especially in south Asia. We aimed to identify differences in the temperature and light intensity, and beetles and vascular epiphytes in the canopy between old-growth and secondary forests. Using sensors, we recorded two key microclimatic variables, the air temperature, and light intensity, in the crowns of 36 *Cullenia exarillata* A. Robyns trees. We sampled beetles (morphospecies) and vascular epiphytes (genera) in the crowns. We provide evidence that canopies of secondary forest stands (intensively logged 60 years ago) (1) continue to show higher canopy air temperatures and light intensity, and (2) have higher beetle abundance (individuals), richness (morphospecies), and diversity but lower vascular epiphyte abundance (individuals), richness (genera), and diversity as compared to primary forest stands. We also show that the beetle communities differ (with greater beta diversity in the primary forest), but the vascular epiphyte communities were similar between the two forest types. We hope that this information begins to bridge the gap in understanding the role of microclimate in driving species communities and the ecology of human-modified forests.

Keywords: canopy, beetles, vascular epiphyte, microclimate, tropical wet forest

Edited by:
Akihiro Nakamura,
Xishuangbanna Tropical Botanical Garden (CAS), China

Reviewed by:
Adrià Barbeta,
University of Barcelona, Spain
Sven Peter Batke,
Edge Hill University, United Kingdom

****Correspondence:***
Rubin Sagar
rubin.sagar@gmail.com

Specialty section:
This article was submitted to Forest Growth, a section of the journal Frontiers in Forests and Global Change

Received: *01 July 2021*
Accepted: *03 March 2022*
Published: *13 April 2022*

Citation:
Sagar R and Devy MS (2022) The Impact of Anthropogenic Disturbance to the Canopy Microclimate of Tropical Forests in the Southern Western Ghats, India. Front. For. Glob. Change 5:734448. doi: 10.3389/ffgc.2022.734448

INTRODUCTION

Microclimates affect species assemblages and functions of tropical forest ecosystems (Chen et al., 1999) and microclimatic conditions experienced by several organisms may be quite different from the macroclimatic conditions (De Frenne et al., 2013; von Arx et al., 2013). With global warming predicted to exceed 1.5°C and 2°C in the 21st century (IPCC, 2021), non-climatic anthropogenic pressures, that is, activities directly resulting in adverse impacts on natural ecosystems, especially forest degradation and fragmentation may worsen microclimatic conditions by reducing the buffering effect (Ewers and Banks-Leite, 2013). Forest degradation has shown to increase the severity of understory microclimatic conditions (Blonder et al., 2018; Jucker et al., 2018). For instance, local air temperatures have been found to be as much as 13.6°C greater in logged forests as compared to primary forests (Senior et al., 2017). Further, the role of intact canopy cover, a feature of old-growth forests (primary forests, henceforth) has a buffering effect on the understory microclimate, offsetting and reducing the severity of macroclimatic variations

(De Frenne et al., 2019; Zellweger et al., 2019). Such effects in the canopy microclimate are not as well explored.

Canopies host a high diversity of arthropods (Erwin, 1982; Basset et al., 2008, 2012), particularly beetles (Stork and Grimbacher, 2006). Species unique to the canopy have been observed in the range of 20–30% (Stork and Grimbacher, 2006; Ulyshen and Hanula, 2007; Schroeder et al., 2009) and display very high microhabitat specialization (Wardhaugh et al., 2013). A characteristic feature of tropical rainforests is the presence of vascular epiphytes, which represent approximately 9% of the extant vascular plant diversity (Zotz, 2013). Larger trees, an indicator of older trees, support complex epiphytic communities (Woods et al., 2015; Woods, 2017). Hence, epiphyte communities in old-growth forests are often more diverse than disturbed and secondary forests (Barthlott et al., 2001; Woods and DeWalt, 2013). Consequently, it is no surprise that the presence of epiphytes positively contributes to arthropod diversity in tropical forests (Cruz-Angón et al., 2009; Díaz et al., 2012). The reasoning for the positive association between epiphytes and arthropod abundance and diversity may lie in the fact that epiphytes may offer refuge and resources to arthropods (Nadkarni, 1994). Thus, old-growth trees with greater diversity, abundance, and even size (biomass) of epiphytes may attract a greater abundance and diversity of arthropods. Stuntz et al. (2002a) report substrates with cooler temperatures in the proximity of certain epiphyte species as compared to exposed branch surfaces of host trees, and indicate that this factor, along with the size (biomass) of canopy epiphytes could contribute to the positive association of epiphytes and arthropods (Stuntz et al., 2002b).

Increased forest degradation and fragmentation are well-known to adversely impacted insect communities (Klein, 1989; Feer and Hingrat, 2005; Nichols et al., 2007) through direct exposure to higher ambient temperatures (Piyaphongkul et al., 2012; Woods, 2013; Zhang et al., 2014) and indirectly, such as through changes in host plant qualities. For instance, the leaf surface temperature increases the body temperature of small arthropods on the leaf (Caillon et al., 2014), and higher temperatures can affect several adult phenotypic aspects both within generations and even between generations (Crill et al., 1996). Further, the light intensity has been found to potentially impact the vertical distribution of insects in forests. For instance, Grossner (2009) found light intensity as one of the potential factors affecting the diversity of Heteroptera across vertical strata in beech and oak dominated forests. The understanding of impacts of forest degradation on beetles remains mixed, as some have highlighted the negative effects of fragmentation on beetle species richness and community composition (Feer and Hingrat, 2005; Jung et al., 2018; Salomão et al., 2019), however, some do not demonstrate a clear direction of the impact of fragmentation (Davies and Margules, 1998).

In tropical forests, secondary stands support fewer epiphytes as compared to primary stands (Barthlott et al., 2001; Nadkarni et al., 2004; Padmawathe et al., 2004; Woods and DeWalt, 2013). Forest clearances also adversely affect vascular epiphytes through changes in the microclimate (Zotz and Bader, 2009), such as drier microclimates in secondary forest (Gradstein et al., 2008) or loss of shade-adapted species (Benítez et al., 2012). For instance, *Psygmorchis pusilla* Dodson and Dressler (Orchidaceae) showed reduced biomass and floral spikes at just 3°C above its optimum temperature (Vaz et al., 2004). Increases in light intensity have shown to reduce relative growth rates of a vascular epiphyte (Laube and Zotz, 2003) as well as photoinhibition (Stancato et al., 2002) which could eventually lead to dieback and reduced numbers in environments with higher light intensity.

The need for prioritizing research on microclimate and its impact on forest biodiversity has been indicated (De Frenne et al., 2021). The importance of the microclimate has been recognized for decades, but our understanding of biotic responses to microclimates in the context of human land-use change is still in its infancy (Santos and Benítez-Malvido, 2012; De Frenne et al., 2021). Further, such work is lacking in Indian rainforests in spite of the existence of two biodiversity hotspots (Myers et al., 2000)—the Western Ghats and the Indo-Burma region. Epiphytes form an important part of the rainforest canopy in the southern Western Ghats including several endemics (Parthasarathy, 1988; Ganesan and Livingstone, 2001). Similarly, beetles also form a major component of the insect taxa (Devy and Davidar, 2003; Mohanraj et al., 2014). The responses of these taxa to environmental change have been little quantified. To provide further insights into the impacts of disturbance on microclimatic variables, beetle and epiphyte communities, we focused on the following two questions:

1) Does the canopy microclimate (temperature and light intensity) differ between primary and secondary forest stands?
2) Do beetles and canopy vascular epiphytes differ between primary and secondary forest stands?

Throughout this paper, we use the word "canopy" or "forest canopy" referring to the overstorey defined as the "stratum of trees that have outgrown the other vegetation in a forest to have their uppermost crown foliage largely of fully in direct sunlight, usually as a relatively continuous layer (excluding gaps)" (Moffett, 2006). We use the word "crown" or "crown-tree" when referring to the top limbs and leaves of individual trees (from the lowermost limb, excluding the trunk). We chose an evergreen wet tropical forest site, with a known history of varying gradients of logging. Hence, there exist intact, old-growth stands, in close proximity to logged stands. The sites were chosen with the expectation of markedly distinct light and temperature levels in the primary and secondary forests, while the elevation and climatic conditions experienced were the largely very similar. To the best of our knowledge, this is the first study in Indian tropical moist forests to study beetle and vascular epiphyte community responses to microclimatic differences in primary and secondary forest canopies.

MATERIALS AND METHODS

Study Location

In this study, we focus on a wet tropical forest in the southern Western Ghats, India. Our study sites were located in Kalakad Mundanthurai Tiger Reserve (KMTR), a protected area in Tamil Nadu, India. Our site was logged in the 1960s in varying gradients and the logged sites are referred to as "secondary forest." The intact, old-growth forest stands, are referred to as "primary forest." All the stands were in close proximity to each other (maximum distance between any two stands, regardless of forest type, was about 1.5 km). These sites have been used in earlier studies to assess the impact on butterflies (Devy and Davidar, 2001). Also, the sites in the primary forest are part of a long-term phenological study, ongoing since the mid-1990s by the Ashoka Trust for Research in Ecology and the Environment (ATREE).

We identified a total of six sampling plots, three in the primary forest and three in the disturbed forest. The primary forest is characterized by a near-continuous canopy of mature trees representative of the landscape—*Cullenia exarillata* A. Robyns, *Palaquium ellipeticum* (Dalz.) Baillon, *Aglaia elaeagnoidea* (Juss.) Benth, and *Myristica dactiloides* Gaertn (based on the Species Importance Value, Ganesh et al., 1996). Gaps in the primary forest are largely due to fallen mature trees and disturbances caused by cyclonic storms. Being in the Western Ghats, the topography is undulating with several exposed rocks throughout the forest. The stands have a distinct midstorey and understorey. Dominant species in the understorey consist of *Nilgirianthus foliosus*, *Nilgirianthus perrotettianus*, *Diotacanthus grandis,* and *Agrostistachys indica* (Ganesh et al., 1996).

The secondary forest was selectively logged in the 1960s, until 1988, however, we do not know the exact extraction volumes. The secondary site we chose was intensively logged with a small proportion of remnant old growth trees. Dominant tree species are *Epiprinus malotiformis*, *Holigarna nigra,* and *Cullenia exarillata* (Devy and Davidar, 2001). The plots are characterized by varying midstorey and understorey, fewer mature trees, and subsequently a discontinuous canopy. The gaps between them are now occupied largely by light-loving pioneer species such as *Macaranga peltata* (Roxb.) Mueller (Thorat et al., 2016).

Selection of Trees

We selected *Cullenia exarillata*, one of the dominant trees of the landscape for the sampling. *C. exarillata* are hosts for epiphytes (Devy, 2006) and the branching makes it a very suitable tree for accessing the crown using the Single Rope Technique (SRT) (Perry, 1978). The locations of the trees in the sites were along the trail used for the long-term phenology surveys (Ganesh et al., 2017). Trees were chosen based on suitability for accessing the crown. The following criteria were used to select a tree—the presence of at least two living branches in the same plane, the height of the branch chosen for installing the rope should not exceed half the length of the climbing rope, and the midstorey should allow a clear line-of-sight to the branch for installing the rope.

A total of 36 trees were selected for sampling (focal tree)—18 trees in the intact primary forest and 18 trees in the secondary forest. We recorded the diameter at breast height (DBH) and estimated the height of each sampled tree.

Canopy Air Temperature and Light Intensity

The canopy air temperature and light intensity was measured using ONSET UA-002-64 HOBO data loggers (Onset Computers, United States). The data loggers are capable of measuring temperature values between −20 and 70°C (accuracy ± 0.53°C for 0–50°C), and light intensity up to 320,000 Lux. A single calibrated HOBO was installed in the crown (inner canopy) of each tree by accessing the crown using the SRT. The HOBO was fixed inside a 6 × 4 × 4 inch stainless steel cage with a mesh size of one square inch, primarily to protect it from damage or tampering by curious Lion-Tailed Macaques. The cage with the HOBO was attached with stainless steel wires (2 mm diameter) to a randomly chosen branch above lower most branch in each tree, but restricted to the inner canopy, and horizontally near the main trunk.

Data loggers were deployed for 86.86 ± 12.56 h, with a logging interval of 6 min (10 readings per hour). Each logger was launched using HOBOware (Onset Computers, United States) such that the start time began at the nearest 30-min mark. We recorded the light intensity (Lux) and ambient air temperature (degrees Celsius) within each sampled tree's crown. The sampling was spread across March and April 2021, since we used a staggered approach—the plots were sampled on different days, keeping in mind that HOBOs were installed on roughly the same number of trees in the primary and secondary sites within a given week. We chose the post-winter monsoon dry season, which is when the weather is consistently clear and warm during these months, with occasional cloudy and rainy days; we avoided data collection on the few days that rain was expected.

Vascular Epiphyte and Beetle Sampling

We accessed the tree crowns using SRT and for the entire crown, we recorded epiphyte genera (and morphospecies for different species in the same genera) and abundances per crown-tree for each epiphyte genera, and took images wherever possible. We also used binoculars to confirm epiphyte genera where necessary.

For collecting beetles, we made baited funnel traps with used 1-L plastic bottles. The top one-third of the bottles were cut, inverted, and attached to the bottom two-thirds using black electrical insulation tape. We used two types of baits—50 ml of banana "extract" and 50 ml of mango juice, both with a teaspoon of active dry yeast. The banana juice extract was prepared using approximately 200 g of ripe bananas mixed with 1 L of water and strained. The mango juice bait was prepared by mixing a commercially available brand of mango juice and was diluted with water in a 1:1 ratio by volume.

We used 2 mm steel wires to attach the traps to the branches, ensuring that the traps faced upwards. The traps were retrieved along with the HOBOs, and the contents were immediately transferred to a closed container. Beetles in the traps were preserved in 70% ethanol within 12 h of retrieval from the trees.

Data Analysis

We calculated the daily mean values for comparing the canopy temperature and light intensity between the primary and secondary forests. We used Levene's test to check for homogeneity of variances (temperature: $F = 0.2666$, $p = 0.609$; light intensity: $F = 0.4152$, $p = 0.5238$) before testing the significance of differences in the daily means of temperature and light intensity between the forest types with an independent t-test.

We compiled the frequency of temperature and light readings in specific range classes. For temperature, the range classes were from 12 to 32.999°C, with an interval of 0.999°C (12–12.999°C, 13–13.999°C and so on), and for light, the range classes were from 0 Lux to 140,000 Lux with an interval of 999.999 lux (0–999.999 lux, 1000–1999.999 lux and so on). To test for differences in the frequency of temperature and light intensity reading in the range classes, we used Fisher's exact test.

We used the R package "hillR" (Li, 2018) to calculate the Shannon Diversity Index values (Hill numbers). The significance of differences in the beetle and epiphyte abundance, richness and diversity (Hill numbers) values per crown-tree were tested using the Mann–Whitney U-test due to non-normality of data, checked using the Shapiro test. Cohen's d-value was used to determine the effect size to support the tests, using the R package "rstatix" (Kassambara, 2020). Lastly, we calculated the Bray Curtis dissimilarity index, non-metric multidimensional scaling (NMDS) analysis, and an ANOSIM test with 9,999 permutations using the R package "vegan" (Oksanen et al., 2019) to evaluate differences in species communities between the primary and secondary forests. Further, we tested the effect of forest type on the beta diversity of the beetle and epiphyte communities using the "betadisper" function with 999 permutations available in the R package "vegan" (Oksanen et al., 2019). An ANOVA was performed to test whether these distances differed.

All the analyses, data management, and plotting were done using RStudio (v1.2.5033). We used the package "ggplot2" (Wickham, 2016) for creating the graphs, and "dplyr" (Wickham et al., 2019) for data management and organization.

RESULTS

We obtained a total of 29,972 (Primary: 14,781, Secondary: 15,191) temperature readings from 36 trees and (Primary: 7,257, Secondary: 8,267) daytime light intensity readings from 35 trees (one HOBO was dislodged from its position from a tree in the disturbed forest, presumably by a Lion-Tailed Macaque). Further, the *C. exarillata* trees we sampled were larger in the primary forest (mean ± SD, tree DBH: 91.58 cm ± 26.93 cm in the primary forest, 73.96 cm ± 21.62 cm in the secondary forest; mean ± SD, tree height: 22.88 m ± 2.98 m in the primary forest; 18.56 m ± 2.56 m in the secondary forest).

Canopy Microclimate

The mean daily temperature of the crowns in the disturbed forest was significantly higher than the mean daily temperature of crowns in the primary forest (mean ± SD: 19.4°C ± 2.58°C vs. 20.5°C ± 3.04°C; t-test $p < 0.01$; df = 34). The mean hourly crown temperature was consistently higher for the disturbed forest (**Figure 1A**). The mean daily crown light intensity was also higher in the disturbed forest, but the difference was not significant (mean ± SD: 7,670 ± 12,424 lux in the primary forest vs. 8,862 ± 11,686 lux in the secondary forest; t-test $p > 0.05$, df = 33). Unlike temperature, the light intensity of the disturbed forest was not higher for all hours of the day (**Figure 1B**).

The frequency of temperatures and light intensity for each sampled crown-tree were consistent with the above findings (**Figure 2**). Lower temperatures were more frequent in the primary forest, as compared to the secondary forest (**Figure 2A**). The difference in the frequency of the hourly mean temperatures was significant (Fisher's exact test p-value = $1e^{-04}$, based on 9,999 Monte-Carlo replicates). Although the light intensity does not vary as dramatically (**Figure 2B**), the difference in the frequency of the hourly mean light intensities was also significant (Fisher's exact test $p = 0.0324$, based on 9,999 Monte-Carlo replicates).

Canopy Beetles and Vascular Epiphytes

We obtained 175 beetle individuals (mean ± SD: 2.6 ± 2.3 individuals per crown-tree in the primary forest; 7.1 ± 5.7 individuals per crown-tree in the secondary forest). 161 individuals were identified in 18 families, however, the family for 14 individuals could not be identified. Also, a total of 66 beetle morphospecies were identified. We collected 28 beetle morphospecies from the primary forest and 49 beetle morphospecies from the secondary forest in our samples. Of the 66 morphospecies, 47 beetle morphospecies were singletons. 11 (16.67%) beetle morphospecies were found in both forest types, whereas 17 (25.75%) were unique to the primary forest and 38 (57.57%) were unique to the secondary forest. The five most abundant families (abundance, relative abundance), with a relative abundance greater than 5% were Mordellidae (63, 36%), Nitidulidae (31, 17.7%), Staphylinidae (13, 7.4%), Elateridae (10, 5.7%), and Chrysomelidae (9, 5.14%). There was one morphospecies in the Mordellidae family, with 56 individuals found in the secondary forest, and 7 individuals in the primary forest. Similarly, we found more Elateridae and Chrysomelidae individuals in the secondary forest. Nitidulidae and Staphylinidae abundances were similar in both forest types (**Supplementary Table 1**).

We also found approximately 2,720 vascular epiphyte individuals (mean ± SD: 104.5 ± 241.05 individuals per crown-tree in the primary forest; 46.83 ± 104.42 individuals per crown-tree in the secondary forest). The most abundant epiphyte genera (abundance, relative abundance) were *Eria* sp. (Orchidaceae) (1,320, 48.45%), *Bulbophyllum* sp.1 (Orchidaceae) (980, 35.97%) and *Bulbophyllum* sp.2 (Orchidaceae) (265, 9.72%). Among these, *Eria* sp. and *Bulbophyllum* sp.2 were largely absent from the secondary forest, except for one tree (D3T1). Whereas *Bulbophyllum* sp.1 was more abundant in the secondary forest, but their abundance was exceptionally high in one tree (D3T1) (**Supplementary Table 2**).

Beetle abundances, richness, and diversities were quite variable across the trees, but consistently and significantly lower in the primary forest (**Table 1** and **Figures 3A–C**). Contrastingly, the epiphyte abundances, richness, and diversity values were higher in the primary forest but only the diversity (Hill numbers) was significantly higher (**Table 2** and **Figures 3D–F**).

The Bray Curtis dissimilarity index was 0.76 for beetle communities and 0.69 for the epiphyte communities between the primary and secondary forest. The NMDS analysis divided the beetle communities in the primary and secondary forests into distinct clusters (**Figure 4A**) with a stress value less than 0.01.

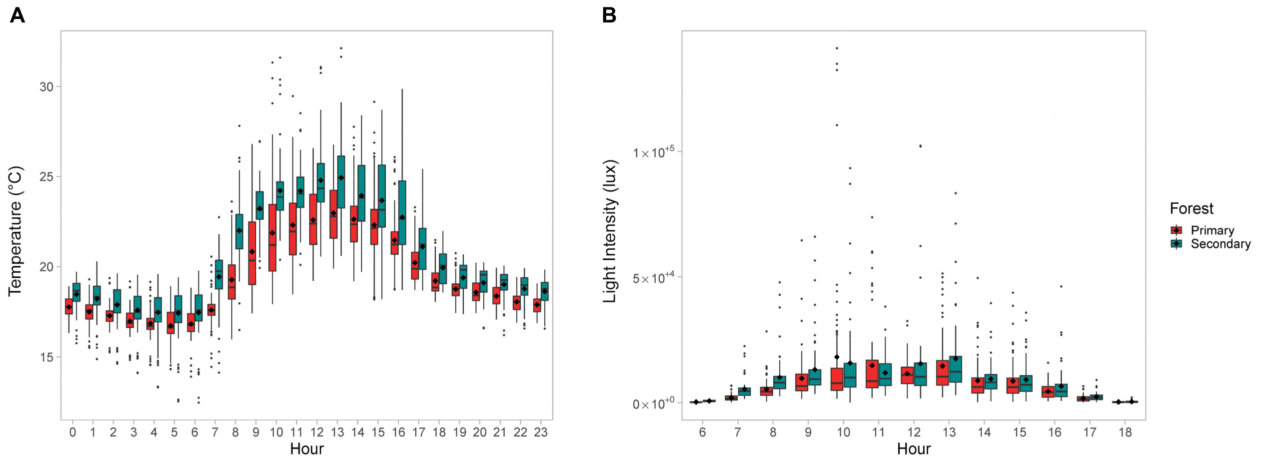

FIGURE 1 | Boxplots showing the **(A)** mean hourly temperature (°C) and **(B)** mean hourly light intensity (lux) for the crown-trees compared by forest type. Black dots inside the boxplot indicate mean values.

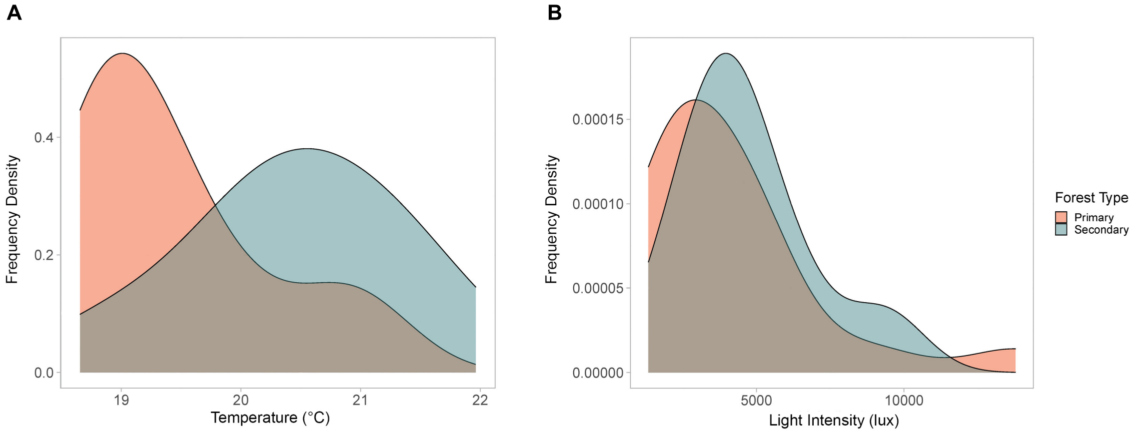

FIGURE 2 | Frequency density charts for **(A)** mean daily temperature and **(B)** mean daily light intensity (6 a.m.–6 p.m.) for the crown-trees, compared by forest type.

TABLE 1 | Summary of beetle abundance (individuals per crown-tree), richness (per crown-tree), and Shannon's diversity values (per crown-tree) between the primary and secondary forests.

	Primary	**Secondary**	**Primary**	**Secondary**			
	Mean		***SD***		**Mann–Whitney *U*-test W**	**Mann–Whitney *U*-test *p*-value**	**Effect size**
Beetle abundance	2.61	7.11	2.29	5.69	70	0.0035*	0.985†
Beetle richness	2	4.22	1.56	2.97	77.5	0.0071*	0.889†
Beetle Shannon diversity (hill numbers)	0.59	1.13	0.53	0.60	77.5	0.0073*	0.839†

**Indicating significance of test at the 0.05 level.*
†Indicating large effect sizes.

Further analysis performed using an ANOSIM test resulted in a statistically significant, but with a low level of dissimilarity (ANOSIM statistic R: 0.3169, $p = 2e^{-04}$). The Betadisper analysis followed by ANOVA showed significant differences in the dispersion of beetles ($F = 9.239$, $P = 0.0049$) indicating greater heterogeneity in beetle communities between the forest types. For the vascular epiphyte communities, the NMDS analysis showed a high degree of overlap with a stress value of less

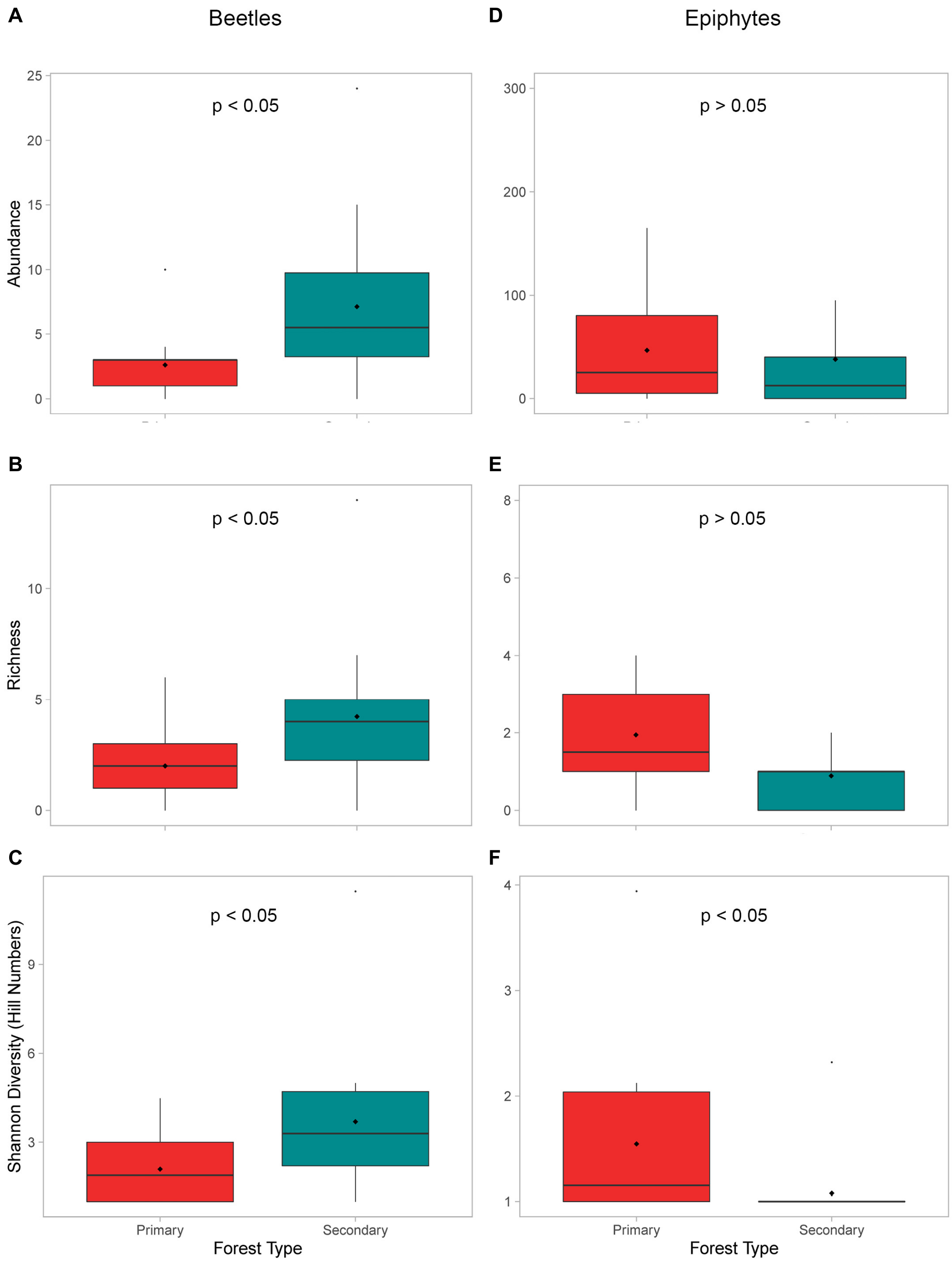

FIGURE 3 | Boxplots of the **(A)** beetle abundances (number of individuals), **(B)** beetle morphospecies richness, **(C)** beetle morphospecies Shannon's diversity (Hill numbers), **(D)** epiphyte abundances (number of individuals), **(E)** epiphyte genera richness, and **(F)** epiphyte genera Shannon's diversity (Hill numbers) across all trees compared by forest type. Black dots inside the boxplots indicate mean values.

than 0.01 (**Figure 4B**). The ANOSIM test resulted in values indicating similar vascular epiphyte communities in the primary and secondary forest (ANOSIM statistic R: 0.0163, $p = 0.2804$). Additionally, the Betadisper analysis followed by ANOVA did not show significant differences in the dispersion of epiphytes in the two forest types ($F = 1.7289$, $P = 0.201$) also indicating similar communities.

DISCUSSION

Research on microclimate in ecology has been gaining considerable attention recently (Bramer et al., 2018). Higher temperatures and more intense microclimates in the understorey of logged or secondary forests as compared to primary forests have been well-quantified (Blonder et al., 2018; Jucker et al., 2018;

TABLE 2 | Summary of epiphyte abundance (individuals per crown-tree), richness (per crown-tree) and Shannon's diversity values (per crown-tree) between the primary and secondary forests.

	Primary	Secondary	Primary	Secondary			
	Mean		***SD***		**Mann–Whitney *U*-test W**	**Mann–Whitney *U*-test *p*-value**	**Effect size**
Epiphyte abundance	104.5	46.833	241.05	104.428	203	0.1949	0.352
Epiphyte richness	1.94	0.89	1.78	0.80	219	0.0618	0.726
Epiphyte Shannon diversity (hill numbers)	0.785	0.568	0.464	0.415	221.5	0.0261*	0.780

**Indicating significance of test at the 0.05 level.*

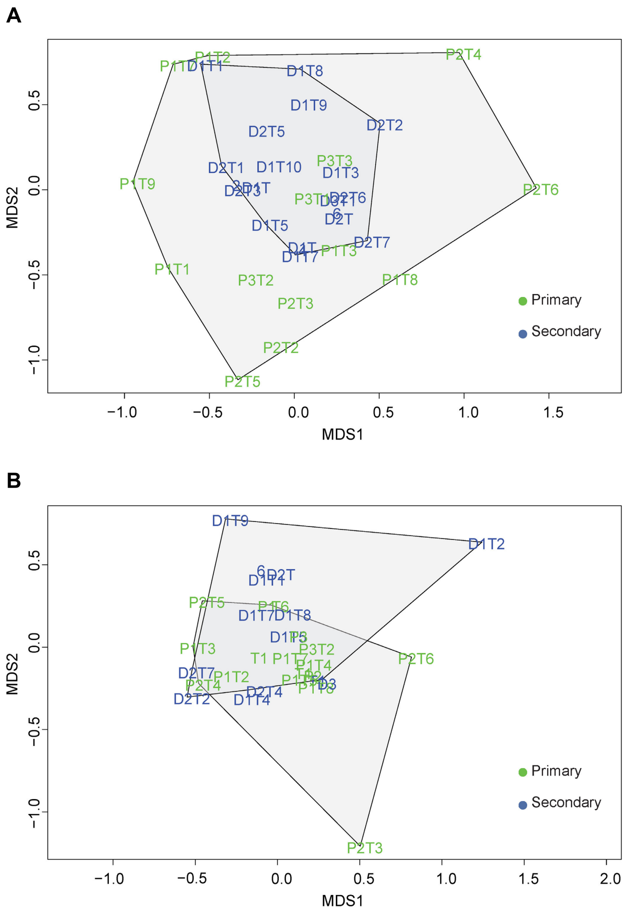

FIGURE 4 | A two-dimensional non-metric multidimensional scaling analysis of the **(A)** beetle and **(B)** vascular epiphytes based on the Bray-Curtis dissimilarity matrix.

De Frenne et al., 2019). Our work was entirely focused on the canopy and extends earlier findings by showing that the canopy microclimate in selectively logged secondary forests is generally warmer than in primary forests. Our results are consistent with Padmawathe et al. (2004), the only other study focused on Indian forests which reported higher temperature and light intensities at heights of 15–16 m. Sampled trees in the primary forest were surrounded by similar, large neighboring trees. In contrast, sampled trees in the logged sites had a greater inter-tree distance with other trees of comparable size, which is a plausible explanation for our findings.

Our beetle data and findings are consistent with those of Wagner (2000), who also reported significantly higher beetle abundances in a secondary forest from a tropical rainforest site in Uganda with some similarities as our site in terms of forest dynamics and characteristics. Thorat et al. (2016) report higher herbivory levels in the leaves of *Macaranga peltata* trees in secondary forests in Kalakad (our study region), suggesting higher numbers of insect herbivores such as *Aphthona macarangae*, a beetle in the Chrysomelidae family. We found eight Chrysomelidae beetles in the secondary forest, whereas only one individual in the primary forest. An explanation for the higher abundance of beetles in the secondary forest could be that more light penetrates the canopy, resulting in higher leaf density and more productivity, which are more palatable to phytophagous beetles (Aide and Zimmerman, 1990). Beetle abundance has also been found to be affected by canopy or crown structure and may be driven by dense and moist foliage (Dial et al., 2006), which is contradictory to our findings. Although we did not account for canopy structure or relative humidity, one would expect higher temperatures and light in secondary forests to result in lower humidity and drier foliage.

Results of the community analysis suggest dissimilar beetle assemblages between the primary and secondary forest types and similar vascular epiphyte assemblages. Wagner (2000) also observed a low degree of overlap in certain beetle families in the canopies of the same tree species between primary and secondary forests. Although we found higher beetle abundances, species richness, and diversity in the secondary forest, the multivariate analysis indicate that the beetle communities are more variable in the primary forests than in the secondary forests. Conversely, the epiphyte community analysis indicated similarity in the communities between both forest types, which was also reported by Barthlott et al. (2001). One reason for the similarity in communities could be because we focused on the same tree species, and host-specificity has been widely observed (Wagner et al., 2015). Studies have reported differences in communities when other host trees were considered, for instance, Woods and DeWalt (2013) report an approximately 40% similarity in epiphyte species composition between a 55-year-old secondary forest (similar age as the secondary forests we sampled) and an old-growth forest.

There is not much work on drivers of beetle communities in the canopy at the microscale, making it difficult to provide insights into drivers of canopy beetle communities. Most of our beetles were small, in the size range of 1–5 mm; Pincebourde et al. (2016) suggest that for small ectotherms, thermal properties of the habitat surface may be drivers of temperature variations experienced by the organism. Disturbances in forests increase the types of microhabitats available, and the more diverse beetle communities may be responding to the vegetation recovery in the under- and midstorey (Perry et al., 2018) rather than the absence of large trees. Further, certain beetles such as dung beetles (which includes Scarabaeidae) may have larger microclimatic variation tolerances than thought (Torppa et al., 2020), and this could extend to other beetle families.

The higher epiphyte abundances and richness were in primary forests were consistent with previous studies (Barthlott et al., 2001; Guzmán-Jacob et al., 2020) and can be explained by the larger and older trees in the primary forest which possess greater microhabitat heterogeneity (Woods et al., 2015). Additionally, the secondary site we chose has seen a higher intensity of logging with higher inter-tree distance (Devy and Davidar, 2001). In India, Padmawathe et al. (2004) observed a strong decline in non-orchid angiosperm epiphyte abundance and richness in secondary forests. However, Seshadri et al. (unpublished) demonstrate a lower epiphyte abundance in primary forest in Kalakad, as compared to selectively logged sites (which were different from the secondary forest plots in this study). Research in the montane tropical rainforests in the Andes has also shown an increase in Orchid abundance and richness and no overall significance in differences in epiphyte abundances and richness in tree canopies with an increase in managed and remnant trees relative to unmanaged mature forest (Larrea and Werner, 2010).

The higher abundances of *Bulbophyllum* sp.1 in the secondary forest trees indicates that it is a colonizing species, and the "bulbs" are a water-conserving adaptation which could contribute to their establishment in secondary forest trees (Seshadri et al., 2021). Previous explanations for lesser epiphytes in disturbed forests (Barthlott et al., 2001) or differences in communities (Larrea and Werner, 2010) have been attributed to drier and more sunlit microhabitats. Our results suggest that light and temperature may not be the primary factors influencing epiphyte communities. However, the effects of relative humidity are yet to be observed.

Our research design employed a "staggered" deployment of HOBOs, i.e., HOBOs in the primary and secondary forest trees were deployed on different days. This was the most practical way to make the best use of our time, with limited manpower as the process of rigging the tree, accessing the crown, and collecting samples is laborious. An ideal research design would have deployed an equal number of HOBOs and funnel traps in the primary and secondary forest canopies with a complete overlap in the deployment duration. Methods in microclimate ecology vary, as sensors and data loggers used for measurements differ in aspects, making comparisons between datasets difficult (Bramer et al., 2018). We used Onset HOBOs (HOBO pendant logger), a commonly used economical sensor with precisions comparable to more sophisticated sensors (Long et al., 2012). Hence, our method can be easily replicated for spatially (both horizontally and vertically) denser measurements. Future research could deploy sensors at a higher density, with multiple sensors on each tree. Research could also examine coupling between air temperature and leaf surface temperature, and their impacts on arthropods (Pincebourde and Woods, 2012).

Our short-term study provides an insight into the microclimatic differences between primary and secondary tropical wet forest canopies, a first for the southern Western Ghats. Further, we provide an important baseline for future research on beetles in the region. Long-term studies on seasonal fluctuations in beetle communities, comparisons with trends in canopy tree phenology, and the role of crown structure should reveal much more about the ecology of beetles in tropical wet forests.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://github.com/atreebangalore/rubinsagar.

AUTHOR CONTRIBUTIONS

RS and MD: conceptualization, funding acquisition, project administration, and writing—review and editing. RS: formal analysis, investigation, visualization, and writing the original draft. Both authors contributed to the article and approved the submitted version.

FUNDING

This study was funded through a Rufford First Small Grant (29821-1) by the Rufford Foundation to RS.

ACKNOWLEDGMENTS

We thank the entire staff at the Agasthyamalai Community Conservation Center (ACCC), ATREE for logistical support and P. Mahesh, Thalavaipandi, Satheesh S., and particularly Tamilalagan for supporting RS with the fieldwork. We thank Chiti Arvind for helping with photographing and storing the beetle specimens, and Naman Goyal for lending camera equipment. Lastly, we are very grateful to the Rufford Foundation for funding RS (29821-1) and the Tamil Nadu Forest Department for allowing the fieldwork to be conducted and Tamil Nadu Electricity Board for providing quarters to the researchers.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2022.734448/full#supplementary-material

Supplementary Figure 1 | A map showing the study area. The deep orange circle in the top left image (inset) shows the study area in India. The bottom left image (inset) shows the sampled site within KMTR. The orange rhombuses represent the trees sampled in the primary forest and the red circles represent the trees sampled in the secondary forest, with the background of a satellite image of the area (Imagery © 2022 CNES/Airbus, Landsat/Copernicus, Maxar Technologies, Map data © 2022).

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