COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Vertical stratification of a temperate forest caterpillar community in eastern North America

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Abstract

Vertical niche partitioning might be one of the main driving forces explaining the high diversity of forest ecosystems. However, the forest's vertical dimension has received limited investigation, especially in temperate forests. Thus, our knowledge about how communities are vertically structured remains limited for temperate forest ecosystems. In this study, we investigated the vertical structuring of an arboreal caterpillar community in a temperate deciduous forest of eastern North America. Within a 0.2-ha forest stand, all deciduous trees ≥ 5 cm diameter at breast height (DBH) were felled and systematically searched for caterpillars. Sampled caterpillars were assigned to a specific stratum (i.e. understory, midstory, or canopy) depending on their vertical position and classified into feeding guild as either exposed feeders or shelter builders (i.e. leaf rollers, leaf tiers, webbers). In total, 3892 caterpillars representing 215 species of butterflies and moths were collected and identified. While stratum had no effect on caterpillar density, feeding guild composition changed significantly with shelter-building caterpillars becoming the dominant guild in the canopy. Species richness and diversity were found to be highest in the understory and midstory and declined strongly in the canopy. Family and species composition changed significantly among the strata; understory and canopy showed the lowest similarity. Food web analyses further revealed an increasing network specialization towards the canopy, caused by an increase in specialization of the caterpillar community. In summary, our study revealed a pronounced stratification of a temperate forest caterpillar community, unveiling a distinctly different assemblage of caterpillars dwelling in the canopy stratum.

Keywords Feeding guilds · Food web · Forest canopy · Lepidoptera · Specialization

Introduction

Forests represent complex and heterogenous habitats (Basset et al. 2003; Floren and Schmidl 2008; Ulyshen 2011). Their canopies are often considered as hotspots for arthropods, especially in tropical regions, where they often harbour unique species (Nakamura et al. 2017). Accordingly, vertical changes in species communities and their interactions can be

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expected to play a key role in maintaining the high biodiversity found in forest ecosystems. Understanding forests as a three-dimensional system is thus essential to gaining a comprehensive view of the driving forces shaping their diversity and community structure. For obvious logistical reasons, the vertical dimension of forests, beyond what can easily be reached from the ground, has remained poorly studied.

Over the past 3 decades, there has been increasing interest in canopy research (Lowman 2009; Nakamura et al. 2017; but see also Basset et al. 2003). However, the ecology of temperate canopies has received little attention, most likely due to the general assumption that temperate communities are less stratified than those in tropical forests (Ulyshen 2011). This would imply that temperate forests reveal a low species turnover with increasing tree height, and that per taxon densities of focal taxa are evenly distributed (see Ulyshen 2011). Nevertheless, recent studies on some insect groups revealed that temperate forests display pronounced stratification (e.g. Stireman et al. 2012; Maguire et al. 2014; Di Giovanni et al. 2015; Weiss et al. 2016; De Smedt et al. 2018; Šigut et al. 2018), with differences attributed to various biotic and abiotic factors including microclimate, resource availability, forest structure, leaf quality, plant composition, and plant seasonality (Basset et al. 2003; Ulyshen 2011; Wardhaugh 2014; Nakamura et al. 2017). Leaves, for instance, generally experience greater sun exposure in the canopy leading to decreased foliage quality from a herbivore's perspective: leaves in the canopy are thicker, tougher, of lower water content, and show higher concentrations of secondary metabolites compared to leaves growing in the shaded understory (Murakami et al. 2005; Ulyshen 2011 and references therein). Consequently, canopy leaves are physically and chemically better protected and thus less suitable for herbivores, negatively affecting herbivore densities, especially those of generalist species (Ali and Agrawal 2012; Volf et al. 2015).

The order Lepidoptera represents an ecologically diversified and taxonomically well-known group for studying changes along environmental gradients (e.g. Summerville and Crist 2003; Summerville et al. 2004; Pellissier et al. 2012). The order comprises about 157,000 described species (Nieukerken et al. 2011) and is one of the largest radiations of phytophagous insects (Menken et al. 2010; Mitter et al. 2017). In temperate forests, caterpillars can reach high densities and thus play an essential role in food webs, both as herbivores and prey or hosts for higher-level consumers. Furthermore, Lepidoptera show a broad range of larval host–plant specificity (Menken et al. 2010), life histories, and habitat affinities (Müller et al. 2011), which makes them an excellent model group to study vertical stratification of insect communities in forest ecosystems.

While several studies have investigated the vertical stratification of adult Lepidoptera (e.g. Beccaloni 1997; DeVries and Walla 1999; Schulze et al. 2001; Brehm 2007; Hacker and Müller 2008; Ashton et al. 2016; dos Santos et al. 2017; De Smedt et al. 2018), few studies have documented the vertical structuring of caterpillar communities (Le Corff and Marquis 1999; Murakami et al. 2005; Šigut et al. 2018). Studies on caterpillars have three main advantages. First, because of their limited mobility, larvae are most likely to be found at or proximate to their feeding site. Second, the stratum where a larva occurs is likely to be a reliable indicator of a species' 'natural' vertical niche used for development. Third, caterpillar communities allow for investigation of host–plant interactions, which provide deeper insights into ecosystem functioning (e.g. how specialization and species richness/diversity are linked).

Here, we investigate various aspects of how an eastern US forest caterpillar community was vertically structured with initial aims to test three hypotheses:

1. We expected the density to decline towards the canopy due to harsher environmental conditions in upper forest strata (Ulyshen 2011) and lower foliage quality (Murakami et al. 2005). We further expected a dominance shift between exposed feeders and shelter-building caterpillars. We assumed the latter would become more prevalent in the upper strata, because shelter builders are more protected from desiccation, weather extremes, and many are known to feed on leaves of lower quality (Barber and Marquis 2011).

2. We expected changes in composition of higher taxonomic levels due to family constrained height preferences as reported for adult Lepidoptera (e.g. DeVries and Walla 1999; Brehm 2007; De Smedt et al. 2018). We further expected species richness and diversity to be lowest in the canopy (Hirao et al. 2009) and highest in the midstory due to overlapping Lepidoptera assemblages in this stratum (Fermon et al. 2005). Concomitantly, we expected the highest similarities between caterpillar assemblages of neighbouring strata as observed in adult Lepidoptera (Intachat and Holloway 2000; Schulze et al. 2001), due to higher overlaps in resource availability (i.e. host plant spp.) and abiotic conditions.

3. We expected increased specialization of plant–caterpillar networks towards the canopy due to the expected shift in guild composition. Shelter-building caterpillars are generally more host specific than exposed feeders (e.g. Menken et al. 2010). Thus, a proportional increase of this guild with tree height should result in a concomitant increase in specialization as well.

By sampling all larval Lepidoptera from a patch of deciduous Appalachian forest, a dominant forest type in eastern North America, this study aims to rigorously document the

extent to which herbivorous insects and their interactions with host plants are vertically structured in temperate forests.

Materials and methods

Study site

We conducted our sampling in a mixed deciduous forest in eastern North America, 2.5 km east of Toms Brook (Shenandoah County, VA, USA; 38°55′ N, 78°25′ W; 220 m a.s.l.). The oak–hickory association of our study site represents one of the region's most widespread forest types, and was chosen to be as similar as possible to the forest at the nearby Smithsonian Conservation Biology Institute (SCBI; ~ 23 km away in Front Royal, VA), which includes a large forest dynamics plot of the Forest Global Earth Observatory (ForestGEO; Anderson-Teixeira et al. 2015) and a core site of the National Ecological Observatory Network (NEON). The research site was owned by a timber company and had already been planned for logging, which allowed us to take advantage of the scheduled logging process for our sampling. Fifteen woody tree species were sampled; *Quercus* spp*., Carya* spp., and *Nyssa sylvatica* Marshall were the most commonly sampled taxa (Table 1). The tallest trees of the forest reached heights up to 30 m. At SCBI, precipitation is year-round (1000 mm of rainfall year^{-1}) and mean annual temperature is 12.9 °C (Anderson-Teixeira et al. 2015). The field work was carried out over two growing seasons, between late April and end of August in 2016 and 2017.

Sampling design

In 2016, we set up two 0.1-ha plots within the forest avoiding forest edges, gaps, and roads. The plots were located about 350 m apart. Due to their close proximity, we treated them as a single forest stand, not as independent replicates.

All deciduous tree individuals with a diameter at breast height (DBH) ≥ 5 cm were felled during both seasons. When two or more individuals of a tree species were to be sampled in the same year, we sampled one tree early in season (spring) and a matched individual late in the season (fall). One tree was felled and exhaustively sampled for Lepidoptera larvae at a time. This design has been used successfully to assess community structure and trophic interactions of non-flying herbivorous insects (see Volf et al. 2019).

Folivorous caterpillars (excluding miners) were collected immediately after the felling procedure from leaves, branches, and stems. For each larva, the exact height (measured from the tree base) was recorded using a measuring tape that was fixed to the stem/trunk base. Each caterpillar was classified into feeding guild as exposed feeder or shelter builder (i.e. leaf roller, leaf tiers, webber, also known collectively as semi-concealed feeders).

For every sampled tree, total height, crown height, and maximum crown width were measured. Furthermore, trees were stripped of leaves (small trees: 100%; mid-size trees:

Table 1 List of the 15 tree species with information on abundance (N_P), sampled leaf area, its proportional assignment to a certain stratum (US = understory, MS = midstory, CA = canopy), as well as average tree height ($\pm$ SE). Furthermore, caterpillar abundance (N_C) and number of caterpillar species (S_C) sampled from each tree species are given

Species	Family	N_P	Leaf area [m²]	US	MS	CA	Height [m]	N_C	S_C
Acer rubrum L.	Sapindaceae	8	167	0.35	0.47	0.18	13.8 (± 2.3)	105	28
Amelanchier arborea (F. Michx.) Fernald	Rosaceae	4	39	1	–	–	7.4 (± 0.7)	40	15
Carya glabra (Mill.) Sweet	Juglandaceae	15	272	0.48	0.39	0.13	11.6 (± 1.5)	315	46
Carya tomentosa (Lam.) Nutt.	Juglandaceae	25	416	0.45	0.47	0.08	13.1 (± 1.0)	168	47
Cornus florida L.	Cornaceae	2	19	1	–	–	5.0 (± 0.4)	15	3
Fraxinus americana L.	Oleaceae	3	66	0.13	0.81	0.06	18.3 (± 3.4)	32	11
Nyssa sylvatica Marshall	Cornaceae	47	572	0.41	0.58	0.01	11.2 (± 0.6)	597	52
Ostrya virginiana (Mill.) K. Koch	Betulaceae	5	81	0.87	0.13	–	10.1 (± 1.3)	70	20
Prunus avium L.	Rosaceae	2	10	0.19	0.81	–	14.5 (± 1.2)	31	9
Prunus serotina Ehrh.	Rosaceae	1	20	0.04	0.96	–	18.1	28	7
Quercus alba L.	Fagaceae	10	608	0.1	0.57	0.33	19.0 (± 2.8)	1194	77
Quercus rubra agg.[a]	Fagaceae	33	1191	0.06	0.74	0.2	19.1 (± 1.0)	1189	117
Quercus montana Willd.	Fagaceae	1	46	0.04	0.92	0.04	21.7	59	16
Sassafras albidum (Nutt.) Nees	Lauraceae	2	27	0.31	0.69	–	12.9 (± 2.4)	14	5
Ulmus americana L.	Ulmaceae	3	53	0.71	0.29	–	12.2 (± 0.4)	35	16
Total		161	3587	–	–	–	–	3892	–

[a]Note that specimens of *Quercus rubra* L. and *Quercus velutina* Lam. as well as their hybrids were pooled together as *Quercus rubra* agg

50%; large trees: 25%) after finishing the sampling process the same day. The respective crown fraction which needed to be defoliated for mid-size and large trees was estimated visually by at least two persons. Collected leaves were weighed to estimate the foliage biomass per tree. Additionally, a random subset of leaves per tree were weighed, spread over a white 50×50 cm background, and photographed. Depending on leaf size, two to four such photographs were taken to measure the leaf area using the software ImageJ v1.48 (Schneider et al. 2012). To calculate the total foliage area per tree, leaf frame values were extrapolated to the whole foliage biomass This approach allowed for further calculations of caterpillar densities (individuals per m^{-2} of leaf area).

Vertical stratification

Based on maximum tree heights within the plots (i.e. 30 m), we divided the forest stand into three strata ('vertical zones') of equal heights, hereafter referred as 'understory' (up to 10 m), 'midstory' (11–20 m), and 'canopy' (21–30 m). As there is no unified way to define forest strata (for review see Parker and Brown 2000), this classification, standardized by equal height intervals, was chosen, as it provided an objective method independent from assumptions about the vegetation structure to investigate stratification patterns within the caterpillar community.

Based on the height in which they were found, caterpillars were assigned to a respective stratum. For some trees, the crown was spread along two or three strata. Here, the leaf area was divided and allocated to the corresponding stratum as well. In these situations, we first calculated the crown volume (V_{Crown}) assuming a crown shape of a spheroid:

$$V_{Crown} = \frac{4}{3}\pi a^2 c,$$

where a is the crown horizontal radius ($0.5 \times$ maximum crown width) and c represents crown vertical radius ($0.5 \times$ crown height).

Spheroids are commonly used to study the architecture of deciduous forests (e.g. Chen et al. 2005; Walcroft et al. 2005). Afterwards, the volume of the upper and/or lower crown cap (V_{Cap}) was calculated using the formula:

$$V_{Cap} = \frac{\pi a^2}{3c^2}h^2(3c - h),$$

where h is the crown cap height (top or bottom part of the crown reaching into a certain stratum), and a and c are defined as for crown volume. This allowed us to partition the crown volume to the respective strata by subtraction of cap volume(s) from the total crown volume. A detailed script on volume calculations is presented in Appendix 1 (Figure S1,

Table S1). Based on the volume proportions, the total foliage area of a tree was divided proportionally into the strata.

Species identification

Host plants: All trees were identified to species with the exception of red oak species (i.e. *Quercus rubra* L. and *Q. velutina* Lam.), which hybridise in our study site. We treat red oak as a species complex, hereafter referred to as '*Quercus rubra* agg.'. Abundances, attributes, and caterpillar abundances for the 15 sampled tree species are given in Table 1.

Caterpillars: The sampled caterpillars were morphotyped, photographed (Canon EOS 700D; 60 mm macro lens), and stored in ethanol (96%) to enable later DNA barcoding. Only a few individuals were reared to adulthood at the beginning of our sampling in April and May, 2016.

For species identification and delimitation, we used an integrative taxonomy approach, drawing from morphological characteristics and molecular information (see Schlick-Steiner et al. 2010).

First, all caterpillar morphospecies found were identified by CLS and DLW as far as possible using external morphological characteristics and available literature (Wagner 2005; Wagner et al. 1998, 2001, 2011). For those caterpillar individuals which were successfully reared, morphological characteristics of the adults were also used for identification. Specimens which could not be reliably identified were barcoded (COI gene fragment; 658 bp) at the Canadian Centre for DNA Barcoding (CCDB; Guelph, Canada), using their standard Sanger sequencing protocols (Wilson 2012). The obtained sequences were cross-referenced with the comprehensive barcode library of North American Lepidoptera being built by the Agriculture Canada, CCDB, and the Smithsonian National Museum of Natural History (Washington, D.C.) (Levesque-Beaudin et al. 2017). In cases where a definite identification based on the integrative approach was still not possible, the 'Barcode Index Number System' (BIN system; Ratnasingham and Hebert 2013) was adopted, which allowed an assignment to a molecular operational taxonomic unit (M-OTU) in the 'Barcode of Life Database' (BOLD)—a convention increasingly applied in ecological studies dealing with Lepidoptera (e.g. Kekkonen and Hebert 2014; Delabye et al. 2018). For simplicity, identified species and M-OTUs are hereafter called 'species' and listed in Table S2. We followed Pohl et al. (2016) for taxonomic nomenclature and family classification.

Preserved caterpillar specimens are deposited at the Institute of Entomology in České Budějovice (Czech Republic). Reared adults are stored in the collection of the Smithsonian National Museum of Natural History (Washington, DC; USA). COI sequences can be accessed from the public

BOLD dataset DS-VERTCAT (https://doi.org/10.5883/ds-vertcat).

Statistical analyses

All statistical analyses were performed using the statistical software R version 3.4.4 (R Development Core Team 2017).

Density distribution and guild composition

Caterpillar densities (individuals m^{-2} leaf area) were calculated for each stratum of a given tree (individual). For a sampled tree individual, only strata with a minimum leaf area of 1 m^2 were considered for density analyses to account for calculation error: 21 out of 266 density values (7.9%) were excluded. Prior to further analyses, density values were log$_{10}$-transformed to meet the assumption of normality.

We developed two linear mixed models (LMMs) to test for differences in caterpillar densities among strata and among feeding guilds. As caterpillar densities vary among tree species (e.g. Nuttle et al. 2011; Farkas and Singer 2013; Šigut et al. 2018), tree individual nested within tree species were included as random effects in both models.

The first LMM tested for the effect of forest stratum on overall caterpillar densities. Here, stratum was fitted as a categorical fixed effect. The P value was estimated using ANOVA where the model of interest was compared against the null model.

The second LMM tested for differences among feeding guild densities within each stratum. Here, the interaction of guild and stratum was used as a fixed effect. We first tested if the interaction had a significant effect on densities of individual guilds by comparing the model of interest against the null model using ANOVA. Afterwards, Tukey post hoc tests were applied to test for dominance shift between the guilds among the strata using the '*emmeans*' package (Lenth 2018).

For both LMMs, residuals were checked for normality (QQ plots, histograms) and homoscedasticity (Levene's test for homogeneity of variance; '*car*' package (Fox and Weisberg 2019). Linear mixed models were developed using the '*lme4*' package (Bates et al. 2015).

Community composition and species diversity

Proportional composition of the most common caterpillar families (min. abundance ≥ 250 individuals) among the contrasted strata was compared using Chi squared contingency tests performed on the family-by-strata abundance matrix. For comparisons of relative abundances within single families among the strata, the abundances of each family were compared with the remaining families in a separate Chi squared test. For these, P values were adjusted using the Bonferroni correction to account for the multiple comparisons (to reduce risk of type I error).

Sample size-based rarefaction and extrapolation curves for species richness were calculated for each stratum across guilds and separately for exposed feeders and shelter builders using the '*iNEXT*' package (Chao et al. 2014; Hsieh et al. 2016). A short-range extrapolation (two times the smallest sample size) was applied. Estimated species richness (S_{Chao}) was calculated based on an asymptotic diversity estimate using the Chao and Jost (2015) method. Calculated confidence intervals further indicated if estimated species richness significantly differ among strata (no CI overlap) or not (CI overlap; see Colwell et al. 2004). Two indices were calculated to compare the diversity of the caterpillar communities among the strata, Fisher's α and Shannon–Wiener index (H').

Overlap of caterpillar assemblages between strata was calculated applying the Morisita–Horn similarity index (S_{MH}). This index is less sensitive to the presence of rare species and robust to variations in sample sizes and diversities (see Wolda 1981; Jost et al. 2011; Beck et al. 2013). We used the '*vegan*' package (Oksanen et al. 2018) to calculate both diversity and similarity indices.

Caterpillar–plant interaction networks

Prior to analyses, for each stratum caterpillar species with less than three individuals per host plant species were removed to avoid including inaccurate host associations, e.g. those resulting from straying larvae. Afterwards, quantitative interaction networks for understory, midstory, and canopy were created using the R package '*bipartite*' (Dormann et al. 2008). For specialization comparisons, we chose four quantitative network indices that account for interaction frequencies. Quantitative indices are less affected by differences in sample size and sampling effort, and thus reflect the network structure in a more realistic way than qualitative indices (Banašek-Richter et al. 2004; Blüthgen et al. 2006). We calculated weighted connectance, H$_2$', weighted generality, and weighted vulnerability by applying the '*networklevel*' function implemented in the R package '*bipartite*' (Dormann et al. 2009) to characterize the interaction networks. Weighted connectance is the proportion of the possible interactions that is realized and was measured as the proportion of links weighted by the interaction frequency. H$_2$' describes the degree of specialization of the whole interaction network and ranges between 0 (no specialization) and 1 (complete specialization). This index is robust to differences in network size and sampling effort (Blüthgen et al. 2006) and thus allows for direct comparisons among interaction networks of our defined strata. Weighted generality and weighted vulnerability indicate the mean number of host plants a caterpillar species is feeding on and the mean number of caterpillar

species feeding on a plant species, respectively. Both indices are weighted by interaction strength. While H_2' is a measure of specialization which considers both trophic levels, generality and vulnerability indicate the specialization of a certain trophic level (resource level: vulnerability; consumer level: generality). For all calculated network metrics, only plant species interacting with at least one caterpillar species were considered.

Additionally, index values of H_2', weighted generality and weighted vulnerability were compared to null model simulations of the respective interaction network. Null model-based approaches enable better interpretations of network properties than the observed index values alone (Dormann et al. 2009). We thus generated 999 random networks in each stratum. In each randomization, the interaction network matrix is reshuffled using the '*vaznull*' function implemented in the '*bipartite*' package (Dormann et al. 2009). These null models are constrained by connectance and marginal totals are proportional to the observed ones (Vázquez et al. 2007). Subsequently, all the network metrics were measured in these random networks, creating a null distribution for each index. Standardized effect sizes (Z-scores) and corresponding P values were calculated for each specialization index. This approach accounts for network size (number of interacting species in both trophic levels) and thus further allows for comparisons among the interaction networks. Increasing Z-scores indicate an increase in specialization between the networks, while decreasing Z-scores indicate that specialization decreases.

Results

Across both seasons, 161 individual trees from 15 species (nine families) were felled and caterpillars were collected from more than 3500 m^2 of foliage (Table 1). In total, 4118 caterpillars were collected of which 226 individuals (5.5%) could not be identified or at least assigned to a molecular operational taxonomic unit (M-OTU) and thus were excluded from subsequent analyses. The analysed dataset contained 3892 caterpillar individuals in 215 species representing 24 families (Table S2). The vast majority of these caterpillars were found in the midstory (2024 individuals), whereas only 1039 and 829 individuals were found in the understory and canopy, respectively. The highest number of caterpillar species was found in the midstory (167 spp.), followed by understory (149 spp.), and canopy (86 spp.).

Density distribution and guild composition

Overall caterpillar density (individuals/m^2 leaf area) was found to be highest in the canopy ($1.56 \pm$ SE 0.27), followed by understory ($1.23 \pm$ SE 0.15) and midstory ($1.03 \pm$ SE 0.12). These differences, however, were not significant ($df = 2$, $\chi^2 = 4.39$, $P = 0.11$; Fig. 1a). Feeding guild densities differed significantly across strata ($df = 9$, $\chi^2 = 21.8$, $P < 0.001$; Fig. 1b). In the understory stratum, the density of exposed feeders was significantly higher than those of shelter-building caterpillars ($df = 343.8$, $t = 2.3$, $P = 0.022$). The opposite was found in the canopy, where densities

Fig. 1 Box plots of caterpillar densities per m^2 of leaf area observed in the understory (US), midstory (MS), and canopy (CA) within a mixed deciduous forest in northeastern USA. Densities are presented **a** across feeding guilds, and **b** for individual guilds. Asterisks indicate significant differences (*$P < 0.05$, ** $P < 0.01$). (Color version of this figure is available online)
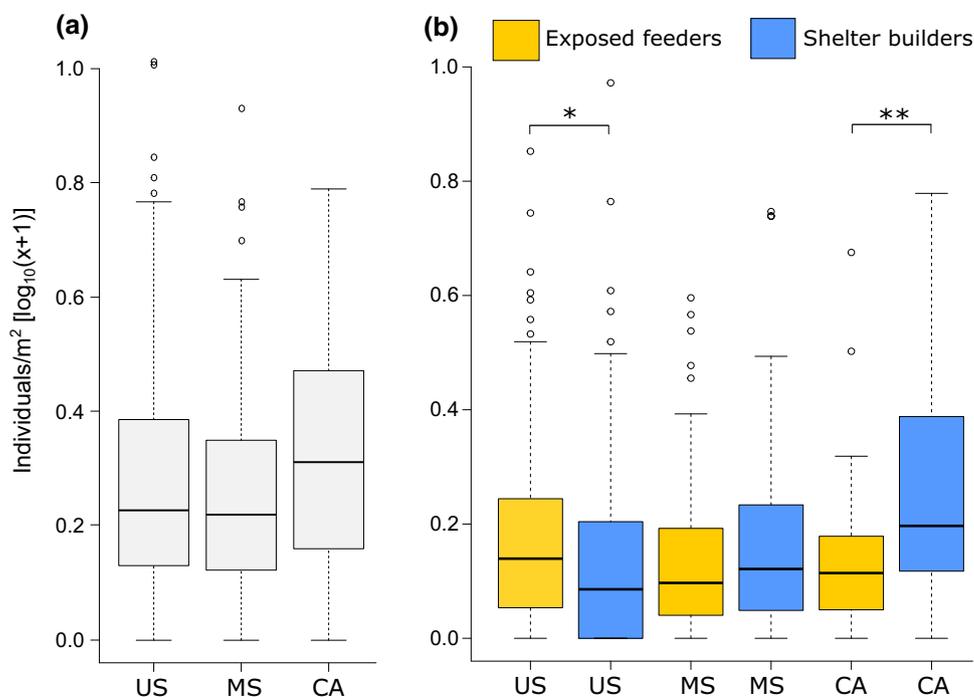
of shelter builders were found to be significantly higher ($df = 343.8$, $t = -3.215$, $P < 0.01$). By contrast, no difference among guild densities was observed for the midstory caterpillar fauna ($df = 343.8$, $t = -1.079$, $P = 0.281$, Fig. 1b). Furthermore, densities of shelter-building caterpillars were significantly higher in the canopy compared to midstory ($df = 407.06$, $t = -2.676$, $P = 0.021$) and understory ($df = 462.92$, $t = -2.579$, $P = 0.027$). Contrary, densities of exposed feeders were significantly higher in the understory compared to midstory ($df = 434.59$, $t = 3.121$, $P = 0.005$) and canopy ($df = 462.92$, $t = 2.357$, $P = 0.049$).

Community composition and species diversity

The most abundant families (together > 83% of all caterpillars) for the whole forest stand as well as for individual strata were, in alphabetic order, Depressariidae, Erebidae, Geometridae, Noctuidae, Notodontidae, and Tortricidae (Fig. 2). These families were also the most abundant ones found at individual strata. Their proportional abundances, however, changed significantly across the three strata ($df = 12$, $\chi^2 = 415.3$, $P < 0.001$, Fig. 2). The proportion of Depressariidae increased towards the canopy (understory: 19.7% vs. canopy: 56%), while those of Erebidae, Geometridae, and Noctuidae decreased ($df = 2$, $\chi^2 > 48.0$, $P < 0.05$ for each family). The proportion of Notodontidae and Tortricidae did not differ significantly among the strata ($df = 2$, $\chi^2 < 4.52$, $P > 0.05$).
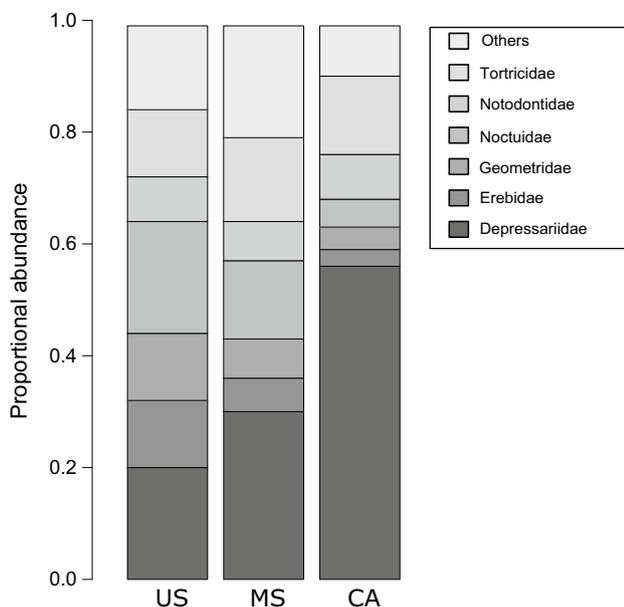

Fig. 2 Proportional family composition of the six most abundant caterpillar taxa separated by understory (US), midstory (MS), and canopy (CA)

The most speciose families were Geometridae (36 spp.; 16.5%), Noctuidae (35 spp.; 16.3%), and Tortricidae (32 spp., 14.7%), which collectively accounted for nearly half (47.9%) of the sampled species. When species with less than three individuals were removed from analyses, 14 species (6.4%) were exclusively found in a single stratum (understory: 10 spp.; midstory: 4 spp.; no species was exclusively associated with the canopy; Table S2). Across both feeding guilds, individual-based species accumulation curves for each stratum revealed significant differences in species richness between canopy vs. understory and canopy vs. midstory, respectively (Fig. 3a, Table S3). Due to overlapping confidence intervals, no significant difference was observed between midstory and understory. When estimated separately for each feeding guild, this pattern stayed constant for shelter builders, whereas no significant differences in species richness among the strata could be detected for exposed feeders (Fig. 3b, c). Furthermore, the species accumulation curves suggested a higher richness of exposed feeding caterpillar species for all three forest strata (Fig. 3b, c). For the entire community, both Fisher's α and Shannon diversity (H') indices indicated highest diversity in the understory and midstory (Table S3). Similar patterns were observed when feeding guilds were analysed separately, although the diversity decline between midstory and canopy was less pronounced for exposed feeders (Table S3).

Based on Morisita–Horn similarity indices (S_{MH}), the overlap of caterpillar communities was greater between understory and midstory ($S_{MH} = 0.74$) followed by midstory vs. canopy ($S_{MH} = 0.61$). High compositional turnover was observed between the understory and canopy assemblages ($S_{MH} = 0.24$).

Caterpillar–plant interaction networks

The canopy network comprised only a small subset (3 spp.) of interacting host–plants, whereas understory and midstory networks involved 14 and 12 plant species, respectively (Fig. 4). Furthermore, the caterpillar species richness (spp. > 2 ind.) was more than twice as high in the understory network (63 spp.) and midstory network (76 spp.) compared to that of the canopy (31 spp.).

Weighted connectance was three times higher in the canopy compared to understory and midstory where it was found to be equal (Table 2). The network specialization (H_2') values were similar among understory and midstory and increased slightly in the canopy. For all strata, the observed H_2' values deviated significantly from null model distributions and thus revealed a higher specialization than expected due to random chance. Furthermore, Z-scores increased towards the canopy indicating an increase in network specialization. Weighted generality decreased with height and was found to be significantly lower than expected
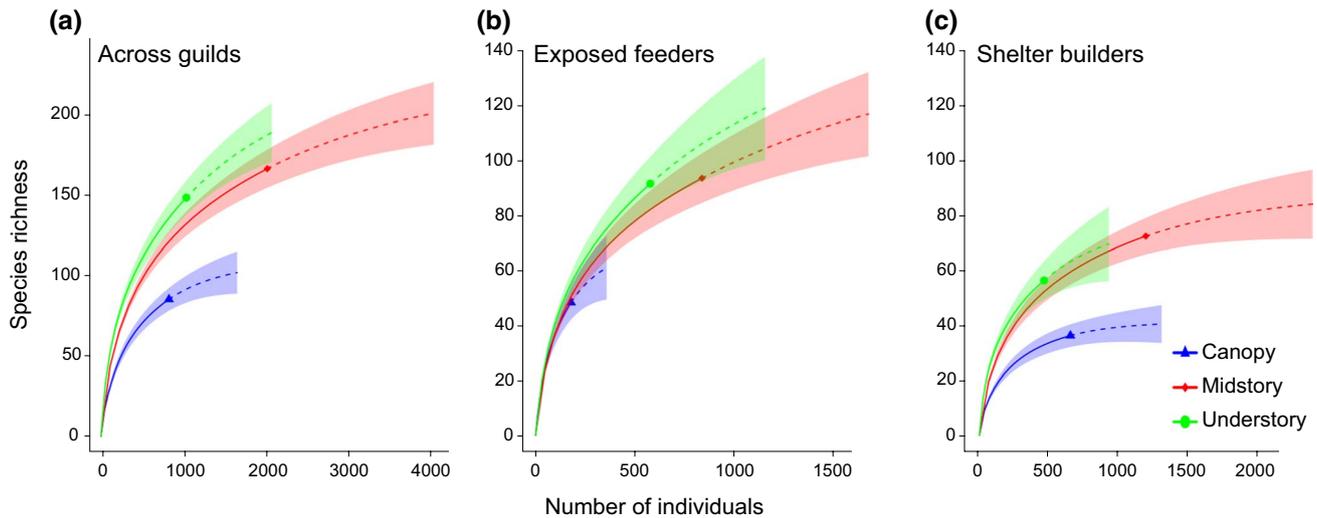
Fig. 3 Individual-based rarefaction curves (solid lines) and short-range extrapolation (dashed lines) for **a** the whole caterpillar community, **b** exposed feeders, and **c** and shelter builders segregated into understory, midstory, and canopy stratum. Shaded areas represent ± 95% confidence intervals. (Color version of this figure is available online)

due to random chance for midstory and canopy. Calculated effect size deviations from null models (Z-scores) further revealed that the specialization of the caterpillar community decreased from canopy towards the understory. Weighted vulnerability showed only small differences between understory and midstory (10.2 vs. 12.3) but dropped strongly in the canopy (5.2). Nevertheless, for all strata, its values did not differ from the respective null model distribution.

Discussion

Our study revealed a pronounced vertical stratification of a temperate forest caterpillar community in terms of guild composition, species richness, and specialization. While overall densities did not vary among strata, our study showed that exposed feeders dominated the understory and shelter builders the canopy. The canopy was further found to be species poor and of low diversity, while midstory and understory were speciose and of higher diversity. Caterpillar assemblages overlapped most among neighbouring strata. Both network and caterpillar specialization were found to be highest in the canopy and to decline towards the understory.

Density distribution and guild composition

Our study revealed that stratification had no effect on caterpillar densities. This is contrary to our hypothesis and many studies on herbivorous insects which reported unequal vertical density distributions in forest ecosystems (e.g. Basset 2001; Ribeiro and Basset 2007; Šigut et al. 2018; for review see Ulyshen 2011). Previous studies have mainly attributed

vertical changes to variations in leaf quality (Murakami et al. 2005; Ribeiro and Basset 2007) or predation (Šigut et al. 2018). Our findings, by contrast, indicate that vertical differences in caterpillar abundances are mainly driven by available foliage. One explanation for this finding could be that factors affecting caterpillar density vary more within than among the defined strata. Leaf quality, for instance, displays significant intra- and interspecific variations among tree individuals, which strongly influences the density of associated insect herbivores and their performance (Hemming and Lindroth 1995; Lill and Marquis 2001; Summerville et al. 2003; Murakami et al. 2005; Barber and Marquis 2011). In temperate regions, leaf quality also changes considerably over the course of the growing season (Schultz et al. 1982; Hunter and Lechowicz 1992; Zehnder et al. 2009). Therefore, seasonal as well as inter- and intraspecific variations in leaf quality could commonly override vertical stratification patterns in caterpillar density in temperate forests. A similar result was obtained in a study by Le Corff and Marquis (1999), who also found no difference in caterpillar densities between understory (< 2.5 m) and canopy (15–20 m) in two oak species of a temperate forest in USA. However, as caterpillar densities differ among tree species (Nuttle et al. 2011; Farkas and Singer 2013; Šigut et al. 2018), vertical density distributions might change considerably among forest ecosystems, depending on plant species composition and particularly on the subset of trees that compose each stratum. Significant changes in caterpillar density patterns might therefore also occur among different successional stages of a forest. For instance, caterpillar species feeding primarily on trees forming the canopy of mature forests might be more abundant in lower strata during forest succession.
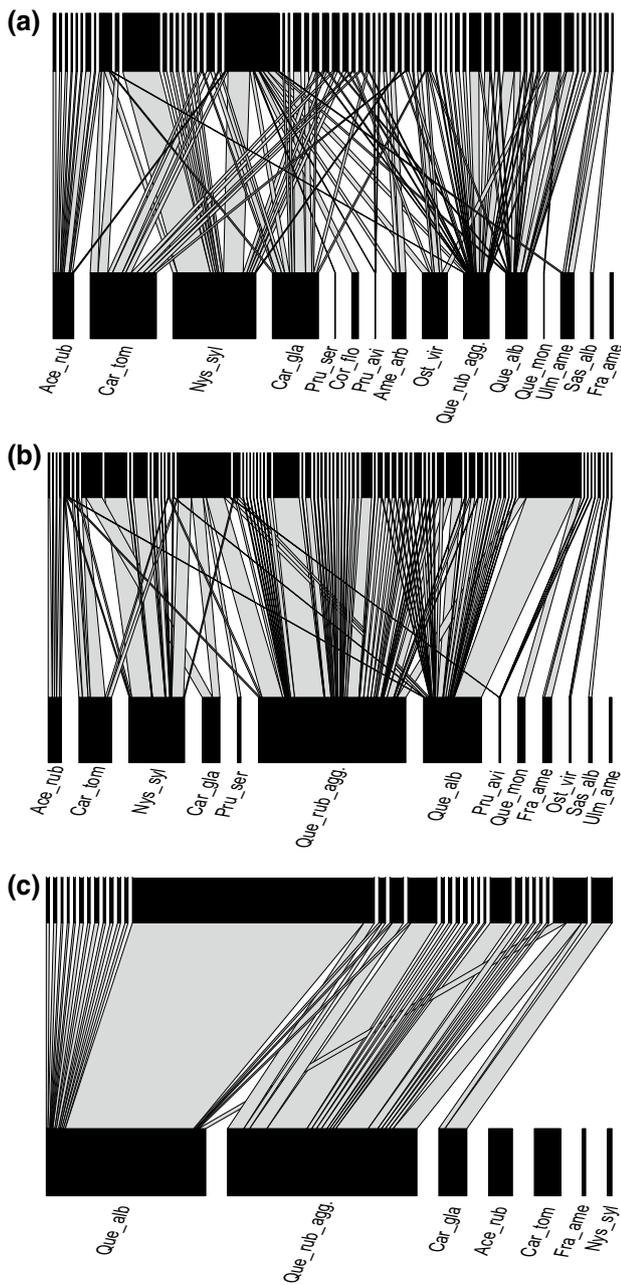
Fig. 4 Quantitative host plant–caterpillar interaction networks for understory **a** understory, **b** midstory, and **c** canopy of a deciduous forest in eastern USA. Each bar represents either a caterpillar species (upper bars), or a plant species (lower bars; taxa indicated by abbreviated name). Widths of upper bars indicate relative caterpillar abundances, while lower bar widths indicate relative plant abundances measured as m^2 leaf area. Linkage width indicates relative interaction frequency of the upper and lower trophic levels

On the guild level, we found dominance shifts among the strata, in accordance with our initial hypotheses. While shelter-building caterpillars revealed higher densities in the canopy, exposed feeders dominated the understory. Similar stratification patterns were observed in two oak species by

Le Corff and Marquis (1999). They also found that the relative abundance of external feeders was lower in the canopy than in the understory, compared to those of shelter-building species. The observed decline in exposed feeders at higher strata is most likely due to desiccating and changing weather conditions, which become harsher towards the canopy (Ulyshen 2011; Nakamura et al. 2017). In such environments, a shelter-forming life style is advantageous, as it prevents larvae from dropping down (during storm events or heavy rainfalls), and desiccation (Greeney et al. 2012), and thus reduces mortality. Another explanation for vertical changes in feeding guild composition could be predation risk. For instance, studies from Costa Rica (Gentry and Dyer 2002) and Papua New Guinea (Hrcek et al. 2013) indicate that shelter-building caterpillars experience a higher predation pressure by parasitoids compared to exposed feeders. Exposed feeders, by contrast, are more easily predated by visual hunters, especially foraging birds (Holmes and Schultz 1988). To reduce guild-specific predator groups, shelter builders and externally feeding Lepidoptera might seek out enemy-reduced niche space in different strata. Most likely both the vertical climate gradient as well as predator avoidance strategy influence the stratification patterns of caterpillar feeding guilds.

Our sampling method could have biased our findings on feeding guild distributions, e.g. caterpillars could have been dislodged during the felling process. Such a risk might have been more pronounced for the upper parts of larger trees and primarily affect external feeders (especially those that thrash upon disturbance). Shelter-building caterpillars, by contrast, tend to retreat into their shelters upon disturbance. However, given that we found few caterpillars on the ground during the sampling process and the distinct proportional differences in guild composition among the strata strongly suggest that our results are not artifactual.

Community composition and species diversity

According to our expectations, lepidopteran family and species composition changed significantly along the vertical gradient with the lowest overlap among the most distant strata (i.e. understory vs. canopy). This reaffirms previous reports of species- and family-specific height preferences for Lepidoptera in forest ecosystems (e.g. Brehm 2007; Hirao et al. 2009; De Smedt et al. 2018). Taxon-specific height preferences in insects can be driven by morphological traits such as body size (Schulze et al. 2001; Grimbacher and Stork 2007; Graça et al. 2017) as well as by natural history traits such as feeding guild or place and stage of hibernation (Brown et al. 1997; Di Giovanni et al. 2015). In Lepidoptera, body size and feeding guild are strongly linked (Holmes and Schultz 1988), and often phylogenetically conserved (Menken et al. 2010; Regier et al. 2012, 2015). Our

Table 2 Network metrics for caterpillar–host plant interaction networks of three equally sized strata of a mixed deciduous forest in eastern USA

Network metric	Understory			Midstory			Canopy		
	Obs.	Z score	P value	Obs.	Z score	P value	Obs.	Z score	P value
Host richness	14	–	–	12	–	–	3	–	–
Caterpillar richness	63	–	–	76	–	–	31	–	–
Connectance	0.12	–	–	0.12	–	–	0.39	–	–
H_2'	0.64	5.20	0.002**	0.65	7.47	0.002**	0.71	9.57	0.002**
Generality	2.68	− 1.14	0.254	2.08	− 2.72	0.016*	1.31	− 4.18	0.010*
Vulnerability	10.24	− 0.60	0.578	12.33	0.88	0.400	5.20	0.31	0.736

Based on comparisons with null models, which are constrained by connectance, Z-scores and respective P values are reported for weighted generality, weighted vulnerability and H_2'

Significance codes: $P \leq 0.001$ '***'; $P \leq 0.01$ '**'; $P \leq 0.05$ '*'; n.s. ''

study suggests that families of small-sized, shelter-building species dominate the upper strata (e.g. Depressariidae and Tortricidae). While, families representing primarily mid-sized or large species, and whose caterpillars are exposed feeders, are commonly more prevalent in the understory (e.g. Geometridae, Erebidae, and Noctuidae). Higher concentrations of small-sized insects in the canopy have been also reported from several tropical forest ecosystems (see Wardhaugh 2014).

Both species richness and diversity were highest in the two lower strata and declined steeply in the canopy. Thus, our findings confirmed the hypothesis of lowest richness and diversity in the canopy, while the expectation of heightened diversity in the midstory (i.e. a mid-domain effect) was not supported. The lack of any species associated uniquely to the canopy was unexpected. This indicates that understory and midstory are more important in maintaining caterpillar species richness in temperate forests. This is in line with the findings of Hacker and Müller (2008) and Hirao et al. (2009) on adult Lepidoptera who also pointed out the importance of lower strata in maintaining diversity for deciduous temperate forests in Germany and Japan, respectively. By contrast, De Smedt et al. (2018) found the canopy to be a key habitat for adult macro-moths in Belgium, which agrees with several studies on butterflies and moths from the tropics (e.g. Brehm 2007; Fordyce and DeVries 2016; dos Santos et al. 2017). However, these studies focused on adults, and thus included taxa which are not associated with forest habitats and/or whose caterpillars are not tree-dwelling. Although Beccaloni (1997; Great Britain, Ecuador, Costa Rica) and Brehm (2007; Costa Rica) found patterns indicating that flight height of butterflies and moths is linked to the heights of their respective host–plant growth forms, strata preferences of adults and their immature stages do not necessarily overlap (Schulze et al. 2001; Basset et al. 2003). Due to their mobility, adult lepidopterans can be found in forest strata which do not serve as habitat for the immature stages (e.g. during dispersal, 'hilltopping', or flights to locate food resources or mating partners). Thus, studies dealing with

Lepidoptera adults would be expected to yield different findings.

Many tree species in our forest plot did not reach the canopy or were represented by a negligible amount of foliage, while the understory and midstory strata harboured nearly all tree taxa. Most caterpillar species, especially dietary specialists, are therefore restricted to the lower strata by default. This would explain much of the high species overlap among understory and midstory and further supports the assumption that resource availability (= host plant richness) is a main driver for spatial distribution patterns in Appalachian and presumably other forest canopies (Wardhaugh 2014).

Besides host plant richness, the feeding guild also affects species richness. Families of primarily exposed feeders generally tend to be more diverse (Menken et al. 2010) and that is supported by our richness and diversity estimates at the guild level as well. Thus, the low caterpillar richness in the canopy most likely results also from the high fraction of caterpillars representing a shelter-forming behaviour. This effect is further strengthened by the high dominance of one shelter-building depressariid species (*Psilocorsis reflexella* Clemens, 1860), which accounts for nearly half (47.4%) of all the recovered individuals in the canopy samples.

Caterpillar–plant interaction networks

A few studies have focused on vertical stratification of antagonistic food webs, primarily between various guilds of herbivorous insects and their associated parasitoids (e.g. Paniagua et al. 2009; Morris et al. 2015; Chaij et al. 2016; Šigut et al. 2018). This is, to our knowledge, the first study addressing specialization patterns of insect herbivore–plant networks along a vertical forest gradient.

In our study, the canopy network was distinctly smaller than those of the lower strata. The higher connectance in the canopy thus probably results from the smaller network size and does not indicate higher redundancy (see Jordano 1987; Olesen and Jordano 2002; Pellissier et al. 2018). According to our prediction, specialization matrices increased

towards the canopy. The caterpillar–plant network metrics revealed an increasing network specialization (H_2') towards the canopy, and a concomitant increasing specialization of the caterpillar community. As we found no significant difference for vulnerability from our null models, the increased H_2' towards the canopy probably reflects the decreasing proportion of dietary generalists across the strata. The high specialization of the caterpillar community in the canopy likely results from the increased fraction of shelter builders in this stratum. This guild generally displays a higher degree of specialization compared to exposed feeders (Powell 1980; Menken et al. 2010). Thus, their greater presence in the canopy might significantly contribute to the higher specialization seen there. The distinctly lower plant richness in the canopy stratum is probably another driving force of the vertical specialization pattern. Here, caterpillars can only interact with a small subset of tree species, whereas they are able to interact with more species at lower strata.

Our results are in line with Paniagua et al. (2009) who found a higher specialization of gall-maker parasitoids in the canopy of a dry forest in Panama. However, most other studies on antagonistic food webs observed no distinct differences in specialization among forest strata (Morris et al. 2015; Chaij et al. 2016; Šigut et al. 2018).

Conclusion

Our study shows pronounced vertical stratification of immature insect herbivores in temperate forest ecosystems. While caterpillar density did not vary with height, vertical stratification patterns of guild structure, species richness, composition, and specialization differed significantly across strata and were closely linked. We showed that natural history traits, such as feeding guild, can play a crucial role in structuring caterpillar assemblages and their host–plant interactions along vertical forest gradients. Understory and midstory strata had greater species richness and diversity than the canopy stratum underpinning their importance for an approbate conservation management of temperate forests. The higher incidence of exposed feeders in lower strata would make them further a more productive foraging habitat for visually hunting predators such as foliage-gleaning birds.

This, to our knowledge, is the first study examining vertical trends in plant–caterpillar networks of a complete arboreal community of folivorous caterpillars. To a degree, increased specialization in the canopy was a consequence of the reduced host–plant richness of this stratum, and to what extent this phenomenon structures other temperate and tropical forest canopies is worthy of additional study. Therefore, our findings provide an essential baseline for further investigation, and will allow spatial and temporal comparisons to other plant–insect associations, especially those examining the vertical stratification of the world's forests.

Author contribution statement VN, SEM, KJAT, and MV established the project. MV, VN, GPAL, and CLS designed the sampling protocols and the experimental approach. GPAL, CLS, and MV lead the field work. CLS, DLW, SEM, and MV processed the data. CLS and LRJ analysed the data. CLS wrote the first draft of the manuscript; all authors discussed the results and significantly contributed in writing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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