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Vertical stratification of leaf physical traits exerts bottom–up pressures on insect herbivory in a sugar maple temperate forest

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Abstract

1. Do light vertical gradients in temperate forest structure insect herbivore communities? We tested the hypothesis that the increase in light intensity from understory to forest canopy drives differences in leaf physical traits and bud burst phenology that impact insect herbivores and thus play a role in structuring both herbivore communities and the leaf damage they cause. Understanding these interactions is essential for addressing knowledge gaps in the dynamics of temperate deciduous forest ecosystems.
2. Twelve sugar maple (*Acer saccharum*) sites were monitored in southern Quebec, examining insect herbivore patterns from understory saplings to mature tree-shaded and sun canopy (where intensity is highest and canopy cover lowest) over the summers of 2020, 2021 and 2022. Additionally, we recorded leaf physical traits and sun exposure.
3. Our findings revealed that leaf thickness increased along the vertical gradient in 2021, making mature tree leaves in the canopy less favourable to herbivores than understory sapling leaves. Accordingly, we recorded a consistent decrease in insect herbivory damage rates from understory to shaded and to sun canopy in 2020 and 2021, driven by leaf cutters, skeletonizers, stipplers and leaf miners. These results support our hypothesis that variation in leaf physical traits contributes to the vertical stratification of insect damage. This variation in leaf traits can be linked to light levels or to tree ontogeny. In 2022, the gradient of insect herbivore abundance corroborated the observed damage trends from the previous years. We calculated an average annual herbivory rate of 9.1% of the leaf surface in our study site.
4. Overall, our study highlights the importance of vertical gradients in structuring insect herbivore communities and emphasizes the role of leaf traits in mediating these interactions. In addition, the average annual herbivory rate suggests limited evidence supporting a significant contribution of background herbivory to the decline of sugar maple forests.

KEYWORDS
forest strata, herbivore community pattern, insect feeding guilds, leaf economic spectrum, plant-insect interaction

INTRODUCTION

The pattern of insect herbivore communities varies in relation to heterogeneity in forest structure, linked to differences in bottom–up and top–down forces on herbivorous insects. While predators and parasitoids act as the top–down pressure in controlling herbivore population density, bottom–up forces on insect herbivores arise from plant defensive traits, which can be particularly essential for plant species to maintain themselves in ecosystems in the face of herbivory (Hairston et al., 1960; Schowalter et al., 1986a; Singer & Stireman, 2005; Aikens, 2008; Ulyshen, 2011; Wilkinson & Sherratt, 2016). However, much of the variation in plant defensive traits occurs in response to light intensity in line with the leaf economic spectrum, in order to maximize photosynthesis under different conditions (Messier et al., 2017). These physical functional traits can also influence leaf resistance to herbivores and can therefore spatially structure bottom–up pressures on herbivores along sunlight gradients (Messier et al., 2017; Morrow et al., 2022; Reich, 2014).

The effect of light level on leaf phenology and leaf physical traits is one of the key processes shaping microhabitats in a vertical gradient of deciduous forest trees throughout the season (Murakami et al., 2005; Thomas et al., 2010; Ulyshen, 2011). Sun-exposed leaves in the upper canopy tend to be smaller, drier and better structurally and chemically defended, resulting in a decline in herbivory damage in upper strata (Murakami et al., 2005; Ulyshen, 2011). Furthermore, saplings in deciduous forests tend to break buds earlier than canopy of mature trees, which is an adaptation to seasonal light availability in the understory. This variation in the phenology of bud burst over the growing season directly impacts leaf quality in each stratum and can increase herbivore damage on saplings (Murakami et al., 2005). Moreover, leaf quality generally declines from spring to summer due to an increase in toughness and tannin content and a decrease in water and nitrogen contents as leaves mature, making spring leaves more favourable for herbivores and emphasizing the importance of bud burst phenology (Murakami et al., 2005; Seifert et al., 2021). This spatio-temporal variation in leaf quality, from understory to the canopy level and from spring to summer, can shape herbivore communities and predict patterns of damage they cause (Eisenring et al., 2021; Hunter & Lechowicz, 1992; Seifert et al., 2020).

The vertical stratification effect on insect herbivores has been well-researched in tropical forests and large differences have been recorded in herbivore richness, assemblage and distribution between the canopy and understory (Ashton et al., 2016; Brehm, 2007; Graça et al., 2017; Grimbacher & Stork, 2007; Yang et al., 2018). The general pattern in tropical forests seems to show a reduction in insect herbivore defoliation in the sun canopy compared with the shade canopy, and higher herbivory in mature upper canopy than young leaves in understory (Basset, 1991; Lowman, 1992; Zhang et al., 2023). However, knowledge about the spatio-temporal pattern of insect herbivores in deciduous forests in relation to heterogeneity in leaf quality is lacking (Thomas et al., 2010; Ulyshen, 2011; Yang, 2014) and this knowledge gap limits our understanding of factors shaping biodiversity in temperate forest ecosystems.

A few studies done in deciduous forests show a decline in herbivory damage and richness going up towards the canopy, linking it to the lower leaf quality in upper forest strata (Corff & Marquis, 1999; Murakami et al., 2005; Rowe & Potter, 1996; Seifert et al., 2020; Thomas et al., 2010). However, insects with different feeding guilds do not react to this heterogeneity of resources in the same way. Insect herbivores can be divided into two groups based on their feeding guilds: the shelter builders including miners, rollers and gall makers in the first group are predicted to survive better in harsh condition in the upper canopy, whereas the exposed feeders composed of cutters, skeletonisers and stippler insects in the second group are expected to prefer the lower canopy (Seifert et al., 2020; Thomas et al., 2010).

We aimed to study this vertical stratified leaf traits effect on insect herbivore community on sugar maples (*Acer saccharum* Marsh) as a crucial species in Eastern Canadian temperate forests, serving as a keystone species and playing a significant role in the local economy and culture (Horsley et al., 2002). Sugar maple is a shade-tolerant tree species that typically grows in late-succession stages, regenerating under a forest canopy. Young sugar maple trees can spend decades in the understory before a gap opens up and allows them to grow to the canopy (Bray, 1956; Godman et al., 1990). This ontogenetic journey from shade to light is crucial for understanding how young saplings grow into mature canopy trees. During this transition, saplings experience different environmental conditions, influencing their vulnerability to herbivore damage. Previous work suggests that leaves of these young trees are more vulnerable to herbivore damage than those in the canopy of mature trees (Thomas et al., 2010).

Since the late 1970s, there has been a significant decline in sugar maple-dominated deciduous forests, and insect defoliation has been identified as one of the factors contributing to this decline (Côté & Ouimet, 1996; Duchesne et al., 2003; Horsley et al., 2002; Payette et al., 1996). Outbreaking defoliators can cause crown deterioration, decreased radial growth and even mortality in sugar maples that are also under abiotic stress (Horsley et al., 2002; Johns et al., 2016). However, background herbivory, which results in mild but continuous damage on host plants, can also cause a progressive weakening in trees, contribute to dieback and increase tree susceptibility to the next abiotic or biotic stress (Cánovas et al., 2018; Horsley et al., 2002). To understand the extent to which this background herbivory could contribute to sugar maple decline, we need to first study insect herbivore community on these maple trees.

To this end, our study examines vertical stratification of insect herbivory damage on sugar maples in an eastern Canadian temperate forest, to understand how it varies between different herbivore guilds and through the seasons and testing the role of bottom–up forces linked to plant ontological and phenological traits in shaping this pattern. Based on previous literature, we hypothesize that leaves become less palatable and flush out later along a vertical gradient from understory to the shade and sun canopy strata, leading to lower overall insect herbivory damage and reduced abundance of insect herbivores in the sun canopy. Additionally, we expect to observe variations in damage patterns among herbivores belonging to different feeding guilds.

MATERIALS AND METHODS

Study site and plant species

We conducted our sampling at the Kenauk Nature property, an old-growth natural forest located in southwestern Quebec, Canada (45°42′N; 74°53′W). The property spans two regions from the Outaouais river valley to the Laurentian Mountains. Based on the provided domains map by the Quebec Ministry of Forests, it is in a maple/bitternut hickory domain of temperate deciduous forest (Quebec Ministry of Forests, 2023). To account for the variation in insect herbivory throughout the growing season, we conducted three sampling sessions at the end of June, the end of July and the middle of August in 2020, 2021 and 2022. During each session, we sampled the same 12 sites. Aside from two sites where the distance between trees was 18 m, the rest were over 40 m apart, with a maximum distance of 9 km between some sites. Tree species composition was relatively uniform in all sites. These sites were selected based on the similarity in tree size and safety considerations for climbing. Each site comprised one mature sugar maple tree and one sapling. We collected samples from the branches on both the sunlit and shaded canopy strata of the mature trees, as well as from the branches of saplings in the understory. Canopy branches were sampled at a height of 10–13 m above ground on mature trees averaging around 16 m in height, and the understory branches were defined as those on surrounding sugar maple saplings ranging from 1 to 3 m in height. To differentiate branches in the shaded canopy from those in the sun canopy, we measured light intensity on the targeted branches, to make sure the average amount of light shows a difference between shaded and sunlit branches. No serious outbreaks of herbivorous insects were observed throughout the study duration. For visual clarity and to present all sampling events and their respective timings in one place, an infographic has been included as Figure 1.

Assessment of insect herbivore damage

Leaf herbivory damage is recognized as a good measure of the structure of insect herbivore communities and their interactions with host plants (Schowalter, 2006; Schowalter et al., 1986b). Among the methods of assessing leaf herbivory damage, visual assessments are accurate enough for comparative analysis (Johnson et al., 2016; Landsberg, 1989). In this study, herbivory damage was visually quantified as the percentage of leaf area damaged by different insect feeding guilds (Houston et al., 1990; Johnson et al., 2016; Thomas et al., 2010; Wint, 1983). We averaged the damage scores for 15 fully expanded leaves per stratum per site in both 2020 and 2021. Nine insect herbivory damage types identified on sugar maples (Thomas et al., 2010) were assessed, including leaf rollers, leaf skeletonisers, leaf cutters, leaf stippling, spindle galls, erineum gall, ocellate gall, maple leaf-cutter miner and maple leaf-blotch miner (Figure 1). Trained interns conducted assessments using the guidebook by Houston et al. (1990), which features clear images of insect herbivores induced damage on sugar maple leaves (Houston et al., 1990). During assessments, each leaf was visually divided into four parts, and the percentage of observed damage per part was estimated. The sum of

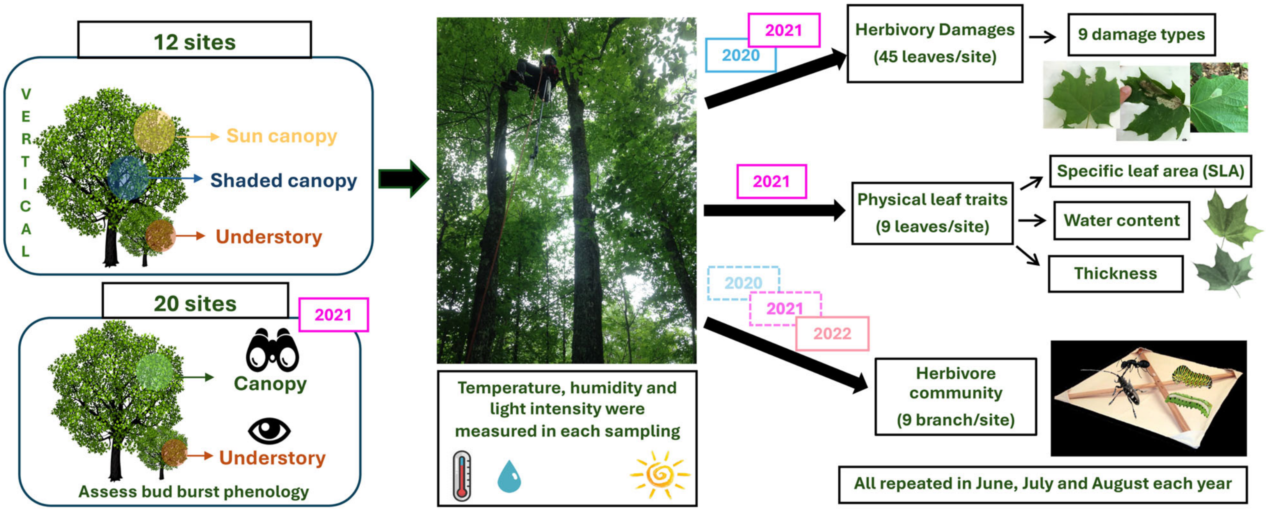

FIGURE 1 Infographic detailing the data collection methods used in the study from 2020 to 2022 in Kenauk nature. This graphic provides an overview of the experimental setup, timeline, data collection techniques and key variables measured. Insect herbivory data collected in 2020 and 2021 are depicted with dashed-line borders on the arrow, indicating that they were excluded from the analysis due to a low number of samples.

these percentages yielded the total percentage of a particular damage type on the leaf. To ensure consistency in assessment methods, subsequent interns received supervision from the previous year's intern, and the first 20–30 leaves were assessed together until their estimations reached the closest possible approximation. To capture both spatial and temporal effects on herbivory damage, we randomly collected 15 leaves per stratum per site at three sampling sessions (June, July and August). In total 1620 leaves were collected (12 sites × 3 strata × 15 leaves × 3 sampling dates) (Schowalter et al., 1986a; Turcotte et al., 2014; Zehnder et al., 2009). For understory, leaves were collected by hand from accessible branches on the ground. Canopy leaves were accessed using single rope technique and climbing gear and were cut with a 2-m pole pruner; shaded leaves were collected from mid-center canopy branches under near-complete canopy cover receiving less light, while sunlit leaves were sampled from higher and more marginal canopy branches with less than 50% canopy cover to ensure greater sun exposure. Due to the limitations of single rope techniques, sampling was often confined to the inner section of the tree crown (Basset, 1991; Maguire et al., 2014; Thomas et al., 2010). Environmental factors such as light intensity, temperature, and humidity were recorded at each sampled branch during the three sampling sessions to confirm microhabitats differences between branches in sun-exposed canopies, shaded canopies, and understory. Light intensity was measured using a Reed light meter model R1930, while temperature and humidity were recorded using a Reed temperature and humidity monitor model R6000.

Measurement of leaf physical traits and phenology of leaf bud burst

In 2021, we evaluated the effect of microhabitat heterogeneity on leaf quality by collecting 36 undamaged, fully expanded leaves per stratum per sampling date (total of 108 leaves per stratum = 3 leaves × 12 sites × 3 sampling dates). Sampling size was determined based on previous studies that measured leaf traits in a vertical gradient of deciduous forests (Corff & Marquis, 1999; Fortin & Mauffette, 2002; Murakami et al., 2005; Zvereva et al., 2020). The leaf sampling was synchronized with the herbivore damage sampling. Fresh leaves were weighed on-site, and their thickness was measured using a digital calliper. Subsequently, the leaves were pressed, labelled, transported to the lab, and dried in an oven for 72 h. Dried leaves were then weighed again, and all leaves were scanned to measure the leaf area using ImageJ software. All efforts were made to ensure that dried leaves remain intact and free from any folding or breaking to maintain measurement accuracy. This allowed us to determine water content and specific leaf area (SLA) for all sampled leaves, facilitating the comparison of leaf traits variation across the three strata.

In 2022, we conducted a comparative study on the phenology of leaf flush between the understory (sugar maple saplings) and the canopy (mature sugar maple trees). On May 10, 2022, we recorded the bud burst ranking by visually inspecting 10 buds on a single sugar maple sapling with the naked eye, and 10 buds on the canopy of mature trees adjacent to the selected sapling using binoculars. The bud burst ranking was assessed on a scale of 0–30; with 0 representing small buds, 10 indicating swollen buds, 20 indicating small leaves and 30 indicating fully expanded leaves (Hannerz, 1999). This recording process was repeated at 20 sites which were distinct from the 12 sites used for herbivory damage and leaf trait measurements but were located in close proximity to those sampled sites and exhibit similar tree species composition. Each site was spaced at least 10 m apart and included one mature sugar maple with a height of 10–15 m to assess canopy level bud burst timing and one sugar maple sapling with a height of 1–3 m representing the understory.

Insect herbivore species identification

To establish a link between the observed herbivory damage types to the actual insect herbivore community on sugar maple trees, we employed the beat sheeting method to collect insects from all three strata at the same 12 sugar maple sites used for herbivory damage assessments. Using the beat sheeting method, three branches from each stratum were struck with a 1-m length stick, while an 85 cm × 85 cm sheet with a detachable jar at the center was positioned beneath the branch. All organisms that dislodged onto the sheet were carefully collected in the jar. Subsequently, the jars containing samples from the canopy strata were lowered to the ground and filled with 70% ethanol to prevent predation on the herbivores within the container. Upon retrieval, the samples were sorted into morphotypes and identified to the lowest possible taxonomic level in the lab. Each sample was meticulously labelled, pinned and deposited at the Lyman Entomological Museum in St-Anne-de-Bellevue, QC, Canada. Although sampling was conducted during the summer of 2020, 2021 and 2022, repeated three times over the sampling season (June, July and August), technical issues resulted in the collection of fewer than 20 individual insect herbivores in the first 2 years, which were subsequently excluded from the analysis. Therefore, only data from 2022 were considered for analysis.

Data analysis

All the statistical analyses were completed using the R program and RStudio version 2024.04.1 Graphical representations of the overall and separate damage types, leaf traits, and insect herbivore abundance were all produced using the ggplot2 package in R (Wickham, 2016).

Insect herbivory damage

The recorded percentages for nine insect herbivory damage types were summed to obtain the total herbivory damage for each leaf. This total damage was then averaged for all 15 leaves providing an estimate of total herbivory damage on each stratum per sampling month. The total and individual percentage damage data were used as response variables for both years. No correlation was observed between damage types in the exploratory data analysis. Given the presence of response variables with percentage values and some zero-inflated damage types, as well as the inclusion of site as random factor, we opted to use generalized mixed models with glmmTMB package (Brooks et al., 2017). The first model with beta distribution included the effects of forest strata (sun canopy, shaded canopy, understory), the time of sampling (late June, late July, and mid-August), and their interaction with insect herbivore damage. Since no interaction was detected, we simplified the model by excluding the interaction between tree strata and sampling time. The total damage types, leaf cutters, leaf skeletonisers and leaf stipplers all followed beta distributions, and so we utilized the beta family in our model. However, the less common damage types such as leaf rollers, gall makers and leaf miners included more than 50% zero values, fitted in a model with the zero-inflated beta family. As no significant trend was shown over tree strata in individual gall maker and individual leaf miner damage types, these were pooled over similar feeding guilds. Therefore, the sum of the percentage of gall damage (spindle gall, erineum gall and ocellate gall) and the sum of the percentage of leaf miners (maple leaf-cutter and maple leaf-blotch miner) were used in the final statistical analysis using the zero-inflated beta family in glmmTMB model. All model assumptions were evaluated by DHARMa package (Hartig, 2022). Separate models were built for damage types in each sampling year.

Leaf physical traits and phenology of leaf bud burst

To observe the trend in leaf traits in 2021 dataset, including SLA, thickness and water content across the vertical strata of sugar maple trees over the sampling season, we used a linear mixed model with lme 4 package including tree stratum and sampling month as fixed factors and site as a random factor (Bates et al., 2015).

Since the leaf trait data were collected at the same time and the same sites with herbivory damage data in 2021, it enabled us to perform redundancy analysis (RDA) to model our damage type response variables as a function of predictor variables including leaf traits and tree strata. RDA results are shown in a correlation triplot with leaf traits as quantitative explanatory variables shown by arrows, strata as qualitative explanatory variables demonstrated by circles, and our damage trait response variables shown by lines (Zuur et al., 2007).

Bud burst phenology of understory and canopy leaves were recorded in 2022 but from different sites from the other leaf traits, therefore it was not included in the RDA analysis. Instead, to test the difference between the bud burst phenology of those two strata, we conducted a Welch's *t*-test for unequal variances (Welch, 1938).

Insect herbivore community

The variation in overall abundance of insect herbivores and abundance of various feeding guilds was assessed across a vertical gradient and over the growing season using generalized linear mixed model regression with glmmTMB package, with the site as a random factor (Brooks et al., 2017). Due to the low abundance of leaf skeletonisers and rollers, they were not included in statistical analysis. Total abundance of insect herbivores, leaf cutters and leaf stipplers were fit in the model with the Gaussian family. Models were simplified when no significant interaction between forest level and sampling date was observed. All model assumptions were evaluated by DHARMa package (Hartig, 2022).

RESULTS

Insect herbivore damage

In 2020, there was a significant increase in overall insect herbivory damage on leaves from sun canopy towards the shade canopy and understory ($z = 5.00$, $p < 0.001$) (Figure 2a,b; Table 1; Figure S1). While sampling periods and interaction between strata and sampling date did not significantly affect total herbivory damage on leaves, certain individual damage types exhibited notable patterns. Specifically, damage from leaf skeletonisers ($z = 3.43$, $p < 0.001$) and miners ($z = 2.28$, $p = 0.022$) significantly increased towards the end of the season, whereas leaf cutter damage ($z = -4.17$, $p < 0.001$) showed the opposite pattern (Figure 2b; Table 1; Figure S1). Regarding vertical stratification, damage caused by leaf cutters ($z = 2.36$, $p = 0.018$), leaf skeletonisers ($z = 4.21$, $p < 0.001$), leaf stipplers ($z = 5.08$, $p < 0.001$), and leaf miners ($z = 2.40$, $p = 0.016$) were significantly higher in the understory compared with the sun canopy. However, gall makers showed a reverse pattern, with significantly higher probability of showing zero values for damage in the understory ($z = -2.77$, $p = 0.005$) than the canopy (Figure 2a; Table 1; Figure S1).

In 2021, total damage did not show any significant changes across a vertical gradient of sugar maple trees. However, herbivory damage significantly increased towards the third sampling period ($z = 3.42$, $p < 0.001$), with no interaction between strata and sampling period (Figure 2c,d; Table 2; Figure S2). Damage caused by leaf stipplers ($z = 6.87$, $p < 0.001$), leaf miners ($z = 2.93$, $p = 0.00331$) and leaf rollers ($z = 7.71$, $p < 0.001$) increased towards the last sampling date, with leaf rollers having significantly higher probability of showing zeros values in the second sampling period ($z = -2.11$, $p = 0.03489$), while leaf skeletonisers caused more damage in the early season ($z = -2.33$, $p = 0.019$) (Figure 2d; Table 2; Figure S2). Significant vertical stratification was observed in damage caused by leaf cutters ($z = 2.53$, $p = 0.011$), leaf skeletonisers ($z = 2.06$, $p = 0.039$) and rollers ($z = 3.94$, $p < 0.001$) with higher values in the understory compared with the canopy level (Figure 2c; Table 2; Figure S2). Box plots for total herbivory and individual damage types for both sampling years are included in the supporting information.

Among the three environmental factors measured for three strata of sugar maple trees in 2021, including temperature, humidity, and light intensity, only light intensity showed a statistically significant difference between three strata ($\mathrm{df} = 96$, $t = -9.63$, $p < 0.001$) with

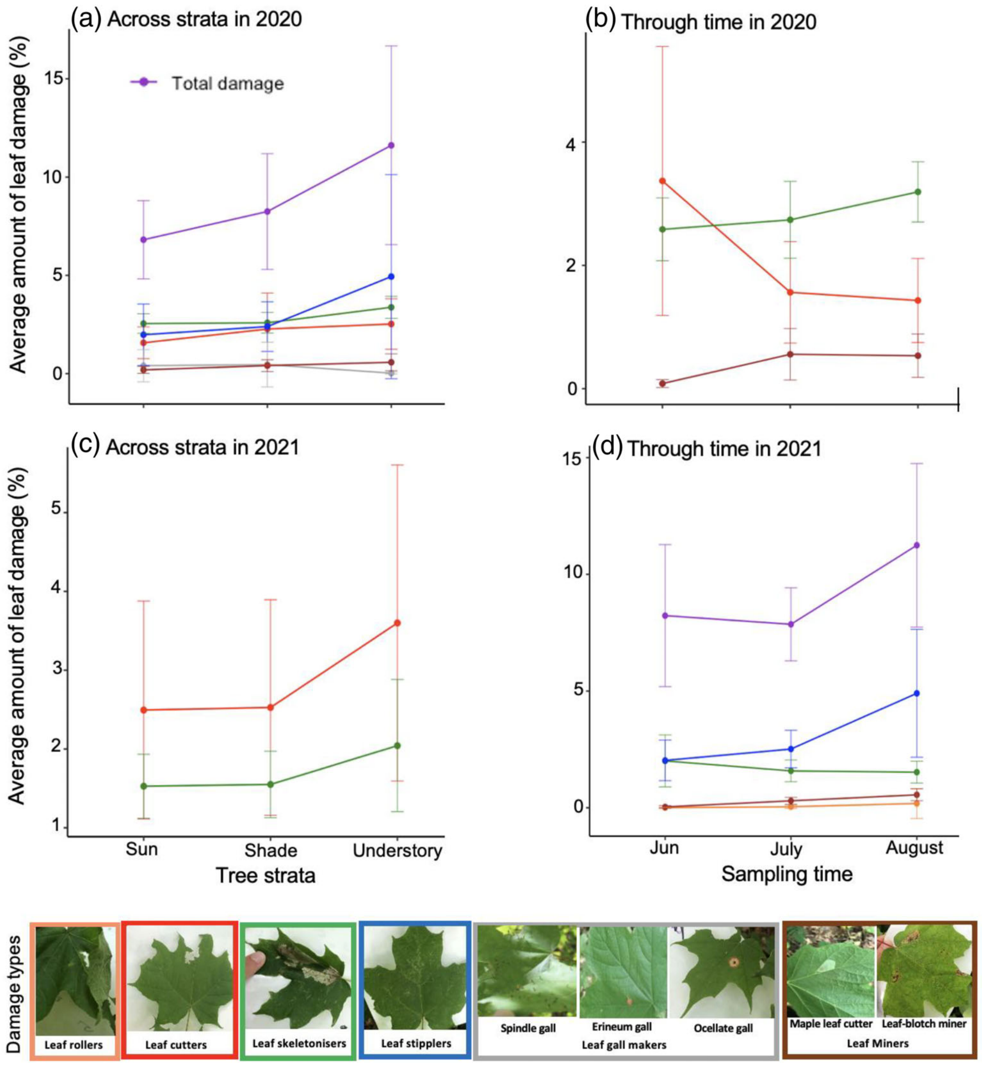

FIGURE 2 Line plots showing the variation in percentage of insect herbivory damage on leaf surface over three strata (sun leaves, shaded leaves, and understory) of sugar maple trees and through the sampling season (June, July and August) in 2020 and 2021(n = 180 leaves per stratum per sampling date = 1620). Only the damage types that significantly change through time and over vertical gradient are presented in these graphs. Error bars represent standard deviation (SD). (a) Insect herbivory damage over three vertical strata of sugar maple trees in 2020, (b) insect herbivory damage through the sampling season in 2020, (c) insect herbivory damage over three vertical strata of sugar maple trees in 2021 and (d) insect herbivory damage through the sampling season in 2021.

average value of 1120 ± SE 191 lux in the understory, 1363 ± SE 118 lux in the shaded canopy and 3187 ± SE 238 lux in the sun canopy for all three sampling sessions.

Insect herbivory damage—leaf traits correlation

Results showed a consistent seasonal pattern of change in leaf physical traits: SLA, water content, and thickness all decreased over the course of the season. This trend was consistent across all tree strata and was not affected by interactions between strata and sampling time (Figure 3; Table S1). Additionally, we found significant vertical variation in leaf traits with lower leaf thickness (df = 96, $t = -7.33$, $p < 0.001$), higher SLA (df = 96, $t = 15.51$, $p < 0.001$) and higher water content (df = 96, $t = 6.23$, $p < 0.001$) observed in the understory compared with the canopy level (Figure 3; Table S1). Detailed result table from the linear mixed model analysis of the effects of strata and time on leaf traits is provided in Supporting Information S1.

The Welch's test results of bud burst phenology recorded on sugar maple saplings and the canopy of mature trees indicate a statistically significant difference between the two groups ($df = 23.74$, $t = -7.96$, $p < 0.001$). On average, sapling exhibited a leaf flush ranking of 15.13 ± SE 0.40 (indicating the observation of very small leaves), while the canopy of mature sugar maples had an average ranking of 11.71 ± SE 0.14 (indicating the presence of swollen buds).

The RDA between herbivore damage and leaf traits demonstrated that the first two axes of the ordination explained a total of 16% of the variation in the data. Axis one explained 13.79%of the variation,

TABLE 1 Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on insect herbivore damage of 12 sugar maple trees at Kenauk nature in 2020.

							GlmmTMB zero-inflation beta distribution model					
	GlmmTMB beta distribution model						Conditional model			Zero-inflation model		
Predictors	Estimates	Std. error	*p*	Estimates	Std. error	*p*	Estimates	Std. error	*p*	Estimates	Std. error	*p*
	Total damage			**Leaf skeletonisers**			**Sum leaf gall makers**					
(Intercept)	−2.54	0.110	**<2e−16**	−3.73	0.067	**<2e−16**	−4.56	0.36	**<2e−16**	0.59	0.59	0.318
Shaded canopy	0.16	0.106	0.120	0.014	0.069	0.837	−0.22	0.33	0.501	−0.29	0.54	0.587
Understory	0.50	0.101	**5.73e−07**	0.27	0.065	**2.93e−05**	−0.34	0.52	0.514	1.99	0.72	**0.00553**
July	−0.07	0.101	0.4808	0.05	0.068	0.399	0.08	0.36	0.817	−0.81	0.58	0.161
August	−0.02	0.100	0.806	0.22	0.066	**0.00063**	0.12	0.43	0.768	0.77	0.63	0.221
	Leaf cutters			**Leaf stipplers**			**Sum leaf miners**					
(Intercept)	−3.70	0.148	**<2e−16**	−3.94	0.191	**<2e−16**	−5.98	0.28	**<2e−16**	1.47	0.55	**0.00859**
Shaded canopy	0.195	0.161	0.224	0.23	0.152	0.128	0.35	0.22	0.114	−0.93	0.62	0.137
Understory	0.372	0.157	**0.018189**	0.74	0.145	**3.63e−07**	0.52	0.21	**0.0163**	−1.58	0.67	**0.01886**
July	−0.507	0.153	**0.00094**	0.03	0.144	0.813	0.48	0.25	0.0633	−3.20	0.73	**1.43e−05**
August	−0.589	0.156	**0.000165**	0.16	0.140	0.235	0.59	0.26	**0.0222**	−2.38	0.60	**9.41e−05**

Note: Significant effects are indicated in bold. The table distinguishes between models with a beta distribution and zero-inflated beta distribution. The results include estimates, standard errors, and *p*-values for different predictors. Intercept is sun canopy strata. Leaf cutter damage, leaf skeletonisers and leaf stipplers, as well as total damage with beta distribution fit in the model: GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), family = beta_family (link = "logit"), data). The sum of leaf gall maker and the sum of leaf miner damage with zero-inflated beta distribution fit in model: GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), ziformula = ~ tree strata + sampling date + (1|site), family = beta_family(), data). The conditional model results account for the predictors' effects on leaf damage considering only the non-zero proportions. The zero-inflated model results account for how the predictors affect the probability of having zero damage. Leaf roller damage showed no significant change over strata and Sampling time so the results for that are not included here.

TABLE 2 Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on insect herbivore damage of 12 sugar maple trees at Kenauk nature in 2021.

							GlmmTMB zero-inflation beta distribution model					
	GlmmTMB beta distribution model						**Conditional model**			**Zero-inflation model**		
Predictors	**Estimates**	**Std. error**	***p***	**Estimates**	**Std. error**	***p***	**Estimates**	**Std. error**	***p***	**Estimates**	**Std. error**	***p***
	Total damage			**Leaf skeletonisers**			**Leaf rollers**					
(Intercept)	−2.45	0.108	**<2e−16**	−4.011	0.116	**<2e−16**	−8.32	0.66	**<2e−16**	2.93	0.86	**0.00067**
Shaded canopy	−0.037	0.097	0.69	0.010	0.109	0.924	−1.56	0.61	**0.0105**	1.865e−08	0.77	1.00
Understory	0.140	0.094	0.13	0.217	0.105	**0.039**	1.71	0.43	**8.12e−05**	−0.27	0.74	0.710
July	−0.008	0.098	0.92	−0.195	0.105	0.064	1.49	0.53	**0.00496**	−1.73	0.82	**0.03489**
August	0.307	0.093	**0.00105**	−0.254	0.108	**0.0196**	3.93	0.50	**1.23e−14**	−1.834e−07	1.02	1.00
	Leaf cutters			**Leaf stipplers**			**Sum leaf miners**					
(Intercept)	−3.69	0.16	**<2e−16**	−3.90	0.157	**<2e−16**	−6.07	0.31	**<2e−16**	2.07	0.67	**0.00224**
Shaded canopy	−0.021	0.13	0.869	−0.04	0.113	0.6833	−0.17	0.19	0.350	−0.81	0.75	0.279
Understory	0.314	0.12	**0.0113**	0.13	0.110	0.2397	0.11	0.17	0.533	−1.37	0.78	0.079
July	−0.018	0.12	0.8841	0.022	0.123	0.628	0.50	0.30	0.09878	−3.28	0.67	**1.38e−06**
August	−0.014	0.12	0.9056	0.78	0.114	**6.26e−12**	0.87	0.29	**0.00331**	−5.04	1.13	**7.99e−06**

Note: Significant effects are indicated in bold. The table distinguishes between models with a beta distribution and zero-inflated beta distribution. The results include estimates, standard errors, and *p*-values for different predictors. Intercept is sun canopy strata. Leaf cutter damage, leaf skeletonisers and leaf stippleres, as well as total damage with beta distribution fit in the model: GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), family = beta_family (link = "logit"), data). The leaf rollers and the sum of leaf miner damage with zero-inflated beta distribution fit in model: GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), ziformula = ~tree strata + sampling date + (1|site), family = beta_family(), data). The conditional model results account for the predictors' effects on leaf damage considering only the non-zero proportions. The zero-inflated model results account for how the predictors affect the probability of having zero damage. Sum of leaf gall maker showed no significant change over strata and sampling time so the results for that are not included here.

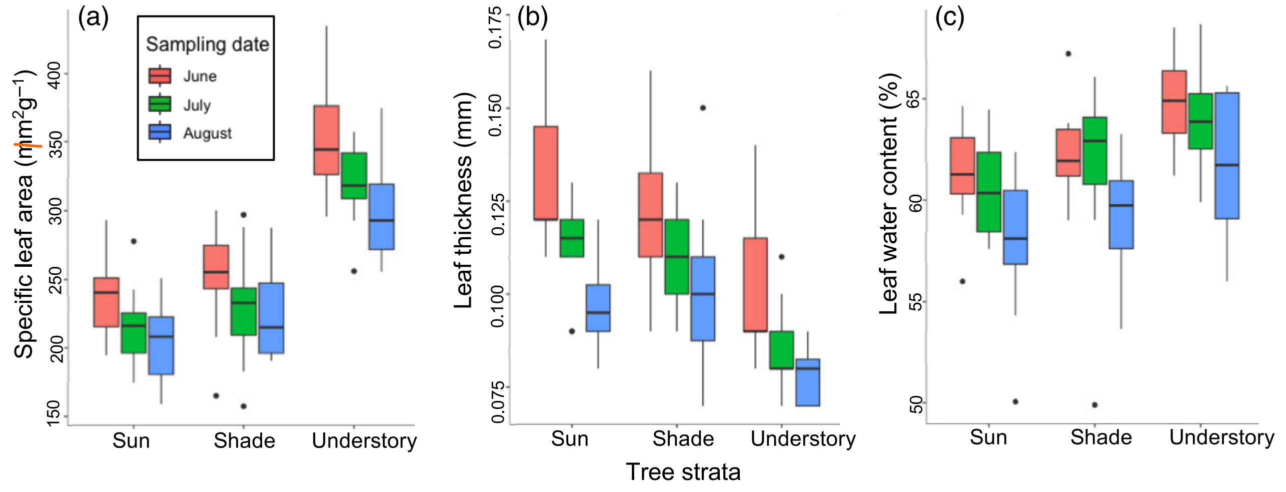

FIGURE 3 Boxplots showing variation of three measured leaf physical traits over three strata (sun leaves, shaded leaves and understory) of 12 sugar maple trees and through sampling season (June, July and August) in 2021 ($n = 36$ leaves per stratum per sampling date) (a) SLA on three strata over the sampling dates, (b) leaf thickness on three strata over the sampling dates and (c) leaf water content on three strata over the sampling dates.

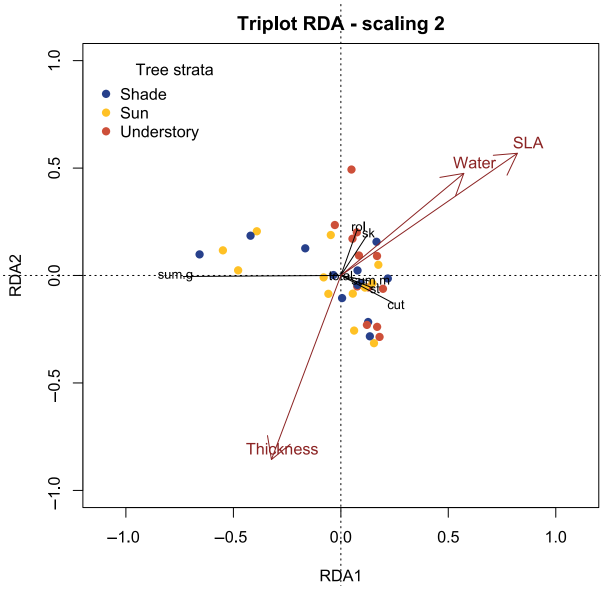

FIGURE 4 Scaling 2 redundancy analysis (RDA) correlation triplot showing the relation between quantitative explanatory variable (sugar maple SLA, leaf water content and leaf thickness demonstrated by brown arrows), the tree strata as the qualitative explanatory variable indicated by coloured circles (yellow circles = sun canopy, blue circles = shaded canopy and red circles = understory), and the six different damage types caused by insect herbivores and total herbivory damage on leaves shown by black lines (cut, leaf cutter; rol, leaf roller; sk, leaf skeletoniser; st, leaf stippler; sum.g, all gall makers; sum.m, all leaf miners; total, total damage). Angles between damage type lines and between leaf trait arrows represent correlation between damage and between leaf traits. Longer arrows mean variable has stronger effect on the variation in the community matrix. Leaf trait arrows pointing in opposite directions of another leaf trait arrow or damage line have a negative relationship with the representative leaf trait and damage type. Arrows and line pointing in the same direction have a positive relationship.

primarily driven by positive correlation with SLA and water content, and a negative correlation with leaf thickness. Similarly, axis 2 explained 2.17% of the variation, with positive correlations with SLA and water content, and a negative correlation with leaf thickness (Figure 4; Table 3).

Analysis of the RDA plot and leaf damage scores (Figure 4; Table 3) revealed the damage caused by cutters, stipplers, skeletonisers, miners and rollers contributed to the variation explained by axis one in descending order. This suggests that their variation is positively correlated with SLA and leaf water content, and negatively correlated with leaf thickness. Conversely, damage caused by gall makers exhibited a strong negative contribution to axis one, indicating that its variation is negatively associated with SLA and water content, and positively associated with leaf thickness. The Permutation test for RDA under the reduced model indicated that the RDA model approached statistical significance (df = 3, $F = 2.043$, $p = 0.052$) suggesting that the explanatory variables included in the model collectively contribute to explaining the variation in the response variables.

Insect herbivore community response

In this study, we collected a total of 604 arthropod individuals across all three strata of sugar maple trees in 2022. Out of these individuals, only 203 were identified as insect herbivores, while the rest consisted primarily of predatory spiders and mites, predatory hemipterans, as well as detrivore Dipterans and Psocopterans, a few fungivore Collembola, and hymenopteran parasitoids. Non-herbivore samples were identified only to the order level. Analysis of the data revealed that the abundance of leaf stippling was slightly higher in understory with no significant difference (Figure 5). Model results further indicated that the abundance of leaf cutters ($z = 3.78$, $p < 0.001$) and total insect herbivores ($z = 2.474$, $p < 0.001$) were significantly higher in

TABLE 3 Redundancy analysis (RDA) results showing the relation between leaf physical traits (thickness, water content, SLA) and insect herbivore damage on tree strata of 12 sugar maple sites at Kenauk Institute in 2021.

	RDA1	RDA2
Variance explained by each constrained axis		
Eigenvalue	0.00561	0.0008825
Proportion explained	0.13789	0.0216913
Cumulative proportion	0.13789	0.1595785
Explanatory variables score		
Thickness	−0.3229	−0.8563
Water content	0.5721	0.4755
SLA	0.8214	0.5690
Scores for insect herbivore damage		
Leaf roller	3.871e−02	1.086e−01
Leaf cutter	1.220e−01	−6.432e−02
Leaf skeletoniser	6.137e−02	9.452e−02
Leaf stippling	7.523e−02	−2.913e−02
Gall makers	−3.666e−01	−2.351e−03
Leaf miners	6.586e−02	−1.259e−02
Total damage	−2.859e−05	−5.434e−05

Note: Canonical axis values show axis contribution to the variance. Constraining variables scores and insect herbivore damage scores indicate how strongly each predictor variables and each damage type are associated with two axes. Damage types with higher scores along a particular axis contribute more to the variation explained by that axis and have strong correlations with the explanatory variables represented by that axis compared with species with lower scores (model: rda(formula= leaf damage ~ thickness + water content + SLA, data)).

the understory. Moreover, the total abundance of insect herbivores exhibits a significant decrease towards the last sampling date, primarily driven by a decline in the abundance of leaf cutters throughout the sampling season (Figure 5; Table S2). Detailed general mixed model results of herbivore abundance over time and between strata (Table S2), as well as a complete list of collected insect herbivore species and their abundance values, are presented in Table S3.

DISCUSSION

The results of our generalized mixed effect and RDA models suggest that vertical stratification in herbivore damage is related to variation in morphological traits of sun and shaded canopy on mature trees and understory leaves on sapling. Previous work on sugar maples has shown that sun canopy leaves have stronger physical defences such as increased toughness and thickness, compared with the more shaded leaves in understory (Fortin & Mauffette, 2002). In our study, consistently lower SLA and water content and thicker leaves in sun canopy over the sampling season support the idea that sun leaves have stronger physical defence and that these traits are linked to the lower damage rates observed on sun leaves compared with understory leaves. Low SLA make leaves thicker (Agrawal & Fishbein, 2006) and less palatable to herbivores (Wright et al., 2004). Leaves with lower SLA are suited to upper canopy conditions due to their compact structure, which helps them withstand strong winds and optimizes photosynthesis in high-light environments (Eisenring et al., 2021). However, our result indicates no significant differences in leaf traits and leaf damage between sun and shaded strata on mature three, while clear differences in leaf traits and herbivory damage were observed between saplings in understory and sun leaves on mature trees. This suggests that ontogeny effect may be stronger than the light intensity effect on insect herbivory.

The timing of bud burst can also influence herbivore damage on leaves in different strata. As expected, bud break in our study occurred earlier in sugar maple saplings than in tree canopies. The timing of bud burst, which depends on weather conditions and light intensity (Seiwa, 1998), has the potential to influence the period when foliage is most nutritionally beneficial for insect herbivores and to contribute to changes in herbivore assemblages (Fortin & Mauffette, 2002). This variation in the phenology of bud burst could lead to higher herbivory in the understory very early in the season, as these are the only leaves available (Murakami et al., 2005), but our sampling did not begin early enough in the season to record any such effect.

Within-species variation in herbivore damage has often been linked to plant defensive strategies, arguing that trees should invest more heavily in defending the most valuable leaves (Eisenring et al., 2021; Fortin & Mauffette, 2002): by this argument, mature trees should defend sun-leaves more than shade leaves (Dudt & Shure, 1994; Eisenring et al., 2021). Our results suggest that a vertical gradient in leaf defence can arise from leaf physical traits in response to photosynthesis, whereby the most valuable photosynthetically active leaves also inherently have highest physical defence without any investment in defence per se. However, these leaves also often have higher nutritional value: in sugar maple, sun leaves have higher sugar and protein content than shade leaves (Fortin & Mauffette, 2002; Levesque et al., 2002; Panzuto et al., 2001). Theory suggests that chemical defences should be invested mostly in these high-value leaves (Dudt & Shure, 1994; Eisenring et al., 2021). However, this is not what is observed in all related literature: in sugar maple, sun leaves contain lower concentrations of phenolics than shade leaves (Fortin & Mauffette, 2002; Levesque et al., 2002). This study showed that physical traits could protect these leaves without additional chemical defence.

Additionally, the vertical gradient implies differences in tree ontogeny, with young trees in understory and only mature trees in the upper canopy (Thomas et al., 2010). Optimal defence theory suggests that saplings with only few leaves should invest more in their defence than many-leaved mature trees (Boege & Marquis, 2006); however, this does not seem to be generally supported (Barton & Koricheva, 2010) and has not been observed in sugar maple (Thomas et al., 2010). Thomas et al. (2010) proposed that the decline in leaf palatability as trees age stems from a combination of selective pressures and physiological effects linked to increased tree size and

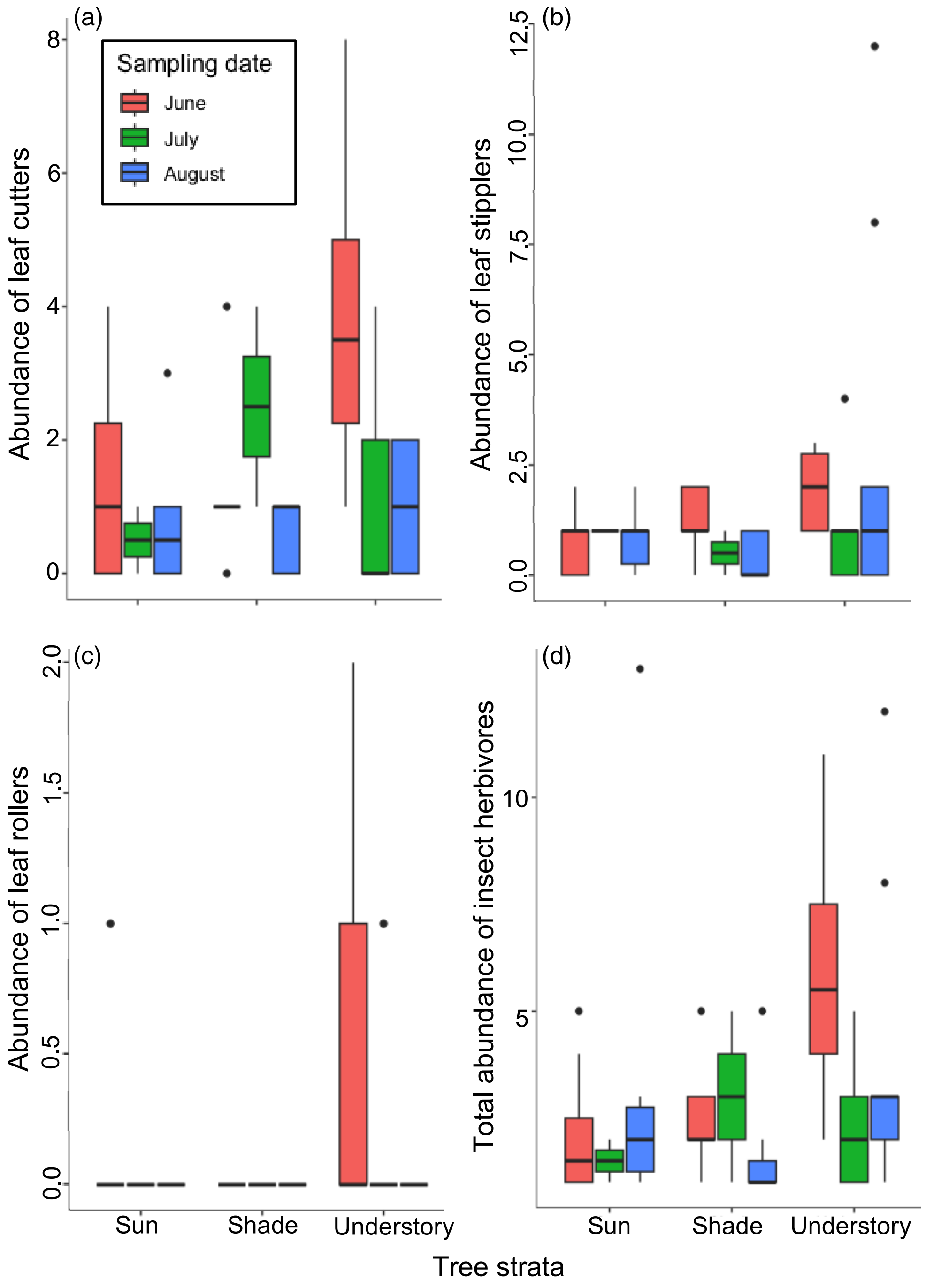

FIGURE 5 Box plots showing the variation of abundance of leaf cutter, leaf stippling, leaf roller and total insect herbivores collected on three strata (understory, shaded canopy, and sun canopy) of 12 sugar maple sites through the sampling season (June, July and August) at Kenauk nature in 2022. (a) Variation in abundance of leaf cutter insect herbivores, (b) variation in the abundance of leaf stippling insect herbivores, (c) variation in the abundance of leaf roller insect herbivores and (d) variation in the total abundance of insect herbivores.

reproductive investment. Consequently, leaves of mature trees are less susceptible to herbivory damage than the leaves of young saplings. Sugar maple saplings show a survivalist strategy by exhibiting a high root-to-shoot ratio and ceasing shoot growth early in the season (Gaucher et al., 2005); in this context, early bud burst phenology is essential to accumulate carbohydrates, but leaf herbivory is perhaps less costly since photosynthetic activity is mostly finished by the time leaf damage occurs.

Other factors that could also contribute to higher leaf protection in sun leaves include direct abiotic effects of high sunlight on insects (e.g. overheating and desiccation) or higher top–down pressure from natural enemies (Zvereva et al., 2020). Habitat heterogeneity linked to vertical stratification affects the interactions between parasitoids and predators and their phytophagous hosts (Sigut et al., 2018; Stireman et al., 2012; Zvereva et al., 2020). Although parasitism and predation rates on herbivores in a vertical gradient in deciduous forests are not well-researched (Sigut et al., 2018; Zvereva et al., 2020), a few studies suggested that stratification exists in both parasitoid and predator communities. We aimed to evaluate this top–down force in our study site as a future project.

Regarding the temporal changes in insect herbivory damage, studies have shown that sugar maple leaf quality changes significantly from spring to summer with an increase in leaf toughness and tannin content and a decrease in water and nitrogen contents (Fortin & Mauffette, 2002; Hunter & Lechowicz, 1992; Schultz et al., 1982). This pattern was confirmed in our study with spring leaves having higher SLA and water content. These variations in leaf quality will influence insect herbivores in diverse ways, notably due to differences in their feeding guilds and life histories (Eisenring et al., 2021; Murakami et al., 2005). Spring leaves are preferred by many leaf cutters (Murakami et al., 2005; Zehnder et al., 2009) and we observed higher cutter damage earlier in the season (Figure 2). Similarly, leaf roller damage is expected to be higher in the early season, which is not consistent with the result we had in 2021. This type of damage is primarily attributed to *Sparganothis* (Tortricidae) species (Houston et al., 1990; Thomas et al., 2010). We encountered six individuals of these species in our early season collections, but none later. These insects create nest and feed within them until pupating by the end of June (Hébert et al., 2017; Murakami et al., 2005; Thomas et al., 2010). By contrast, leaf skeletoniser damage, as was observed in 2020, increased through August due to the emergence of the maple trumpet skeletoniser larvae (*Castastega aceriella*) at the end of June and its increasing leaf consumption until pupation in September (Hébert et al., 2017; Thomas et al., 2010). These larvae were not yet active during the first sampling done in June. Similarly, leaf miner damage from species like *Paraclemensia acerifoliella*, which produces pale spots on sugar maple leaves, also increased until the end of summer before pupation (Hébert et al., 2017).

Our results overall supported the hypotheses: damage was generally highest in the understory. In 2020, total damage was higher in the understory and shaded canopy leaves, and most damage types, including leaf cutters and skeletonisers showed the same pattern. In 2021, similar patterns were seen in fewer damage types. There was also a strong seasonal pattern of increase in total damage from spring to summer. Looking at the monthly overall average temperature of 2020 and 2021 reported from the Kenauk weather station, it is shown that 2020 had a warmer July with an average of 21.75°C than July 2021 with 18.85°C. This may have contributed to differences in vertical stratification in herbivory patterns in the 2 years.

Based on a dataset of the percentage of leaf herbivory damage on many vascular plant species (Turcotte et al., 2014), the recorded annual rate of standing insect herbivory damage on sugar maple leaves in natural forests in the USA and Canada was 8.4% ($N = 6$ studies). To record insect herbivory damage in these studies, leaf samples ranged from 30 to 630, and excluded damage caused by gall makers (Turcotte et al., 2014). Another study by Thomas et al. (2010) on sugar maple reported a total insect herbivory damage of 11.5% on leaves and 7.7% insect herbivory damage excluding gall maker damage. This assessment was based on the examination of 1800 leaves exclusively from the canopy level (Thomas et al., 2010). In our study, we observed an average annual insect herbivore damage rate of 9.1% ± SE 0.35 (excluding gall maker damage, the rate was 8.1% ± SE 0.33). Our assessment involved a total of 1620 leaves, collected from both the canopy (1080 leaves) and understory (540 leaves) of sugar maple trees. The recorded annual herbivory damage rate in this study is consistent with the overall rate reported in Turcotte et al.'s dataset, despite the much smaller sample size in those studies. When examining canopy level insect herbivory only, our damage rate of 8.6% is slightly lower than that reported by Thomas et al. (2010).

An annual rate of 9.1% of background herbivory on sugar maples provides limited evidence to support a significant contribution of this type of herbivory to the decline of sugar maple forests. However, the higher levels of herbivory damage on saplings in the understory (10.5%) could impact the growth of these very small trees that already face challenging conditions of low light levels and high competition. In this context, even moderate levels of herbivory could contribute to decreasing sugar maple regeneration (Henry et al., 2021). However, previous research does not support an important role for background herbivory on the growth of sugar maple saplings (Henry et al., 2021; Strong et al., 2000).

Finally, sugar maple supports considerable invertebrate diversity (Maguire et al., 2014; Turgeon, 2019) and the diversity of insect herbivores collected from the understory, including various groups of insects, indicates the significance of this stratum in providing a habitat for herbivorous insects and preserving biodiversity. These insects play a crucial role as key trophic levels affecting forest ecosystem functions and production. While some of these insect herbivores may be pests, high insect diversity tends to decrease the severity of pest outbreaks and to improve forest resilience (Mina et al., 2022; Stemmelen et al., 2022).

CONCLUSION

Overall, this study shows that vertical stratification in maple trees affects herbivory patterns, with the most damage in the understory, followed by shade, then sun leaves in the canopy. This pattern varies among different herbivore feeding guilds: exposed feeders such as skeletonisers and cutters were more abundant in the understory, while gall makers as shelter builders were more present in the sun canopy. Leaf physical traits varied as expected according to the leaf economics spectrum showing greater thickness, lower SLA, lower water content and later phenology with more sun exposure; they were also strong predictors of herbivore damage. These results suggest that a defensive gradient arises from leaf functional traits in response to photosynthesis generating stronger physical defences in the most photosynthetically valuable sun leaves. Ontogenetic changes as trees grow from saplings in the understory to mature canopy trees, including earlier phenology in the understory than in the canopy, can also contribute to vertical gradients in leaf physical traits. We also show a temporal gradient in leaf physical traits as leaves become thicker and tougher during the growing season, which is linked to a shift in the insect herbivore community. Leaf functional traits in response to light gradients and tree age can thus contribute to structuring patterns of herbivory across vertical microhabitats in a temperate forest.

AUTHOR CONTRIBUTIONS

Mahsa Hakimara: Writing – original draft; formal analysis; visualization; methodology; software; data curation; conceptualization; project administration; investigation. **Emma Despland:** Writing – review and editing; funding acquisition; supervision; resources; methodology; investigation; conceptualization; project administration; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.4mw6m90h6.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on leaf traits of 12 sugar maple trees at Kenauk nature in 2021. Leaf thickness, leaf water content and leaf SLA fit in the model: lmer (leaf trait ∼ tree strata + sampling date + (1|site), data).

Table S2. Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on insect herbivore abundance on 12 sugar maple trees at Kenauk nature in 2022. Total abundance of insect herbivores and leaf cutters fit in the model: GlmmTMB (total abundance ∼ tree strata + sampling date + (1|site), family = gaussian, data). No significant change in skeletoniser and roller insects were detected so they are not included in the table. No gall maker and leaf miners were collected.

Table S3. Abundance of Insect herbivore species collected from three strata (sun leaves, shaded leaves, and understory) of 12 sugar maple trees through the sampling season at Kenauk Institute in 2022.

Figure S1. Box plots showing the variation of average percentage of leaf surface damaged by different insect herbivores between three strata (sun leaves, shaded leaves and understory) and through the sampling season on 12 sugar maple trees at Kenauk Institute in 2020 a) average of leaf cutters damage b) average of leaf skeletonisers damage c) average of leaf rollers damage d) average of leaf stippling damage e) average of leaf miners f) average of leaf gall makers and g) average of total insect herbivore damage.

Figure S2. Box plots showing the variation of average percentage of leaf surface damaged by different insect herbivores between three strata (sun leaves, shaded leaves and understory) and through the sampling season on 12 sugar maple trees at Kenauk Institute in 2021 a) average of leaf cutters damage b) average of leaf skeletonisers damage c) average of leaf rollers damage d) average of leaf stippling damage e) average of leaf miners f) average of leaf gall makers and g) average of total insect herbivore damage.

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