Spatial variation in leaf traits and herbivore community within the beech canopy between two different latitudes

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A concise and informative title: Spatial and latitudinal variation in beech leaf traits and herbivore community

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Abstract

To understand how the herbivore community on beech canopies varies between two different latitudes, we assessed leaf traits and herbivory by three major feeding types (chewing, mining, and galling) at different positions in the canopy using a scaffolding system along a 1400 km latitudinal gradient between Kuromatsunai (north) and Shiiba (south) in Japan. The chemical and morphological traits of the canopy foliage differed significantly between latitudes and between canopy parts. The leaf mass per area (LMA), leaf nitrogen, and carbon/nitrogen (CN) ratio was higher at south latitude than at north latitude. The upper canopy had a greater LMA, leaf nitrogen, and CN ratio than the lower canopy at both latitudes. On the other hand, herbivory by the three major feeding types differed significantly between latitudes and between canopy parts. The miner and galler densities were higher at south latitude than at north latitude, while the chewing herbivory was lower, showing different latitudinal patterns among feeding types. Among these feeding types, only chewing herbivory was higher in the lower canopy than in the upper canopy at both latitudes. The stepwise regression models showed that LMA and CN ratio explained spatial variation in chewing herbivory. Our study demonstrates that the latitudinal and spatial variations in leaf traits can play an important role in determining the latitudinal and spatial variation in the herbivore community on beech canopies via different responses of each feeding type.

Key words

canopy foliage; chewers; feeding types; gallers, miners; scaffolding system

Introduction

Herbivory generally increases toward the tropics (Coley and Aide 1991; Coley and Barone 1996). According to the literature reviews that used a variety of methodologies and plant species, the more favorable climatic conditions in the tropics throughout the year should allow insect herbivores to feed constantly, compared to temperate zones. Moreover, many studies that assessed insect communities along a latitudinal gradient compared samples that were fundamentally different not only in climate but also from varied habitats and evolutionary lineages (e.g., Moran and Southwood 1982; Majer et al. 2001). Therefore, it is possible that the purported latitudinal gradient in herbivory is an artefact of comparing different plant species (Andrew and Hughes 2005). To reduce the confounding factors that may influence results along latitudinal gradients, target samples should be collected from a single host plant species.

Recent several studies have used standardized methods on a single plant species to test for the existence of latitudinal variation in herbivory (Andrew and Hughes 2005; Kozlov 2008; Adams and Zhang 2009; Hiura and Nakamura 2013). Diverse empirical work suggests that a latitudinal gradient in herbivory does not exist, or that the trend is counter (reviewed by Moles et al. 2011) to what would generally be expected by Coley and coauthors (Coley and Aide 1991; Coley and Barone 1996). Biological and physicochemical factors (top-down, bottom-up, and climatic factors) vary with latitude and this variability may promote latitudinal variation in herbivory (Roininen et al. 2006; Bairstow et al. 2010; Marczak et al. 2011; Andrew et al. 2012). However, how these factors contribute to latitudinal variation in herbivory is poorly understood.

Host plant traits are key determinants (bottom-up factors) of the feeding preference, performance, and diversity of herbivorous insects (e.g. Feeny 1970; Strong et al. 1984). For example, carbon-based secondary metabolites (e.g. condensed tannin and total phenolics) may inhibit the digestion of herbivores (Rhoades and Cates 1976). Tougher leaves are better defended against herbivores physically (Coley 1983; Clissold et al. 2009). In contrast, nitrogen is a basic structure material and leaves with high nitrogen have more food value for herbivores (Schoonhoven et al. 1998), although a recent framework has pointed out that food value of nitrogen depends on environmental conditions (Simpson et al. 2009; Simpson and Raubenheimer 2012). In Japan, a geographic cline in *Fagus crenata* is recognized in which leaf size decreases gradually from northeast to southwest populations (Hagiwara 1977). A latitudinal gradient in tree crown shape related to leaf size has also been reported (Hiura 1998). These patterns may be related to the local environment to maximize assimilative efficiency (Parkhurst and Loucks 1972; Kuuluvainen 1992). Therefore, we postulate that a latitudinal variation in leaf traits that are adapted to the local environment may cause a difference in herbivory along a latitudinal gradient. Furthermore, because herbivore damage to plants is directly related to the total abundance and biomass of associated herbivores (Fox and Morrow 1983), the abundance of herbivorous insects may also vary along a latitudinal gradient.

In mature forests, most biological activity and species diversity appear to be concentrated in the canopy (Mulkey et al. 1996; Basset et al. 2003). Previous studies that have estimated herbivore damage in the canopy at a single point and time have probably underestimated the damage. Spatial variation in leaf traits is often observed within the canopy of a single tree (Murakami et al. 2005; Nakamura et al. 2008). Light decreases quickly from the external surface to a few centimeters inside the canopy (Mulkey et al. 1996). Since light is one of the major factors that regulate photosynthesis, spatial variation in light conditions causes spatial variation in leaf traits (Mulkey et al. 1996; Yamasaki and Kikuzawa 2003). Such spatial variation in leaf traits should change with time (Yamasaki and Kikuzawa 2003). However, few studies have examined the temporal pattern of the spatial variation in leaf traits along a latitudinal gradient.

Therefore, we examined how the major feeding types (chewing, mining, and galling) of herbivorous insects varied along a 1400-km latitudinal gradient in Japan during two seasons (spring and summer). We used two standardized methods to make our assessments: direct observation of the canopy using a scaffolding system and canopy knock-down. We addressed the following questions: 1) How do the chemical and morphological traits of canopy foliage differ between latitudes and between canopy parts? 2) How does herbivory (chewing, mining, and galling) differ between latitudes and between canopy parts? 3) What leaf traits are responsible for spatial variation in herbivory (chewing, mining, and galling)?

Materials and Methods

Study Area

Fagus crenata is distributed throughout the cool temperate zone of Japan. We chose two mature forests located at two different latitudes: Kuromatsunai (42°39'N, 140°18'E, north latitude) and Shiiba (32°28'N, 130°48'E, south latitude). Kuromatsunai is located at the northern distributional limit of the beech forest, while Shiiba is located 100 km north of the southern distributional limit of the beech forest on Mt. Takakuma. The average annual temperature is 7.1 and 9.2°C at Kuromatsunai and Shiiba, respectively. In 2005, builders scaffolding (length 6 m, width 6 m, height 18 m) was constructed to observe the canopy crown at each study site. Scaffolding was built around canopy trees of *F. crenata* to gain access to the canopy. In total, we observed six canopy trees (height = 10–20 m) at Kuromatsunai and four at Shiiba.

Measurements

In 2007, we randomly selected 5 to 10 branches from both the upper and lower canopies of each tree, and labeled these branches. To determine the latitudinal and spatial variations in chemical and morphological traits of the canopy foliage that may be associated with herbivores, we sampled a leaf with minimal herbivory (less than 10%

leaf loss) from each labeled branch in spring (Kuromatsunai, 16 May; Shiiba, 12 May) and summer (Kuromatsunai, September 14; Shiiba, August 27). After returning to the laboratory, we punched 10 disks (5 mm in diameter) out of each leaf. These samples were oven-dried at 40°C for at least 3 days. The mean leaf mass per area (LMA) was calculated for each leaf. The concentrations of carbon and nitrogen in each leaf were measured using a CN analyzer (NC-900, SUMITOMO, Japan), and the carbon/nitrogen (CN) ratios were then calculated. According to the carbon nutrient balance hypothesis, an increase in the CN ratio implies that carbon becomes relatively more available for carbon-based secondary metabolites (Bryant et al. 1983), which are thought to inhibit digestion by herbivores (Rhoades and Cates 1976).

To identify latitudinal and spatial variation in the herbivore community, we observed herbivores on each labeled branch directly. When assessing the herbivore community, classifying species into feeding types that are ecologically and evolutionary relevant (Root 1973; Simberloff and Dayan 1991) allows comparisons and generalizations to be made that are impossible using taxonomic grouping alone (Landsberg et al. 1989; Andrew and Hughes 2004, 2005). We classified the herbivores into one external (chewing herbivory) and two internal (miners and gall-forming or gallers) feeding types. We scored chewing herbivory for each leaf visually and translated the percentages into six ranked indices as follows: 0% = 0, 1–10% = 1, 11–25% = 2, 26–50% = 3, 51–75% = 4, and 76–100% = 5. In addition, we counted the numbers of miners and gallers on each leaf to calculate miner and galler densities.

In 2008, to determine the latitudinal variation in chewer abundance and order composition, we conducted canopy knock-down at two plots at each latitude in spring (Kuromatsunai, 16 May; Shiiba, 4–6 May) and summer (Kuromatsunai, 4–5 September; Shiiba, 26 August). Canopy knock-down is a very effective technique for collecting herbivorous insects from the forest canopy (Stork et al. 1997). We used the technique outlined in the protocol manual for DIWPA-IBOY (Toda and Kitching 2002). In each plot, we sprayed the selected tree with 10 L of pyrethrum–water solution (the concentration of pyrethrum = 0.2 mL/L) using a fogging machine (Portable Mister 423 Port, Solo, Germany) suspended in the tree with a rope-pulley system. Fallen arthropods were collected for 3 h after fogging using 20 collecting trays (80 cm in diameter). These samples were stored in 80% alcohol and then identified in the laboratory.

Statistical Analyses

For response variables of miners and gallers, miner and galler numbers per leaf were log (n+1)-transformed to satisfy the assumption of normal distribution. For response variable of chewers, the median of each class was used for statistical analysis of chewing herbivory (i.e. 0%, 5%, 18%, 38%, 63%, and 88%). Average response variable per individual tree of each feeding type was calculated. Individual trees were used as replicates. A two-way analysis of variance (ANOVA) was used to examine the effects of latitude, canopy part, and their interaction on leaf traits and feeding types of herbivorous insects in spring and summer. At each latitude, a stepwise multiple regression model was used to determine the leaf traits that contributed most to the observed spatial variation in herbivory (chewing, mining, and galling) within the canopy in summer. We removed from the stepwise models leaf traits for which values of the variation inflation factor (VIF) exceeded 10 because VIF provides a measure of the extent to which variance of an estimated regression coefficient is increased by multi-collinearity. On the other hand, no statistical analysis was applied to chewer abundance because canopy knock-down was conducted at only two plots (replicates) at each latitude and in each season.

Results

Leaf traits

The chemical and morphological traits of the canopy foliage differed significantly between latitudes (Table 1). In spring, all of the measured leaf traits (LMA, Nitrogen, and CN ratio) were higher at south latitude than at north latitude (LMA, $d.f. = 1$, $F < 54.199$, $P < 0.001$; Nitrogen, $d.f. = 1$, $F < 24.640$, $P < 0.001$; CN, $d.f. = 1$, $F < 11.473$, $P < 0.001$; Fig. 1a-c). In summer, LMA and leaf nitrogen also was higher at south latitude than at north latitude (LMA, $d.f. = 1$, $F = 11.115$, $P = 0{,}005$; Nitrogen, $d.f. = 1$, $F = 32.524$, $P < 0.001$; Fig. 1d, e), while the CN ratio did not change between latitudes ($d.f. = 1$, $F = 0.004$, $P = 0.952$; Fig. 1f). In addition, there were significant spatial variations in leaf traits (Table 1). In spring, LMA and leaf nitrogen were higher in the upper canopy than in the lower canopy (LMA, $d.f. =1$, $F = 30.181$, $P < 0.001$; Nitrogen, $d.f. = 1$, $F = 21.751$, $P < 0.001$; Fig. 1a, b). In summer, all of the measured leaf traits were higher in the upper canopy than in the lower canopy (LMA, $d.f. = 1$, $F = 29.910$, $P < 0.001$; Nitrogen, $d.f. = 1$, $F = 47.668$, $P < 0.001$; CN, $d.f. = 1$, $F = 10.048$, $P = 0.006$; Fig. 1d-f).

Insect herbivory

Herbivory by the three different types of herbivores differed significantly between latitudes (Table 2). In spring, chewing herbivory did not change between latitudes (*d.f.* = 1, F = 1.652, P = 0.218; Fig. 2a), while miner and galler densities were higher at south latitude than at north latitude, although the densities were very low (miner, *d.f.* = 1, F = 5.563, P =0.032; galler, *d.f.* = 1, F = 8.066, P = 0.012; Fig. 2b, c). In summer, gall density was also higher at south latitude than at north latitude, while chewing herbivory was lower (galler, *d.f.* = 1, F = 14.217, P = 0.002; chewer, *d.f.* =1, F = 71.243, P < 0.001; Fig. 2d, f). There was a significant spatial variation in chewing herbivory only in summer (Table 2). Chewing herbivory was greater in the lower canopy than in the upper canopy at both latitudes (*d.f.* =1, F = 14.629, P =0.002; Fig. 2d).

Relationships between herbivory and leaf traits at each latitude

The stepwise multiple regression models showed that LMA explained spatial variation in chewing herbivory at north latitude (Table 3). LMA was negatively correlated with chewing herbivory (F = 6.777, P = 0.029). On the other hand, CN ratio was selected for explaining spatial variation in chewing herbivory at south latitude and was negatively correlated with chewing herbivory (F = 43.807, P = 0.001). However, any leaf traits did not explain spatial variation in miner and galler densities at both latitudes (P > 0.05).

Chewer abundance

In summer, chewer abundance clearly was higher at north latitude (plot 1 = 50 individuals, plot 2 = 19) than at south latitude (plot 1 = 11, plot 2 = 8), while there was no latitudinal difference in spring (Table 5). Lepidoptera (larvae) was the dominant order of chewers collected in both spring and summer.

Discussion

Latitudinal variation in leaf traits and herbivory

Consistent with previous findings that plant defenses increased toward the tropics (Coley and Aide 1991), we found that LMA and the CN ratio were higher at south latitude than at north latitude. Such leaf traits likely form under the light and moisture environment that occurs during leaf flushing: high light irradiance and drought conditions in southwestern Japan result in smaller, thicker leaves, while low light irradiance and humid conditions in northeastern Japan result in larger, thinner leaves (Parkhurst and Loucks 1972; Koike et al. 1990; Hiura 1993). Furthermore, we found that leaf nitrogen per area was higher at south latitude than at north latitude. This is probably due to the increase in leaf thickness, which can influence area-based photosynthetic rates (Kuuluvainen 1992; Hiura 1998) and is strongly correlated with leaf nitrogen per area (Hikosaka 2004). In addition, temperature may be related to leaf nitrogen through a change in nutrient availability in soil because high soil temperatures at south latitudes can promote mineralization causing nutrient release from mineral soil and biological nitrogen fixation (Körner 1999).

In general, herbivory is thought to increase toward the tropics (Coley and Aide

1991; Coley and Barone 1996). We found that the densities of both gallers and miners were higher at south latitude than at north latitude, while chewing herbivory was lower. These different latitudinal patterns among feeding types can be explained by different responses to the latitudinal variation in leaf traits. In spring, we found that galler density and LMA were simultaneously higher at south latitude than at north latitude, probably because the greater amount of mesophyll enabled larvae to make galls inside the leaves. The latitudinal variation in gall density was maintained until summer because it is likely that most gall insects oviposit in spring (Komatsu and Akimoto 1995; Yukawa and Masuda 1997) and appear in summer. Furthermore, miner density and LMA were simultaneously higher at south latitude than at north latitude, likely because miners can increase in body size within a thicker leaf and potentially bypass the chemical and mechanical defenses affecting external feeders (Feath et al. 1981; Connor and Taverner 1997). The galler and miner densities and leaf nitrogen showed same latitudinal patterns. Foliage with high nitrogen is likely a higher-quality food for these herbivores (Schoonhoven et al. 1998).

In comparison, we found that chewing herbivory was lower at south latitude than at north latitude in 2007. Hiura and Nakamura (2013) also reported that chewing herbivory decreased toward low latitudes in 2005 and 2006, suggesting that among-year variation in the herbivore population density did not affect the latitudinal patterns in chewing herbivory. LMA was higher at south latitude than at north latitude. This implies that the increased physical defense of leaves decreased chewing herbivory. Tougher leaves are reported to be better defended against herbivores physically (Coley 1983; Clissold et al. 2009). Hiura and Nakamura (2013) also showed that latitudinal variation in one constitutive leaf trait (LMA) best explained latitudinal variation in chewing herbivory in the common-garden experiment of Japanese beech. Moreover, chewing herbivory and leaf nitrogen showed different latitudinal patterns. This result is inconsistent with the previous findings that nitrogen is positively correlated with herbivory (Schoonhoven et al. 1998). Two potential mechanisms explain this paradox. First, it is possible that herbivory is an artificial correlate with leaf nitrogen because leaf nitrogen was strongly correlated with LMA (r = 0.961, $P < 0.0001$) (Hikosaka 2004). Second, it is possible that herbivorous insects increase their consumption rate to compensate for the reduced nitrogen content (Slanshy and Scriber 1985). In addition to the effect of leaf traits, there is another possibility that tree diversity may reduce chewing herbivory (Jaxtel et al. 2007), because tree diversity is higher at Shiiba (south) than at Kuromastunai (north) (Suzuki et al. 2012).

Temperature is a very important factor directly affecting insect herbivore abundance through the modulation of survival, development rates, and dispersal (reviewed by Bale *et al.*, 2002). This leads to a prediction that insect herbivore abundance may increase toward south. However, we found that chewer abundance was lower at south latitude than at north latitude in summer, similar to the pattern in chewing herbivory. Herbivore damage to plants is directly related to the total abundance of associated herbivores (Fox and Morrow 1983). This suggests that the latitudinal variation in leaf traits (LMA and leaf nitrogen) was also responsible for chewer abundance.

Spatial variation in leaf traits and herbivory among canopy parts

The canopy is a spatially heterogeneous environment (Murakami et al. 2005; Nakamura et al. 2008). We found spatial variation in the LMA, leaf nitrogen, and CN ratio within the canopy at both latitudes. The spatial variation in light conditions within the canopy likely causes the spatial variation in leaf traits (Mulkey et al. 1996; Yamasaki and Kikuzawa 2003). In contrast, no temporal change in spatial variation was found, except for the CN ratio. Because the spring census was conducted several weeks after leaf flushing, the leaves in both the upper and lower canopies may have already matured. If we conducted our observations immediately after leaf flushing, a temporal change in the spatial variation in leaf traits might have been detected, similar to a previous study (Yamasaki and Kikuzawa 2003).

Yamasaki and Kikuzawa (2003) reported that herbivores started eating *F. crenata* leaves immediately after leaf flushing at all canopy levels, but further increases in herbivory after June were observed mainly in the lower canopy. Similarly, the pattern of spatial variation in chewing herbivory changed with time at both latitudes: no spatial variation in herbivory was observed in spring, while herbivory was higher in the lower canopy than in the upper canopy in summer. Such spatial variation in summer may be related to food available for chewer herbivores. The stepwise regression models showed that LMA explained spatial variation in chewing herbivory at north latitude, while CN ratio explained at south latitude. Such different leaf traits selected by the model between latitudes may be related with a latitudinal variation in tree crown shapes of Japanese beech, which change from domed at south latitude to even columnar shaped at north latitude (Hiura 1998). Larger spatial variation in light conditions within the canopy due to columnar crown shape at north latitude may strength physical defense due to the 98%

greater LMA in the upper canopy than in the lower canopy. However, in case of smaller spatial variation in light conditions due to domed crown shape at south latitude, chemical defense (CN ratio) may be higher than physical defense because the upper canopy had only 62% increased LMA.

In conclusion, we demonstrated that herbivore communities differed between latitudes. Previously, Coley and Aide (1991) reported that herbivory generally increased towards the tropics. They suggested that higher herbivory in the tropics could result from greater herbivore pressure because of the more favorable climatic conditions for herbivores in the tropics throughout the year. In contrast to previous studies, we studied a single deciduous species, *F. crenata*, in which leaf lifetime differs little between north and south latitudes (Hiura and Nakamura 2013). Our results suggest that the latitudinal gradient for each feeding type on a single plant species can be explained by leaf traits rather than herbivore pressure, as suggested by Coley and Aide (1991). At both latitudes, spatial variation in leaf traits and chewing herbivory was also observed within the canopy. Therefore, to understand the latitudinal and spatial variation in herbivore communities on a single plant species, future studies should focus on the latitudinal and spatial variation in leaf traits, which acclimate or adapt to each environment.

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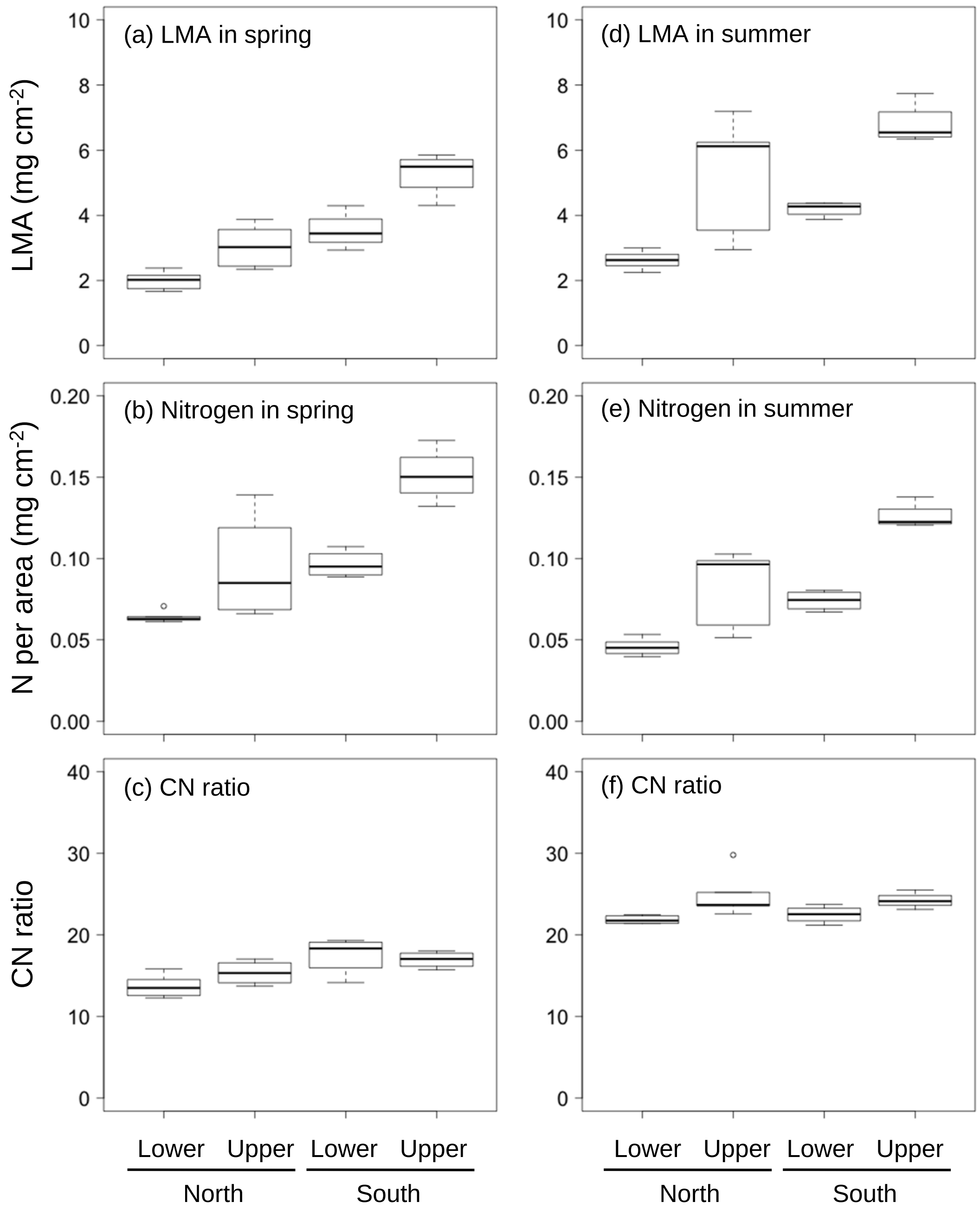
Figure legend

Figure 1. (a, d) LMA, (b, e) leaf nitrogen, (c, f) CN ratio of leaves on lower and upper canopy parts at north (Kuromastunai, n = 6) and south (Shiiba, n = 4) latitudes in spring and summer.

Figure 2. (a, d) cheweing herbivory (%), (b, e) the log (n +1)-transformed miner number per leaf, (c, f) the log (n + 1)-transformed galler number per leaf on lower and upper canopy parts at north (Kuromastunai, n = 6) and south (Shiiba, n = 4) latitudes in spring and summer.

Nakamura et al. Figure 1



Nakamura et al. Figure 2

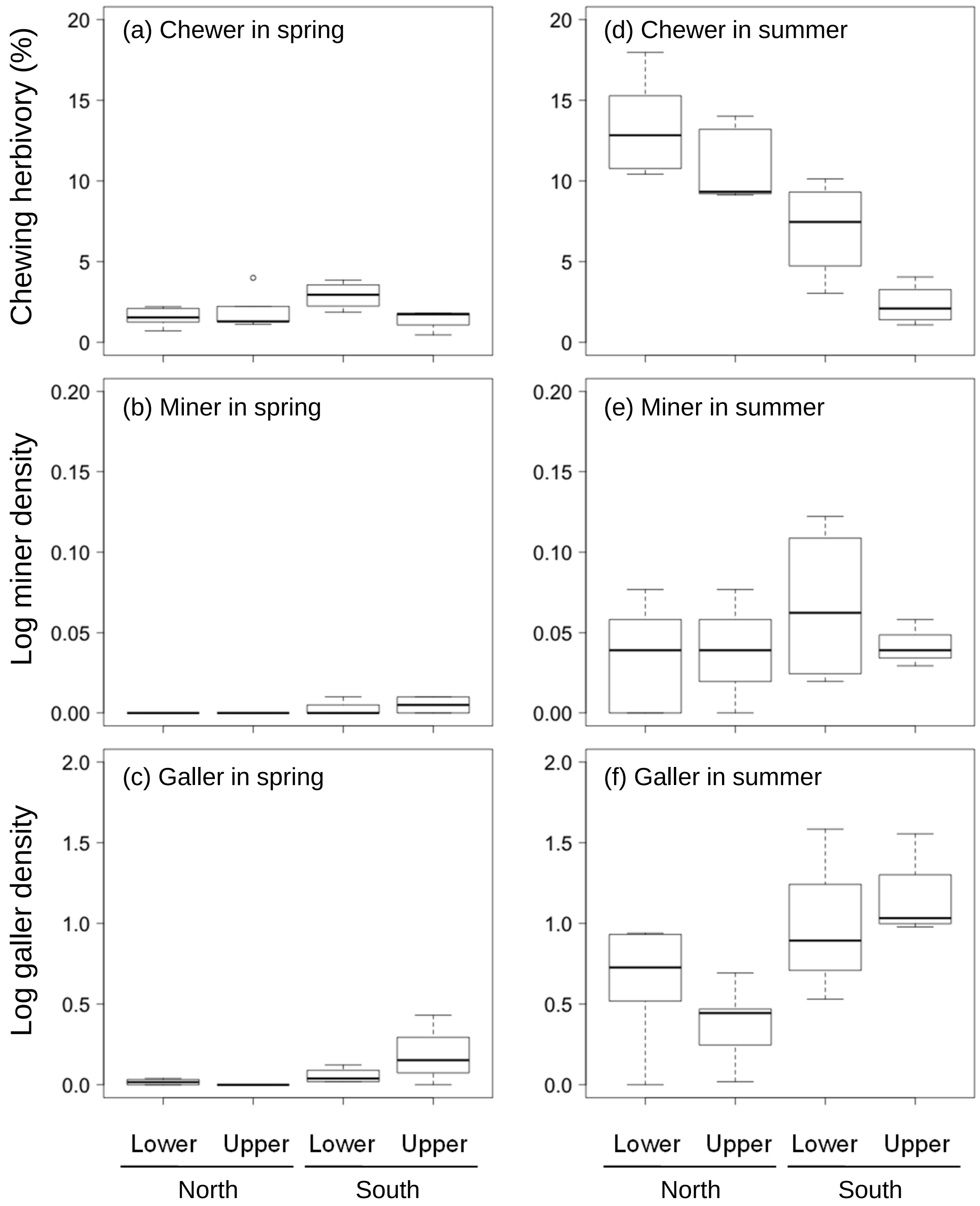


Table1 Two-way ANOVA for the effects of latitude, canopy part, and their interaction on LMA, nitrogen, and CN ratio in spring and summer. Significant values are in bold.

Season	Leaf traits	Factor	SS	d.f.	MS	*F* value	*P* value
Spring	LMA	Latitude	16.336	1	16.336	54.199	**< 0.001**
		Canopy part	9.097	1	9.097	30.181	**< 0.001**
		L x C	0.574	1	0.574	1.904	0.188
		Error	4.521	15	0.301		
	Nitrogen	Latitude	0.0085	1	0.0085	24.640	**< 0.001**
		Canopy part	0.0075	1	0.0075	21.751	**< 0.001**
		L x C	0.0007	1	0.0007	1.848	0.186
		Error	0.0044	15	0.0003		
	CN ratio	Latitude	30.689	1	30.689	11.473	**0.005**
		Canopy part	1.138	1	1.138	0.425	0.526
		L x C	5.051	1	5.051	1.888	0.193
		Error	34.775	15	2.318		
Summer	LMA	Latitude	7.979	1	7.979	11.115	**0.005**
		Canopy part	21.471	1	21.471	29.910	**< 0.001**
		L x C	0.000	1	0.000	0.044	0.995
		Error	10.996	15	0.733		
	Nitrogen	Latitude	0.0061	1	0.0061	32.524	**< 0.001**
		Canopy part	0.0089	1	0.0089	47.668	**< 0.001**
		L x C	0.0003	1	0.0003	1.526	0.236
		Error	0.0028	15	0.0002		
	CN ratio	Latitude	0.010	1	0.0100	0.004	0.952
		Canopy part	27.003	1	27.0030	10.048	**0.006**
		L x C	2.198	1	2.1980	0.818	0.380
		Error	40.310	15	2.6873		

Table 2 Two-way ANOVA for the effects of latitude, canopy part, and their interaction on chewing herbivory (%), the log (n + 1)-transferred miner number per leaf, and the log (n + 1)-transferred galler number per leaf in spring and summer. Significant values are in bold.

Season	Feeding types	Factor	SS	d.f.	MS	*F* value	*P* value
Spring	Chewing herbivory	Latitude	0.015	1	0.015	1.652	0.218
		Canopy part	0.025	1	0.025	2.628	0.126
		L x C	0.046	1	0.046	4.875	**0.043**
		Error	0.140	15	0.009		
	Log mine density	Latitude	0.000064	1	0.000064	5.563	**0.032**
		Canopy part	0.000007	1	0.000007	0.618	0.444
		L x C	0.000007	1	0.000007	0.618	0.444
		Error	0.000173	15	0.000012		
	Log gall density	Latitude	0.057	1	0.057	8.066	**0.012**
		Canopy part	0.014	1	0.014	2.048	0.173
		L x C	0.025	1	0.025	3.533	0.080
		Error	0.105	15	0.007		
Summer	Chewing herbivory	Latitude	2.934	1	2.934	71.243	**< 0.001**
		Canopy part	0.603	1	0.603	14.629	**0.002**
		L x C	0.037	1	0.037	0.900	0.358
		Error	0.618	15	0.041		
	Log mine density	Latitude	0.0024	1	0.0024	2.124	0.166
		Canopy part	0.0013	1	0.0013	1.157	0.299
		L x C	0.0003	1	0.0003	0.288	0.600
		Error	0.0169	15	0.0011		
	Log gall density	Latitude	1.673	1	1.673	14.217	**0.002**
		Canopy part	0.040	1	0.040	0.339	0.569
		L x C	0.329	1	0.329	2.798	0.115
		Error	1.765	15	0.118		

Table 3 Stepwise multiple regression model for chewing herbivory, the log (n + 1)-transferred miner number per leaf, and the log (n + 1)-transferred galler number per leaf at each latitude in summer. We deleted leaf traits with vales of VIF exceeding 10 to reduce the impact of multicolinearity. Significant values are in bold.

Latitude	Model	Effect	VIF	F-value	P-value
North	Chewing herbivory = -0.105*LMA	CN	3.935	0.007	0.789
		LMA	3.935	6.777	**0.029**
	Log mine density	CN	3.935	0.292	0.602
		LMA	3.935	0.473	0.510
	Log gall density	CN	3.935	2.519	0.147
		LMA	3.935	0.068	0.802
South	Chewing herbivory = -0.216*CN	CN	2.851	43.807	**0.001**
		LMA	2.851	0.448	0.533
	Log mine density	CN	2.851	0.451	0.527
		LMA	2.851	0.654	0.450
	Log gall density	CN	2.851	0.100	0.763
		LMA	2.851	0.067	0.805

Table 4 The number of chewer individuals on each order with canopy knock-down at two plots each in north (Kuromatsunai) and south (Shiiba) latitudes, in spring and summer.

	Spring				Summer			
	North		South		North		South	
Taxon	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Lepidoptera (larva)	6	19	15	18	48	19	6	5
Hymenoptera (larva)		6	2	4				
Coleoptera	1	1	6	1	1		5	2
Orthoptera				1				1
snail					1			
Total	7	26	23	24	50	19	11	8