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1 **Spatial variation in leaf traits and herbivore community within the beech canopy**

2 **between two different latitudes**

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

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17

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

36 community on beech canopies via different responses of each feeding type.

37

38 **Key words**

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

40

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

77 may be related to the local environment to maximize assimilative efficiency (Parkhurst
78 and Loucks 1972; Kuuluvainen 1992). Therefore, we postulate that a latitudinal
79 variation in leaf traits that are adapted to the local environment may cause a difference
80 in herbivory along a latitudinal gradient. Furthermore, because herbivore damage to
81 plants is directly related to the total abundance and biomass of associated herbivores
82 (Fox and Morrow 1983), the abundance of herbivorous insects may also vary along a
83 latitudinal gradient.
84
85 In mature forests, most biological activity and species diversity appear to be
86 concentrated in the canopy (Mulkey et al. 1996; Basset et al. 2003). Previous studies
87 that have estimated herbivore damage in the canopy at a single point and time have
88 probably underestimated the damage. Spatial variation in leaf traits is often observed
89 within the canopy of a single tree (Murakami et al. 2005; Nakamura et al. 2008). Light
90 decreases quickly from the external surface to a few centimeters inside the canopy
91 (Mulkey et al. 1996). Since light is one of the major factors that regulate photosynthesis,
92 spatial variation in light conditions causes spatial variation in leaf traits (Mulkey et al.
93 1996; Yamasaki and Kikuzawa 2003). Such spatial variation in leaf traits should change
94 with time (Yamasaki and Kikuzawa 2003). However, few studies have examined the

95 temporal pattern of the spatial variation in leaf traits along a latitudinal gradient.
96
97 Therefore, we examined how the major feeding types (chewing, mining, and
98 galling) of herbivorous insects varied along a 1400-km latitudinal gradient in Japan
99 during two seasons (spring and summer). We used two standardized methods to make
100 our assessments: direct observation of the canopy using a scaffolding system and
101 canopy knock-down. We addressed the following questions: 1) How do the chemical
102 and morphological traits of canopy foliage differ between latitudes and between canopy
103 parts? 2) How does herbivory (chewing, mining, and galling) differ between latitudes
104 and between canopy parts? 3) What leaf traits are responsible for spatial variation in
105 herbivory (chewing, mining, and galling)?
106

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%

125 leaf loss) from each labeled branch in spring (Kuromatsunai, 16 May; Shiiba, 12 May)
126 and summer (Kuromatsunai, September 14; Shiiba, August 27). After returning to the
127 laboratory, we punched 10 disks (5 mm in diameter) out of each leaf. These samples
128 were oven-dried at 40°C for at least 3 days. The mean leaf mass per area (LMA) was
129 calculated for each leaf. The concentrations of carbon and nitrogen in each leaf were
130 measured using a CN analyzer (NC-900, SUMITOMO, Japan), and the carbon/nitrogen
131 (CN) ratios were then calculated. According to the carbon nutrient balance hypothesis,
132 an increase in the CN ratio implies that carbon becomes relatively more available for
133 carbon-based secondary metabolites (Bryant et al. 1983), which are thought to inhibit
134 digestion by herbivores (Rhoades and Cates 1976).
135
136 To identify latitudinal and spatial variation in the herbivore community, we
137 observed herbivores on each labeled branch directly. When assessing the herbivore
138 community, classifying species into feeding types that are ecologically and evolutionary
139 relevant (Root 1973; Simberloff and Dayan 1991) allows comparisons and
140 generalizations to be made that are impossible using taxonomic grouping alone
141 (Landsberg et al. 1989; Andrew and Hughes 2004, 2005). We classified the herbivores
142 into one external (chewing herbivory) and two internal (miners and gall-forming or

143 gallers) feeding types. We scored chewing herbivory for each leaf visually and
144 translated the percentages into six ranked indices as follows: 0% = 0, 1–10% = 1, 11–
145 25% = 2, 26–50% = 3, 51–75% = 4, and 76–100% = 5. In addition, we counted the
146 numbers of miners and gallers on each leaf to calculate miner and galler densities.
147
148 In 2008, to determine the latitudinal variation in chewer abundance and order
149 composition, we conducted canopy knock-down at two plots at each latitude in spring
150 (Kuromatsunai, 16 May; Shiiba, 4–6 May) and summer (Kuromatsunai, 4–5 September;
151 Shiiba, 26 August). Canopy knock-down is a very effective technique for collecting
152 herbivorous insects from the forest canopy (Stork et al. 1997). We used the technique
153 outlined in the protocol manual for DIWPA-IBOY (Toda and Kitching 2002). In each
154 plot, we sprayed the selected tree with 10 L of pyrethrum–water solution (the
155 concentration of pyrethrum = 0.2 mL/L) using a fogging machine (Portable Mister 423
156 Port, Solo, Germany) suspended in the tree with a rope-pulley system. Fallen arthropods
157 were collected for 3 h after fogging using 20 collecting trays (80 cm in diameter). These
158 samples were stored in 80% alcohol and then identified in the laboratory.
159
160 *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.

178 **Results**

179 *Leaf traits*

180 The chemical and morphological traits of the canopy foliage differed significantly
181 between latitudes (Table 1). In spring, all of the measured leaf traits (LMA, Nitrogen,
182 and CN ratio) were higher at south latitude than at north latitude (LMA, $d.f. = 1$, $F <$
183 54.199, $P < 0.001$; Nitrogen, $d.f. = 1$, $F < 24.640$, $P < 0.001$; CN, $d.f. = 1$, $F < 11.473$, P
184 < 0.001; Fig. 1a-c). In summer, LMA and leaf nitrogen also was higher at south latitude
185 than at north latitude (LMA, $d.f. = 1$, $F = 11.115$, $P = 0{,}005$; Nitrogen, $d.f. = 1$, $F =$
186 32.524, $P < 0.001$; Fig. 1d, e), while the CN ratio did not change between latitudes ($d.f.$
187 $= 1$, $F = 0.004$, $P = 0.952$; Fig. 1f). In addition, there were significant spatial variations
188 in leaf traits (Table 1). In spring, LMA and leaf nitrogen were higher in the upper
189 canopy than in the lower canopy (LMA, $d.f. = 1$, $F = 30.181$, $P < 0.001$; Nitrogen, $d.f. =$
190 1, $F = 21.751$, $P < 0.001$; Fig. 1a, b). In summer, all of the measured leaf traits were
191 higher in the upper canopy than in the lower canopy (LMA, $d.f. = 1$, $F = 29.910$, $P <$
192 0.001; Nitrogen, $d.f. = 1$, $F = 47.668$, $P < 0.001$; CN, $d.f. = 1$, $F = 10.048$, $P = 0.006$;
193 Fig. 1d-f).
194
195 *Insect herbivory*

196 Herbivory by the three different types of herbivores differed significantly between
197 latitudes (Table 2). In spring, chewing herbivory did not change between latitudes (*d.f.* =
198 1, $F = 1.652$, $P = 0.218$; Fig. 2a), while miner and galler densities were higher at south
199 latitude than at north latitude, although the densities were very low (miner, *d.f.* = 1, $F =$
200 5.563, $P = 0.032$; galler, *d.f.* = 1, $F = 8.066$, $P = 0.012$; Fig. 2b, c). In summer, gall
201 density was also higher at south latitude than at north latitude, while chewing herbivory
202 was lower (galler, *d.f.* = 1, $F = 14.217$, $P = 0.002$; chewer, *d.f.* =1, $F = 71.243$, $P <$
203 0.001; Fig. 2d, f). There was a significant spatial variation in chewing herbivory only in
204 summer (Table 2). Chewing herbivory was greater in the lower canopy than in the upper
205 canopy at both latitudes (*d.f.* =1, $F = 14.629$, $P = 0.002$; Fig. 2d).
206
207 *Relationships between herbivory and leaf traits at each latitude*
208 The stepwise multiple regression models showed that LMA explained spatial variation
209 in chewing herbivory at north latitude (Table 3). LMA was negatively correlated with
210 chewing herbivory ($F = 6.777$, $P = 0.029$). On the other hand, CN ratio was selected for
211 explaining spatial variation in chewing herbivory at south latitude and was negatively
212 correlated with chewing herbivory ($F = 43.807$, $P = 0.001$). However, any leaf traits did
213 not explain spatial variation in miner and galler densities at both latitudes ($P > 0.05$).

214

215 *Chewer abundance*

216 In summer, chewer abundance clearly was higher at north latitude (plot 1 = 50

217 individuals, plot 2 = 19) than at south latitude (plot 1 = 11, plot 2 = 8), while there was

218 no latitudinal difference in spring (Table 5). Lepidoptera (larvae) was the dominant

219 order of chewers collected in both spring and summer.

220

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

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

275

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

285

286 *Spatial variation in leaf traits and herbivory among canopy parts*
287 The canopy is a spatially heterogeneous environment (Murakami et al. 2005; Nakamura
288 et al. 2008). We found spatial variation in the LMA, leaf nitrogen, and CN ratio within
289 the canopy at both latitudes. The spatial variation in light conditions within the canopy
290 likely causes the spatial variation in leaf traits (Mulkey et al. 1996; Yamasaki and
291 Kikuzawa 2003). In contrast, no temporal change in spatial variation was found, except
292 for the CN ratio. Because the spring census was conducted several weeks after leaf

293 flushing, the leaves in both the upper and lower canopies may have already matured. If
294 we conducted our observations immediately after leaf flushing, a temporal change in the
295 spatial variation in leaf traits might have been detected, similar to a previous study
296 (Yamasaki and Kikuzawa 2003).
297
298 Yamasaki and Kikuzawa (2003) reported that herbivores started eating *F.*
299 *crenata* leaves immediately after leaf flushing at all canopy levels, but further increases
300 in herbivory after June were observed mainly in the lower canopy. Similarly, the pattern
301 of spatial variation in chewing herbivory changed with time at both latitudes: no spatial
302 variation in herbivory was observed in spring, while herbivory was higher in the lower
303 canopy than in the upper canopy in summer. Such spatial variation in summer may be
304 related to food available for chewer herbivores. The stepwise regression models showed
305 that LMA explained spatial variation in chewing herbivory at north latitude, while CN
306 ratio explained at south latitude. Such different leaf traits selected by the model between
307 latitudes may be related with a latitudinal variation in tree crown shapes of Japanese
308 beech, which change from domed at south latitude to even columnar shaped at north
309 latitude (Hiura 1998). Larger spatial variation in light conditions within the canopy due
310 to columnar crown shape at north latitude may strength physical defense due to the 98%

311 greater LMA in the upper canopy than in the lower canopy. However, in case of smaller
312 spatial variation in light conditions due to domed crown shape at south latitude,
313 chemical defense (CN ratio) may be higher than physical defense because the upper
314 canopy had only 62% increased LMA.
315
316 In conclusion, we demonstrated that herbivore communities differed between
317 latitudes. Previously, Coley and Aide (1991) reported that herbivory generally increased
318 towards the tropics. They suggested that higher herbivory in the tropics could result
319 from greater herbivore pressure because of the more favorable climatic conditions for
320 herbivores in the tropics throughout the year. In contrast to previous studies, we studied
321 a single deciduous species, *F. crenata*, in which leaf lifetime differs little between north
322 and south latitudes (Hiura and Nakamura 2013). Our results suggest that the latitudinal
323 gradient for each feeding type on a single plant species can be explained by leaf traits
324 rather than herbivore pressure, as suggested by Coley and Aide (1991). At both latitudes,
325 spatial variation in leaf traits and chewing herbivory was also observed within the
326 canopy. Therefore, to understand the latitudinal and spatial variation in herbivore
327 communities on a single plant species, future studies should focus on the latitudinal and
328 spatial variation in leaf traits, which acclimate or adapt to each environment.
329

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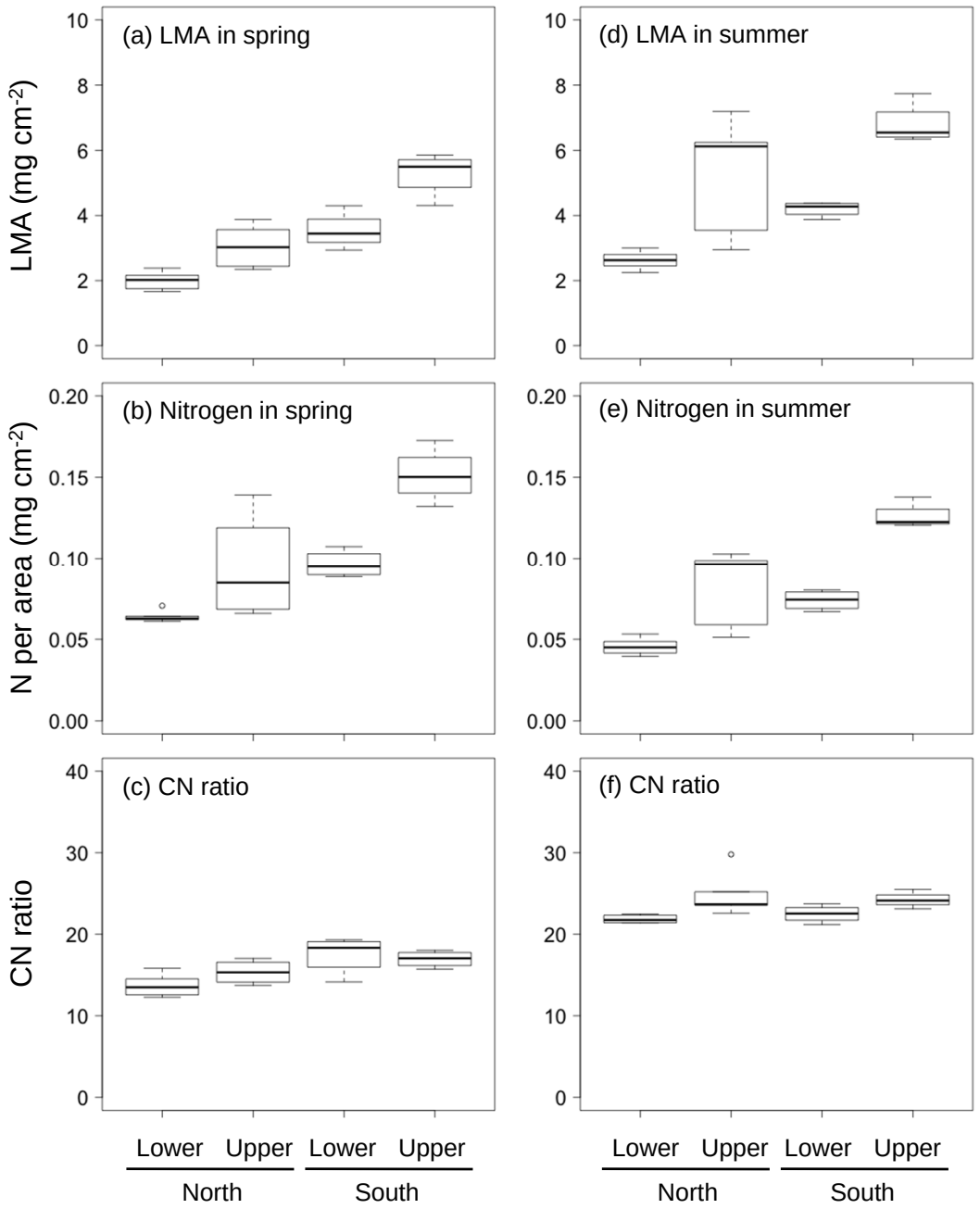
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Figure legend

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

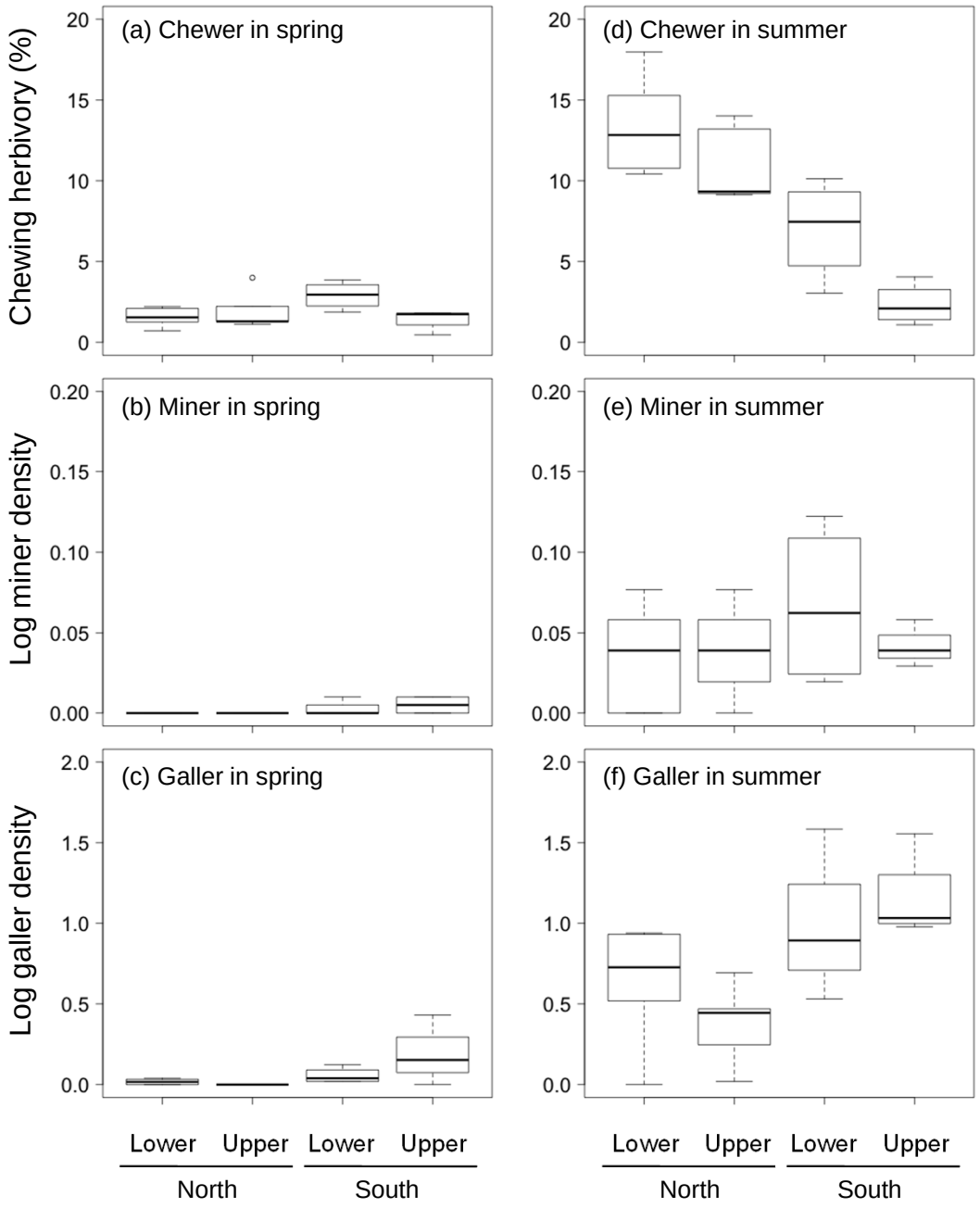


Table1 Two-way ANOVA for the effects of latitude, canopy part, and their interaction on LMA, nitrogen, and CN ratio in spring and summer. Significant values are in bold.

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