

Cost-benefit Quantification of Leaf Carbon Economics to Disentangle Responses of Plant Assemblages to Deer Herbivory

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3 **Cost-benefit quantification of leaf carbon economics to disentangle responses of plant**
4 **assemblages to deer herbivory**

5

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18 authors analyzed the data. YW wrote the manuscript; other authors provided editorial advice.

19

20 **Abstract**

21 Although the plant carbon cost-benefit balance is known to be related to individual plant
22 growth, reproduction, and population expansion, the association with plant community
23 differences is not well understood. In this study, we examined how the leaf carbon cost-
24 benefit metrics were associated with the assembly process of forest understory plant
25 communities in areas highly affected by deer browsing. We calculated these metrics from
26 plant physiological parameters for 14 forest floor plant species growing in deer
27 presence/absence site to detect the relationship between species dominance and leaf carbon
28 cost-benefit metrics. As a result, the patterns of interspecific variation in benefit along the
29 plant dominance rank differed in deer presence/absence sites, contributing to the marked
30 differences in species composition and diversity observed at the two sites. In the absence of
31 deer, where competition was the dominant determinant of plant community composition,
32 carbon benefits among species were positively related to the plant dominance rank, indicating
33 that species able to acquire more carbon were at an advantage. On the other hand, under deer
34 herbivory, differences in carbon benefit between species were not strongly apparent and were
35 not related to the plant dominance rank, indicating few differences in reproductive and
36 expansion ability (plant fitness) between species. This process contributes to the high species
37 diversity of plant communities observed in the presence of deer. Our results emphasize the
38 possibility of connecting different fields of studies, physiological ecology, community
39 ecology, and the plant carbon cost-benefit balance of single leaves to explain plant
40 community composition differences.

41

42 **Keywords:** Cost-benefit, Plant strategy, Physiological traits, Herbivory, Community

43 **Introduction**

44 One of the major goals of plant community ecology is to gain a mechanistic understanding of
45 how a wide variety of factors contribute to the determination of community diversity,
46 richness, and composition in a spatiotemporally heterogeneous environment (Cadotte et al.,
47 2009; Pavoine & Bonsall, 2011; Spasojevic & Suding, 2012; HilleRisLambers et al., 2012).
48 Assessing the determinants of community composition can also help us understand the
49 response of ecosystems to human-induced environmental changes and disturbances, which
50 can lead to the development of appropriate management approaches (Webb et al., 2010; Mori,
51 2011; Mori et al., 2013, 2015; Bjorkman *et al.*, 2018). Currently, the trait-based approach is
52 becoming increasingly important in understanding plant communities' responses to
53 environmental conditions (Cadotte et al. 2013; Mori et al. 2013). Each plant trait, which is
54 realized through many ecological and evolutionary processes, fundamentally reflects the
55 strategies of plant growth, survival, and reproduction (Kattge *et al.*, 2020).

56 Individual plants respond to their growing environments by altering their forms to
57 maximize plant carbon benefit, which is the amount of carbon acquired via photosynthesis
58 subtracted from the costs of producing and maintaining the individual plants. Plant carbon
59 benefit determines the growth and reproduction of individuals and ultimately drives
60 population expansion (Field, 1983; Kudo, 1992; Kikuzawa, 1995a; Ackerly, 1999).
61 Importantly, plant carbon benefits are often quantified on the basis of individual leaves.
62 Researchers have been trying to build the “cost-benefit model” of leaf photosynthetic carbon
63 production to estimate the optimal plant strategies for leaf longevity in growing environments
64 (Chabot & Hicks, 1982; Kikuzawa & Kudo, 1995; Eamus et al., 2000). Because of the high
65 degree of autonomy of individual plant organs, realizing a positive carbon balance at the level

66 of individual leaves fundamentally contributes to optimal whole-plant performance (Sprugel
67 et al. 1991). However, much uncertainty still exists regarding how carbon assimilation and the
68 resultant cost through intrinsic (e.g., respiration) and extrinsic (e.g., herbivory) can be
69 summed up to the community-wide consequences through the responses of individual plants
70 and each of the constituent species. To fill this knowledge gap, functional trait ecology needs
71 to further acknowledge the dynamic features of plant traits. For example, the photosynthetic
72 ability of individual leaves changes over time to achieve positive outcomes of net carbon
73 balance. Combining this information with well-measured plant traits is the first step.

74 Plant strategies generally vary according to one principal axis of the trade-off
75 between resource acquisition and conservation (fast and slow), which is inferred from plant
76 characteristics, especially leaf traits (Wright et al., 2004; Reich, 2014; Onoda et al., 2017).
77 The acquisitive plant strategy is characterized by low investment in leaf structure and high
78 photosynthetic efficiency. This strategy is advantageous when the risk of losing leaves is
79 relatively high (a short leaf lifespan), especially under disturbance pressure, because it allows
80 for the offset of carbon investment in leaf structure in a short time considering the carbon
81 cost-benefit methods. This strategy is often considered the fast end of a plant economic
82 spectrum. In contrast, the slow end of the spectrum is characterized by a conservative strategy
83 with high investment in leaf construction and maintenance, requiring a long leaf lifespan.
84 These features, based on a longer time horizon, enable plants to acquire more carbon per leaf,
85 which is advantageous in competition for light under stable environmental conditions. This
86 fast-slow axis in strategies is observed both inter- and intraspecifically (Wakatsuki et al.
87 2021), importantly guiding the discipline to gain a comprehensive understanding of how the
88 structure and composition of plant species assemblages respond to the fluctuating
89 environment (Kraft et al., 2008, 2015).

90 Forest floor plant communities are known to account for much of the diversity in
91 forests due to their spatial and temporal environmental heterogeneity (Gilliam, 2007). In
92 recent years, there has been an increasing recognition that anthropogenic influences can
93 substantially alter these ecological communities due to land conversion, biological invasion,
94 and an overabundance of herbivores (Whigham, 2004; Gilliam, 2006; Landuyt et al. 2019). In
95 particular, the increasing overpopulation of ungulates is causing major problems worldwide
96 (Côté et al., 2004). They are known to affect ecosystems directly and indirectly through
97 browsing, trampling, and altering carbon and nutrient cycling (Hobbs, 1996; Augustine &
98 McNaughton, 1998; Rooney & Waller, 2003; Kasahara et al., 2016). Here, we focused on the
99 physiological response of forest floor plants to deer herbivory, represented by the amount of
100 carbon acquired by leaves, in order to examine the mechanisms that determine community
101 composition under deer herbivory. We infer differences in the mechanisms of determining
102 plant species composition between deer presence and absence by observing the carbon cost-
103 benefit balance related to species-specific strategies.

104 First, we estimated the annual leaf carbon benefit of multiple forest floor plant
105 species and quantified how the benefits related to plant species dominance in the presence and
106 absence of deer. We assumed that species with larger leaf carbon benefits would have an
107 advantage over other species by allowing them to invest more carbon in species expansion
108 and reproduction. Second, we examined how deer herbivory alters the leaf carbon balance of
109 species and how this affects the determination of the species composition of the community
110 under deer herbivory. We hypothesized that the optimal strategies represented by the carbon
111 cost-benefit balance would be different in the presence and absence of deer, resulting in
112 different species composition. To address the above issues, we selected deer enclosure fences
113 established in a cool temperate forest in northern Japan, where deer overabundance is

114 currently an issue, as our study area (Nishizawa et al. 2016; Fujii et al. 2017; Wakatsuki et al.
115 2021). We compared plant community composition between deer presence and absence sites.
116 Our goal was to expand our understanding of the establishment process of plant communities
117 from the physiological indicators directly related to plant dominance based on plant carbon
118 cost-benefit balance during the growing season instead of the traditional indirect approach
119 using snapshot functional traits.

120

121 **Methods**

122 **Study Site**

123 The study was conducted in the Horobetsu area of Shiretoko National Park (44°06'00"N
124 145°01'42"E) in Hokkaido, Japan. This park was registered as a World Natural Heritage Site
125 by the United Nations Educational, Scientific, and Cultural Organization (UNESCO) because
126 of its high biodiversity due to the relationship between the marine and terrestrial ecosystems
127 (<https://whc.unesco.org/en/list/1193>). The mean annual temperature is approximately 6.69 °C,
128 and the mean annual precipitation is approximately 1322 mm (at Utoro Sharigun, between
129 2011 and 2020, <http://www.data.jma.go.jp/obd/stats/etrn/index.php>). The forest here is a
130 boreal forest characterized by a mixed coniferous and broad-leaved natural forest dominated
131 by *Abies sachalinensis*, *Quercus crispula*, and *Kalopanax septemlobus* (The Forestry Agency
132 of Japan 2004). The forest floor is usually covered with snow from the end of November to
133 early May (<https://www.jma-net.go.jp/sapporo/index.html>).

134 In this area, deer (*Cervus nippon yesoensis*) over-abundance has been a problem
135 since the late 1980s, and the herbivores have dramatically changed the vascular plant
136 community (Tokida et al., 2004). Our study site, Horobetsu, has one of the highest deer

137 densities in the area. Since 2010, the density has been as high as approximately 10 deer/km,
138 as estimated by light censuses conducted in spring (<http://dc.shiretoko-whc.com>). To recover
139 the ground-layer plant community, a “deer enclosure fence” was built in this area in 2003, and
140 since then, there has been a noticeable increase in understory plants inside the fence.

141

142 **Field data collection**

143 The enclosure plot was protected from deer herbivory by the fences, and the control plot was
144 exposed to herbivory. The size of the enclosure and control plots were 80 m × 120 m (0.96
145 ha) and 100 m × 100 m (1.00 ha), respectively. These plots were located close to each other
146 so there was little difference in the woody plant community composition (The Forestry
147 Agency of Japan 2004). Both plots were originally similar forest floor plant communities and
148 were under equivalent browsing pressure by deer before the enclosure fence was built. In
149 these plots, quadrats (1 m × 1 m) were placed at 10m intervals; there were 95 enclosure
150 quadrats and 100 control quadrats. One quadrat was not included because of a fallen tree in
151 the enclosure plot. These quadrats were established in 2014 (Nishizawa et al., 2016). In each
152 quadrat, forest floor plant species were identified, and percent cover of each species was
153 visually estimated in units of 1%. Here, we defined “forest floor plants” as herbaceous plants,
154 ferns, and vines, and excluded the seedlings of semi-tall tree species. In the case of species
155 coverage less than 1%, we recorded the appearance. We conducted this study during the plant
156 growth period (from the end of June 2020).

157

158 **Calculation of expected leaf carbon benefit and cost metrics**

159 We used 14 forest floor species that appeared in both plots in the field study (supplementary
160 material: S1) to evaluate the difference in leaf carbon gain and cost metrics in the deer
161 presence and absence sites. We collected five leaf samples for each species for each treatment
162 (control and exclosure), during the plant growth period (as of the end of August in 2018 and
163 2019). We selected only the fully formed leaves. We estimated the total leaf carbon gain
164 (*TCG*: g), total leaf carbon cost (*cost*: g), and leaf carbon benefit (*benefit*: g) of the collected
165 leaves. These are expected values that take into account leaf life span and leaf growth during
166 the plant growing season in the calculation. These three metrics were calculated from six
167 parameters: expected leaf longevity (*LL*: %), leaf area (*LA*: m²) change with growth, daily
168 light intensity (*PAR*), daily leaf carbon gain (*DCG*: g), leaf producing carbon cost (*LPC*: g),
169 and daily leaf maintaining carbon cost (*LMC*: g). We set the plant growing season as the
170 period from May to November (210 days) when the ground was usually not covered with
171 snow.

172

173 *Parameter 1. Expected leaf longevity*

174 The probability of leaf persistence during the growing season was used as the leaf longevity
175 (*LL*: %) (Supplementary materials: S2). We marked the leaves in May 2019. After that, we
176 checked the counts of the remaining leaves three times (at the end of June, August, and
177 October in 2019). We calculated the probability of leaf persistence per day (%) for each
178 species and treatment (control and exclosure). We confirmed that the leaf mortality rate and
179 browsing opportunity by deer did not change depending on whether they were marked. We
180 assumed that all species would be defoliated by the end of the plant growing season (the end
181 of November).

182

183 *Parameter 2. Leaf area change with growth*

184 We measured the leaf area of collected samples from scanned images using ImageJ software
185 (Rasband 1997–2014), and this size collected in August was assumed to be the fully grown
186 leaf size. To account for the increase in leaf size in calculating leaf carbon gain and cost
187 metrics, we estimated the change in leaf area of collected samples by the mean values of the
188 size expansion rate of marked leaves for each species and each treatment (used in the
189 estimation of leaf longevity [*LL*]). We measured the length and width of marked leaves for
190 each species and each treatment three times during the growing season (the end of May, June,
191 and August). To estimate the leaf area from the leaf length and width, equations between leaf
192 area and the values multiplied by the leaf length and width were obtained for each species in
193 advance using our scanned image data (Supplementary material: S3). We calculated the leaf
194 area (*LA*: m²) for each day during the growing season from the mean values of the size
195 expansion rate during each measurement period (from the end of May to the end of June and
196 from the end of June to the end of August). For all species, it was assumed that leaf area
197 started at zero and that there would be no change in leaf area after September.

198

199 *Parameter 3. Light intensity (photosynthetically active radiation)*

200 Photosynthetically active radiation (*PAR*) was measured using a sensor (Quantum Sensor
201 MIJ-14PAR Type2/K2, Environmental Measurement Japan) for one year from September
202 2019, and data for May–November (plant growing season) were used for the analysis
203 (Supplementary materials: S4). Sensors were placed on the ground in both plots (control and
204 enclosure) at the same level as the height of the understory plants. The *PAR* was recorded

205 once every 10 min (*i*: 144 times per day) and used as *PAR* for a 10-minute period. The
206 average *PAR* of the two locations was used as the annual value, and the average of each of the
207 five days was used for the analysis to improve normality.

208

209 *Parameter 4. Daily leaf carbon gain*

210 Daily leaf carbon gain (*DCG*) was calculated from the net CO₂ assimilation rate (*A*: μmol
211 CO₂ m⁻² s⁻¹), which was inferred from the photosynthetically active radiation (*PAR*) response
212 curve of linear electron flow (*LEF*) (Supplementary material: S5), and leaf longevity (*LL*).
213 We measured *LEF* using Multispeq v.2.0, (Kuhlgert et al., 2016) in study plots from the end
214 of June until early July in 2019. Multispeq can measure the photosynthetic efficiency index at
215 the current light intensity in a short time without damaging the leaves. To obtain the *PAR*
216 response curve of *LEF* for each species, we measured the *LEF* at various light intensities for
217 each species (Supplementary material: S5). The curve equations were derived using a
218 nonlinear regression.

$$219 \quad LEF = a \times \log \left(PAR + \exp \left(-\frac{b}{a} \right) \right) + b \quad \dots (1.1)$$

220 where *a* and *b* are species-specific values. From equation 1.1, we estimated the *PAR* response
221 curve of *A*, referring to Flexas et al. (2002):

$$222 \quad A = c \times LEF \quad \dots (1.2)$$

223 where *c* ($\doteq 0.103$) is the coefficient for calculating *A* from *LEF*, calculated based on Flexas et
224 al. (2002), which shows the relationship between *ETR* ($\doteq LEF$) and *A*. We calculated the *DCG*
225 of each leaf sample using equation 1.2, *PAR* data recorded once every 10 min (144 times per

226 day, details in parameter 4), the change in leaf area with growth (LA : m^2 , details in parameter
227 2), and leaf longevity (LL : %, details in parameter 1):

$$228 \quad DCG_t = \sum_i^{1-144} A_i \times LA_t \times LL_t \times d \quad \dots (1.3)$$

229 where d is the value used to convert the unit of measure from $\mu\text{mol CO}_2 \text{ s}^{-1}$ to grams. The
230 DCG estimates daily carbon gain (g) on day t using PAR at every 10 minutes interval (i).

231

232 *Parameter 5. Leaf-producing carbon cost*

233 Leaf carbon content was used as the leaf-producing carbon cost (C : g) (Supplementary
234 materials: S6). We calculated it by multiplying leaf dry mass by the ratio of carbon in
235 collected leaves measured by an NC analyzer (Sumigraph NCH–22 F, Sumika Chemical
236 Analysis Service, Japan).

237

238 *Parameter 6. Daily leaf maintaining carbon cost*

239 Daily leaf maintaining carbon cost (LMC : g) is defined as the amount of carbon consumed per
240 day through respiration. It was calculated from dark leaf respiration rate (R : $\text{nmol g}^{-1} \text{ s}^{-1}$),
241 which was inferred from leaf nitrogen concentration (N : mmol g^{-1}) referring to Reich et al.
242 (2008):

$$243 \quad \log R = 1.078 \times \log(N) + 0.911$$

$$244 \quad R = 10^{1.078 \times \log(N) + 0.911} \quad \dots (2.1)$$

245 We calculated N by using the ratio of nitrogen in the dry mass of collected leaves measured
246 by an NC analyzer (Sumigraph NCH–22 F, Sumika Chemical Analysis Service, Japan). Leaf

247 mass per area (LMA ; g m^{-2}) was calculated by dividing the leaf area of collected samples by
 248 the respective leaf dry masses to estimate the change in leaf dry mass (DM : g) with the
 249 change in leaf area with growth (LA : m^2 , details in parameter 2):

$$250 \quad DM_t = LA_t \times LMA \quad \dots (2.2)$$

251 DM estimates leaf dry mass on day t . We assumed that N and LMA measured in the collected
 252 leaf samples did not change during the growing periods. We calculated *the LMC* of each leaf
 253 sample using equations 2.1 and 2.2:

$$254 \quad LMC_t = R \times DM_t \times LL_t \times e \quad \dots (2.3)$$

255 where e is the value used to convert the unit of measure from nmol s^{-1} to gram. *The LMC*
 256 estimates the carbon cost (g) by leaf respiration on day t .

257

258 We calculated total leaf carbon gain (TCG : g), total leaf carbon cost ($cost$: g), and leaf carbon
 259 benefit ($benefit$: g) of collected leaf samples using the above six parameters, referring to
 260 Kikuzawa (1995b). TCG is the sum of the daily leaf carbon gain (DCG) for the plant-growing
 261 season.:

$$262 \quad TCG = \sum_t^{1-210} DCG_t$$

263 TCG estimates the total carbon gain during the growing season by the sum of carbon gains on
 264 day t (210 days: during growth periods).

265 $Cost$ is the sum of the daily leaf-maintaining carbon cost (LMC) during the growing season
 266 and the leaf producing carbon cost (LPC):

$$267 \quad Cost = \sum_t^{1-210} LMC_t + LPC$$

268 *Cost* estimates the expected total carbon cost of producing and maintaining leaves during the
269 growing season by the sum of carbon cost on day t and leaf-producing carbon cost.

270

271 *Benefit* was the net profit of the leaf obtained during the growing season by subtracting *the*
272 *cost* from *TCG*.:

$$273 \qquad \qquad \qquad \textit{Benefit} = \textit{TCG} - \textit{Cost}$$

274

275 **Data analysis**

276 We first compared plant community composition between the control and exclosure plots to
277 clarify their differences in the presence and absence of deer. Species richness (number of
278 species in quadrats) was used as a measure of diversity. We constructed a rank abundance
279 curve, which is a chart depicting both species richness and species evenness, to evaluate the
280 differences between the two communities.

281 Next, we confirmed the correlation between total carbon gain and benefit along
282 species dominance rank for each plot to examine how leaf carbon gain was related to plant
283 dominance. The species dominance rank for each plot was determined based on the
284 abundance of species in each plot. We assumed carbon cost as an indicator of species' carbon
285 resource investment strategies and confirmed the correlation in carbon cost among species
286 along the species dominance rank. The larger and smaller carbon cost implies that more and
287 less carbon is invested in the production and maintenance of leaves, respectively (more
288 conservative and acquisitive strategy, respectively).

289

290 **Results**

291 *Plant community composition*

292 In the two plots (including 100 quadrats in the control plot and 95 quadrats in the enclosure
293 plot), we recorded 51 species. There were 41 plant species in the control plot and 39 plant
294 species in the enclosure plot. Species richness was higher in the control plot than in the
295 enclosure plot (Supplementary material: S7). The mean (\pm SE) species richness was 7.03 (\pm
296 0.29) and 5.05 (\pm 0.19) in control and enclosure, respectively. *Toxicodendron orientale* was
297 the most frequently observed species in both plot types. Enclosure plots were characterized by
298 many individuals of *Maianthemum dilatatum* and *Sasa kurilensis*. Conversely, control plots
299 were characterized by the abundance of *Hydrangea petiolaris* and *Sasa spiculosa* in the
300 understory stratum. The predominance of several unpalatable species, often observed under
301 deer browsing pressure, was not seen in the control plots. The rank-abundance curve showed
302 a steeper gradient in the enclosure than in the control (Supplementary material: S8). This
303 means a large difference in dominance between dominant species (high-ranking species) and
304 rare species (low-ranking species) in the enclosure plots. The order of abundance of the 14
305 targeted species in each plot (Supplementary materials: S1) showed that the frequency of each
306 species differed between the inside and outside of the fences. The targeted species in our
307 study accounted for 61.7 percent and 71.6 percent of the total coverage in the control plots
308 and the enclosure plots, respectively. *Sasa sp.*, one of the dominant species in both plots, was
309 excluded from the target species because it is not deciduous in one year, making it difficult to
310 estimate its carbon gain during the growing season.

311

312 *Leaf carbon gain*

313 Comparing each parameter that we used to calculate the metrics of leaf carbon benefit and
314 cost between inside and outside the fence for each species, the leaf life span was longer, and
315 the leaf carbon concentration was higher in deer absence plots than in deer presence plots
316 (Supplementary materials: S2 and S6).

317 Figure 1 shows that species with higher total carbon gain and carbon benefit were
318 more dominant in the enclosure plots. On the other hand, in control plots, total leaf carbon
319 gain was higher for less dominant species than inside the fences, and there was no significant
320 relationship between leaf carbon benefit and the dominance rank. Figure 2 shows that species
321 with higher carbon costs in the leaves were more dominant in the enclosure plot. On the other
322 hand, the opposite trend was observed in the control plot. The total carbon gain, leaf carbon
323 benefit, and cost were greater in the enclosure plot than in the control plot, and the plasticity
324 of these metrics was also greater in the enclosure plot (Supplementary material: S9).

325

326 **Discussion**

327 **Plant community composition**

328 Species composition and diversity differed between the inside and outside of the enclosure
329 fences (supplementary materials: S1, S7 and S8). In the deer-excluded environment, plant
330 diversity was lower than that in the deer-browsed sites. Previous studies here (Nishizawa et al.
331 2016; Wakatsuki et al. 2021) have suggested that the release of plants from deer herbivore
332 pressure (by establishing the fences) leads to an environment with high plant coverage, where
333 competition for light can be significantly intensified, leading to a low-diversity community
334 dominated by a limited set of species that are superior in their ability to acquire light. In
335 contrast, although plants are prone to herbivory disturbance, the presence of deer relaxes

336 competition for light among species, enabling the coexistence of multiple species. In
337 summary, the determinants of assembly processes change due to the presence or absence of
338 herbivores, which shape local plant communities.

339 These trends, which were seen when comparing sites with and without deer, have
340 been reported in other studies, and not only in this study area (Webster et al., 2017; Li et al.,
341 2018; Sabo et al., 2019). We could infer that moderate browsing pressure contributes to the
342 maintenance and enhancement of the diversity of the forest plant community (Stokely &
343 Betts, 2020). Therefore, excluding deer with the aim of restoring plant diversity would not
344 necessarily be effective and would move the system to an alternative, undisturbed ‘natural’
345 state resulting from the termination of herbivore browsing.

346

347 **Plant assembly mechanisms**

348 In the enclosure plots, species with a more significant total carbon gain and benefit had a
349 higher dominance rank (Figure 1). Previous studies addressing invasive plants suggested that
350 a greater leaf carbon gain allows for more investment in reproduction and expansion for
351 species, leading to enhanced species dominance (Osunkoya *et al.*, 2010; Shen *et al.*, 2011).
352 The existence of these species, which have advantages relative to others, is likely to result in
353 low diversity environments dominated by certain species. Dominant species inside the fences
354 had high carbon content (Figure 2); that is, they had a more conservative strategy. In addition,
355 the leaf life span and leaf carbon concentration were greater in plants within the fences
356 (Supplementary materials: S2 and S6). The absence of deer browsing pressure may allow for
357 a longer leaf lifespan, resulting in a more carbon-invested leaf (Wright et al., 2004). These
358 results suggest that species investing more in their leaf structures dominate environments

359 without ungulate browsing. Features of carbon-invested leaves, such as large leaf size and
360 strong leaf structure, are advantageous in competition for resources (Smith, 1982; Bartemucci
361 *et al.*, 2006; Kunstler *et al.*, 2016). At excluded deer browsing sites, species with a
362 conservative strategy (investing carbon in leaf structure) have an advantage over other species
363 in competition for resources, resulting in greater carbon acquisition for these species. We
364 assume that this process leads to the establishment of an environment suitable for certain
365 competitive species that is relatively low in diversity.

366 In contrast, in the control plots, total carbon gain was higher in lower dominance
367 rank species, and there were no differences in benefit among species along the dominance
368 rank (Figure 1). This finding indicates that the amount of total carbon gain does not lead to
369 species dominance; in contrast to the enclosure plot, the species with larger total carbon gains
370 are less dominant. In addition, interspecific differences in benefits are smaller in the control
371 than in the enclosure (Supplementary material: S9). It seems that deer herbivory pressure
372 reduces the interspecific difference in carbon benefit, and as a result, the difference in
373 reproductive and expansion ability (plant fitness) among species is also reduced. This
374 equalizing mechanism that reduces interspecific differences in fitness might be a factor
375 enabling multispecies coexistence under deer herbivory (Chesson, 2000; Leibold and McPeck
376 2006). The dominant species in the control plot had leaves with low carbon costs (Figure 2),
377 suggesting that these species have a fast strategy. Previous studies have suggested that species
378 with less costly leaves, i.e., small leaf size and low leaf toughness, are advantageous under
379 browsing pressure (Webster *et al.*, 2017; Boulanger *et al.*, 2018). These features allow plants
380 to achieve a positive carbon balance in leaves within a short time (Kikuzawa, 1995b; Navas *et*
381 *al.*, 2003). Moreover, given that deer browsing pressure can substantially shorten leaf
382 lifespan—the potential of investment failure (Supplementary materials: S2), the strategy to

383 quickly reach out to the positive leaf carbon balance as much as possible within a limited time
384 is likely to be advantageous under deer herbivory (Chabot & Hicks, 1982; Matsuki & Koike,
385 2006). It is considered that herbivory reduces the difference in survival advantage among
386 species, resulting in the establishment of a highly diverse environment. This effect could also
387 be the fundamental mechanism underlying the intermediate disturbance hypothesis, in which
388 intermediate disturbance contributes to the maintenance of diversity (Connell, 1978; Hobbs &
389 Huenneke, 1992; Olf & Ritchie, 1998).

390

391 In conclusion, our results suggest the significance of observing the carbon balance of plants in
392 clarifying the community assembly process. Differences in carbon cost-benefit metrics among
393 species could be a more direct indicator of survival strategies than others, reflecting
394 differences in plant morphological, physiological, and phenological features, such as leaf size,
395 photosynthetic efficiency, and leaf longevity. Furthermore, this metrics can provide a measure
396 that reflects differences in the fitness of individuals and species, which is crucial for
397 understanding community assembly (Saito et al. 2021). Plant carbon cost-benefit analysis is a
398 topic that has long been studied as an indicator of survival strategies, but the present study
399 demonstrates its novel importance in understanding plant community composition. The
400 evaluation of community composition based on individual leaves in this study might be an
401 overestimation because we could not confirm the carbon balance of the whole plant, including
402 aboveground and belowground parts. Nevertheless, we believe that our approach, which
403 focuses on cost-benefit analysis based on individual leaf level, provides a more realistic
404 explanation of species dominance than the trait-based approach and is useful for a more
405 delineated understanding of plant community assembly processes. The study results

406 emphasize that connecting research from different fields, physiological and community
407 ecology, can lead to fresh ecological insights.

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412 **Declarations**

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416 **Conflicts of interest/Competing interests**

417 Authors declare that they have no conflict of interest.

418 **Ethics approval**

419 Not applicable

420 **Consent to participate**

421 Not applicable

422 **Consent for publication**

423 Not applicable

424 **Availability of data and material**

425 These are available from the corresponding author upon reasonable request.

426 **Code availability**

427 These are available from the corresponding author upon reasonable request.

428

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Figures

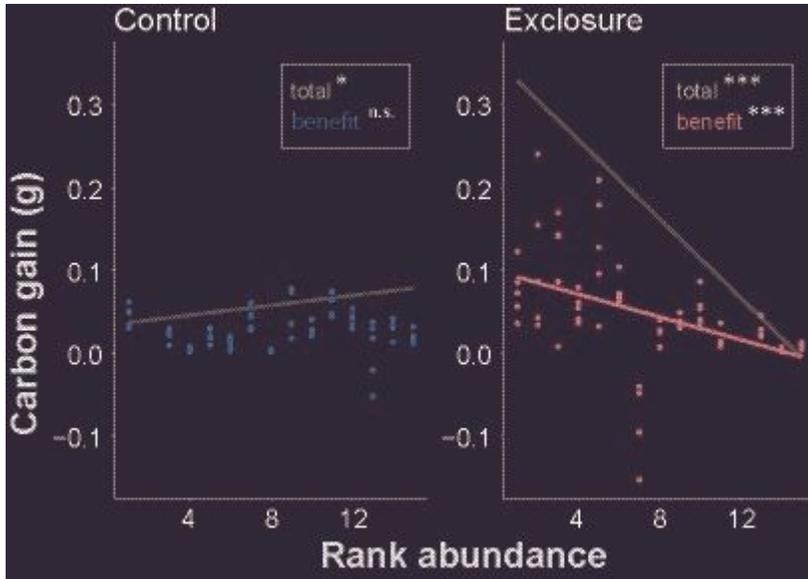


Figure 1

Correlation between total carbon gain (gray line) and benefit (colored line) and plant species abundance rank. Orange and green points show the data measured in the control plot and in exclosure plot, respectively. Significant are evaluated by Kendall rank correlation coefficient (n.s: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

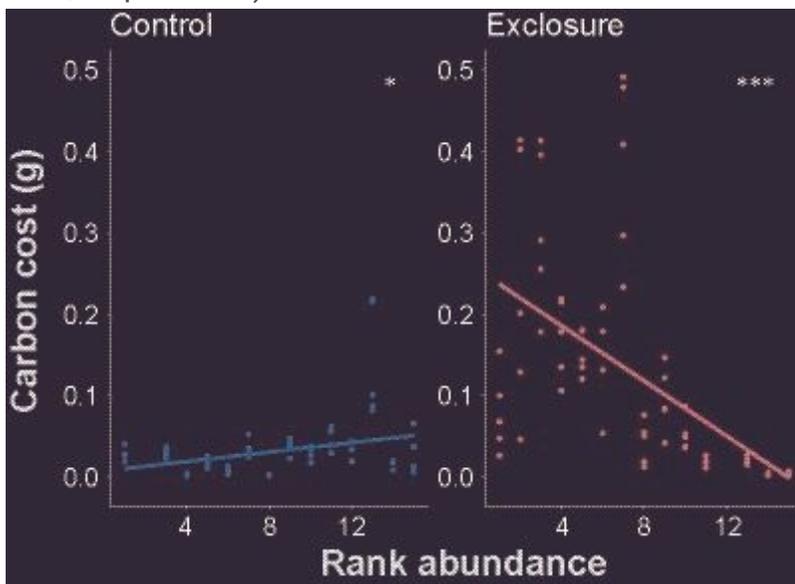


Figure 2

Correlation between carbon cost and plant species abundance rank. Orange and green points show the data measured in the control plot and in exclosure plot, respectively. Significant are evaluated by Kendall rank correlation coefficient (n.s: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

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