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3 **Bottom-up effects of woodland eutrophication: interacting**  
4 **limiting nutrients determine herbivory frequency in**  
5 **northwestern Patagonia**

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23 **Abstract**

24 Nutrient enrichment disrupts plant–animal interactions and ecosystem functioning  
25 globally. In woodland systems, the mechanisms of bottom-up turnover on plant–  
26 herbivore interactions remain understudied. Here, we performed a full-factorial field  
27 experiment to evaluate the interactive effects of nutrient addition (nitrogen, phosphorus,  
28 and/or potassium) on the assemblage of foliar herbivores and the interaction frequency  
29 with *Berberis microphylla*, a dominant shrub species in Patagonian woodlands.  
30 Additionally, we assessed whether these effects could be mediated by changes in  
31 vegetative traits and microhabitat characteristics (i.e., canopy cover) that may ultimately  
32 influence the foraging behavior of herbivores. The addition of nitrogen reduced the  
33 herbivory frequency by 41%, yet this effect was diluted in the presence of potassium. We  
34 found no effects of phosphorus addition. Our results suggest that the impact of multiple  
35 nutrient additions (N and K) on herbivory patterns could be mediated by changes in two  
36 important foliar traits, leaf size and leaf density. This study shows how multiple nutrient  
37 addition can change the magnitude of antagonistic plant–animal interactions in  
38 woodlands. Since herbivory by arthropods has a relevant role in net primary productivity,  
39 our results highlight the importance of buffering human-driven woodland eutrophication  
40 to maintain important ecological functions (e.g., herbivory) associated with antagonistic  
41 plant–animal interactions and avoiding ecosystem dysfunction.

42

43 **Keywords:** Eutrophication, herbivores, nitrogen, potassium, phosphorus, Patagonia

44

45 **Introduction**

46 Human activity in the Anthropocene is driving multiple changes at a global scale,  
47 including woodland eutrophication (i.e., excessive nutrient enrichment) (Smith et al.  
48 1999). The deposition of nitrogen (N), phosphorus (P), or potassium (K) derived from  
49 industrial and agricultural activities (Sardans and Peñuelas 2015, Penuelas et al. 2020)  
50 causes changes to biological and physicochemical soil conditions that regulate plant  
51 populations at both the community and species levels (Smith et al. 1999, Elser et al. 2007,  
52 Verheyen et al. 2012, Harpole et al. 2016, Stevens et al. 2018). Recent experimental  
53 evidence shows that an increase in the number of different limiting nutrients added to  
54 soils depauperates diversity and promotes changes in the community structure of  
55 grasslands (Harpole et al. 2016). At the species level, nutrient enrichment has been shown  
56 to change the response of vegetative and reproductive traits of plants which in turn  
57 influence interacting patterns with both mutualistic (e.g., mycorrhizal fungi, pollinators  
58 or seed dispersers) (Carvalho et al. 2020b, 2021) and antagonistic partners (herbivores  
59 or parasites) (Lind et al. 2017, Pöyry et al. 2017a). However, the evidence is scarce and  
60 scattered, and there is little mechanistic knowledge about how the effects of  
61 eutrophication propagate toward higher trophic levels and ultimately shape plant–animal  
62 interaction patterns and ecosystem functioning.

63 Foliar herbivory by insects drives plant–animal coevolution and modulates key  
64 ecological processes such as primary productivity, plant community composition or  
65 nutrient cycling (Schmitz 2008). Soil nutrient enrichment has been shown to modify  
66 growth rates and a wide range of vegetative traits of plants, such as leaf density, leaf size,  
67 specific leaf area or nutrient content in leaves. Consequently, soil eutrophication may  
68 shape microhabitat characteristics (e.g., light availability) by modifying the canopy cover  
69 of coexisting plant species (Borer et al. 2014). Changes in microhabitat and vegetative

70 traits could influence foraging behavior and plant selection patterns by herbivorous  
71 insects with different nutritional needs, thus potentially entailing changes in the  
72 composition of herbivore assemblages and interaction frequency with plants (Maiorana  
73 1981, Throop and Ler dau 2004, Pöyry et al. 2017b). However, most of this evidence  
74 derives from studies evaluating the impact of N or NPK enrichment, but it remains  
75 unclear how multiple additions of different limiting nutrients (i.e., N, P, and K) can  
76 impact herbivory patterns. Understanding the mechanisms underlying the bottom-up  
77 effects of soil nutrient enrichment on herbivore assemblages and herbivory patterns is  
78 relevant to better anticipate their potential consequences in ecosystem (mis)functioning  
79 (Schmitz 2008). While most research to address this problem was developed in grasslands  
80 (Borer et al. 2014, Harpole et al. 2016, Lind et al. 2017, Anderson et al. 2018), woodland  
81 ecosystems where shrub species dominate the community have rarely been evaluated  
82 (Campo and Dirzo 2003, Andersen et al. 2010).

83         Patagonian temperate woodlands harbor a diversity of shrub species that grow in  
84 nutrient-limited soils (Diehl et al. 2008), making them a good study system to test a  
85 nutrient-related hypothesis experimentally. In this study, we performed a manipulative  
86 field experiment to assess the bottom-up effects of soil nutrient enrichment on the  
87 interaction patterns between *Berberis microphylla*, a dominant shrub species in the area,  
88 and the assemblage of its foliar herbivores. We also evaluated whether bottom-up effects  
89 (if present) could be related to changes in canopy cover and vegetative traits that can  
90 change the foraging decisions of herbivorous insects. We used a full-factorial design  
91 encompassing eight fertilization treatments (i.e., no nutrient addition, N, P, K, NP, NK,  
92 PK, and NPK) that represent a gradient of progressive addition of the three most  
93 important limiting nutrients in the Patagonian woodlands. We evaluated the effects of  
94 nutrient addition on *i*) the guild diversity of foliar herbivores and *ii*) the interaction

95 frequency with foliar herbivores. We then explored whether these effects might be  
96 mediated by changes in canopy cover, plant volume, and foliar traits (leaf density, leaf  
97 size, and specific leaf area). Although multiple responses may arise from the addition of  
98 a different combination of nutrients, we overall hypothesize that if herbivores have  
99 different nutritional needs we should find a parallel increase in both the diversity of  
100 functional guilds and the interaction frequency with *B. microphylla* as the number of  
101 limiting resources added increases.

102

### 103 **Material and Methods**

#### 104 Study system

105 The study was carried out during spring 2018 (October–December) at a 4.5-ha site  
106 embedded within a temperate woodland in northwestern Patagonia (El Foyel,  
107 41°38'37''S, 71°26'54''W, Rio Negro Province, Argentina). The study site is located at  
108 an altitude between 790 m and 880 m a.s.l. with annual precipitation of 920–1300 mm,  
109 mainly concentrated during the fall and winter seasons. The average annual temperature  
110 is 3 °C in winter (range: -2–9 °C) and 15°C in summer (range: 6–24 °C), and frosts are  
111 present approximately 80 days per year, mainly during June–August. Soils are  
112 Hapludands (Diehl et al. 2008), usually limited both by N and P with minimal  
113 atmospheric pollution (Perakis and Hedin 2002). Vegetation is characterized by a high-  
114 density mixed woodland where species are typical of the secondary succession stage.  
115 Dominant species include *Nothofagus antarctica* (Nothofagaceae), *Lomatia hirsuta*,  
116 *Embothrium coccineum* (Proteaceae), *Diostea juncea* (Verbenaceae), *Schinus patagonica*  
117 (Anacardiaceae), *Discaria chacaya* (Rhamnaceae) and *Berberis microphylla*  
118 (Berberidaceae), which are all widely distributed throughout the study site. The  
119 community of foliar herbivores in the study site is broadly diverse and includes at least

120 nine different functional guilds: leaf chewers, bud feeders, hole feeders, skeletonizers,  
121 sticky feeders, surface abrasion feeders, miners, sap-sucking, and galls (Garibaldi et al.  
122 2011, Nacif et al. 2020)

123         Among all local plant species, we selected *B. microphylla* as model species  
124 because *i*) it grows in all experimental plots, *ii*) it is one of the most abundant shrub  
125 species at the study site and *iii*) it is an important plant species that provides feeding  
126 resources to a wide broad range of organisms (e.g., herbivores, pollinators or frugivores).  
127 Furthermore, *B. microphylla* is an evergreen shrub that can grow up to 2 m tall and can  
128 be easily recognized by its thorns located in the stems in groups of three. It presents simple  
129 oval leaves with dimensions up to 40 mm × 14 mm and an area up to 2.8 cm<sup>2</sup>.

### 130 Experimental design

131 To evaluate the bottom-up effects of soil nutrient enrichment (i.e., a progressive addition  
132 of different limiting nutrients to woodland soils) on the interaction patterns of *B.*  
133 *microphylla* with foliar herbivores, we performed a fertilization field experiment with a  
134 full factorial design. We applied eight different fertilization treatments resulting from a  
135 factorial combination of N, P, and K (Fig. 1). Each of the eight treatments (N, P, K, NP,  
136 NK, PK, NPK, Control) was replicated with four blocks, resulting in a total of thirty-two  
137 31.5 × 45 m experimental plots (Fig. 1). After consulting local soil experts and following  
138 recommendations of previous research, we fertilized at a nutrient concentration of 100  
139 kg/ha of time-release urea for nitrogen (Lindberg and Persson 2004), 75 kg/ha of triple-  
140 superphosphate for phosphorus, and 56 kg/ha of potassium sulfate for potassium (Kim  
141 2008). Experimental plots were fertilized three times during the three years prior to the  
142 first sampling date (fertilizer application dates: November 2016, September 2017 and  
143 2018). Nutrients were applied by hand, resulting in an increment of 5%, 180% and 75%  
144 of nitrogen, phosphorus and potassium, respectively, when compared to soils in

145 nonfertilized plots. Although some variation in nutrient content among pre-treatment  
146 plots may exist, we expect that our randomized block design capture and account for that  
147 variation.

148

149 Characterization of vegetative plant traits, canopy cover, guild diversity and herbivory  
150 frequency.

151 We considered four vegetative traits relevant for interactions with foliar herbivores that  
152 might be sensitive to nutrient availability: plant volume, leaf density, leaf size and specific  
153 leaf area (SLA hereafter). We characterized these four traits at the plot level as follows.

154 Plant volume ( $m^3$ ) was estimated from 4–8 individuals per experimental plot (mean = 5.5,  
155  $n = 178$ ) as  $Height \cdot D_{max} \cdot D_{per}$ , where  $D_{max}$  is the largest diameter of the plant and

156  $D_{per}$  is its perpendicular diameter. To characterize the rest of the vegetative traits, we  
157 additionally sampled four random branches from each plant individual ( $n = 538$   
158 branches). Leaf density was estimated as the  $\frac{\text{number of leaves}}{\text{branch length, m}}$ .

159 We selected 16 green leaves per individual (4 random leaves per branch) for estimating  
160 leaf size and SLA. Individual leaf size ( $cm^2$ ,  $n = 2084$  leaves) was estimated by using  
161 ImageJ software (Rueden et al. 2017). Then, selected leaves were oven dried at 60 °C for  
162 two days and weighed by using a digital balance ( $\pm 0.1$  mg). SLA was defined as

163  $\frac{\text{leaf area, } cm^2}{\text{leaf dry mass, g}}$  for each leaf. We also characterized the canopy cover

164 (%) of the tree layer for each sampled plant as a surrogate for light availability by using  
165 the *Canopeo* app for Android (Patrignani and Ochsner 2015). Light availability was  
166 estimated as it is known to influence the vegetative traits of plants and the foraging  
167 behavior of herbivorous animals (Maiorana 1981).



168 We followed the approach proposed by Nacif et al (2020) for characterizing  
169 herbivore guild diversity, e.g., leaf chewers, bud feeders, hole feeders, miners (Nacif et  
170 al. 2020), yet we only observed foliar damage mediated by leaf chewers. Therefore, we  
171 finally did not include the diversity of herbivore guilds as an interesting variable in our  
172 analyses as no variation was found. Finally, the interaction of *B. microphylla* with leaf  
173 chewers was estimated as the frequency of damaged leaves (i.e.,  
174  $\frac{\text{damaged leaves}}{\text{total leaves}}$ ) for each sampled branch ( $n = 538$ ). The leaf area  
175 damage (i.e., the damaged surface of each leaf) was not considered as we found little  
176 variation in this variable.

177

#### 178 Data analysis

179 To evaluate the fertilizer effects on herbivory patterns and to assess whether they are  
180 mediated by changes in foliar traits and/or canopy cover, we followed a multi-step  
181 GLMM approach (Fig S1). It is worth noting that we used this multistep GLMM approach  
182 instead of a structural equation model (SEM) approach because current analytical  
183 development does not allow us to properly accommodate interactions among three factors  
184 (e.g.,  $N \times P \times K$  interactions) in SEMs (Lefcheck 2016). To assess the impact of nutrient  
185 amendment on the herbivory frequency, we first applied a generalized linear mixed-effect  
186 model (GLMM) (Pinheiro and Bates 2000, Gelman and Hill 2006). The model (GLMM-  
187 1) included the interaction among the different fertilizers (N, P, K) as fixed factors that  
188 take two possible values (1= fertilized, 0 = not fertilized), and the experimental block was  
189 included as a random effect. We fitted the model using a binomial distribution with a  
190 *logit*-link function and applied a Tukey post hoc contrast to assess pairwise differences  
191 among fertilization treatments that were involved in significant interactions. Given that  
192 we were interested in identifying those mediator variables that can potentially mediate

193 the effects of fertilizer amendment on herbivory patterns, we then applied an additional  
194 GLMM (GLMM-2). We used herbivory frequency as a response, potential mediator  
195 variables (i.e., plant volume, leaf density, SLA and canopy cover) as predictors and the  
196 experimental block as a random factor. The independent SLA variable was standardized  
197 to allow model convergence. All mediator variables were included as model predictors  
198 except leaf size since it correlated with leaf density (Spearman's  $r = -0.45$ ,  $p < 0.001$ ;  
199 Fig. 2) and canopy cover (Spearman's  $r = -0.50$ ,  $p < 0.001$ ; Fig. 2), therefore entailing  
200 potential multicollinearity issues. Note that the  $N \times P \times K$  interactions were not included  
201 in this model as we were interested in assessing the direct effect of the mediator variables  
202 on the herbivory frequency independent of the influence of the fertilizer application.  
203 Finally, we applied a set of GLMMs (GLMM-3) to assess the influence of nutrient  
204 enrichment on the significant mediator variables detected in GLMM-2. All models  
205 included the significant interactions among nutrients (if any) detected in the GLMM-1  
206 (i.e., in the herbivory frequency GLMM, Fig. S1). The experimental block was also  
207 included as a random effect across the different models. All models were fitted using a  
208 Gaussian distribution of errors with a *log*-link function, and the *DHARMA* R package  
209 (Hartig 2020) was used to verify that model residuals were normally distributed. Finally,  
210 when a statistically significant interaction was detected in any of the previous GLMMs,  
211 we also applied Tukey's post hoc contrast to assess pairwise differences among  
212 fertilization treatments involved in that interaction. We used the *lme4* R package (Bates  
213 et al. 2015) to perform all the abovementioned GLMMs and the *emmeans* R package to  
214 apply the post-hoc analyses (Lenth 2020).

215

216 **Results and discussion**

217 Northwestern Patagonian woodlands host a high diversity of generalist herbivorous  
218 insects, including different functional guilds such as leaf chewers, miners, skeletonizers  
219 or sticky feeders (Garibaldi et al. 2011, Nacif et al. 2020). However, we found that leaf  
220 damage in *B. microphylla* was mediated exclusively by leaf chewers, which include  
221 several lepidopteran larvae from the Geometridae, Noctuidae and Saturniidae families in  
222 our study system (Garibaldi et al. 2011). Similarly, other coexisting evergreen shrub  
223 species with similar ecological requirements and leaf traits such as *Maytenus chubutensis*  
224 (Celastraceae), showed a low diversity assemblage of herbivores dominated by leaf  
225 chewers (Nacif et al. 2020). Generalist herbivore species (e.g., leaf chewers) tend to select  
226 plants with high N content (Whitfield et al. 2012), which is higher in deciduous than  
227 evergreen species in our study system (Diehl et al. 2008). Therefore, the low diversity of  
228 herbivores in *B. microphylla* is most likely explained by the low palatability of leaves  
229 (i.e., low N content) when compared to deciduous plant species that dominate the plant  
230 community (e.g., *Nothofagus* sp.) (Diehl et al. 2008, Nacif et al. 2020).

231 Overall, herbivory frequency was low ( $0.05 \pm 0.006$ ; mean  $\pm$  SE) but consistent  
232 with values reported for other evergreen shrub species of the community (Nacif et al.  
233 2020). As stated above, generalist herbivores tend to avoid low palatable leaves in favor  
234 of more nutritional leaves produced by alternative plant species (Nacif et al. 2020). The  
235 addition of N fertilizer further accentuated this pattern by decreasing the herbivory  
236 frequency by 41% when compared to the control plots ( $\chi^2 = 12.9$ ,  $p < 0.01$ ; Table 1, Fig.  
237 3). However, as shown by the significant N  $\times$  K interaction detected ( $\chi^2 = 22.5$ ,  $p < 0.01$ ;  
238 Table 1), herbivory reduction mediated by nitrogen addition was only evident when  
239 potassium was not added to the experimental plots (Table 1; Fig. 3). Finally, we found no  
240 effect of phosphorus addition on herbivory patterns (Table 1), most likely because this  
241 nutrient was not limited in our study site (Diehl et al. 2008).

242 Previous studies suggest that the effects of nutrient enrichment on plant–animal  
243 interactions, rather than direct interactions, might be explained by changes in foliar traits  
244 and microhabitat characteristics (Borer et al. 2014, Lind et al. 2017). Accordingly, our  
245 GLMM analyses showed that leaf density had a marked negative effect on herbivory  
246 frequency ( $Z = -6.1, p < 0.01$ ; Table S1, Fig. 4a), which in turn was highly influenced by  
247 nitrogen and potassium addition in opposite directions (Table 1, Fig. 4b). On the one  
248 hand, individuals located in N-fertilized plots exhibited small leaves growing at high  
249 densities (N-fertilized plots:  $0.70 \pm 0.11$  leaves/cm vs. nonfertilized plots:  $0.56 \pm 0.17$   
250 leaves/cm; Table 1), as shown by the negative correlation among leaf density and leaf  
251 size (*Spearman's*  $r = -0.45, p < 0.001$ ; Fig. 2). As reported in previous research, the  
252 reduced herbivory detected in N-fertilized plots suggests that herbivorous insects might  
253 be avoiding small (and high dense) leaves, in favor of larger leaves (and low dense) as  
254 they are usually more palatable (Stiling and Moon 2005). Alternative nonexclusive  
255 explanations cannot, however, be discarded; for example, nitrogen enrichment might  
256 promote a more efficient synthesis of nitrogenous anti-herbivory compounds such as  
257 alkaloids, which would discourage herbivore foraging (Mattson 1980). Alternatively, a  
258 high foliar N content might reduce herbivory frequency if herbivores are able to meet  
259 their nutritional requirements at low *per capita* consumption rates.

260 On the other hand, our results show that potassium enrichment counteracted the  
261 negative effect of nitrogen addition on the herbivory frequency, as shown by the similar  
262 values in the NK-fertilized ( $0.056 \pm 0.006$ ) and the nonfertilized plots ( $0.061 \pm 0.006, p$   
263  $> 0.05$ ; Fig. 3, Table 1). This counteracting effect of potassium when added  
264 simultaneously with nitrogen might be mediated by the reduced leaf density (and  
265 increased leaf size; Fig. 2) observed in these NK plots (Table 1, Fig. 4b). As discussed  
266 above, herbivores preferentially selected individuals with sparse large leaves (NK-

267 fertilized:  $0.39 \pm 0.06$  leaves/cm vs. N-fertilized:  $0.70 \pm 0.11$  leaves/cm,  $p < 0.001$ ; Fig.  
268 4b; Table 1), most likely for optimizing their energy uptake from more palatable leaves  
269 (Mattson 1980). In addition, if the increase in leaf size was not accompanied by an  
270 increase in photosynthetic capacity with NK addition, then the production of defensive  
271 compounds (e.g., phenolics, terpenes) could be hampered and foliar herbivory promoted  
272 (Herms 2002, Glynn et al. 2003).

273 Unexpectedly, nutrient addition did not influence canopy cover (Table 1) as would  
274 be expected if canopy-dominant species of the community (e.g., *Nothofagus* sp., *Lomatia*  
275 *hirsuta*, *Embothrium coccineum*) increased their growth rate in response to the addition  
276 of limiting nutrients (Magnani et al. 2007, Thomas et al. 2010). However, canopy cover  
277 was still relevant to mediating herbivory patterns of *B. microphylla*, since individuals  
278 located under high canopy cover showed higher levels of herbivory ( $Z = 18.8$ ,  $p < 0.01$ ;  
279 Table S1, Fig. 4c). Despite a lack of a clear general pattern, increased herbivory in shaded  
280 microhabitats has been found in other research (Lincoln and Langenheim 1979, Shure  
281 and Wilson 1993, Muth et al. 2008). This preference for shaded microhabitats by  
282 herbivorous arthropods could be due to *i*) lower predation risks and *ii*) lower stressful  
283 abiotic conditions (e.g., more stable temperatures, higher humidity). Alternative  
284 explanations may rest on the response of key plant trait responses to modified  
285 microhabitats (Lincoln and Mooney 1984, Henriksson et al. 2003, Muth et al. 2008).  
286 Accordingly, canopy cover was positively correlated with leaf size (Spearman's  $r = 0.50$ ,  
287  $p < 0.001$ ) and negatively correlated with leaf density (Spearman's  $r = -0.29$ ,  $p < 0.001$ ,  
288 Fig. 2), which was previously shown to also increase the herbivory frequency.

289

290 **Conclusions**

291 Here, we demonstrate that soil nitrogen enrichment entails marked bottom-up effects on  
292 herbivory patterns of an important shrub species of the Patagonian woodlands, yet these  
293 effects are diluted when potassium is added simultaneously. Potassium, usually  
294 disregarded as a limiting nutrient in woodlands, has an interesting role in the regulation  
295 of nitrogen impacts on ecosystem functioning (Sardans and Peñuelas 2021). Our results  
296 further suggest that the impact of multiple nutrient additions on the interaction patterns  
297 between herbivores and *B. microphylla* might be mediated by changes in leaf density and  
298 leaf size. In conclusion, our results show that woodland eutrophication modifies  
299 antagonistic plant–animal interactions, ultimately affecting key ecosystem functions such  
300 as herbivory. We expect similar effects in other temperate woodlands hosting plant  
301 communities adapted to nutrient-limited soils (Verheyen et al. 2012, Carvalheiro et al.  
302 2020a). Buffering woodland eutrophication effects driven by human activities seems  
303 therefore essential to maintain important ecological functions associated with  
304 antagonistic plant–animal interactions and avoid ecosystem dysfunctioning.

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306

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311 Incorporación program from the Ministry of Science and Innovation.

312 **Table 1:** Analysis of deviance ( $\chi^2$ ) for generalized linear mixed effects models  
 313 assessing changes in herbivory frequency, leaf density and canopy cover under the  
 314 factorial nutrient addition experiment. Note that for leaf density and canopy cover we  
 315 only included the N  $\times$  K interaction in the models as P was not found to influence the  
 316 herbivory frequency in GLMM-1. Intercepts were allowed to vary by block (random  
 317 effect). Bold values represent statistically significant effects (\*\*\*) p-value < 0.001; \*\* <  
 318 0.01; \* < 0.05).

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 320  
 321  
 322

	<i>Analysis of deviance (<math>\chi^2</math>)</i>		
	Herbivory frequency	Leaf Density	Canopy cover
Control	-	-	-
N	<b>12.91</b> ***	0.1149	1.108
P	0.50	-	-
K	3.23	<b>4.9364</b> *	2.582
NP	3.15	-	-
NK	<b>22.50</b> ***	<b>2.9697</b> *	0.135
PK	2.25	-	-
NPK	0.05	-	-

323 **Figures**

324 **Figure 1.** Study site and experimental design: A) Map of geographical location of “El  
325 Foyel” temperate woodland; B) Illustrative picture of the physiognomy of “El Foyel”  
326 temperate woodland C) Experimental setup consisted of 32 experimental plots distributed  
327 across 8 fertilization treatments (factorial combination of N, P and K addition and a  
328 control non-fertilization treatment) and 4 replication blocks.

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332 **Figure 2:** Correlogram showing correlations (Spearman's correlations) among all  
333 variables characterized. Positive correlations are displayed in blue and negative  
334 correlations in red. Circle size and intensity of the color are proportional to the  
335 correlation coefficients (see the legend at the right side of the plot to associate colors  
336 and correlation coefficients). Only significant correlations are displayed ( $p < 0.01$ ).  
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340

341 **Figure 3.** Impact of fertilizers (N-K) on the herbivory frequency of *Berberis microphylla*.  
342 Note that phosphorus addition was not included as it did not influence the frequency of  
343 herbivory. Points indicate the estimates of GLMMs while error bars show the estimated  
344 Standard Errors (SE). Different letters indicate statistical differences (*p-value* < 0.05)  
345 among treatments after applying a Tukey post-hoc contrast.

346

347

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349

350 **Figure 4.** *Panel A:* Relationships between leaf density and the herbivory frequency (i.e.,  
351 the proportion of damaged leaves) resulting from the fitted GLMM. *Panel B:* Impact of  
352 nitrogen and potassium amendment (N:K) on leaf density. Points indicate the estimates  
353 of GLMMs while error bars show the estimated Standard Errors (SE). Different letters  
354 indicate statistical differences ( $p$ -value < 0.05) among treatments after applying a Tukey  
355 post-hoc contrast. Note that phosphorus addition was not included as it did not influence  
356 the frequency of herbivory. *Panel C:* Relationship between the canopy cover and the  
357 herbivory frequency resulting from the fitted GLMM.

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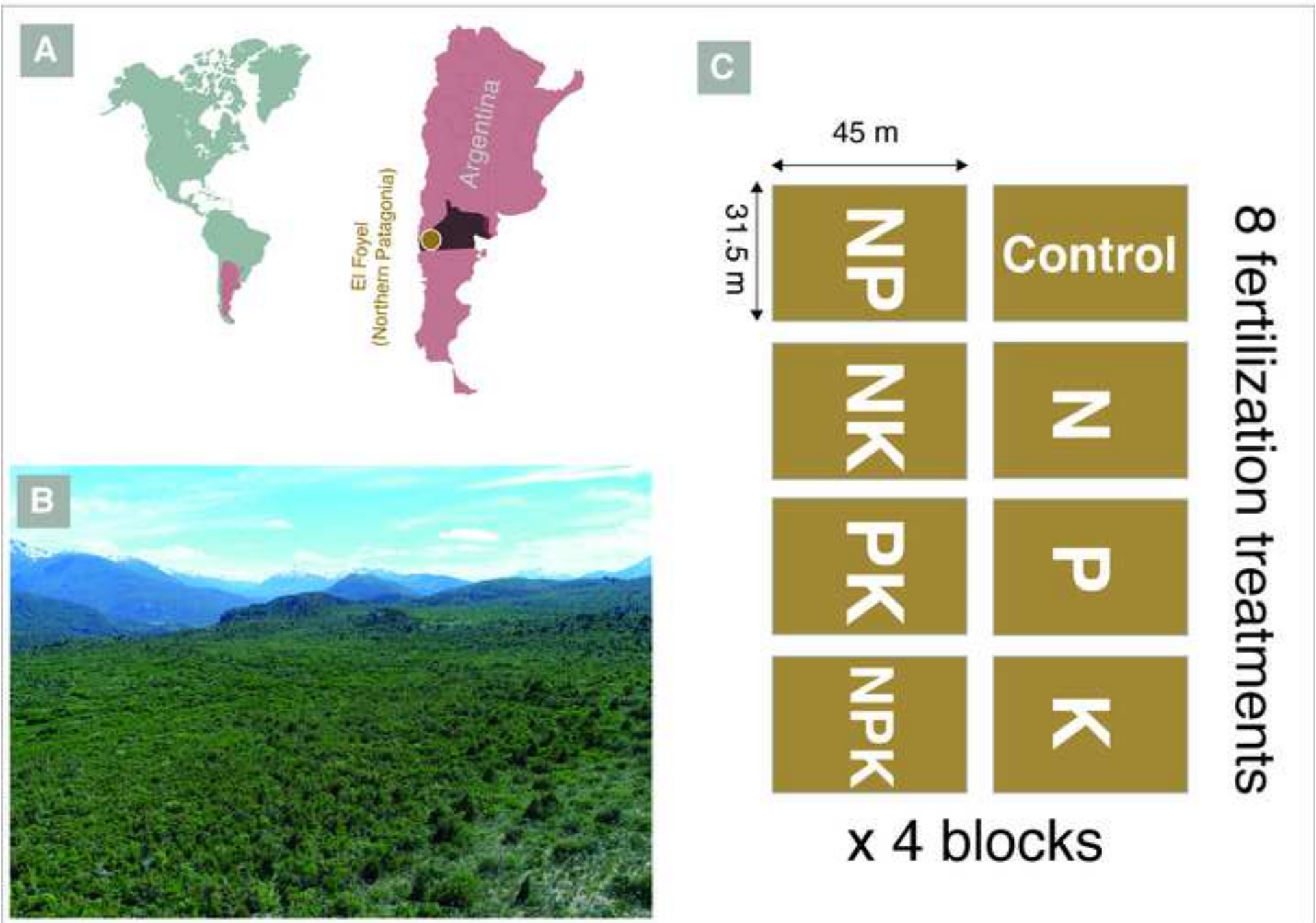


Figure 2

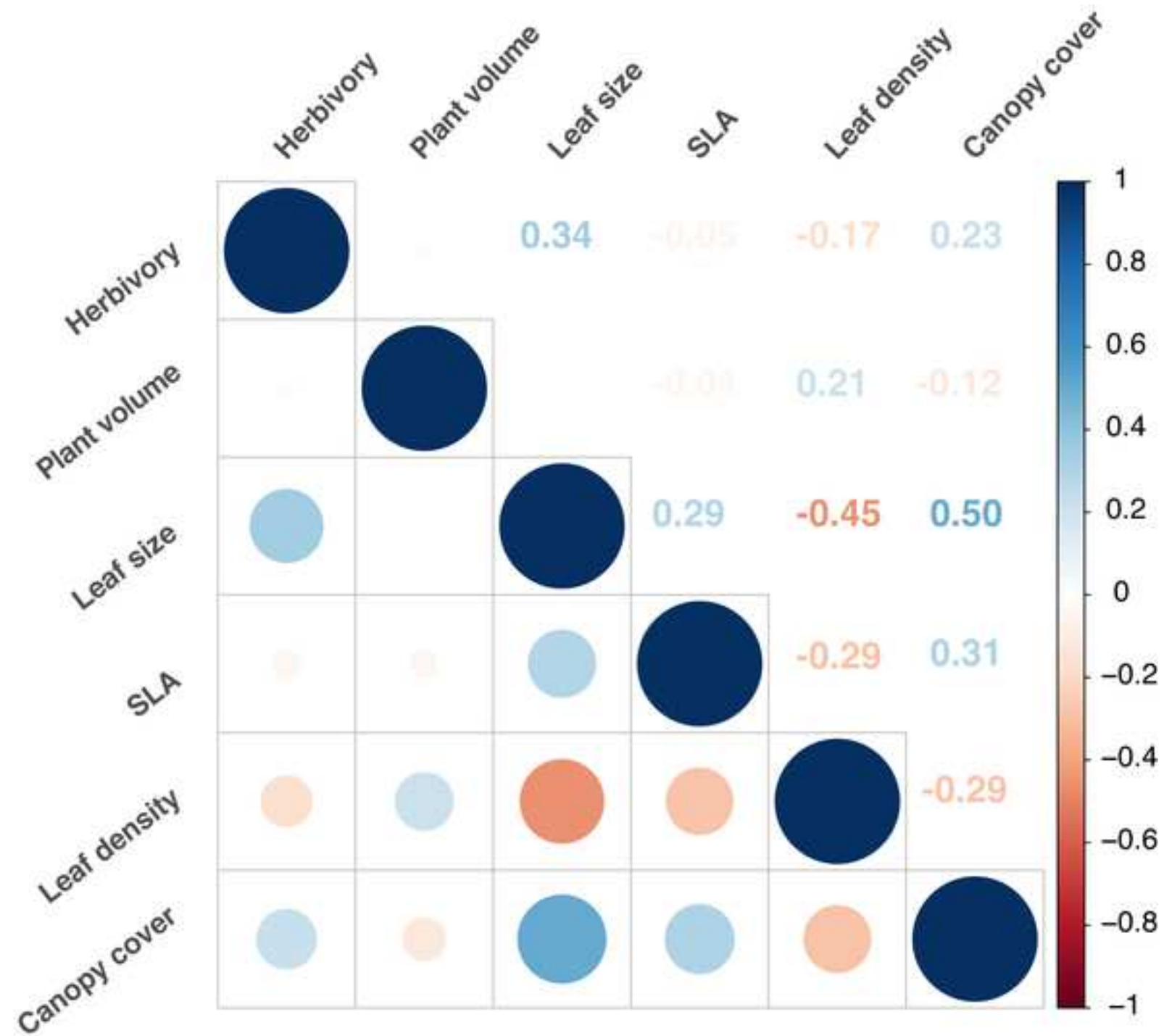
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Figure 3

