

1 **Evolutionary history of grazing and resources determine herbivore exclusion effects on plant**  
2 **diversity**

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52 **Ecological models predict that the effects of mammalian herbivore exclusion on plant diversity**  
53 **depend on resource availability and plant exposure to ungulate grazing over evolutionary time.**  
54 **Using an experiment replicated in 57 grasslands on six continents, with contrasting evolutionary**  
55 **history of grazing, we tested how resources (mean annual precipitation and nutrient addition)**  
56 **determine herbivore exclusion effects on plant diversity, decomposed into richness and evenness.**  
57 **We tested the hypothesis that in sites with a long history of ungulate grazing, plant diversity**  
58 **decreases with herbivore exclusion in resource-rich sites; whereas in short-history sites the effect**  
59 **of herbivore exclusion depends on plant species origin (native or exotic from long-history sites).**  
60 **We found that in long-history sites, herbivore exclusion reduced plant diversity by reducing both**  
61 **richness and evenness, and the responses of richness and diversity to herbivore exclusion**  
62 **decreased with mean annual precipitation. In short-history sites, the effects of herbivore exclusion**  
63 **differed for native and exotic plant richness and depended on fertilization; native species richness**  
64 **was unaffected by herbivore exclusion, whereas exotic species richness declined with herbivore**  
65 **exclusion in fertilized plots. In sum, herbivore exclusion caused loss of plant diversity in grasslands**  
66 **that evolved with ungulates, but these findings do not simply extrapolate to other grasslands,**  
67 **rather they depend on grazing history and plant provenance. Thus, plant species' evolutionary**  
68 **history of grazing continues to shape the response of the world's grasslands to changing**  
69 **mammalian herbivory.**

70

71 Grasslands cover 30% of the Earth's terrestrial surface and provide livelihoods for ca. 800 million  
72 people <sup>1</sup>. Human activities are altering the plant and herbivore diversity of grasslands worldwide by  
73 increasing nutrient availability and changing the species composition, abundance, and foraging  
74 patterns of domestic and wild mammalian herbivores <sup>2-4</sup>. Given the ongoing losses of wild herbivores  
75 <sup>5</sup>, determining the mechanisms by which nutrients and large mammalian herbivores regulate plant  
76 diversity is critical for grassland conservation and management <sup>6</sup>.

77           The effect of large, mammalian herbivores (hereafter herbivores) on plant diversity is  
78 predicted to depend on three potentially interacting factors—the evolutionary history of grazing by  
79 herds of large, hoofed herbivores (i.e., ungulates); resource availability for plant growth, especially  
80 water and nutrients; and modern-era grazing intensity<sup>7-14</sup>. Fluctuations in grazing intensity in sites  
81 with a relatively long evolutionary history of ungulate occurrence (i.e., grazing present for >500 to  
82 10,000 years, hereafter long-history sites) have selected for two different pools of plant species—  
83 short-statured species that are tolerant or resistant to grazing and tall-statured species that are less  
84 grazing-resistant but better at capturing light<sup>9,15</sup>. In these long-history sites, models predict that the  
85 effect of herbivores on plant diversity depends on the availability of resources for plant growth. In  
86 sites with high rainfall or high nutrient availability, a few tall plant species dominate in the absence  
87 of grazing (i.e., low richness and low evenness), thereby reducing ground-level light available for  
88 seedlings and short-statured species<sup>8,9,13</sup>. In this case, herbivores promote plant diversity by  
89 increasing light availability for shorter, more grazing-tolerant species<sup>8,9,16,17</sup>. Hence, herbivores  
90 increase diversity both through increased species richness (more grazing-tolerant species) and  
91 evenness (lower dominance of tall, less grazing-resistant species). In resource-poor sites, where light  
92 is generally less limiting, herbivores may have little effect on plant diversity, or negative effects if  
93 resource limitation (water, nutrients) prevents regrowth or plant reproduction after grazing<sup>6</sup>.  
94 Exclusion of herbivores from long-history sites is, therefore, expected to have no effect or increase  
95 plant diversity at low resource availability and decrease diversity at high resource availability (Box 1).

96           For sites with a relatively short evolutionary history of ungulate grazing (i.e., <500 years,  
97 hereafter short-history sites), it is predicted that herbivores generally reduce plant diversity<sup>9</sup>. Here,  
98 native plant species are assumed to lack mechanisms for tolerating or resisting grazing, instead  
99 possessing upright growth-forms with elevated buds that are more vulnerable to grazing and  
100 trampling<sup>13</sup>. These grazing impacts on diversity are likely to be especially relevant in grasslands  
101 recently exposed to Eurasian-derived animal husbandry practices. In this case, grazing intensity is  
102 shaped by non-migratory domestic grazers whose stocking densities and duration of grazing per unit

103 area may lack evolutionary precedents<sup>8,9,13</sup>. These effects will likely be further compounded by any  
104 management-based resource augmentation involving watering, fertilization, or offsite feed  
105 supplementation that increases herd capacity above what the sites could otherwise sustain. Under  
106 these circumstances, herbivore exclusion should, in theory, increase the diversity of plants with  
107 grazer-sensitive growth-forms relative to grazed plots (Box 1). One exception might be at low grazing  
108 intensity in resource-rich sites, where herbivore exclusion may decrease diversity due to competitive  
109 release (see also Fig. 1 in Cingolani et al.<sup>8</sup>). However, empirical evidence and alternative models  
110 (e.g., state-and-transition models<sup>18</sup>) demonstrate irreversible reductions in plant diversity due to  
111 local extinction of grazing-sensitive species<sup>8,19,20</sup> (R1 wanted more empirical refs, can folks from  
112 short-history regions suggest some key citations supporting this point). Recovery of plant species  
113 following herbivore exclusion can be limited by seed availability in the soil seedbank, propagule  
114 dispersal, or both, as well as invasion by exotic plants, soil erosion and elevated soil nutrients<sup>18,20</sup>.  
115 Hence, we expect neutral responses (if recovery of grazing-sensitive species is not possible) to small  
116 increases in native diversity (due to increased plant species richness) in response to herbivore  
117 exclusion at short-history sites (Box 1).

118 Many short-history sites are now dominated by non-native, potentially invasive, plant  
119 species originating from regions with a long evolutionary history of grazing, which were often  
120 intentionally sown for forage<sup>8,9,13</sup>. Short-history grasslands can be transformed from native to exotic  
121 dominance through a combination of introduced livestock grazing, introduced plants from regions  
122 with long-term ungulate husbandry, increased temporal and spatial offtake caused by fencing or  
123 provision of water, and sometimes fertilizer addition<sup>8,9,21-23</sup>. Hence, plant community diversity  
124 responses to herbivores in short-history sites may reflect the evolutionary history of the non-native  
125 species, and grazing-adapted non-native species will often increase if the native community does not  
126 contain grazing-adapted plants<sup>24,25</sup>. We therefore predict that responses of exotic species in short-  
127 history sites to herbivore exclusion will match responses of native species from long-history sites  
128 (Box 1).

129 Two recent meta-analyses provide limited support for model predictions that the plant  
130 diversity response to herbivore exclusion depends on resource availability (proxied by precipitation  
131 or aridity and plant biomass)<sup>26,27</sup> or evolutionary history of grazing<sup>27</sup>. Both studies found that, in  
132 general, herbivore exclusion tends to reduce plant species richness<sup>26,27</sup>. However, while meta-  
133 analysis provides a quantitative synthesis of published studies, differences in design and  
134 methodology among experiments obscures inference about the relative importance of the biological  
135 and methodological factors underlying the observed patterns<sup>6,28</sup>. For example, increasing light  
136 availability at ground level is a direct mechanism by which herbivores support plant diversity<sup>29</sup>, but  
137 often data on light capture by the canopy is not measured. Instead a range of proxies for light  
138 availability (e.g., precipitation, aboveground plant biomass) have been employed in past studies to  
139 test model predictions<sup>9,30</sup>, likely leading to variation among results because these proxies vary in  
140 explanatory power<sup>29</sup>. We argue direct measures of resource availability (light, moisture, nutrients)  
141 should be used to determine how the relationship between grazing and diversity is mediated by  
142 resource availability.

143 Here, we use an unprecedented herbivore exclusion experiment using a standardised  
144 experimental and sampling design, in 57 grasslands on six continents (Fig. 1), to examine responses  
145 of plant diversity, richness and evenness to herbivore exclusion. We test mechanistic hypotheses  
146 related to light availability, grazing history and current grazing intensity. This is the first large-scale  
147 distributed experiment to explore these effects across sites differing in evolutionary history of  
148 ungulate grazing. To compare our results with previous studies and conceptual models, we include  
149 precipitation as a measure of resource availability, with sites spanning an order of magnitude of  
150 annual precipitation (192-1,877 mm). We also modified resources through addition of nitrogen,  
151 phosphorus, potassium plus micronutrients (NPK $\mu$ ). The experimental nutrient addition enables us  
152 to provide a more mechanistic understanding of the role of resources in mediating grazing effects  
153 within sites when climate, soil type and herbivore communities are kept constant. Additionally, we  
154 have direct measures of light availability at ground level from 47 of the 57 sites. We classified

155 grasslands as having long (24 sites) or short (33 sites) evolutionary history of grazing according to  
156 local experts and definitions in Milchunas and Lauenroth<sup>30</sup> (Fig. 1; Supplementary Note 1;  
157 Supplementary Table 1). All plant species were classified as native or exotic by experts at each site.  
158 We compared plots that were fenced to exclude all vertebrate herbivores (body mass >50 g) for  
159 three years with unfenced plots that were subject to variable grazing by the contemporary suite of  
160 herbivores present at each site (Supplementary Table 3). Our hypotheses, arising from the  
161 generalised grazing models<sup>8,9</sup>, are described in Box 1.

162

### 163 **Results and discussion**

164 Across 57 globally distributed grasslands, the evolutionary history of grazing was critical for  
165 predicting plant diversity responses to herbivore exclusion. Our results highlight the importance of  
166 grazing for maintaining diversity (Fig. 2; particularly native species richness, Fig. 3a) in long-history  
167 sites, especially at higher precipitation (Table 1; Fig. 4). In contrast, current grazing had a negligible  
168 effect on native species richness in short-history sites (Fig. 3a). However, exotic species that mostly  
169 originated from long-history sites profited from grazing under fertilized conditions, responding  
170 similarly to native species from long-history sites (Fig. 3a,b). Hence, we show that the evolutionary  
171 history of individual species also regulates plant diversity responses. Together, these results suggest  
172 that conservation of native plant diversity may be facilitated by maintaining grazing herbivores in  
173 long-history sites, whereas short-history sites show muted (and variable) responses to herbivore  
174 exclusion.

175 In long-history sites, herbivore exclusion decreased diversity (measured as inverse Simpson's  
176 or  $ENS_{PIE}$ ; unfertilized:  $t = -3.73$ ,  $P < 0.001$ , fertilized:  $t = -4.62$ ,  $P < 0.001$ ), plant richness (unfertilized:  
177  $t = -1.70$ ,  $P = 0.046$ , fertilized:  $t = -2.64$ ,  $P = 0.005$ ) and Simpson's evenness (unfertilized:  $t = -3.10$ ,  $P <$   
178  $0.001$ , fertilized:  $t = -2.68$ ,  $P = 0.004$ ) (Fig. 2; Supplementary Fig. 1). Hence, herbivore exclusion  
179 decreased the number of native plant species (Fig. 3a) and increased dominance of a few plant  
180 species, consistent with other studies in which herbivores also promoted diversity<sup>12,16,31,32</sup>. In long-

181 history sites, dominant plant species are often palatable, and grazing reduces their dominance,  
182 thereby increasing evenness, and promoting more grazing-tolerant native species<sup>33</sup>. Reductions in  
183 native richness with herbivore exclusion were due to a reduction in light availability in fenced plots,  
184 confirming the mechanism by which herbivores maintain plant diversity (Fig. 5)<sup>28</sup>.

185 Consistent with our first hypothesis (Box 1) and model predictions<sup>8,9</sup> for long-history sites,  
186 herbivore exclusion reduced plant species richness, and thereby diversity, more at sites with greater  
187 precipitation (Table 1; Fig. 4a). Contrary to what we expected, herbivore exclusion decreased plant  
188 species richness across the whole of the studied precipitation gradient (Fig. 4a), which was driven by  
189 the response of native species to herbivore exclusion (Fig. 4b). Hence, herbivores are important in  
190 maintaining native plant richness at long-history sites, even at sites with low rainfall. However, it is  
191 possible that increases in plant richness with herbivore exclusion were not found because  
192 precipitation was not low enough, with few sites being classified as semi-arid (only three sites with  
193 <250 mm MAP). We found similar results using an index of aridity for diversity (Supplementary Table  
194 7).

195 Contrary to our expectation of a stronger reduction in diversity with nutrient addition for  
196 long-history sites, fertilization did not alter the effect of herbivore exclusion on inverse Simpson's  
197 diversity, species richness or Simpson's evenness (Fig. 2; error bars of unfertilized and fertilized  
198 treatments overlap), nor the relationship between the *LRR* (log response ratio) of richness and  
199 rainfall (no significant interactions with fertilization; Table 1). Here, we predicted an overall stronger  
200 reduction in diversity in fertilized compared to unfertilized conditions, independent of precipitation,  
201 as nutrient addition increases resource availability (i.e., shift toward the resource-rich end in Box 1).  
202 Nutrient addition alone decreased inverse Simpson's diversity and plant richness, and we found the  
203 lowest diversity and richness in fertilized plots from which herbivores were excluded  
204 (Supplementary Fig. 1). However, fertilization did not alter the effect of herbivore exclusion on  
205 diversity, likely because some sites are water-limited and unable to respond to the additional  
206 nutrients sufficiently to move to a light-limited state<sup>29</sup>. Additionally we found ambient soil nitrogen



207 (from control plots) had no effect of the relationship between grazing and diversity, richness, or  
208 evenness suggesting soil nutrients have little effect on this relationship (Supplementary Table 8).

209 The theoretical models underpinning our hypotheses predict that the effect of grazing on  
210 plant diversity will depend on current grazing intensity<sup>8,9</sup>. Here, we used an index of grazing  
211 intensity that incorporated importance values of all current herbivore species at 43 sites, estimated  
212 by site experts<sup>34</sup>. Then, we tested whether the *LRRs* of inverse Simpson's diversity, plant richness  
213 and Simpson's evenness to herbivore exclusion were related to this index. In the long-history sites,  
214 herbivore exclusion decreased diversity more when current grazing intensity was higher, driven  
215 mainly by changes in evenness (Supplementary Fig. 2). As few sites had high herbivore densities,  
216 these results suggest that grazing even at intermediate intensities is sufficient to decrease plant  
217 dominance and maintain plant diversity (Fig. 2; Supplementary Fig. 2).

218 Consistent with our predictions for sites with a short evolutionary history of grazing (Box 1),  
219 herbivore exclusion had no effect on overall inverse Simpson's diversity (unfertilized:  $t = -0.72$ ,  $P =$   
220  $0.472$ , fertilized:  $t = -0.94$ ,  $P = 0.351$ ), plant richness (unfertilized:  $t = -0.25$ ,  $P = 0.805$ ; fertilized:  $t = -$   
221  $1.10$ ,  $P = 0.273$ ) or Simpson's evenness (unfertilized:  $t = -0.57$ ,  $P = 0.568$ ; fertilized:  $t = 0.29$ ,  $P =$   
222  $0.771$ ) (Fig. 2; Supplementary Fig. 1), and these responses were not related to precipitation (Fig. 4a).  
223 The results of the total species pool do not support the Milchunas et al.<sup>9</sup> model that diversity should  
224 increase with herbivore exclusion (Fig. 4a). However, the response of total species diversity reflects  
225 the combined responses of both native and exotic species, and species in these groups differ in their  
226 evolutionary history of grazing (Supplementary Table 4). At short-history sites, an average of 32% of  
227 species were exotic (compared to 13% in long-history sites), and 93% of these species originated  
228 from regions with a long evolutionary history of ungulate grazing (Supplementary Table 4); hence,  
229 the species pool comprised species with mixed evolutionary exposure to grazing.

230 In sites with a short evolutionary history of grazing, we predicted different responses for  
231 native and exotic species, because native species lack tolerance or resistance mechanisms to  
232 ungulate grazing; whereas exotic species mostly originate from long-history sites<sup>8,9,21,22</sup>. We found

233 support for our prediction (Box 1) that diversity responses in short-history sites reflected the  
234 evolutionary grazing history of the plant species present. We expected recovery of native species to  
235 be limited (neutral to small richness increases) with herbivore exclusion, especially under eutrophied  
236 conditions because native species are often disadvantaged by high soil nutrients<sup>35</sup>. Indeed, we  
237 found no overall response of native species richness to herbivore exclusion in unfertilized ( $t = 0.90$ ,  $P$   
238  $= 0.371$ ) or fertilized treatments ( $t = 0.11$ ,  $P = 0.913$ ) (Fig. 3a). However, the response of native  
239 species to herbivore exclusion depended on the extent that herbivores changed light availability,  
240 similarly to the long-history sites (Fig. 5). We also predicted that exotic species in short-history sites,  
241 which mostly have a longer evolutionary exposure to ungulate grazing, would decrease in diversity  
242 with herbivore exclusion, as with native species from long-history sites. We found partial support for  
243 this prediction, as exotic species richness decreased with herbivore exclusion under fertilized  
244 conditions ( $t = -2.98$ ,  $P = 0.002$ ; Fig. 3b), as we had expected at high resource availability (Box 1), and  
245 in sites with lower grazing intensity (Supplementary Figures 2b and 3). However, exotic species  
246 richness did not decrease with herbivore exclusion under unfertilized conditions ( $t = -1.24$ ,  $P = 0.218$ ;  
247 Fig. 3b), and the response to herbivore exclusion was not related to precipitation (Fig. 4b). These  
248 results indicate that in short-history sites, nutrient enrichment and low-intensity grazing promotes  
249 exotic species richness.

250         The neutral responses of native plant species to herbivore exclusion suggest little recovery  
251 from grazing in short-history sites, which conflicts with the single equilibrium model proposed by  
252 Milchunas et al.<sup>9</sup>. However, the results support our predictions that short-history sites may diverge  
253 from the single equilibrium model and are consistent with previous studies (reviewed in Cingolani et  
254 al.<sup>8</sup> Table 2) and conceptual models<sup>18</sup>. There are several possible interpretations of our findings.  
255 First, recovery of grazing-sensitive species may require longer timeframes than our study (three  
256 years). Second, recovery of native species may not be possible because they have become locally  
257 extinct, or because grazing and exotic invasion have altered ecological processes resulting in stable,  
258 degraded states<sup>19,33,36</sup>. In this case, *current grazing* may not be impacting diversity as effects

259 occurred in the past. This can be the case despite significant negative effects of historical livestock  
260 grazing (e.g., if grazing intensity now is lower than previous levels)<sup>33</sup>. It is also possible that neutral  
261 responses to herbivore exclusion indicate grazing never affected native species as we do not have  
262 historical data on the impacts of grazing in these sites. However, for the short-history regions  
263 included in the study extensive evidence has reported negative effects of introduced stock grazing  
264 on plant diversity<sup>37</sup>(here also please suggest references from other short history regions).

265         Whilst we could not distinguish the contribution of the above factors to the muted  
266 responses of native species to herbivore exclusion in short-history sites, we were able to explain  
267 some of the variability in the responses. We found that herbivore exclusion decreased species  
268 richness at sites with lower grazing intensity (Supplementary Fig. 2b), driven by declines in exotic  
269 species richness (Supplementary Fig. 3). While the models predict this pattern in both short- and  
270 long-history sites, we only detected this relationship for exotic species (that originate from long-  
271 history sites) in short-history sites. We found this pattern was reversed at higher grazing intensity,  
272 where herbivore exclusion increased exotic species richness. At least 28 of the 33 short-history sites  
273 have a history of domestic ungulate grazing (Supplementary Table 5), but few were grazed by  
274 ungulates during the experiment. Our variable diversity responses to herbivore exclusion may be  
275 partly due to differences in the recovery trajectory. That is, we are removing herbivores at different  
276 points along a longer time span of removal of domestic grazing at these sites. In this case, the  
277 control plots might also be on a trajectory of recovery from higher levels of ungulate grazing. It is  
278 difficult to determine what measures of grazing intensity are most relevant, especially for the short-  
279 history sites where historical grazing may have been much higher than current grazing. In some  
280 cases, historical grazing impacts may be difficult to reverse<sup>16</sup>.

281         By combining evolutionary history of grazing and species origin, our empirical results from  
282 57 sites spanning six continents extends understanding of herbivore effects on grassland diversity  
283 beyond that of recent meta-analyses. Consistent with previous studies, we report reductions in plant  
284 diversity with herbivore exclusion<sup>26,27</sup>, but only in long-history sites. Consistent with theoretical

285 predictions but contrasting with recent meta-analyses <sup>26,27</sup>, our distributed experiment  
286 demonstrated that the response of diversity to herbivore exclusion depended on precipitation <sup>26</sup>,  
287 albeit again only in long-history sites. These contrasting results between short- and long-history sites  
288 were as we predicted, based on theoretical and empirical models that suggest muted responses of  
289 native species to herbivore exclusion in short-history sites due to irreversibility of grazing effects <sup>8,18</sup>.  
290 The meta-analysis that incorporated evolutionary history of grazing did not find this factor to be  
291 important <sup>27</sup>, pointing to the importance of our study's identical methods and directly comparable  
292 responses across sites and, likely, the importance of allocation of sites to long- and short-history of  
293 grazing. The meta-analysis classified all sites in the Americas as long-history, whereas some regions  
294 in the USA (e.g., in California, Florida and Oregon) and in South America are better characterized as  
295 short-history (e.g., the Pampas in Argentina) (Fig. 1; Supplementary Note 1). Moreover, our data  
296 allowed us to decompose richness into native and exotic species, enabling us to determine that  
297 decreases in richness with herbivore exclusion were limited to exotic species in short-history sites,  
298 providing resolution that has not been possible in other datasets <sup>27</sup>.

299         Although we found the diversity responses to herbivore exclusion depended on evolutionary  
300 history of grazing, the mechanisms by which grazing can promote diversity were the same regardless  
301 of grazing history. We found the alleviation of light limitation as a mechanism through which  
302 herbivores promote native species richness, regardless of evolutionary history of grazing (Fig. 5) <sup>29</sup>.  
303 Here, the response of light availability to herbivore exclusion was related to aboveground biomass in  
304 both long- and short-history sites (Supplementary Fig. 4), and not to precipitation. In sites with  
305 greater plant biomass, herbivore exclusion decreased light availability more than in sites with lower  
306 plant biomass. In contrast, Koerner et al. <sup>26</sup> concluded that changes in dominance, measured with  
307 the Berger-Parker Dominance Index, was the main mechanism through which herbivore exclusion  
308 reduces plant richness. In this study, we did not use the Berger-Parker Dominance Index as it was  
309 correlated with plant richness (Pearson's  $r = -0.6$ ). Instead, we decomposed diversity into richness  
310 and evenness (i.e., the inverse of dominance) and determined that herbivore exclusion reduced

311 diversity in long-history sites via reductions in both of these variables. Thus, our results are broadly  
312 consistent with this meta-analysis<sup>26</sup>, but rather than treating dominance as a mechanism, we view it  
313 as an intermediate response variable, with an underlying mechanism.

314

## 315 **Conclusions**

316 Our findings confirm predictions of the Milchunas et al.<sup>9</sup> model for regions with a long-history of  
317 grazing, while for short-history sites our results support non-equilibrium models such as that  
318 proposed by Cingolani et al.<sup>8</sup> in their modification of the Milchunas model. The effect of herbivores  
319 on biodiversity has been contentious<sup>26,27,38</sup>, and contrasting results in previous studies may stem  
320 from analysing long- and short-history sites together. By separating sites that have a long- and short-  
321 history of ungulate grazing, we found some support for long-held theories about the response of  
322 plant diversity to herbivore exclusion in relation to resource availability. Consistent with conceptual  
323 model predictions, we found stronger reductions in plant species richness with herbivore exclusion  
324 at higher precipitation in sites that have a long evolutionary history of grazing. This relationship did  
325 not exist for short-history sites. We found that one of the mechanisms by which herbivores can  
326 promote plant richness is through alleviating light limitation, and this is generalizable across sites  
327 regardless of the evolutionary history of grazing. In sites with a short evolutionary exposure to  
328 ungulate grazing, diversity responses to herbivore exclusion were related to species origin with some  
329 evidence that exotic species respond similarly to native species from long-history sites, as most  
330 exotic species originate from long-history sites. Native species in short-history sites showed little  
331 recovery following herbivore exclusion, suggesting that short-history grasslands might be in a stable  
332 (and potentially degraded) state. While fencing alone would be insufficient, restoration  
333 interventions in addition to fencing, such as seeding, planting, or fire, may increase native plant  
334 diversity at these sites. However, studies have found persistent land-use legacies from grazing, and  
335 that re-instating a more historical disturbance (like fire) does not necessarily increase native diversity  
336 if ecosystems are in a degraded, but stable state after grazing<sup>19</sup>. More generally, our findings

337 highlight the importance of evolutionary history and species provenance in interpreting and  
338 predicting the effects of human land-use and global change.

339

## 340 **Methods**

341 **Site selection and experimental treatments.** This study used data from the Nutrient Network  
342 ([www.nutnet.org](http://www.nutnet.org)) a globally replicated experimental study of herbivore exclusion and nutrient  
343 addition in grassland ecosystems. A fully factorial combination of herbivore exclusion ('unfenced' or  
344 'fenced') and nutrient addition ('unfertilized' or 'fertilized') was applied at each site, totalling four  
345 treatment plots (5 m x 5 m) in three blocks (although some sites have a different number of blocks;  
346 Supplementary Table 2). Fences (up to 2.30 m high) were designed to exclude aboveground  
347 mammalian herbivores (> approximately 50 g). The fertilized plots received annual applications of 10  
348 g m<sup>-2</sup> year<sup>-1</sup> N, P and K as time-released urea [(NH<sub>2</sub>)<sub>2</sub>CO], triple-super phosphate [Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>], and  
349 potassium sulphate [K<sub>2</sub>SO<sub>4</sub>]. Micronutrients (μ) were applied once, at the start of the experiment, as  
350 100 g m<sup>-2</sup> mix of Fe (15%), S (14%), Mg (1.5%), Mn (2.5%), Cu (1%), Zn (1%), B (0.2%) and Mo (0.05%).  
351 More details on the experimental design and nutrient sources are available in Borer et al. <sup>28</sup>.

352 For this study, 57 NutNet sites were included that met two conditions: (i) experimental data  
353 had been collected for the four treatments for at least three years of treatment applications; and (ii)  
354 mammalian herbivores were present in the sites, and were excluded by the fences (see  
355 Supplementary Table 2 for an overview of the sites included and Supplementary Table 6 for sites  
356 with exceptions to the NutNet fence design). Sites that only had herbivore species with a body  
357 weight <50 g (e.g. voles, mice, rats, squirrels, gophers) were not included, as these herbivores are  
358 not likely to be excluded by the fences. The mammalian herbivores in the sites ranged from  
359 domestic ungulates, such as sheep and cattle, to wild ungulates such as deer, wild macropods like  
360 kangaroo and other medium-sized mammals (for an overview of all herbivore species see  
361 Supplementary Table 3). The study sites represent a wide range of herbaceous ecosystems including  
362 prairie, montane grassland, shrub steppe, alpine grassland and savanna. The sites also encompassed

363 varying environmental conditions; e.g., mean annual precipitation (192–1,877 mm) and mean annual  
364 temperature (-3–24°C) (Fig. 1; Supplementary Table 2).

365 We classified our sites as subject to a long (grazing present >500 to 10,000 years ago; 24  
366 sites) or short (<500 years; 33 sites) evolutionary history of grazing according to site experts and  
367 Milchunas and Lauenroth<sup>30</sup> (see Supplementary Note 1 for details). We used three years of post-  
368 treatment data at all sites (i.e., plant data, light; see data collection below), which was sufficient to  
369 observe herbivore impacts on aboveground biomass<sup>39</sup>.

370

371 **Data collection and calculations.** All NutNet sites followed standard sampling protocols, with yearly  
372 sampling at peak biomass. In a permanently marked 1 m x 1 m subplot that was randomly  
373 designated, cover was estimated visually to the nearest 1% for all plant species. Studies have  
374 reported variable responses of diversity to grazing effects at different scales, and it is possible our  
375 results would differ if using larger sample sizes. However, a recent study using NutNet data found no  
376 consistent effects of fencing on species area curves<sup>40</sup>. Adjacent to this subplot, aboveground  
377 biomass of all plants was clipped at ground level within two 1 m x 0.1 m strips. Biomass was sorted  
378 to live (current year's growth) and dead (previous years' growth), dried at 60 °C to constant mass  
379 and weighed to the nearest 0.01 g. Lead scientists at each site provided data, based on site-level  
380 knowledge, on the provenance (native or exotic) of each plant species at their sites.

381 Photosynthetically active radiation (PAR, mmol photons m<sup>-2</sup> s<sup>-1</sup>) was determined at the time of  
382 biomass clipping at approximately solar noon (between 11:00 and 14:00). In the same 1 m<sup>2</sup> subplots  
383 used for plant cover, two light availability measurements were taken at ground level and one was  
384 taken above the canopy. Light availability was calculated as the ratio of PAR below and above the  
385 canopy. We used data from the third year post-treatment at each site. Data on mean annual  
386 precipitation at each site was derived from the WorldClim database (version 1.4)<sup>41</sup>, which provides  
387 high resolution interpolated global climate data from stations with 10 to 30 years of data. We  
388 focused on precipitation as our measure of resource availability but we also tested aridity index

389 (which was strongly correlated with precipitation:  $P < 0.001$ ,  $r = 0.69$ ), which gave qualitatively  
390 similar results for diversity and tended to do so for richness (Supplementary Table 7). In addition, we  
391 also tested the effect of soil nitrogen in control plots (Supplementary Table 8).

392

393 **Calculations and statistical analyses.** We calculated the inverse Simpson's diversity index or  
394 Effective Number of Species and Probability of Interspecific Encounter ( $ENS_{PIE}$ ) as

395 
$$ENS_{PIE} = \frac{1}{\sum_{i=1}^S p_i^2}$$

396 where  $p_i$  is the proportion of species  $i$  and  $S$  is the number of species (i.e., species richness) in each  
397 treatment plot. The Simpson's evenness index was calculated by dividing Simpson's diversity ( $ENS_{PIE}$ )  
398 by richness ( $S$ ). In this way we decomposed diversity into changes in number of species (richness)  
399 and changes in dominance (evenness).

400 We calculated the effect of herbivore exclusion on inverse Simpson's diversity, richness  
401 (total, native and exotic), Simpson's evenness, light availability, and aboveground biomass as the log  
402 response ratio ( $LRR$ ) =  $\ln(\text{fenced/unfenced})$ . We calculated separate  $LRR$ s for the unfertilized and the  
403 fertilized (NPK $\mu$ ) plots within each block per site. If  $LRR = 0$  herbivore exclusion had no effect on, for  
404 example species richness, while  $LRR < 0$  and  $LRR > 0$  indicate that herbivore exclusion respectively  
405 decreased or increased species richness. For the  $LRR$ s of inverse Simpson's diversity, richness and  
406 Simpson's evenness we had  $n = 152$  (76 per fertilization treatment) for the long-history sites and  $n =$   
407 206 (103 per fertilization treatment) for the short-history sites. Light data were not available for ten  
408 sites, so here we had  $n = 119$  for the long-history sites and  $n = 182$  for the short-history sites.

409 We started our analyses by examining the overall effect of herbivore exclusion on inverse  
410 Simpson's diversity, richness (total, native and exotic) and Simpson's evenness in the long- and  
411 short-history sites separately. We did this independently of resources to see if evolutionary history  
412 of grazing is critical for predicting plant diversity responses to herbivore exclusion. Therefore, we  
413 performed one-sample  $t$  tests on the  $LRR$ s under unfertilized and fertilized conditions in the long-



414 and short-sites separately. If the 95% confidence interval values of the *LRRs* did not overlap with  
415 zero, there was a significant decrease or increase with herbivore exclusion. We then used linear  
416 mixed effects models (LMMs) to test the effects of evolutionary history of grazing, mean annual  
417 precipitation (MAP), fertilization, and their interactions, on the *LRR* of inverse Simpson's diversity,  
418 richness and Simpson's evenness to herbivore exclusion. For these models, we included block within  
419 site as a random factor. Using linear regressions, we tested whether the effect of herbivore  
420 exclusion on native and exotic species richness was related to changes in light availability.

421 To estimate current grazing intensity, we used a grazing index which accounted for variation  
422 in diversity and abundance of herbivores across the sites. All herbivore species (>2 kg) that consume  
423 aboveground biomass throughout the year were documented by the lead scientist of each site and  
424 assigned an importance value; from 1 (very low impact and frequency) to 5 (very high impact and  
425 frequency). The index value was calculated for each site as the sum of herbivore importance values  
426 for all herbivores<sup>34,39</sup>. This grazing index, based on a standardized rubric completed for each sites,  
427 accounts for site-level variation in herbivore abundance and diversity, integrated across seasons and  
428 years. Due to missing data, we were unable to estimate index values for four long- and ten short-  
429 history sites, so these were excluded from the analysis. We used LMMs to test the effects of  
430 evolutionary history of grazing and grazing intensity, and their interactions, on the *LRRs* of inverse  
431 Simpson's diversity, richness, and Simpson's evenness to herbivore exclusion, with block within site  
432 as random factor. We simplified the models to not include precipitation because there were no  
433 significant interactions between precipitation and grazing intensity when running the full LMMs. All  
434 data analyses were performed in R, version 3.6.1<sup>42</sup>.

435

#### 436 **Data availability**

437 Source data are provided with this paper. The data presented in this study are also available in **xxx**  
438 (will update if accepted). The WorldClim database (version 1.4) is available at  
439 <http://www.worldclim.org/bioclیم>.

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551

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576

### 577 **Author contributions**

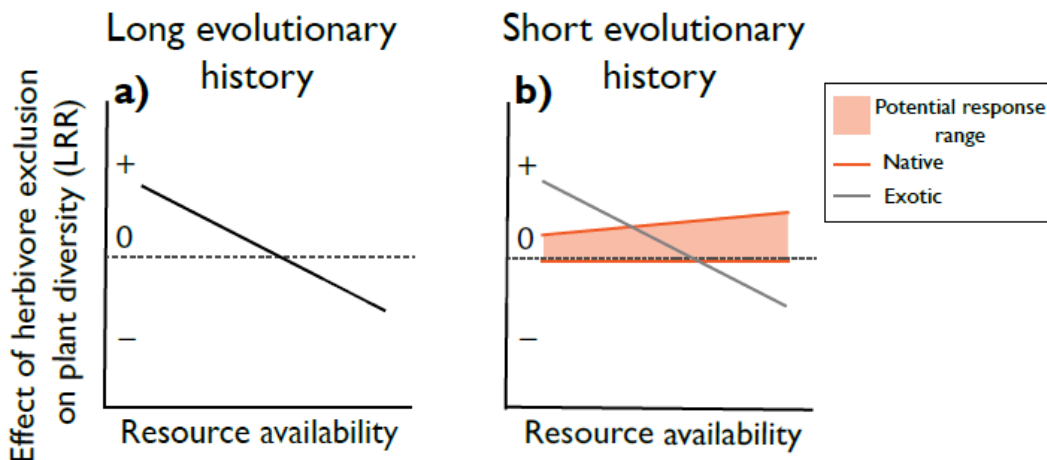
578 J.N.P. and J.S. developed and framed research questions, analyzed the data and wrote the paper.  
579 T.O. and P.T. developed and framed research questions and contributed to data analyses. C.S.B, S.P.  
580 and E.S.B. developed and framed research questions. E.W.S. and E.T.B. developed and framed  
581 research questions and coordinate the Nutrient Network collaboration. S.B. contributed to analyses.  
582 J.P., J.S., C.S.B., E.W.S., E.T.B., S.P., E.S.B., A.S.M., L.Y., D.S.G., H.O.V., I.C.B., P.G., C.A.A., J.D.B.,  
583 D.M.B., E.H.B., L.A.B., M.N.B., M.W.C., M.C.C., C.R.D., I.D., S.G., Y.H., I.S.J., L.S.L., R.L.M., J.L.M., S.A.P.,  
584 A.C.R., M.S., R.S., C.J.S., G.F.V., R.V., G.M.W. contributed data. All authors contributed to paper  
585 writing.

586

### 587 **Competing interests**

588 The authors declare no competing interests.

589 **Box 1.**



590

591

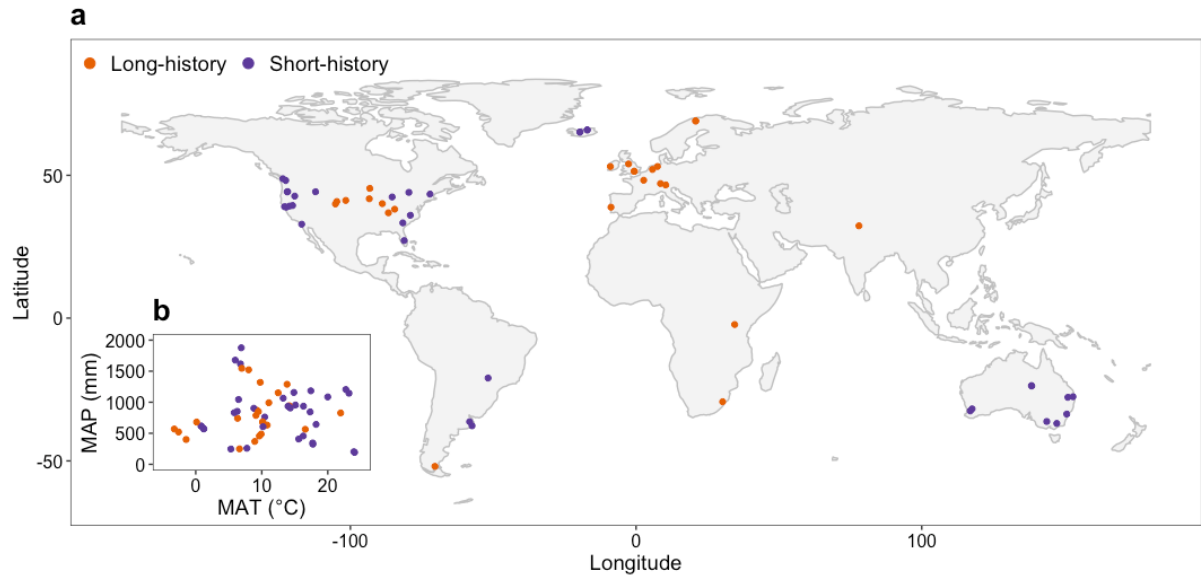
592 The conceptual figure outlines our hypotheses for the response of plant diversity to herbivore  
593 exclusion expressed as a log response ratio ( $LRR: \ln [\text{exclusion}/\text{grazed}]$ ) in long- and short-history  
594 sites, defined as, respectively, greater than or less than 500 years of evolutionary history with  
595 ungulate grazers (see Supplementary Note 1). These hypotheses are based upon theory predicting  
596 responses contingent on evolutionary grazing history, current grazing intensity and ecosystem  
597 productivity (Milchunas et al. <sup>9</sup> and Cingolani et al. <sup>8</sup>). Here, we have adapted the model predictions  
598 to focus on resource availability generalised across grazing intensities. We also decomposed plant  
599 diversity into richness (number of species) and evenness (inverse of species dominance).

600 In a single equilibrium ecosystem (as expected for long-history sites), we hypothesize that,  
601 with the exclusion of herbivores, plant diversity will increase at low resource availability (positive  
602  $LRR$ ) and decrease at high resource availability (negative  $LRR$ ) (a). At low resource availability, we  
603 expect plant diversity to increase both through gains in native, grazing-intolerant species (increase in  
604 richness) and decreases in dominance of native, grazing-tolerant species (increase in evenness). At  
605 high resource availability, we expect tall, native species not adapted to grazing to dominate when  
606 herbivores are excluded, suppressing other plant species. In the long-history sites, these changes are

607 driven by native species, and exotic species are less common with lower abundance than in short-  
608 history sites.

609 We expect short-history sites to diverge from the single equilibrium model, with some native  
610 species unable to recover due to lack of seed supply or altered ecosystem conditions (e.g., altered  
611 nutrient cycling). Hence, (b) indicates potential for zero or more restricted recovery of native species  
612 at low-mid resource availability and greater increases at high resource availability if changes are  
613 reversible. We expect the response of exotic species (which mostly originate from long-history  
614 regions) to be closer to the single equilibrium ecosystem in the long-history sites (grey line).

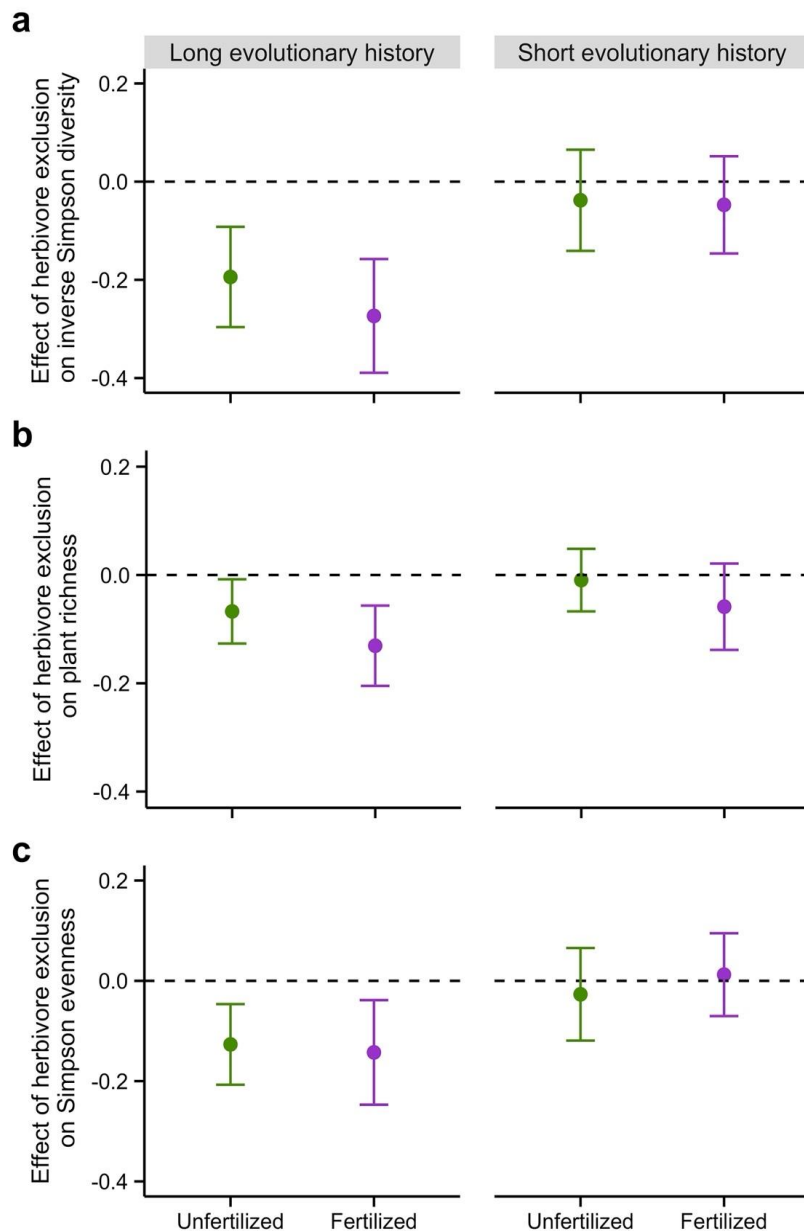
615 We test these hypotheses using a natural precipitation gradient and experimentally  
616 increased nutrients as measures of resource availability. As nutrient addition increases resource  
617 availability regardless of rainfall, we expect fertilization to shift sites to the right of the resource  
618 availability axes, and to show a more negative effect of herbivore exclusion than at unfertilized sites.  
619 As our fertilization rates were high, we expect most sites to shift to the far-right of the resource  
620 availability axis with potentially a weaker or minimal relationship with rainfall owing to the  
621 shortened gradient.



622

623 **Fig. 1 | Geographic and climatic distribution of experimental sites.** Location of the 57 NutNet sites  
 624 at which the full factorial experiment of herbivore exclusion and nutrient addition was replicated. (a)  
 625 Sites were classified as subject to a long (large herds of ungulates present >500 to 10,000 years ago;  
 626 24 sites) or short (<500 years; 33 sites) evolutionary history of grazing. (b) The 57 sites represent a  
 627 wide range of mean annual temperature (MAT) and mean annual precipitation (MAP) conditions.  
 628 Additional site details are provided in Supplementary Note 1 and Supplementary Table 2.

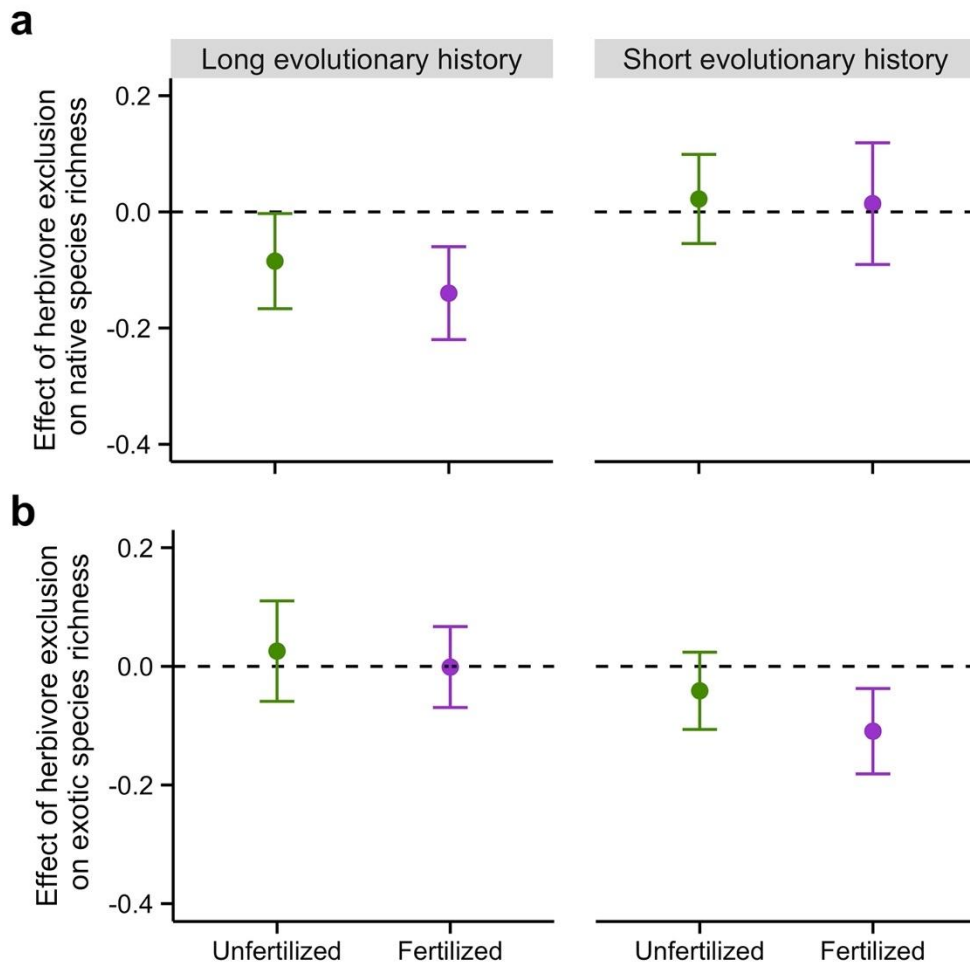




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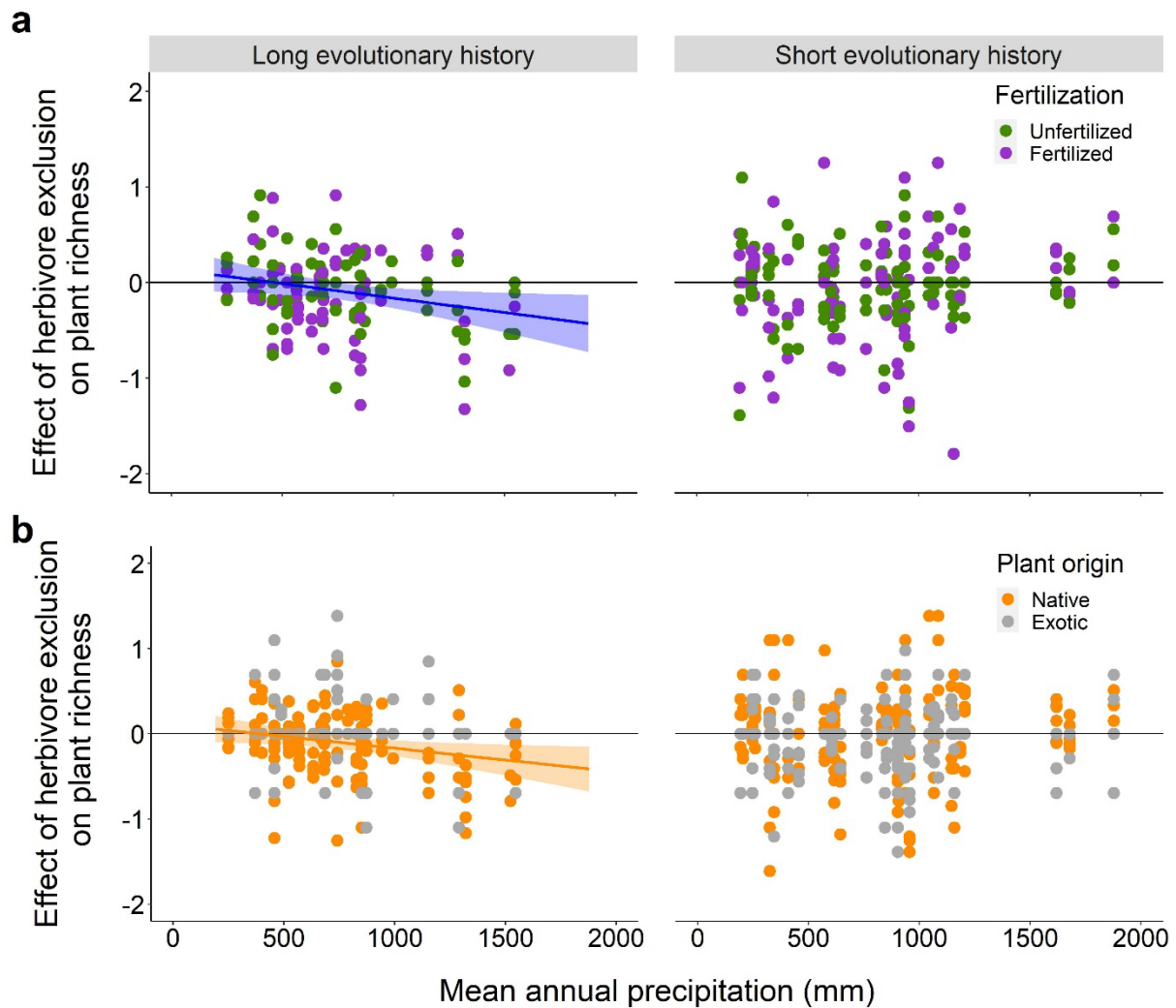
631 **Fig. 2 | Herbivore exclusion effects on plant diversity, richness and evenness.** Effect of herbivore  
 632 exclusion on (a) inverse Simpson's diversity, (b) richness and (c) Simpson's evenness calculated as  
 633  $LRR = \ln(\text{fenced/unfenced})$  for unfertilized (green) and fertilized (NPK $\mu$ ) plots (purple) in sites with a  
 634 long- or short-history of grazing. Points represent the mean effect across all 57 sites with the *LRRs*  
 635 calculated per block ( $n = 76$  per fertilization treatment for the long-history sites and  $n = 103$  for the  
 636 short-history sites) and error bars the range of 95% confidence intervals. Effects are considered  
 637 significant when error bars do not overlap with zero.



638

639

640 **Fig. 3 | Herbivore exclusion effects on native and exotic species richness.** Effect of herbivore  
 641 exclusion on (a) native species richness and (b) exotic species richness calculated as  $LRR =$   
 642  $\ln(\text{fenced/unfenced})$  for unfertilized (green) and fertilized (NPK $\mu$ ) plots (purple) in sites with a long-  
 643 or short-history of grazing. Points represent the mean effect across all 57 sites with the  $LRR$ s  
 644 calculated per block ( $n = 76$  per fertilization treatment for the long-history sites and  $n = 103$  for the  
 645 short-history sites) and error bars the range of 95% confidence intervals. Effects are considered  
 646 significant when error bars do not overlap with zero.



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648

649 **Fig. 4 | Herbivore exclusion effects on plant richness related to rainfall.** Relationship between

650 mean annual precipitation and the *LRR* of (a) total plant richness and (b) native (orange points) and

651 exotic (grey points) plant richness to herbivore exclusion in sites with a long ( $n = 152$  plots from 24

652 sites) or short ( $n = 206$  plots from 33 sites) evolutionary history of grazing. Sites included unfertilized

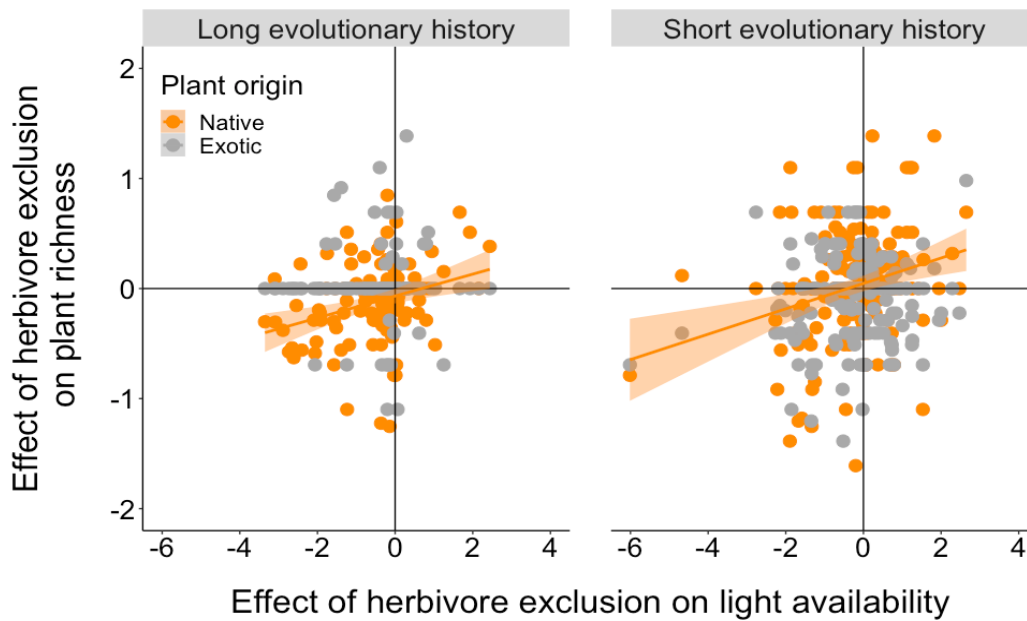
653 control plots and plots fertilized with  $\text{NPK}_\mu$ , which are green and purple in (a) but not defined with a

654 colour in (b). For the long-history sites, there were significant relationships across the precipitation

655 gradient, so trendlines were produced using predicted values of the linear mixed effects models. In

656 (a) this line was predicted to all points regardless of fertilization as this experimental treatment did

657 not have a significant effect (see Table 1), while in (b) the line was predicted for native richness.



658

659

660 **Fig. 5 | Herbivore exclusion effects on plant richness related to changes in light availability.**

661 Relationship between the *LRR* of light availability to herbivore exclusion and the *LRR* of native

662 (orange points) and exotic (grey points) species richness to herbivore exclusion. Sites had a long or

663 short evolutionary history of grazing and included unfertilized control plots and plots fertilized with

664 NPK $\mu$ . Per plant provenance category,  $n = 119$  for long-history sites and  $n = 182$  for short-history

665 sites. For native richness, there was a significant relationship in both long- ( $P < 0.001$ ,  $R^2 = 0.08$ ) and

666 short-history sites ( $P < 0.001$ ,  $R^2 = 0.06$ ), so trendlines were produced using `geom_smooth` (*ggplot2*

667 package in R).

668 **Table 1** | Results of linear mixed model analysis testing the effects of evolutionary history of grazing  
669 (EVO), mean annual precipitation (MAP), fertilization (FERT), and their interactions on the *LRR* of  
670 plant species diversity, richness and evenness to herbivore exclusion. Random effects in the models  
671 were 'site' (s.d.= 0.20 for diversity model, s.d. = 0.16 for richness, and s.d. = 0.09 for evenness) and  
672 'block nested within site' (s.d. = 0.45 for diversity model, s.d. = 0.40 for richness, and s.d. = 0.42 for  
673 evenness). The intercept is the mean value of the unfertilized long-history sites. Table shows  
674 parameter estimate, standard error, degrees of freedom, test statistic, and *P*-value for the 2-tailed  
675 test. Variables in bold had a significant effect (*P*-value < 0.05).

	Estimate	Std. Error	DF	t-value	P-value
<i>Inverse Simpson's diversity</i>					
Intercept	0.16	0.17	175	0.90	0.368
EVO	-0.25	0.21	53	-1.19	0.238
<b>MAP</b>	<b>-4.49 × 10<sup>-4</sup></b>	<b>2.05 × 10<sup>-4</sup></b>	<b>53</b>	<b>-2.20</b>	<b>0.033</b>
FERT	-0.35	0.19	175	-1.87	0.063
<b>EVO × MAP</b>	<b>5.18 × 10<sup>-4</sup></b>	<b>2.47 × 10<sup>-4</sup></b>	<b>53</b>	<b>2.10</b>	<b>0.041</b>
EVO × FERT	0.25	0.23	175	1.10	0.273
MAP × FERT	3.53 × 10 <sup>-4</sup>	2.25 × 10 <sup>-4</sup>	175	1.57	0.119
EVO × MAP × FERT	-2.48 × 10 <sup>-4</sup>	2.71 × 10 <sup>-4</sup>	175	-0.92	0.361
<i>Plant species richness</i>					
Intercept	0.27	0.14	175	1.87	0.063
EVO	-0.31	0.18	53	-1.71	0.093
<b>MAP</b>	<b>-4.34 × 10<sup>-4</sup></b>	<b>1.73 × 10<sup>-4</sup></b>	<b>53</b>	<b>-2.51</b>	<b>0.015</b>
FERT	-0.27	0.16	175	-1.68	0.096
<b>EVO × MAP</b>	<b>4.64 × 10<sup>-4</sup></b>	<b>2.09 × 10<sup>-4</sup></b>	<b>53</b>	<b>2.22</b>	<b>0.031</b>
EVO × FERT	0.09	0.20	175	0.46	0.646
MAP × FERT	2.77 × 10 <sup>-4</sup>	1.98 × 10 <sup>-4</sup>	175	1.40	0.163
EVO × MAP × FERT	-1.14 × 10 <sup>-4</sup>	2.39 × 10 <sup>-4</sup>	175	-0.48	0.633
<i>Simpson's evenness</i>					
Intercept	-0.11	0.14	175	-0.81	0.419
EVO	0.05	0.17	53	0.28	0.777
MAP	-2.33 × 10 <sup>-5</sup>	1.63 × 10 <sup>-4</sup>	53	-0.14	0.887
FERT	-0.07	0.18	175	-0.42	0.677
EVO × MAP	6.54 × 10 <sup>-5</sup>	1.96 × 10 <sup>-4</sup>	53	0.33	0.741
EVO × FERT	0.16	0.22	175	0.74	0.463
MAP × FERT	7.54 × 10 <sup>-5</sup>	2.13 × 10 <sup>-4</sup>	175	0.35	0.723
EVO × MAP × FERT	-1.35 × 10 <sup>-5</sup>	2.56 × 10 <sup>-4</sup>	175	-0.53	0.599

676