



# Evolutionary history of grazing and resources determine herbivore exclusion effects on plant diversity

Jodi N. Price<sup>1,35</sup>  , Judith Sitters<sup>2,3,35</sup>  , Timothy Ohlert<sup>4</sup>, Pedro M. Tognetti<sup>5</sup> , Cynthia S. Brown<sup>6</sup> , Eric W. Seabloom<sup>7</sup> , Elizabeth T. Borer<sup>7</sup> , Suzanne M. Prober<sup>8</sup>, Elisabeth S. Bakker<sup>9</sup> , Andrew S. MacDougall<sup>10</sup>, Laura Yahdjian<sup>5</sup> , Daniel S. Gruner<sup>11</sup> , Harry Olde Venterink<sup>12</sup> , Isabel C. Barrio<sup>12</sup> , Pamela Graff<sup>5</sup> , Sumanta Bagchi<sup>13</sup>, Carlos Alberto Arnillas<sup>14</sup> , Jonathan D. Bakker<sup>15</sup> , Dana M. Blumenthal<sup>16</sup>, Elizabeth H. Boughton<sup>17</sup> , Lars A. Brudvig<sup>18</sup>, Miguel N. Bugalho<sup>19</sup>, Marc W. Cadotte<sup>20</sup> , Maria C. Caldeira<sup>21</sup> , Chris R. Dickman<sup>22</sup> , Ian Donohue<sup>23</sup> , Sonnier Grégory<sup>17</sup> , Yann Hautier<sup>24</sup>, Ingibjörg S. Jónsdóttir<sup>25</sup> , Luciola S. Lannes<sup>26</sup> , Rebecca L. McCulley<sup>27</sup> , Joslin L. Moore<sup>28</sup> , Sally A. Power<sup>29</sup> , Anita C. Risch<sup>30</sup> , Martin Schütz<sup>30</sup>, Rachel Standish<sup>31</sup> , Carly J. Stevens<sup>32</sup> , G. F. Veen<sup>33</sup> , Risto Virtanen<sup>34</sup>  and Glenda M. Wardle<sup>22</sup> 

**Ecological models predict that the effects of mammalian herbivore exclusion on plant diversity depend on resource availability and plant exposure to ungulate grazing over evolutionary time. Using an experiment replicated in 57 grasslands on six continents, with contrasting evolutionary history of grazing, we tested how resources (mean annual precipitation and soil nutrients) determine herbivore exclusion effects on plant diversity, richness and evenness. Here we show that at sites with a long history of ungulate grazing, herbivore exclusion reduced plant diversity by reducing both richness and evenness and the responses of richness and diversity to herbivore exclusion decreased with mean annual precipitation. At sites with a short history of grazing, the effects of herbivore exclusion were not related to precipitation but differed for native and exotic plant richness. Thus, plant species' evolutionary history of grazing continues to shape the response of the world's grasslands to changing mammalian herbivory.**

Grasslands cover 30% of the Earth's terrestrial surface and provide livelihoods for about 800 million people<sup>1</sup>. Human activities are altering the plant and herbivore diversity of grasslands worldwide by increasing nutrient availability either unintentionally through the combustion of fossil fuels or intentionally through the use of artificial fertilizers and by changing the species composition and abundance and foraging patterns of domestic and wild mammalian herbivores<sup>2–6</sup>. Given the ongoing losses of wild herbivores<sup>7</sup>, determining the mechanisms by which nutrients and large mammalian herbivores regulate plant diversity is critical for grassland conservation and management<sup>8</sup>.

The effect of large, mammalian herbivores (hereafter herbivores) on plant diversity is predicted to depend on three potentially interacting factors—the evolutionary history of grazing by herds of large, hooved herbivores (ungulates); resource availability for plant growth, especially water and nutrients; and modern-era grazing intensity<sup>9–16</sup>. Fluctuations in grazing intensity in sites with a relatively long evolutionary history of ungulate occurrence (grazing present for >500–10,000 years, hereafter long-history sites) have selected for two different pools of plant species—short-statured species that are more tolerant of grazing and tall-statured species that are susceptible to grazing but are better at capturing light than are short-statured species<sup>11,17</sup>. In these long-history sites, models

predict that the effect of herbivores on plant diversity depends on the availability of resources for plant growth. In sites with high rainfall or high nutrient availability, a few tall plant species dominate in the absence of grazing (low richness and low evenness), thereby reducing ground-level light available for seedlings and short-statured species<sup>10,11,15</sup>. In this case, herbivores promote plant diversity by increasing light availability for shorter, more grazing-tolerant species<sup>10,11,18,19</sup>. Hence, herbivores increase diversity both through increased species richness (more grazing-tolerant species) and evenness (lower dominance of tall, less grazing-tolerant species). In resource-poor sites, where light is generally less limiting, herbivores may have little effect on plant diversity or a negative effect if resource limitation (water, nutrients) prevents regrowth or plant reproduction after grazing<sup>6</sup>. Exclusion of herbivores from long-history sites is, therefore, expected to have no effect or to increase plant diversity at low resource availability and decrease diversity at high resource availability (Fig. 1).

For sites with a relatively short evolutionary history of ungulate grazing (<500 years, hereafter short-history sites), it is predicted that herbivores generally reduce plant diversity<sup>11</sup>. Here native plant species are assumed to lack mechanisms for tolerating or resisting grazing, instead possessing upright growth-forms with elevated buds that are more vulnerable to grazing and trampling<sup>15</sup>. These grazing

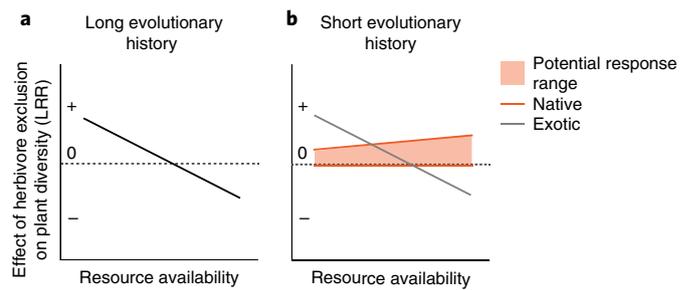
A full list of affiliations appears at the end of the paper.

impacts on diversity are likely to be especially relevant in grasslands recently exposed to Eurasian-derived animal husbandry practices. In this case, grazing intensity is shaped by non-migratory domestic grazers whose stocking densities and duration of grazing per unit area may lack evolutionary precedents<sup>10,11,15</sup>. These effects will probably be further compounded by any management-based resource augmentation involving watering, fertilization or offsite feed supplementation that increases herd capacity above what the sites could otherwise sustain. Under these circumstances, herbivore exclusion should, according to theory, increase the diversity of plants with grazer-sensitive growth-forms relative to grazed plots (Fig. 1). One exception might be at low grazing intensity in resource-rich sites, where herbivore exclusion may decrease diversity due to competitive release (see also Fig. 1 in ref. <sup>10</sup>). However, empirical evidence and alternative models (for example, state-and-transition models<sup>20</sup>) demonstrate irreversible reductions in plant diversity due to local extinction of grazing-sensitive species<sup>10,21–23</sup>. Recovery of plant species following herbivore exclusion can be limited by seed availability in the soil seedbank, propagule dispersal, or both, as well as invasion by exotic plants, soil erosion and elevated soil nutrients<sup>20,21</sup>. Hence, we expect neutral responses (if recovery of grazing-sensitive species is not possible) to small increases in native diversity (due to increased plant species richness) in response to herbivore exclusion at short-history sites (Fig. 1)<sup>10</sup>.

Many short-history sites are now dominated by non-native, potentially invasive, plant species originating from regions with a long evolutionary history of grazing, many of which were intentionally sown for forage<sup>10,11,15</sup>. Short-history grasslands can be transformed from native to exotic dominance through a combination of introduced livestock grazing, introduced plants from regions with long-term ungulate husbandry, increased temporal and spatial offtake caused by fencing, provision of water or fertilizer addition<sup>10,11,24–27</sup>. Hence, plant community diversity responses to herbivores in short-history sites may reflect the evolutionary history of the non-native species and grazing-adapted, non-native species will often increase if the native community does not contain grazing-adapted plants<sup>28,29</sup>. We therefore predict that responses to herbivore exclusion of exotic species in short-history sites will match responses of native species from long-history sites (Fig. 1).

Two recent meta-analyses provide limited support for model predictions that the plant diversity response to herbivore exclusion depends on resource availability (proxied by precipitation or aridity and plant biomass)<sup>30,31</sup> or evolutionary history of grazing<sup>31</sup>. However, these studies did not examine plant provenance which is needed to explore the evolutionary history of the whole plant community. Both studies found that, in general, herbivore exclusion tends to reduce plant species richness<sup>30,31</sup>. However, while meta-analyses provide a quantitative synthesis of published studies, differences in design and methodology among experiments obscure inference about the relative importance of the biological and methodological factors underlying the observed patterns<sup>8,32</sup>. For example, increasing light availability at ground level is a direct mechanism by which herbivory maintains plant diversity<sup>33</sup> but often light capture by the canopy is not measured. Instead, a range of proxies for light availability (for example, precipitation and aboveground plant biomass) have been used in past studies to test model predictions<sup>11,34</sup>, probably leading to variation among results because these proxies vary in explanatory power<sup>33</sup>. We suggest that direct measures of resource availability (light, precipitation and nutrients) will provide a more consistent assessment of how the relationship between grazing and diversity is mediated by resource availability.

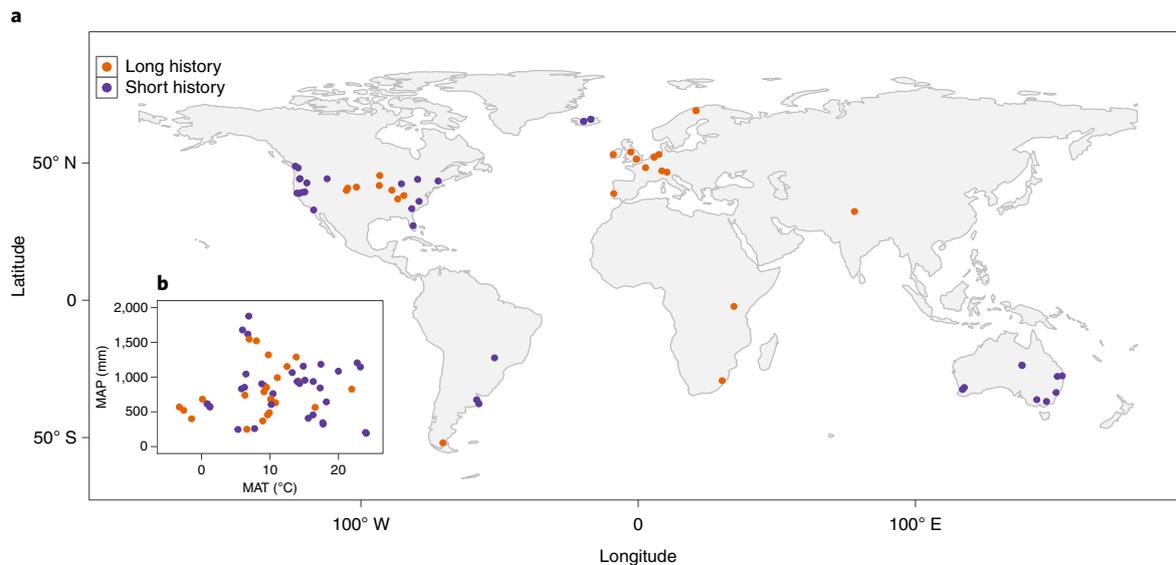
Here we use a herbivore exclusion experiment using a standardized experimental and sampling design, in 57 grasslands on six continents (Fig. 2), to examine responses of plant diversity, richness and evenness to herbivore exclusion. We test mechanistic hypotheses related to light availability, grazing history and current



**Fig. 1 | Conceptual figure of the hypotheses for the response of plant diversity to herbivore exclusion. a, b.** The conceptual figure outlines our hypotheses for the response of plant diversity to herbivore exclusion expressed as a log response ratio (LRR:  $\ln(\text{exclusion}/\text{grazed})$ ) in long-history sites (**a**) and short-history sites (**b**), defined as, respectively, greater than or less than 500 years of evolutionary history with ungulate grazers (Supplementary Note 1). These hypotheses are based upon theory predicting responses contingent on evolutionary grazing history, current grazing intensity and ecosystem productivity<sup>10,11</sup>. Here we have adapted the model predictions to focus on resource availability generalized across grazing intensities. We test our hypotheses using a natural precipitation gradient and a soil nitrogen gradient as measures of resource availability. We also decomposed plant diversity into richness (number of species) and evenness (inverse of species dominance). In a single equilibrium ecosystem (as expected for long-history sites), we hypothesize that, with the exclusion of herbivores, plant diversity will increase at low resource availability (positive LRR) and decrease at high resource availability (negative LRR) (**a**). At low resource availability, we expect plant diversity to increase both through gains in native, grazing-intolerant species (increase in richness) and decreases in dominance of native, grazing-tolerant species (increase in evenness). At high resource availability, we expect tall, native species not adapted to grazing to dominate when herbivores are excluded, suppressing other plant species. In the long-history sites, these changes are driven by native species and exotic species are less common with lower abundance than in short-history sites. We expect short-history sites to diverge from the single equilibrium model, with some native species unable to recover due to lack of seed supply or altered ecosystem conditions (for example, altered nutrient cycling). Hence, **b** indicates potential for zero or more restricted recovery of native species at low–mid resource availability and greater increases at high resource availability if changes are reversible. We expect the response of exotic species (which mostly originate from long-history regions) to be closer to the single equilibrium ecosystem in the long-history sites (grey line).

grazing intensity. In a large-scale distributed experiment, we explore these effects across sites differing in evolutionary history of ungulate grazing. To compare our results with previous studies and conceptual models, we include precipitation as a measure of resource availability, with sites spanning an order of magnitude of annual precipitation (192–1,877 mm). We also included soil nitrogen from control plots as a measure of inherent soil resource availability and have measured light availability at ground level at 47 of the 57 sites. Additionally, we modified soil resource availability through addition of nitrogen, phosphorus, potassium and micronutrients (NPKμ). With the experimental nutrient treatment, we can test whether the same ecological principles apply to grazing responses in our increasingly common eutrophied grasslands.

We classified grasslands as having long (24 sites) or short (33 sites) evolutionary history of grazing according to local experts and definitions in ref. <sup>34</sup> (Fig. 2, Supplementary Note 1 and Supplementary Tables 1 and 2). All plant species were classified as native or exotic by experts at each site. We compared plots that were fenced to exclude all vertebrate herbivores (body mass >50 g) for 3 years with unfenced plots that were subject to variable



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

grazing by the contemporary suite of herbivores present at each site (Supplementary Table 3). Our main hypotheses, arising from the generalized grazing models<sup>10,11</sup>, are described in Fig. 1. Additionally, we used the fertilization treatment to test whether eutrophication (in this case intentionally through fertilization) causes grasslands to respond more like historically productive grasslands.

## Results and discussion

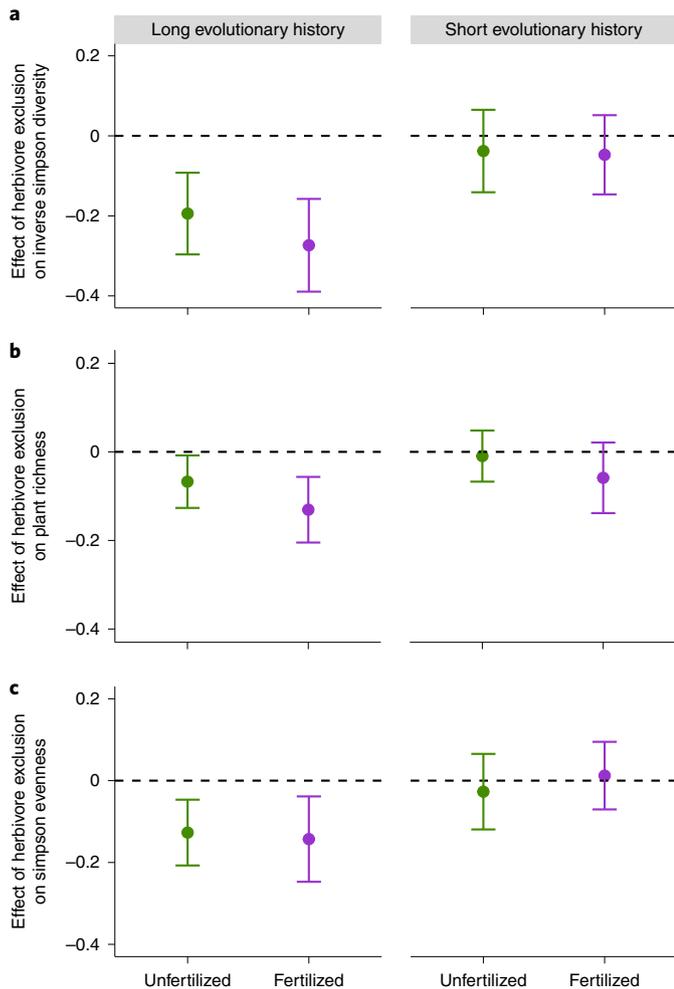
Across 57 grasslands spanning six continents, the evolutionary history of grazing was critical for predicting plant diversity responses to herbivore exclusion. Our results highlight the importance of grazing for maintaining diversity (Fig. 3), particularly native species richness in long-history sites (Fig. 4a), especially at higher precipitation (Table 1 and Fig. 5). In contrast, current grazing had a negligible effect on native species richness in short-history sites (Fig. 4a). Eutrophication, a common occurrence in many grasslands, altered patterns in short-history sites, leading to reductions in exotic species richness with herbivore exclusion. Most exotic species originated from long-history sites and responded similarly to native species from long-history sites (Fig. 4a,b). Hence, we show that the evolutionary history of individual plant species also regulates plant diversity responses as a function of resource availability, which is an important advance over existing papers. Together, these results suggest that conservation of native plant diversity may be facilitated by maintaining grazing herbivores in long-history sites, whereas short-history sites show muted and variable responses to herbivore exclusion.

In long-history sites, herbivore exclusion decreased diversity (measured as inverse Simpson's diversity or effective number of species and probability of interspecific encounter ( $ENS_{PIE}$ ); unfertilized:  $t = -3.73$ ,  $P < 0.001$ ), plant richness (unfertilized:  $t = -1.70$ ,  $P = 0.046$ ) and Simpson's evenness (unfertilized:  $t = -3.10$ ,  $P < 0.001$ ) (Fig. 3 and Extended Data Fig. 1). Hence, herbivore exclusion decreased the number of native plant species (Fig. 4a) and increased dominance of a few plant species, consistent with other studies in which herbivores also promoted diversity<sup>14,18,35,36</sup>. In long-history sites, dominant plant species are often palatable and grazing reduces their dominance, thereby increasing evenness and promoting more grazing-tolerant native species<sup>37</sup>. Reductions in

native richness with herbivore exclusion were due to a reduction in light availability in fenced plots, confirming the mechanism by which herbivores maintain plant diversity (Extended Data Fig. 2)<sup>32</sup>.

Consistent with our first hypothesis (Fig. 1) and model predictions<sup>10,11</sup> for long-history sites, herbivore exclusion reduced plant species richness, and thereby diversity, more at sites with greater precipitation (Table 1 and Fig. 5a). Contrary to what we expected, herbivore exclusion decreased plant species richness across the whole of the studied precipitation gradient (Fig. 5a), which was driven by the response of native species to herbivore exclusion (Fig. 5b). Hence, herbivores are important in maintaining native plant richness at long-history sites, even at sites with low rainfall. However, it is possible we did not find increases in plant richness with herbivore exclusion because the low end of the precipitation gradient was not low enough, with three sites being classified as semi-arid (<250 mm MAP). We found similar diversity responses using an index of aridity (Supplementary Table 4). Additionally, we found ambient soil nitrogen (from control plots) had no effect on the relationship between herbivore exclusion and diversity, richness or evenness (Supplementary Table 5). The theoretical models underpinning our hypotheses predict that the effect of grazing on plant diversity will depend on current grazing intensity<sup>10,11</sup>. Here we used an index of grazing intensity that incorporated importance values of all current herbivore species at 43 sites, estimated by site experts<sup>38</sup>. We tested whether the log response ratios (LRRs) of inverse Simpson's diversity, plant richness and Simpson's evenness to herbivore exclusion were related to the grazing intensity index. In the long-history sites, herbivore exclusion decreased diversity more when current grazing intensity was higher, driven mainly by changes in evenness (Extended Data Fig. 3). As few sites were as heavily grazed as intensively managed rangelands, these results suggest that grazing even at intermediate intensities is sufficient to decrease plant dominance and maintain plant diversity (Fig. 3 and Extended Data Fig. 3).

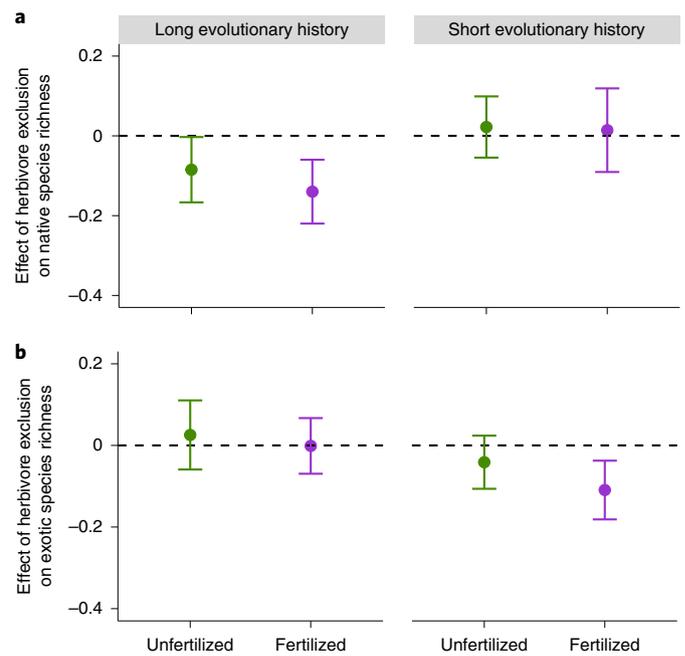
Consistent with our predictions for sites with a short evolutionary history of grazing (Fig. 1), herbivore exclusion had no effect on overall inverse Simpson's diversity (unfertilized:  $t = -0.72$ ,  $P = 0.472$ ), plant richness (unfertilized:  $t = -0.25$ ,  $P = 0.805$ ) or Simpson's evenness (unfertilized:  $t = -0.57$ ,  $P = 0.568$ ) (Fig. 3 and



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

Extended Data Fig. 1) and these responses were not related to precipitation (Fig. 5a). The results of the total species pool do not support the Milchunas et al.<sup>11</sup> model that diversity should increase with herbivore exclusion (Fig. 3a). However, the response of total species diversity reflects the combined responses of both native and exotic species and species in these groups differ in their evolutionary history of grazing (Supplementary Table 6). At short-history sites, an average of 32% of species were exotic (compared to 13% in long-history sites) and 93% of these species originated from regions with a long evolutionary history of ungulate grazing (Supplementary Table 6); hence, the species pool comprised species with mixed evolutionary exposure to grazing.

In sites with a short evolutionary history of grazing, we predicted different responses for native and exotic species because native species lack tolerance to ungulate grazing, whereas exotic species mostly originate from long-history sites (Supplementary Table 6)<sup>10,11,24,27</sup>. We found partial support for our prediction (Fig. 1) that



**Fig. 4 | Herbivore exclusion effects on native and exotic species richness.** **a, b**, Effect of herbivore exclusion on native species richness (**a**) and exotic species richness (**b**) calculated as  $LRR = \ln(\text{fenced}/\text{unfenced})$  for unfertilized (green) and fertilized (NPK $\mu$ ) plots (purple) in sites with a long- or short-history of grazing. Points represent the mean effect across all 57 sites with the LRRs calculated per block ( $n = 76$  per fertilization treatment for the long-history sites and  $n = 103$  for the short-history sites) and error bars the range of 95% confidence intervals. Effects are considered significant when error bars do not overlap with zero.

diversity responses in short-history sites reflected the evolutionary grazing history of the plant species present. We expected recovery of native species to be limited (neutral to small richness increases) with herbivore exclusion. Indeed, we found no overall response of native species richness to herbivore exclusion in unfertilized treatments ( $t = 0.90$ ,  $P = 0.371$ ) (Fig. 4a). We also predicted that exotic species in short-history sites, which mostly have a longer evolutionary exposure to ungulate grazing, would decrease in diversity with herbivore exclusion, as with native species from long-history sites. However, exotic species richness did not decrease with herbivore exclusion under unfertilized conditions ( $t = -1.24$ ,  $P = 0.218$ ; Fig. 4b) and the response to herbivore exclusion was not related to precipitation (Fig. 5b). Similarly to the long-history sites, we found the response of native species to herbivore exclusion depended on the extent that herbivores changed light availability<sup>39</sup>, which did not consistently change in one direction due to herbivore exclusion (Extended Data Fig. 2).

The lack of a directional response of native plant species to herbivore exclusion suggests little recovery from grazing in short-history sites, which does not support the single equilibrium model proposed by Milchunas et al.<sup>11</sup>. However, the results support our predictions that short-history sites may diverge from the single equilibrium model and are consistent with previous studies (reviewed in ref.<sup>10</sup> Table 2) and conceptual models<sup>20</sup>. There are several possible interpretations of our findings. First, recovery of grazing-sensitive species may require longer timeframes than our study (3 years). Second, recovery of native species may not be possible because they have become locally extinct<sup>22</sup> or because grazing and exotic invasion have altered ecological processes resulting in stable, degraded states<sup>23,37,40</sup>. In this case, current grazing may not be affecting diversity as effects occurred in the past. This can be the case despite substantial

**Table 1 | Results of linear mixed model analysis testing the effects of evolutionary history of grazing (EVO), mean annual precipitation (MAP), fertilization (FERT) and their interactions on the LRR of plant species diversity, richness and evenness to herbivore exclusion**

	Estimate	Standard error	d.d.f.	t-value	P value
<b>Inverse Simpson's diversity</b>					
Intercept	0.16	0.17	175	0.90	0.368
EVO	-0.25	0.21	53	-1.19	0.238
<b>MAP</b>	<b><math>-4.49 \times 10^{-4}</math></b>	<b><math>2.05 \times 10^{-4}</math></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><math>5.18 \times 10^{-4}</math></b>	<b><math>2.47 \times 10^{-4}</math></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	$3.53 \times 10^{-4}$	$2.25 \times 10^{-4}$	175	1.57	0.119
EVO × MAP × FERT	$-2.48 \times 10^{-4}$	$2.71 \times 10^{-4}$	175	-0.92	0.361
<b>Plant species richness</b>					
Intercept	0.27	0.14	175	1.87	0.063
EVO	-0.31	0.18	53	-1.71	0.093
<b>MAP</b>	<b><math>-4.34 \times 10^{-4}</math></b>	<b><math>1.73 \times 10^{-4}</math></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><math>4.64 \times 10^{-4}</math></b>	<b><math>2.09 \times 10^{-4}</math></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	$2.77 \times 10^{-4}$	$1.98 \times 10^{-4}$	175	1.40	0.163
EVO × MAP × FERT	$-1.14 \times 10^{-4}$	$2.39 \times 10^{-4}$	175	-0.48	0.633
<b>Simpson's evenness</b>					
Intercept	-0.11	0.14	175	-0.81	0.419
EVO	0.05	0.17	53	0.28	0.777
MAP	$-2.33 \times 10^{-5}$	$1.63 \times 10^{-4}$	53	-0.14	0.887
FERT	-0.07	0.18	175	-0.42	0.677
EVO × MAP	$6.54 \times 10^{-5}$	$1.96 \times 10^{-4}$	53	0.33	0.741
EVO × FERT	0.16	0.22	175	0.74	0.463
MAP × FERT	$7.54 \times 10^{-5}$	$2.13 \times 10^{-4}$	175	0.35	0.723
EVO × MAP × FERT	$-1.35 \times 10^{-5}$	$2.56 \times 10^{-4}$	175	-0.53	0.599

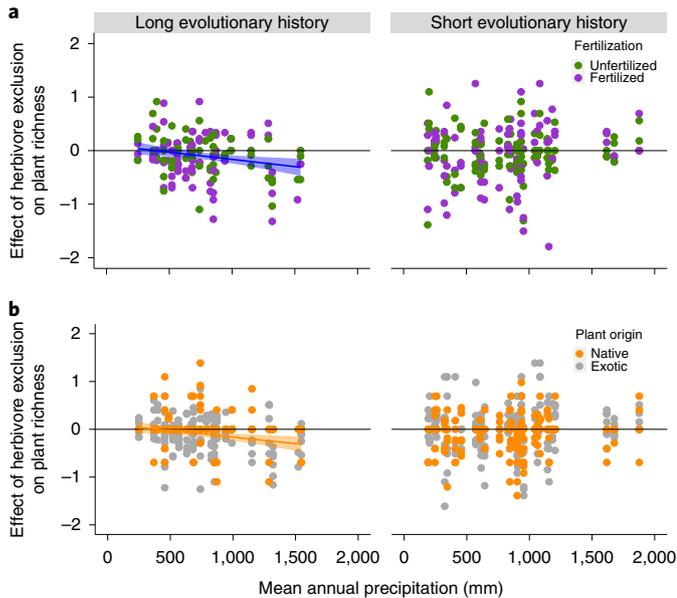
Random effects in the models were 'site' (s.d. = 0.20 for diversity model, s.d. = 0.16 for richness and s.d. = 0.09 for evenness) and 'block nested within site' (s.d. = 0.45 for diversity model, s.d. = 0.40 for richness and s.d. = 0.42 for evenness). The intercept is the mean value of the unfertilized long-history sites. Table shows parameter estimate, standard error, denominator degrees of freedom (d.d.f.), test statistic (t-value) and P value for the two-tailed test. Variables in bold had a significant effect (P value < 0.05).

negative effects of historical livestock grazing (for example, if grazing intensity now is lower than previous levels)<sup>26,37</sup>. It is also possible that neutral responses to herbivore exclusion indicate that grazing never affected native species, as we do not have historical data on the impacts of grazing in these sites. However, for the short-history regions included in the study, evidence suggests negative effects of introduced stock grazing on plant diversity<sup>22,41</sup>.

Whilst we could not distinguish the contribution of the above factors to the muted responses of native species to herbivore exclusion in short-history sites, we were able to explain some of the variability in the responses. We found that herbivore exclusion decreased species richness at sites with lower grazing intensity (Extended Data Fig. 3b), driven by declines in exotic species richness (Extended Data Fig. 3). While the models predict this pattern in both short- and long-history sites, we only detected this relationship for exotic species (that originate from long-history sites) in short-history sites. We found that this pattern was reversed at higher grazing intensity, where herbivore exclusion increased exotic species richness. At least 28 of the 33 short-history sites have a history of domestic ungulate grazing (Supplementary Table 7) but few were grazed by ungulates during the experiment. Our variable diversity responses to herbivore

exclusion may be partly due to differences in the recovery trajectory. That is, we are removing herbivores at different points along a longer time span of removal of domestic grazing at these sites. In this case, the control plots might also be on a trajectory of recovery from higher levels of ungulate grazing. It is difficult to determine what measures of grazing intensity are most relevant, especially for the short-history sites where historical grazing may have been much higher than current grazing. In some cases, historical grazing impacts may be difficult to reverse<sup>16,26</sup>.

We also explored diversity responses to herbivore exclusion in fertilized treatments to determine if nutrient addition caused grasslands to respond more like historically high resource sites. We found fertilization did not alter the effect of herbivore exclusion on total plant diversity, richness or evenness, showing similar reductions with exclusion as unfertilized plots (Fig. 3; error bars of unfertilized and fertilized treatments overlap). Further, the relationship between the LRR of richness and rainfall (no significant interactions with fertilization; Table 1) in both long- and short-history sites remained the same as for unfertilized plots. Hence, for total diversity measures, eutrophication did not cause grasslands to respond more like historically productive grasslands.



**Fig. 5 | Herbivore exclusion effects on plant richness related to rainfall.**

Relationship between mean annual precipitation and the LRR of total plant richness plotted by **a**, treatment, unfertilized (green points) and fertilized (purple points), and **b**, plant origin, native (orange points) and exotic (grey points) plant richness to herbivore exclusion in sites with a long ( $n=152$  plots from 24 sites) or short ( $n=206$  plots from 33 sites) evolutionary history of grazing. Sites included unfertilized control plots and plots fertilized with  $\text{NPK}\mu$ , which are green and purple in **a** but not defined with a colour in **b**. For the long-history sites, there were significant relationships across the precipitation gradient, so trendlines were produced using predicted values of the linear mixed effects models. In **a**, this line was predicted to all points regardless of fertilization as this experimental treatment did not have a significant effect (see Table 1), while in **b** the line was predicted for native richness. The coloured bands represent the 95% confidence intervals.

This lack of change may be due to the short period of fertilization (3 years) in our plots. With limited changes in species composition, communities may still respond to herbivore exclusion according to their prefertilization level of soil resources. It is possible that after a longer period of fertilization, communities would gain taller species and lose shorter species and start to respond to herbivore exclusion more similarly to communities that were historically more productive. Indeed, it has been shown that nutrient effects on plant diversity and biomass continue to increase at nearly all NutNet sites for at least 10 years<sup>42</sup>. However, we also found that ambient soil nutrients did not affect the diversity response to herbivore exclusion, suggesting that precipitation may be a more important resource governing the grazing–diversity relationship. Additionally, the lack of response may be because some sites are water-limited and unable to respond to the additional nutrients sufficiently to move to a light-limited state<sup>33,43</sup>. Fertilization also creates new conditions for plants that may not be easily predictable based simply on resource addition (for example, changes in microbial interactions).

Notably, we found exotic species richness to decrease with herbivore exclusion in short-history sites, when they were fertilized ( $t=-2.98$ ,  $P=0.002$ ; Fig. 4b) or subject to lower grazing intensity (Extended Data Fig. 4). Hence, exotic species responded similarly to native species from long-history sites but only under limited conditions. These results suggest that in short-history sites, both nutrient enrichment and low-intensity grazing can promote exotic species richness.

By combining evolutionary history of grazing, resource availability and species origin, our empirical results from 57 sites spanning six continents extends understanding of herbivore effects on grassland diversity beyond that of recent meta-analyses. Consistent with previous studies, we report reductions in plant diversity with herbivore exclusion<sup>30,31</sup> but only in long-history sites. Consistent with theoretical predictions, but contrasting with two recent meta-analyses<sup>30,31</sup>, our distributed experiment demonstrated that the response of diversity to herbivore exclusion depended on precipitation<sup>30</sup>, albeit again only in long-history sites. These contrasting results between short- and long-history sites were, as we predicted, based on theoretical and empirical models that suggest muted responses of native species to herbivore exclusion in short-history sites due to irreversibility of grazing effects<sup>10,20</sup>. The meta-analysis that incorporated evolutionary history of grazing did not find this factor to be important<sup>31</sup>, pointing to the importance of our study's identical methods and directly comparable responses across sites and, probably, the importance of allocation of sites to long- and short-history of grazing. The meta-analysis classified all sites in the Americas as long-history, whereas some regions in the United States (for example, in California, Florida and Oregon) and in South America (for example, the Pampas in Argentina) are better characterized as short-history (Fig. 2 and Supplementary Note 1). Moreover, our data allowed us to decompose richness into native and exotic species, enabling us to determine that decreases in richness with herbivore exclusion were limited to exotic species in fertilized plots in short-history sites, providing resolution that has not been possible in previous studies<sup>31</sup>.

Although we found that the diversity responses to herbivore exclusion depended on evolutionary history of grazing, the mechanisms by which grazing can promote diversity were the same regardless of grazing history. We identified the alleviation of light limitation as a mechanism through which herbivores promote native species richness, regardless of evolutionary history of grazing (Extended Data Fig. 2)<sup>33</sup>. Here the response of light availability to herbivore exclusion was related to aboveground biomass in both long- and short-history sites but not to precipitation (Extended Data Fig. 5). In sites with greater plant biomass, herbivore exclusion decreased light availability more than in sites with lower plant biomass. In contrast, others<sup>30</sup> concluded that changes in dominance, measured with the Berger–Parker Dominance Index, was the main mechanism through which herbivore exclusion reduces plant richness. In this study, we did not use the Berger–Parker Dominance Index as it was correlated with plant richness (Pearson's  $r=-0.6$ ). Instead, we decomposed diversity into richness and evenness (the inverse of dominance) and determined that herbivore exclusion reduced diversity in long-history sites via reductions in both variables. Thus, our results are broadly consistent with this meta-analysis<sup>30</sup> but, rather than treating dominance as a mechanism, we view it as an intermediate response, with light availability as an underlying mechanism.

## Conclusions

Our findings support the predictions of the Milchunas et al.<sup>11</sup> model for regions with a long-history of grazing, while for short-history sites our results support non-equilibrium models such as that proposed by Cingolani et al.<sup>10</sup> in their modification of the Milchunas model. The effect of herbivores on biodiversity has been contentious<sup>30,31,44</sup> and contrasting results in previous studies may stem from analysing long- and short-history sites together. By separating sites that have a long- and short-history of ungulate grazing, we found some support for long-held theories about the response of plant diversity to herbivore exclusion in relation to resource availability. Consistent with conceptual model predictions, we found stronger reductions in plant species richness with herbivore exclusion at higher precipitation in sites that have a long evolutionary history of grazing. This relationship did not exist for short-history sites.

We found that one of the mechanisms by which herbivores can promote plant richness is through alleviating light limitation and this is generalizable across sites regardless of the evolutionary history of grazing. In sites with a short evolutionary exposure to ungulate grazing, diversity responses to herbivore exclusion were related to species origin with some evidence that exotic species respond similarly to native species from long-history sites, as most exotic species originate from long-history sites. Native species in short-history sites showed little recovery following herbivore exclusion, suggesting that short-history grasslands might be in a stable (and potentially degraded) state. While fencing alone would be insufficient, restoration interventions in addition to fencing, such as seeding, planting or fire, may increase native plant diversity at these sites. However, grazing can have persistent land-use legacies and re-instating a more historical disturbance, such as fire, does not necessarily increase native diversity if ecosystems are in a degraded but stable state after grazing<sup>23</sup>. More generally, our findings highlight the importance of evolutionary history and species provenance in interpreting and predicting the effects of human land-use and global change.

## Methods

**Site selection and experimental treatments.** This study used data from the Nutrient Network ([www.nutnet.org](http://www.nutnet.org)), a globally replicated experimental study of herbivore exclusion and nutrient addition in grassland ecosystems. A fully factorial combination of herbivore exclusion ('unfenced' or 'fenced') and nutrient addition ('unfertilized' or 'fertilized') was applied at each site, totalling four treatment plots (5 × 5 m<sup>2</sup>) in three blocks (although some sites have a different number of blocks; Supplementary Table 2). Fences (up to 2.30 m high) were designed to exclude aboveground mammalian herbivores (more than about 50 g). The fertilized plots received annual applications of 10 g m<sup>-2</sup> year<sup>-1</sup> of 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 potassium sulfate [K<sub>2</sub>SO<sub>4</sub>]. Micronutrients (μ) were applied once, at the start of the experiment, as 100 g m<sup>-2</sup> of mix of Fe (15%), S (14%), Mg (1.5%), Mn (2.5%), Cu (1%), Zn (1%), B (0.2%) and Mo (0.05%)<sup>32</sup>.

For this study, 57 NutNet sites were included that met two conditions: (1) experimental data had been collected for the four treatments for at least 3 years of treatment applications and (2) mammalian herbivores were present in the sites and were excluded by the fences (see Supplementary Table 2 for an overview of the sites included and Supplementary Table 8 for sites with exceptions to the NutNet fence design). Sites that only had herbivore species with a body weight <50 g (for example, voles, mice, rats, squirrels and gophers) were not included, as these herbivores are not likely to be excluded by the fences. The mammalian herbivores in the sites ranged from domestic ungulates, such as sheep and cattle, to wild ungulates such as deer, wild macropods like kangaroo and other medium-sized mammals (for an overview of all herbivore species see Supplementary Table 3). The study sites represent a wide range of herbaceous ecosystems including prairie, montane grassland, shrub steppe, alpine grassland and savanna. The sites also encompassed varying environmental conditions, for example, mean annual precipitation (192–1,877 mm) and mean annual temperature (−3–24 °C) (Fig. 2 and Supplementary Table 2).

We classified our sites as subject to a long evolutionary history of grazing (grazing present >500–10,000 years ago; 24 sites) or a short one (<500 years; 33 sites), according to site experts and ref.<sup>34</sup> (Supplementary Note 1). We used 3 years of post-treatment data at all sites (plant data and light; see data collection below), which was sufficient to observe herbivore impacts on aboveground biomass<sup>45</sup>.

**Data collection and calculations.** All NutNet sites followed standard sampling protocols, with yearly sampling at peak biomass. In a permanently marked 1 × 1 m<sup>2</sup> subplot that was randomly designated, cover was estimated visually to the nearest 1% for all plant species. Studies have reported variable responses of diversity to grazing effects at different scales and it is possible that our results would differ if using larger sample sizes. However, a recent study using NutNet data found no consistent effects of fencing on species area relationships<sup>46</sup>. Adjacent to this subplot, aboveground biomass of all plants was clipped at ground level within two 1 × 0.1 m<sup>2</sup> strips. Biomass was sorted to live (current year's growth) and dead (previous years' growth), dried at 60 °C to constant mass and weighed to the nearest 0.01 g. Lead scientists at each site provided data, based on site-level knowledge, on the provenance (native or exotic) of each plant species at their sites. Photosynthetically active radiation (PAR, mmol photons m<sup>-2</sup> s<sup>-1</sup>) was determined at the time of biomass clipping at approximately solar noon (between 11:00 and 14:00). In the same 1 m<sup>2</sup> subplots used for plant cover, two light availability measurements were taken at ground level and one was taken above the canopy. Light availability was calculated as the ratio of PAR below and above the canopy. We used data from the third-year post-treatment at each site. Data on mean annual precipitation at each site was derived from the WorldClim database (v.1.4)<sup>47</sup>, which provides

high-resolution interpolated global climate data from stations with 10–30 years of data. We focused on precipitation as our measure of resource availability and also tested aridity index (which was strongly correlated with precipitation:  $P < 0.001$ ,  $r = 0.69$ ), which gave similar results for diversity and tended to do so for richness (Supplementary Table 4). In addition, we also tested the effect of soil nitrogen in control plots (Supplementary Table 5).

**Calculations and statistical analyses.** We calculated the inverse Simpson's diversity index or ENS<sub>PIE</sub> as

$$\text{ENS}_{\text{PIE}} = \frac{1}{\sum_{i=1}^S p_i^2}$$

where  $p_i$  is the proportion of species  $i$  and  $S$  is the number of species (species richness) in each treatment plot. The Simpson's evenness index was calculated by dividing the inverse Simpson's diversity (ENS<sub>PIE</sub>) by richness ( $S$ ). In this way, we decomposed diversity into changes in number of species (richness) and changes in dominance (evenness).

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

We started our analyses by examining the overall effect of herbivore exclusion on inverse Simpson's diversity, richness (total, native and exotic) and Simpson's evenness in the long- and short-history sites separately. We did this independently of resources to see if evolutionary history of grazing is critical for predicting plant diversity responses to herbivore exclusion. Therefore, we performed one-sample  $t$ -tests on the LRRs under unfertilized and fertilized conditions in the long- and short-sites separately. If the 95% confidence interval values of the LRRs did not overlap with zero, there was a significant decrease or increase with herbivore exclusion. We then used linear mixed effects models (LMMs) to test the effects of evolutionary history of grazing, mean annual precipitation (MAP), fertilization, and their interactions, on the LRR of inverse Simpson's diversity, richness and Simpson's evenness to herbivore exclusion. For these models, we included block within site as a random factor. Using linear regressions, we tested whether the effect of herbivore exclusion on native and exotic species richness was related to changes in light availability.

To estimate current grazing intensity, we used a grazing index which accounted for variation in diversity and abundance of herbivores across the sites. All herbivore species (>2 kg) that consume aboveground biomass throughout the year were documented by the lead scientist of each site and assigned an importance value; from 1 (very low impact and frequency) to 5 (very high impact and frequency). The index value was calculated for each site as the sum of herbivore importance values for all herbivores<sup>38,45</sup>. This grazing index, based on a standardized rubric completed for each site, accounts for site-level variation in herbivore abundance and diversity, integrated across seasons and years. Due to missing data, we were unable to estimate index values for four long- and ten short-history sites, so these were excluded from the analysis. Estimating grazing intensity across a wide range of ecosystems is a difficult task and having direct measures of intensity may have improved our estimates. However, the index value provides a comparable measure of intensity across sites. We used LMMs to test the effects of evolutionary history of grazing and grazing intensity and their interactions, on the LRRs of inverse Simpson's diversity, richness and Simpson's evenness to herbivore exclusion, with block within site as random factor. We simplified the models to not include precipitation because there were no significant interactions between precipitation and grazing intensity when running the full LMMs. All data analyses were performed in R v.3.6.1 (ref. <sup>48</sup>).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

The data presented in the current study and R code used to analyse the data are available in the Environmental Data Initiative (EDI) repository with the identifier <https://doi.org/10.6073/pasta/96436e301c9578230a0fa851ea13af77>. The WorldClim database (v.1.4) is available at <http://www.worldclim.org/bioclim>. Source data are provided with this paper.

## Code availability

R code to reproduce all statistical analyses is available in the EDI repository with the identifier <https://doi.org/10.6073/pasta/96436e301c9578230a0fa851ea13af77>.

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## Author contributions

J.N.P. and J.S. developed and framed research questions, analysed the data and wrote the paper. T.O. and P.T. developed and framed research questions and contributed to data analyses. C.S.B., S.M.P. and E.S.B. developed and framed research questions and

established and managed the grazing history database. E.W.S. and E.T.B. developed and framed research questions and coordinate the Nutrient Network collaboration. S.B. contributed to analyses. J.N.P., J.S., C.S.B., E.W.S., E.T.B., S.M.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., 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., A.C.R., M.S., R.S., C.J.S., G.F.V., R.V. and G.M.W. contributed data. All authors contributed to paper writing.

### Competing interests

The authors declare no competing interests.

### Additional information

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**Correspondence and requests for materials** should be addressed to Jodi N. Price or Judith Sitters.

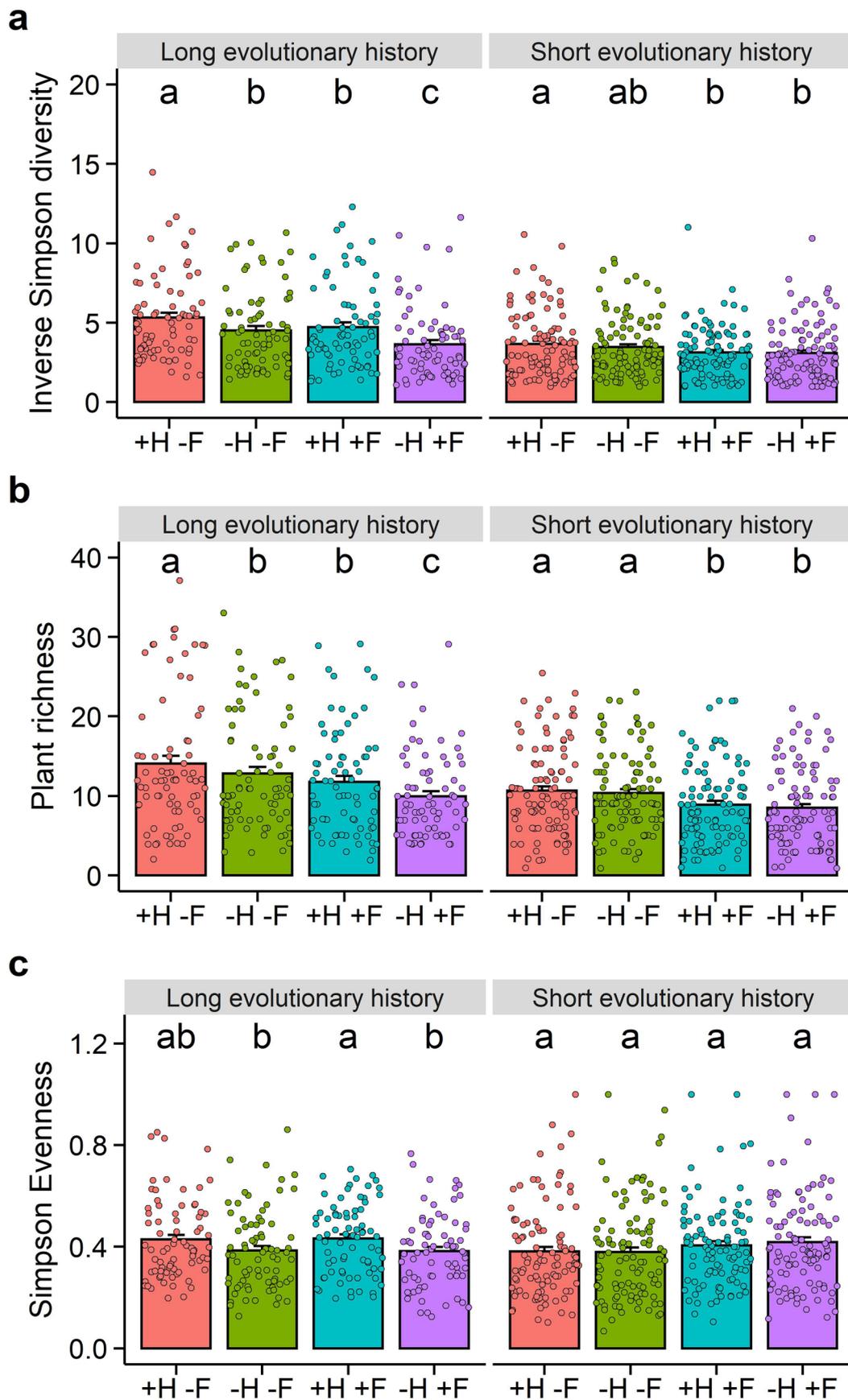
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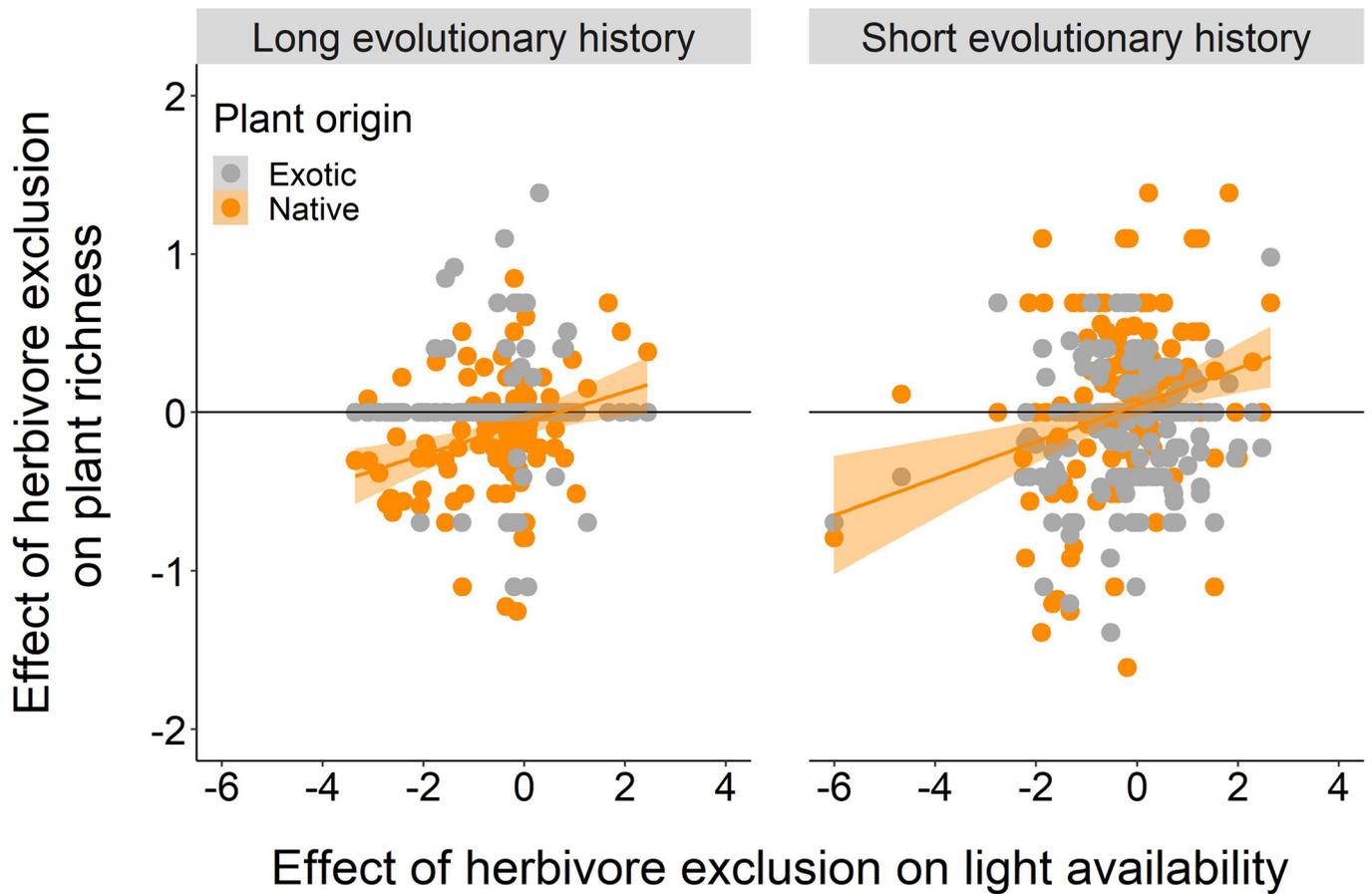
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<sup>1</sup>Gulbali Institute, Charles Sturt University, Albury, New South Wales, Australia. <sup>2</sup>Ecology and Biodiversity, Department Biology, Vrije Universiteit Brussel, Brussels, Belgium. <sup>3</sup>Wageningen Environmental Research, Wageningen University and Research, Wageningen, the Netherlands. <sup>4</sup>Department of Biology, University of New Mexico, Albuquerque, New Mexico, USA. <sup>5</sup>IFEVA-CONICET, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina. <sup>6</sup>Department of Agricultural Biology and Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA. <sup>7</sup>Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA. <sup>8</sup>CSIRO Land and Water, Wembley, Western Australia, Australia. <sup>9</sup>Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, the Netherlands. <sup>10</sup>Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada. <sup>11</sup>Department of Entomology, University of Maryland, College Park, MD, USA. <sup>12</sup>Faculty of Environmental and Forest Sciences, Agricultural University of Iceland, Reykjavik, Iceland. <sup>13</sup>Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India. <sup>14</sup>Department of Physical and Environmental Sciences, University of Toronto-Scarborough, Toronto, Ontario, Canada. <sup>15</sup>School of Environmental and Forest Sciences, University of Washington, Seattle, WA, USA. <sup>16</sup>Rangeland Resources & Systems Research Unit, USDA Agricultural Research Service, Fort Collins, CO, USA. <sup>17</sup>Archbold Biological Station, Buck Island Ranch, Lake Placid, FL, USA. <sup>18</sup>Department of Plant Biology and Program in Ecology, Evolution, and Behavior, Michigan State University, East Lansing, MI, USA. <sup>19</sup>Centre for Applied Ecology 'Prof. Baeta Neves' (CEABN-InBIO), School of Agriculture, University of Lisbon, Lisbon, Portugal. <sup>20</sup>Department of Biological Sciences, University of Toronto-Scarborough, Toronto, Ontario, Canada. <sup>21</sup>Forest Research Centre, School of Agriculture, University of Lisbon, Lisbon, Portugal. <sup>22</sup>Desert Ecology Research Group, School of Life & Environmental Sciences, University of Sydney, Sydney, New South Wales, Australia. <sup>23</sup>Department of Zoology, School of Natural Sciences, Trinity College Dublin, Dublin, Ireland. <sup>24</sup>Ecology and Biodiversity Group, Department of Biology, Utrecht University, Utrecht, the Netherlands. <sup>25</sup>Institute of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland. <sup>26</sup>Department of Biology and Animal Sciences, São Paulo State University-UNESP, Ilha Solteira, Brazil. <sup>27</sup>Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA. <sup>28</sup>School of Biological Sciences, Monash University, Clayton, Victoria, Australia. <sup>29</sup>Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia. <sup>30</sup>Community Ecology, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland. <sup>31</sup>Environmental and Conservation Sciences, Murdoch University, Murdoch, Western Australia, Australia. <sup>32</sup>Lancaster Environment Centre, Lancaster University, Lancaster, UK. <sup>33</sup>Department of Terrestrial Ecology, Netherlands Institute of Ecology, Wageningen, the Netherlands. <sup>34</sup>Ecology and Genetics, University of Oulu, Oulu, Finland. <sup>35</sup>These authors contributed equally: Jodi N. Price, Judith Sitters. ✉e-mail: [joprice@csu.edu.au](mailto:joprice@csu.edu.au); [judith.sitters@wur.nl](mailto:judith.sitters@wur.nl)

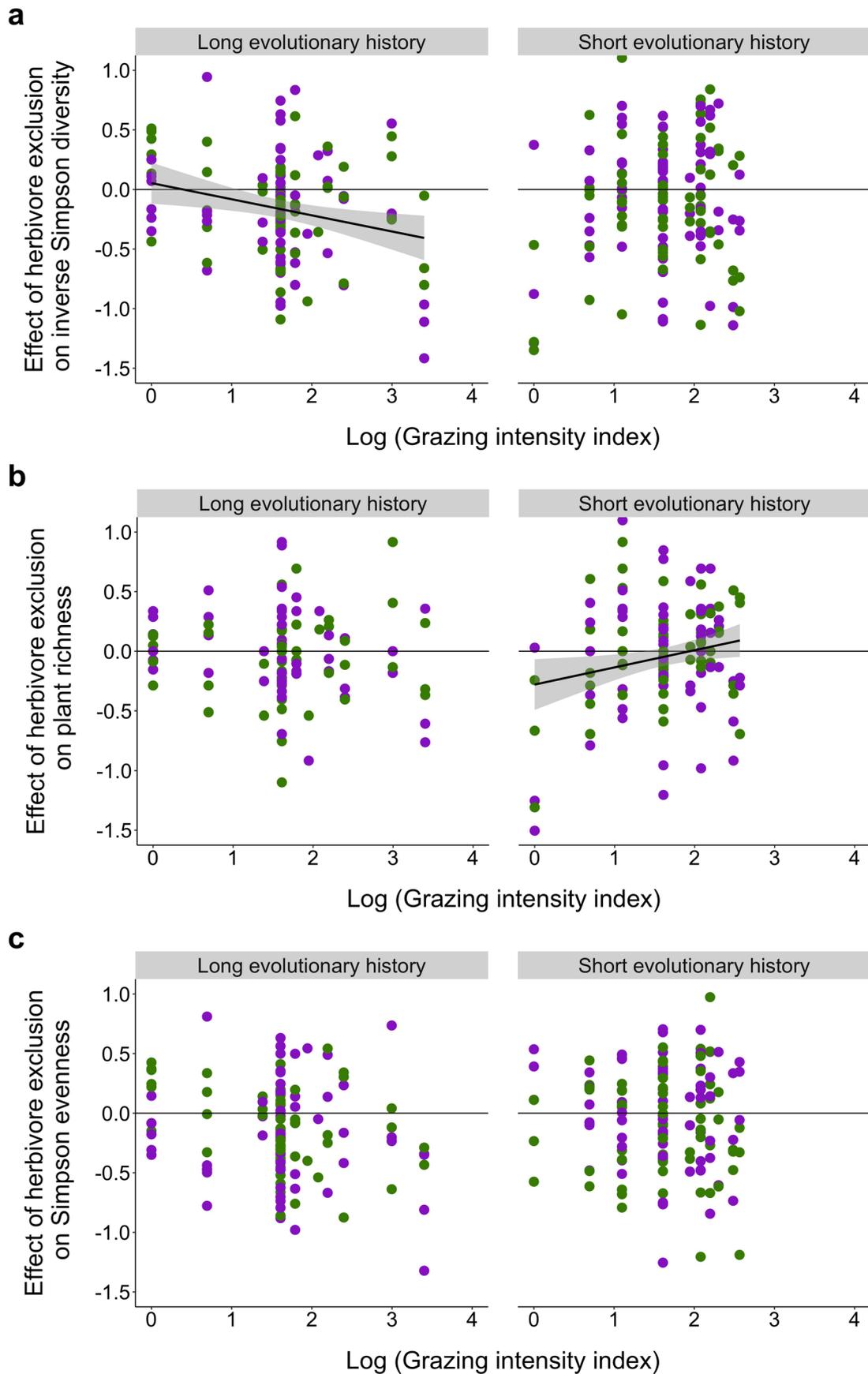


Extended Data Fig. 1 | See next page for caption.

**Extended Data Fig. 1 | Effect of herbivore exclusion and fertilization on plant diversity, richness and evenness.** Effect of herbivore exclusion (+H: herbivores present; –H: herbivores excluded) and fertilization (+F: fertilized with NPK $\mu$ ; –F: unfertilized) on **(a)** plant diversity **(b)** richness and **(c)** evenness in sites with a long or short evolutionary history of grazing. Shown are sample points and bars representing means  $\pm 1 SE$  ( $n = 76$  for long-history sites and  $n = 103$  for short-history sites per bar). Different letters indicate significant differences among the treatment means based on LMMs with herbivore exclusion and fertilization as fixed factors, and block nested within site as random effect.

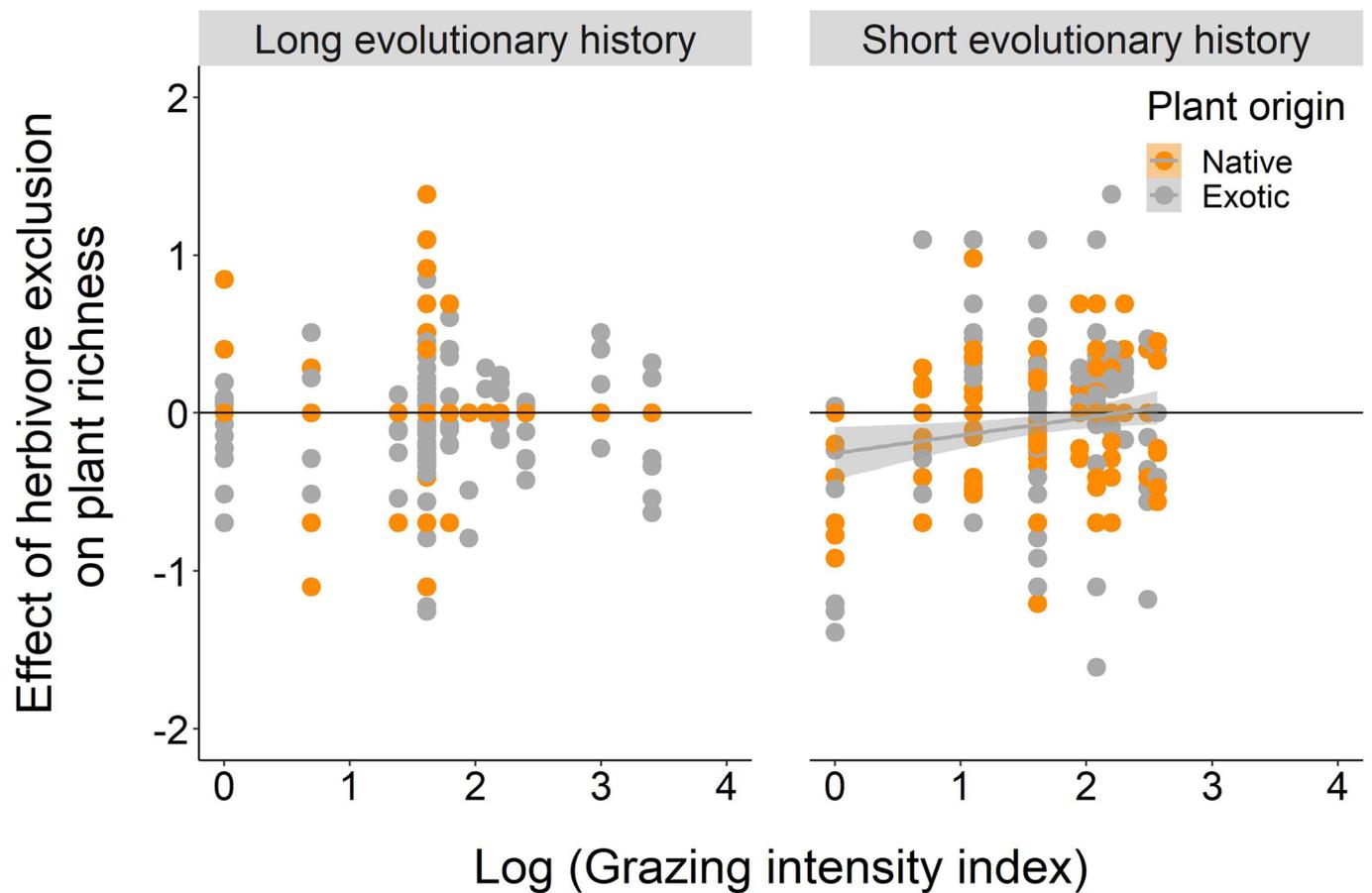


**Extended Data Fig. 2 | Herbivore exclusion effects on plant richness related to changes in light availability.** Relationship between the *LRR* of light availability to herbivore exclusion and the *LRR* of native (orange points) and exotic (grey points) species richness to herbivore exclusion. Sites had a long or short evolutionary history of grazing and included unfertilized control plots and plots fertilized with NPK $\mu$ . Per plant provenance category,  $n=119$  for long-history sites and  $n=182$  for short-history sites. For native richness, there was a significant linear regression in both long- ( $P=0.0008$ ,  $R^2=0.08$ ) and short-history sites ( $P=0.0004$ ,  $R^2=0.06$ ), so trendlines were produced using `geom_smooth` (*ggplot2* package in R). The coloured bands represent the 95% confidence intervals.

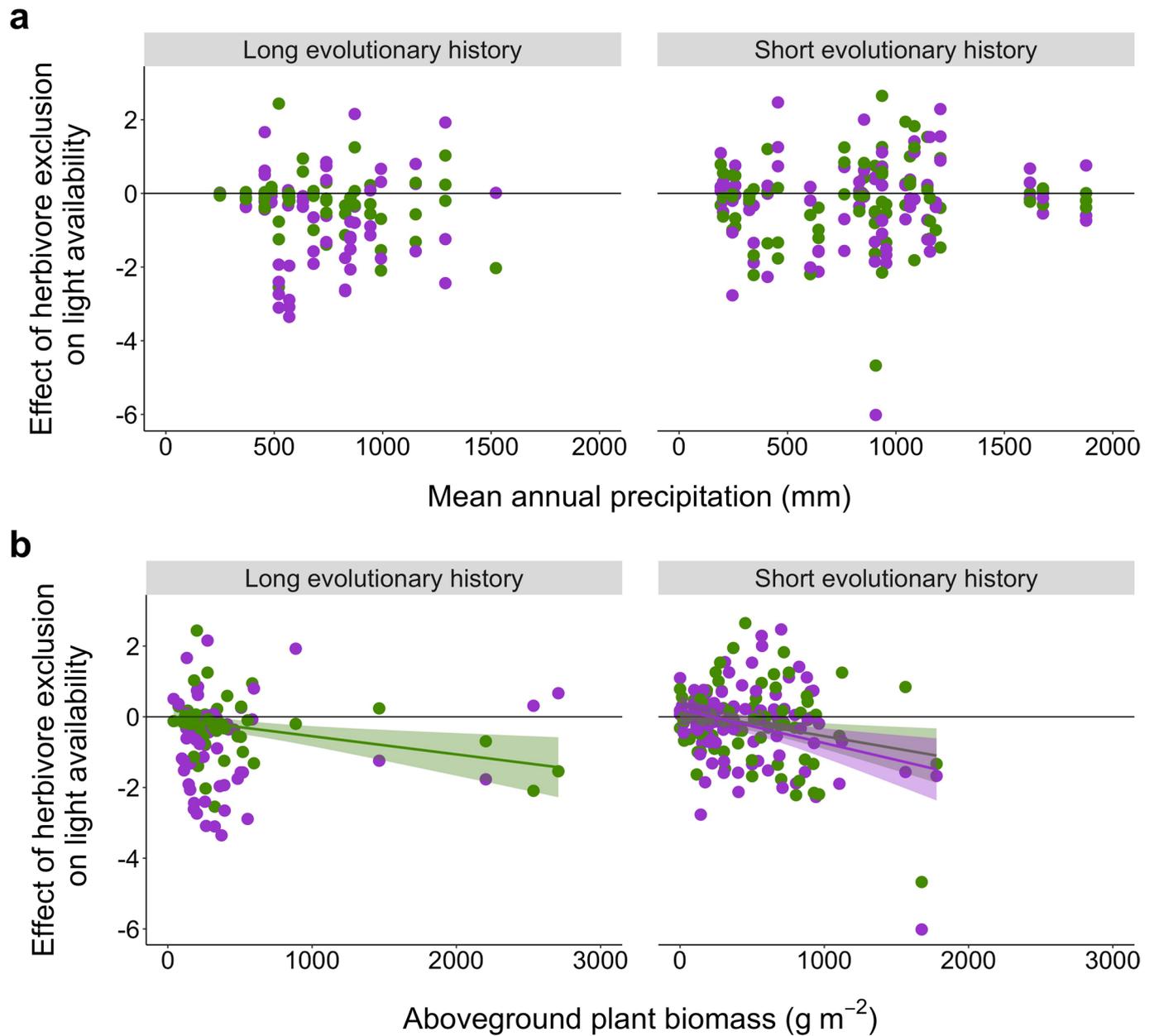


Extended Data Fig. 3 | See next page for caption.

**Extended Data Fig. 3 | Relationship between grazing intensity and herbivore exclusion effects on plant diversity, richness and evenness.** Relationship between the log-transformed grazing intensity index and the *LRR* of (a) plant diversity, (b) richness and (c) evenness to herbivore exclusion ( $\ln(\text{fence/control})$ ) in sites with a long ( $n=20$ ) or short ( $n=23$ ) evolutionary history of grazing. Sites included unfertilized control plots (green points) and plots fertilized with NPK $\mu$  (purple points). Trendlines were added when there were significant linear regressions between the *LRRs* and the grazing index and were produced using `geom_smooth` (*ggplot2* package in R) regardless of fertilization, as this experimental treatment did not have a significant effect. The coloured bands represent the 95% confidence intervals.



**Extended Data Fig. 4 | Relationship between grazing intensity and herbivore exclusion effects on native and exotic plant richness.** Relationship between the log-transformed grazing intensity index and the *LRR* of native (orange points) and exotic (grey points) species richness to herbivore exclusion ( $\ln(\text{fence/control})$ ) in sites with a long ( $n=20$ ) or short ( $n=23$ ) evolutionary history of grazing. A significant linear regression was found for exotic species richness in short-history sites and a trendline was produced using `geom_smooth` (*ggplot2* package in R). The coloured band represents the 95% confidence intervals.



**Extended Data Fig. 5 | Relationship between productivity and herbivore exclusion on light availability.** Relationship between the *LRR* of light availability to herbivore exclusion and **(a)** mean annual precipitation and **(b)** aboveground plant biomass in sites with a long ( $n=119$ ) or short ( $n=182$ ) evolutionary history of grazing. Plant biomass is from the fenced ungrazed plots. Sites included unfertilized control plots (green points) and plots fertilized with NPK $\mu$  (purple points). Trendlines were added when there were significant linear regressions between the *LRR*s and the aboveground plant biomass and were produced using `geom_smooth` (*ggplot2* package in R). The coloured bands represent the 95% confidence intervals.

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*Give  $P$  values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's  $d$ , Pearson's  $r$ ), indicating how they were calculated

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

**Data collection** Data come from the Nutrient Network, a coordinated, multi-site and multi-year herbivore exclusion and nutrient (nitrogen, phosphorus, potassium) enrichment experiment. Data were retrieved on 2 April 2021. No software was used for data collection.

**Data analysis** All analyses were conducted in R, version 3.6.1. Code is available in the Environmental Data Initiative (EDI) repository with the identifier doi:10.6073/pasta/96436e301c9578230a0fa851ea13af77.

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Source data are provided with this paper. The data presented in this study are also available in the Environmental Data Initiative (EDI) repository with the identifier doi:10.6073/pasta/96436e301c9578230a0fa851ea13af77. The WorldClim database (version 1.4) is available at <http://www.worldclim.org/bioclim>.

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## Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	The 57 grassland sites are part of the Nutrient Network (NutNet) Global Research Cooperative, covering a wide range of grassland habitats and relevant gradients of fine-scale and site-level variation. Herbivore exclusion and fertilization treatments were randomly assigned to the plots and were replicated in three blocks at most sites (some sites had fewer/more blocks as indicated in Supplementary Table 1).
Research sample	Plant species richness/diversity and light availability from 57 grassland sites across the globe ( <a href="https://nutnet.org/">https://nutnet.org/</a> ).
Sampling strategy	Sampling was done in 1 m <sup>2</sup> subplots and followed a standardized protocol at all sites. The number of samples was chosen to allow for analyses of all data, but to minimize the efforts for sampling at each site.
Data collection	Treatments and sampling followed a standardized protocol at all sites, detailed in ref. 26 (Borer, E. T. et al. Finding generality in ecology: a model for globally distributed experiments. <i>Methods in Ecology and Evolution</i> 5, 63-73 (2013)). In a permanently marked 1 m <sup>2</sup> subplot plant cover was estimated visually to the nearest 1% for all plant species. Adjacent to this subplot, aboveground biomass was clipped at ground level within two 1 m x 0.1 m strips. Photosynthetically active radiation (PAR) was determined at the time of biomass clipping in the subplot, whereby two light availability measurements were taken at ground level and one was taken above the canopy. Light availability was calculated as the ratio of PAR below and above the canopy. We quantified the inverse Simpson's diversity index across the three replicated 1 m <sup>2</sup> subplots for each site, treatment and duration period using cover data. Data collection was done by the principal investigator at each site.
Timing and spatial scale	We selected sites that had a minimum of three years post-treatment data, which is the year we used for all analyses. Plots at all sites were 5 x 5 m (separated by at least 1 m walkways) spread over an area of at least 1000 m <sup>2</sup> . Sampling was done in 1 m <sup>2</sup> plots grouped into spatial blocks spread over 320 m <sup>2</sup> .
Data exclusions	We selected NutNet sites that met two conditions: (i) experimental data had been collected for the four treatments (herbivore excluded or not, fertilized or not) for at least three years of treatment applications; and (ii) mammalian herbivores were present in the sites, and were excluded by the fences. Sites that only had herbivore species with a body weight <50 g (e.g. voles, mice, rats, squirrels, gophers) were not included, as these herbivores are not likely to be excluded by the fences. Exclusion criteria were pre-established.
Reproducibility	Data were retrieved on 2 April 2021 from the NutNet dropbox (updated regularly by the data manager). Plots were replicated in three blocks at most sites used in this study.
Randomization	Treatments were randomly assigned.
Blinding	Data were analyzed using all replicates per site, but without knowing which site had which influence on the overall results.
Did the study involve field work?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

## Field work, collection and transport

Field conditions	<p>ur sites were distributed over 6 continents and 17 countries. Site name, continent, grassland type, number of blocks, latitude, longitude, mean annual precipitation, mean annual temperature and evolutionary history of ungulate grazing can be found below.</p> <p>Site code, Continent, Grassland type, No. of blocks, Latitude(°N), Longitude (°E), MAP (mm), MAT (°C), Evolutionary history of grazing</p> <p>ahth.is Europe Heathland 3 65.13 -19.67 615 0.8 Short</p> <p>amlr.is Europe Desert grassland 3 65.13 -19.67 615 0.8 Short</p> <p>arch.us North America Mixed grass prairie 3 27.15 -81.18 1205 22.7 Short</p> <p>bldr.us North America Shortgrass prairie 2 39.97 -105.23 487 9.9 Long</p> <p>bnch.us North America Montane grassland 3 44.28 -121.97 1618 6.8 Short</p> <p>bogong.au Australia Alpine grassland 3 -36.87 147.25 1678 6.0 Short</p> <p>burrawan.au Australia Semiarid grassland 3 -27.73 151.14 643 18.2 Short</p> <p>burren.ie Europe Calcareous grassland 3 53.07 -8.99 1320 9.8 Long</p> <p>cbgb.us North America Tallgrass prairie 3 41.79 -93.39 871 9.3 Long</p> <p>cdcr.us North America Tallgrass prairie 5 45.43 -93.21 740 6.3 Long</p> <p>cdpt.us North America Shortgrass prairie 6 41.20 -101.63 456 9.6 Long</p> <p>cereep.fr Europe Old field 3 48.28 2.66 632 10.8 Long</p> <p>chilcas.ar South America Mesic grassland 3 -36.28 -58.27 955 15.1 Short</p> <p>comp.pt Europe Annual grassland 3 38.83 -8.79 564 16.6 Long</p>
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cowi.ca North America Old field 3 48.81 -123.63 762 10.4 Short  
 elliot.us North America Annual grassland 3 32.88 -117.05 344 17.7 Short  
 ethamc.au Australia Desert grassland 3 -23.76 138.47 192 24.1 Short  
 ethass.au Australia Desert grassland 3 -23.64 138.40 203 24.0 Short  
 frue.ch Europe Pasture 3 47.11 8.54 1546 7.0 Long  
 gilb.za Africa Montane grassland 3 -29.28 30.29 943 14.1 Long  
 hall.us North America Tallgrass prairie 3 36.87 -86.70 1289 13.8 Long  
 hart.us North America Shrub steppe 3 42.72 -119.50 259 7.7 Short  
 hero.uk Europe Mesic grassland 3 51.41 -0.64 668 10.2 Long  
 hnv.us North America Old field 3 43.42 -72.14 1044 6.5 Short  
 hopl.us North America Annual grassland 3 39.01 -123.06 1065 13.2 Short  
 kbs.us North America Old field 5 42.41 -85.39 903 8.8 Short  
 kibber.in Asia Alpine grassland 3 32.32 78.01 400 -1.5 Long  
 kilp.fi Europe Tundra grassland 4 69.06 20.87 569 -3.3 Long  
 kiny.au Australia Semi-arid grassland 3 -36.20 143.75 408 15.6 Short  
 koffler.ca North America Pasture 3 44.02 -79.54 853 6.3 Short  
 lagoas.br South America Cerrado 3 -20.98 -51.80 1145 23.2 Short  
 lancasater.uk Europe Mesic grassland 1 53.99 -2.62 1522 8.0 Long  
 look.us North America Montane grassland 3 44.21 -122.13 1877 6.9 Short  
 marc.ar South America Grassland 1 -37.72 -57.42 907 14.3 Short  
 mcla.us North America Annual grassland 3 38.86 -122.41 936 14.0 Short  
 mtca.au Australia Savanna 4 -31.78 117.61 324 17.7 Short  
 pape.de Europe Old field 1 53.09 7.47 788 9.1 Long  
 ping.au Australia Old field 3 -32.50 116.97 456 16.3 Short  
 pinj.au Australia Pasture 3 -27.53 152.92 1085 20.0 Short  
 potrok.ar South America Semi-arid grassland 3 -51.92 -70.41 249 6.6 Long  
 rook.uk Europe Mesic grassland 3 51.41 -0.64 685 10.1 Long  
 saana.fi Europe Montane grassland 4 69.04 20.84 521 -2.6 Long  
 sage.us North America Montane grassland 3 39.43 -120.24 831 5.8 Short  
 sava.us North America Savanna 2 33.34 -81.65 1184 17.4 Short  
 sereng.tz Africa Savanna 3 -2.25 34.51 827 21.9 Long  
 sgs.us North America Shortgrass prairie 3 40.82 -104.77 369 8.9 Long  
 shps.us North America Shrub steppe 4 44.24 -112.20 246 5.3 Short  
 sier.us North America Annual grassland 5 39.24 -121.28 936 16.3 Short  
 smith.us North America Mesic grassland 3 48.21 -122.63 605 10.2 Short  
 spin.us North America Pasture 3 38.14 -84.50 1152 12.5 Long  
 thth.is Europe Heathland 3 65.90 -17.09 572 1.2 Short  
 tmlr.is Europe Desert grassland 3 65.90 -17.09 572 1.2 Short  
 trel.us North America Tallgrass prairie 3 40.08 -88.83 992 11.1 Long  
 unc.us North America Old field 3 36.01 -79.02 1157 14.9 Short  
 valm.ch Europe Alpine grassland 3 46.63 10.37 681 0.1 Long  
 veluwe.nl Europe Old field 5 52.06 5.75 851 9.5 Long  
 yarra.au Australia Mesic grassland 4 -33.61 150.73 844 17.3 Short

Location Location (latitude and longitude) of the 57 sites is reported in the Supplementary Table 1 and above.

Access & import/export Access to sites and data collection followed standard practices and complied with laws. No permits were required.

Disturbance At each sites walkways were established to minimize trampling efforts on the sampling plots.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

### Methods

- | n/a                                 | Included in the study                                  |
|-------------------------------------|--|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern  |

- | n/a                                 | Included in the study                           |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |