

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

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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 nutrient addition) determine herbivore exclusion effects on plant diversity, decomposed into richness and evenness. We tested the hypothesis that in sites with a long history of ungulate grazing, plant diversity decreases with herbivore exclusion in resource-rich sites; whereas in short-history sites the effect of herbivore exclusion depends on plant species origin (native or exotic from long-history sites). We found that in long-history sites, 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. In short-history sites, the effects of herbivore exclusion differed for native and exotic plant richness and depended on fertilization; native species richness was unaffected by herbivore exclusion, whereas exotic species richness declined with herbivore exclusion in fertilized plots. In sum, herbivore exclusion caused loss of plant diversity in grasslands that evolved with ungulates, but these findings do not simply extrapolate to other grasslands, rather they depend on grazing history and plant provenance. 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 ca. 800 million people¹. Human activities are altering the plant and herbivore diversity of grasslands worldwide by increasing nutrient availability and changing the species composition, abundance, and foraging patterns of domestic and wild mammalian herbivores²⁻⁴. Given the ongoing losses of wild herbivores⁵, determining the mechanisms by which nutrients and large mammalian herbivores regulate plant diversity is critical for grassland conservation and management⁶.

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, hoofed herbivores (i.e., ungulates); resource availability for plant growth, especially water and nutrients; and modern-era grazing intensity⁷⁻¹⁴. Fluctuations in grazing intensity in sites with a relatively long evolutionary history of ungulate occurrence (i.e., grazing present for >500 to 10,000 years, hereafter long-history sites) have selected for two different pools of plant species—short-statured species that are tolerant or resistant to grazing and tall-statured species that are less grazing-resistant but better at capturing light^{9,15}. 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 (i.e., low richness and low evenness), thereby reducing ground-level light available for seedlings and short-statured species^{8,9,13}. In this case, herbivores promote plant diversity by increasing light availability for shorter, more grazing-tolerant species^{8,9,16,17}. Hence, herbivores increase diversity both through increased species richness (more grazing-tolerant species) and evenness (lower dominance of tall, less grazing-resistant species). In resource-poor sites, where light is generally less limiting, herbivores may have little effect on plant diversity, or negative effects if resource limitation (water, nutrients) prevents regrowth or plant reproduction after grazing⁶. Exclusion of herbivores from long-history sites is, therefore, expected to have no effect or increase plant diversity at low resource availability and decrease diversity at high resource availability (Box 1).

For sites with a relatively short evolutionary history of ungulate grazing (i.e., <500 years, hereafter short-history sites), it is predicted that herbivores generally reduce plant diversity⁹. 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 trampling¹³. These grazing 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^{8,9,13}. These effects will likely 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, in theory, increase the diversity of plants with grazer-sensitive growth-forms relative to grazed plots (Box 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 Cingolani et al.⁸). However, empirical evidence and alternative models (e.g., state-and-transition models¹⁸) demonstrate irreversible reductions in plant diversity due to local extinction of grazing-sensitive species^{8,19,20} (R1 wanted more empirical refs, can folks from short-history regions suggest some key citations supporting this point). 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^{18,20}. 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 (Box 1).

Many short-history sites are now dominated by non-native, potentially invasive, plant species originating from regions with a long evolutionary history of grazing, which were often intentionally sown for forage^{8,9,13}. 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 or provision of water, and sometimes fertilizer addition^{8,9,21-23}. 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^{24,25}. We therefore predict that responses of exotic species in short-history sites to herbivore exclusion will match responses of native species from long-history sites (Box 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)^{26,27} or evolutionary history of grazing²⁷. Both studies found that, in general, herbivore exclusion tends to reduce plant species richness^{26,27}. However, while meta-analysis provides a quantitative synthesis of published studies, differences in design and methodology among experiments obscures inference about the relative importance of the biological and methodological factors underlying the observed patterns^{6,28}. For example, increasing light availability at ground level is a direct mechanism by which herbivores support plant diversity²⁹, but often data on light capture by the canopy is not measured. Instead a range of proxies for light availability (e.g., precipitation, aboveground plant biomass) have been employed in past studies to test model predictions^{9,30}, likely leading to variation among results because these proxies vary in explanatory power²⁹. We argue direct measures of resource availability (light, moisture, nutrients) should be used to determine how the relationship between grazing and diversity is mediated by resource availability.

Here, we use an unprecedented herbivore exclusion experiment using a standardised experimental and sampling design, in 57 grasslands on six continents (Fig. 1), to examine responses of plant diversity, richness and evenness to herbivore exclusion. We test mechanistic hypotheses related to light availability, grazing history and current grazing intensity. This is the first large-scale distributed experiment to 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 modified resources through addition of nitrogen, phosphorus, potassium plus micronutrients (NPKμ). The experimental nutrient addition enables us to provide a more mechanistic understanding of the role of resources in mediating grazing effects within sites when climate, soil type and herbivore communities are kept constant. Additionally, we have direct measures of light availability at ground level from 47 of the 57 sites. We classified

grasslands as having long (24 sites) or short (33 sites) evolutionary history of grazing according to local experts and definitions in Milchunas and Lauenroth³⁰ (Fig. 1; Supplementary Note 1; Supplementary Table 1). 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 three years with unfenced plots that were subject to variable grazing by the contemporary suite of herbivores present at each site (Supplementary Table 3). Our hypotheses, arising from the generalised grazing models^{8,9}, are described in Box 1.

Results and discussion

Across 57 globally distributed grasslands, 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. 2; particularly native species richness, Fig. 3a) in long-history sites, especially at higher precipitation (Table 1; Fig. 4). In contrast, current grazing had a negligible effect on native species richness in short-history sites (Fig. 3a). However, exotic species that mostly originated from long-history sites profited from grazing under fertilized conditions, responding similarly to native species from long-history sites (Fig. 3a,b). Hence, we show that the evolutionary history of individual species also regulates plant diversity responses. 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 or ENS_{PIE} ; unfertilized: $t = -3.73$, $P < 0.001$, fertilized: $t = -4.62$, $P < 0.001$), plant richness (unfertilized: $t = -1.70$, $P = 0.046$, fertilized: $t = -2.64$, $P = 0.005$) and Simpson's evenness (unfertilized: $t = -3.10$, $P < 0.001$, fertilized: $t = -2.68$, $P = 0.004$) (Fig. 2; Supplementary Fig. 1). Hence, herbivore exclusion decreased the number of native plant species (Fig. 3a) and increased dominance of a few plant species, consistent with other studies in which herbivores also promoted diversity^{12,16,31,32}. In long-

history sites, dominant plant species are often palatable, and grazing reduces their dominance, thereby increasing evenness, and promoting more grazing-tolerant native species³³. 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 (Fig. 5)²⁸.

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

Contrary to our expectation of a stronger reduction in diversity with nutrient addition for long-history sites, fertilization did not alter the effect of herbivore exclusion on inverse Simpson's diversity, species richness or Simpson's evenness (Fig. 2; error bars of unfertilized and fertilized treatments overlap), nor the relationship between the *LRR* (log response ratio) of richness and rainfall (no significant interactions with fertilization; Table 1). Here, we predicted an overall stronger reduction in diversity in fertilized compared to unfertilized conditions, independent of precipitation, as nutrient addition increases resource availability (i.e., shift toward the resource-rich end in Box 1). Nutrient addition alone decreased inverse Simpson's diversity and plant richness, and we found the lowest diversity and richness in fertilized plots from which herbivores were excluded (Supplementary Fig. 1). However, fertilization did not alter the effect of herbivore exclusion on diversity, likely because some sites are water-limited and unable to respond to the additional nutrients sufficiently to move to a light-limited state²⁹. Additionally we found ambient soil nitrogen

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

The theoretical models underpinning our hypotheses predict that the effect of grazing on plant diversity will depend on current grazing intensity^{8,9}. Here, we used an index of grazing intensity that incorporated importance values of all current herbivore species at 43 sites, estimated by site experts³⁴. Then, we tested whether the *LRRs* of inverse Simpson's diversity, plant richness and Simpson's evenness to herbivore exclusion were related to this index. In the long-history sites, herbivore exclusion decreased diversity more when current grazing intensity was higher, driven mainly by changes in evenness (Supplementary Fig. 2). As few sites had high herbivore densities, these results suggest that grazing even at intermediate intensities is sufficient to decrease plant dominance and maintain plant diversity (Fig. 2; Supplementary Fig. 2).

Consistent with our predictions for sites with a short evolutionary history of grazing (Box 1), herbivore exclusion had no effect on overall inverse Simpson's diversity (unfertilized: $t = -0.72$, $P = 0.472$, fertilized: $t = -0.94$, $P = 0.351$), plant richness (unfertilized: $t = -0.25$, $P = 0.805$; fertilized: $t = -1.10$, $P = 0.273$) or Simpson's evenness (unfertilized: $t = -0.57$, $P = 0.568$; fertilized: $t = 0.29$, $P = 0.771$) (Fig. 2; Supplementary Fig. 1), and these responses were not related to precipitation (Fig. 4a). The results of the total species pool do not support the Milchunas et al.⁹ model that diversity should increase with herbivore exclusion (Fig. 4a). 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 4). 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 4); 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 or resistance mechanisms to ungulate grazing; whereas exotic species mostly originate from long-history sites^{8,9,21,22}. We found

support for our prediction (Box 1) that 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, especially under eutrophied conditions because native species are often disadvantaged by high soil nutrients³⁵. Indeed, we found no overall response of native species richness to herbivore exclusion in unfertilized ($t = 0.90$, $P = 0.371$) or fertilized treatments ($t = 0.11$, $P = 0.913$) (Fig. 3a). However, the response of native species to herbivore exclusion depended on the extent that herbivores changed light availability, similarly to the long-history sites (Fig. 5). 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. We found partial support for this prediction, as exotic species richness decreased with herbivore exclusion under fertilized conditions ($t = -2.98$, $P = 0.002$; Fig. 3b), as we had expected at high resource availability (Box 1), and in sites with lower grazing intensity (Supplementary Figures 2b and 3). However, exotic species richness did not decrease with herbivore exclusion under unfertilized conditions ($t = -1.24$, $P = 0.218$; Fig. 3b), and the response to herbivore exclusion was not related to precipitation (Fig. 4b). These results indicate that in short-history sites, nutrient enrichment and low-intensity grazing promotes exotic species richness.

The neutral responses of native plant species to herbivore exclusion suggest little recovery from grazing in short-history sites, which conflicts with the single equilibrium model proposed by Milchunas et al.⁹. 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 Cingolani et al.⁸ Table 2) and conceptual models¹⁸. There are several possible interpretations of our findings. First, recovery of grazing-sensitive species may require longer timeframes than our study (three years). Second, recovery of native species may not be possible because they have become locally extinct, or because grazing and exotic invasion have altered ecological processes resulting in stable, degraded states^{19,33,36}. In this case, *current grazing* may not be impacting diversity as effects

occurred in the past. This can be the case despite significant negative effects of historical livestock grazing (e.g., if grazing intensity now is lower than previous levels)³³. It is also possible that neutral responses to herbivore exclusion indicate 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 extensive evidence has reported negative effects of introduced stock grazing on plant diversity³⁷(here also please suggest references from other short history regions).

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 (Supplementary Fig. 2b), driven by declines in exotic species richness (Supplementary 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 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 5), 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 reverse¹⁶.

By combining evolutionary history of grazing 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^{26,27}, but only in long-history sites. Consistent with theoretical

predictions but contrasting with recent meta-analyses ^{26,27}, our distributed experiment demonstrated that the response of diversity to herbivore exclusion depended on precipitation ²⁶, 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 ^{8,18}. The meta-analysis that incorporated evolutionary history of grazing did not find this factor to be important ²⁷, pointing to the importance of our study's identical methods and directly comparable responses across sites and, likely, 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 USA (e.g., in California, Florida and Oregon) and in South America are better characterized as short-history (e.g., the Pampas in Argentina) (Fig. 1; 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 short-history sites, providing resolution that has not been possible in other datasets ²⁷.

Although we found 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 found the alleviation of light limitation as a mechanism through which herbivores promote native species richness, regardless of evolutionary history of grazing (Fig. 5) ²⁹. Here, the response of light availability to herbivore exclusion was related to aboveground biomass in both long- and short-history sites (Supplementary Fig. 4), and not to precipitation. In sites with greater plant biomass, herbivore exclusion decreased light availability more than in sites with lower plant biomass. In contrast, Koerner et al. ²⁶ 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 (i.e., the inverse of dominance) and determined that herbivore exclusion reduced

diversity in long-history sites via reductions in both of these variables. Thus, our results are broadly consistent with this meta-analysis²⁶, but rather than treating dominance as a mechanism, we view it as an intermediate response variable, with an underlying mechanism.

Conclusions

Our findings confirm predictions of the Milchunas et al.⁹ 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.⁸ in their modification of the Milchunas model. The effect of herbivores on biodiversity has been contentious^{26,27,38}, 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, studies have found persistent land-use legacies from grazing, and that re-instating a more historical disturbance (like fire) does not necessarily increase native diversity if ecosystems are in a degraded, but stable state after grazing¹⁹. 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) 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 m x 5 m) 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 (> approximately 50 g). The fertilized plots received annual applications of 10 g m⁻² year⁻¹ N, P and K as time-released urea [(NH₂)₂CO], triple-super phosphate [Ca(H₂PO₄)₂], and potassium sulphate [K₂SO₄]. Micronutrients (μ) were applied once, at the start of the experiment, as 100 g m⁻² mix of Fe (15%), S (14%), Mg (1.5%), Mn (2.5%), Cu (1%), Zn (1%), B (0.2%) and Mo (0.05%). More details on the experimental design and nutrient sources are available in Borer et al. ²⁸.

For this study, 57 NutNet sites were included that met two conditions: (i) experimental data had been collected for the four treatments for at least three years of treatment applications; and (ii) 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 6 for sites with exceptions to the NutNet fence design). 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. 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; e.g., mean annual precipitation (192–1,877 mm) and mean annual temperature (–3–24°C) (Fig. 1; Supplementary Table 2).

We classified our sites as subject to a long (grazing present >500 to 10,000 years ago; 24 sites) or short (<500 years; 33 sites) evolutionary history of grazing according to site experts and Milchunas and Lauenroth³⁰ (see Supplementary Note 1 for details). We used three years of post-treatment data at all sites (i.e., plant data, light; see data collection below), which was sufficient to observe herbivore impacts on aboveground biomass³⁹.

Data collection and calculations. All NutNet sites followed standard sampling protocols, with yearly sampling at peak biomass. In a permanently marked 1 m x 1 m 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 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 curves⁴⁰. Adjacent to this subplot, aboveground biomass of all plants was clipped at ground level within two 1 m x 0.1 m 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⁻² s⁻¹) was determined at the time of biomass clipping at approximately solar noon (between 11:00 and 14:00). In the same 1 m² 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 (version 1.4)⁴¹, which provides high resolution interpolated global climate data from stations with 10 to 30 years of data. We focused on precipitation as our measure of resource availability but we also tested aridity index

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

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

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

where p_i is the proportion of species i and S is the number of species (i.e., species richness) in each treatment plot. The Simpson's evenness index was calculated by dividing Simpson's diversity (ENS_{PIE}) 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 LRR s for the unfertilized and the fertilized (NPK μ) 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 LRR s 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 LRR s 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^{34,39}. This grazing index, based on a standardized rubric completed for each sites, 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. 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, version 3.6.1⁴².

Data availability

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

440

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577 **Author contributions**

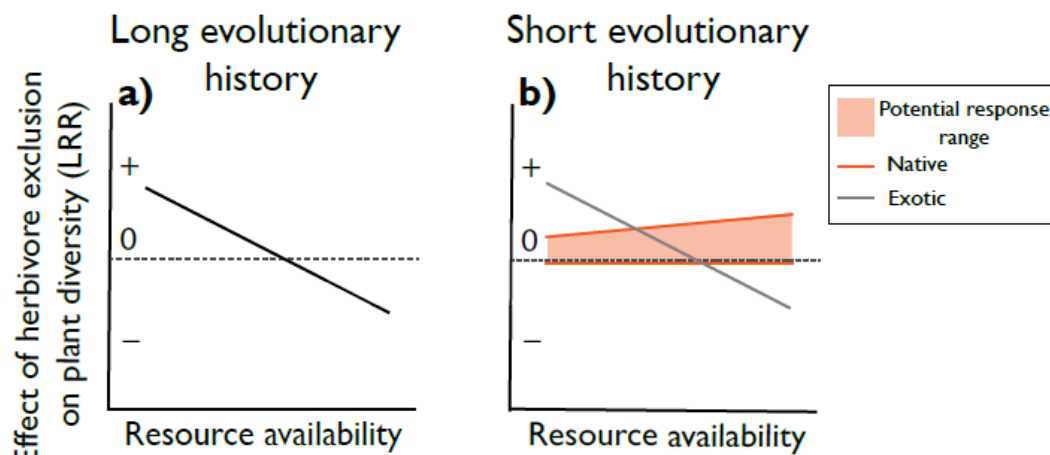
578 J.N.P. and J.S. developed and framed research questions, analyzed the data and wrote the paper.
579 T.O. and P.T. developed and framed research questions and contributed to data analyses. C.S.B, S.P.
580 and E.S.B. developed and framed research questions. E.W.S. and E.T.B. developed and framed
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582 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.,
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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.

Box 1.



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

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.

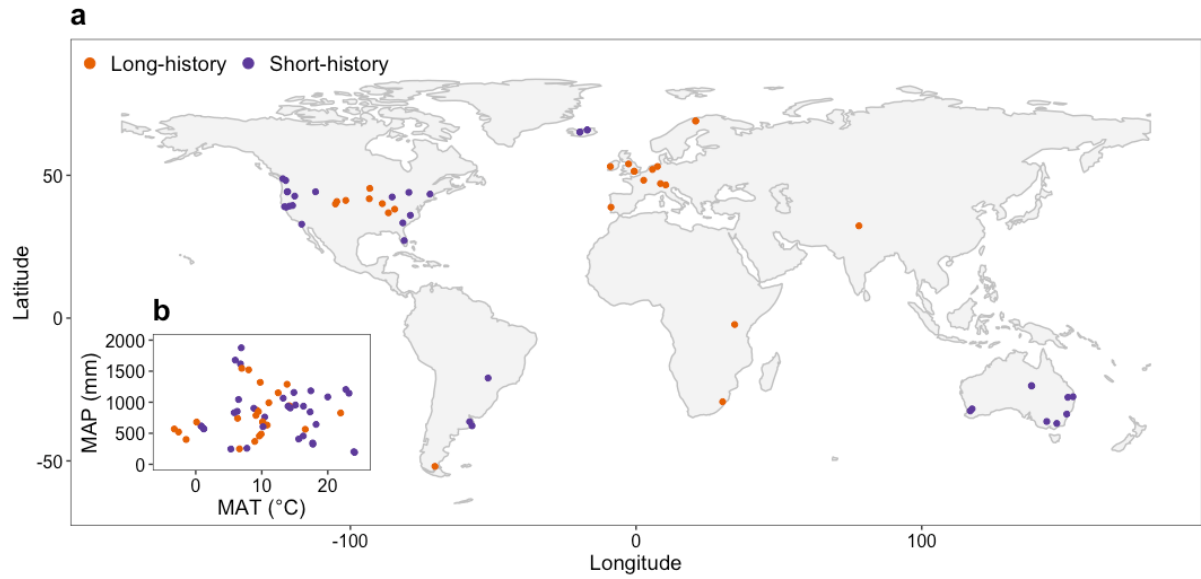


Fig. 1 | Geographic and climatic distribution of experimental sites. Location of the 57 NutNet sites at which the full factorial experiment of herbivore exclusion and nutrient addition was replicated. (a) Sites were classified as subject to a long (large herds of ungulates present >500 to 10,000 years ago; 24 sites) or short (<500 years; 33 sites) evolutionary history of grazing. (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.

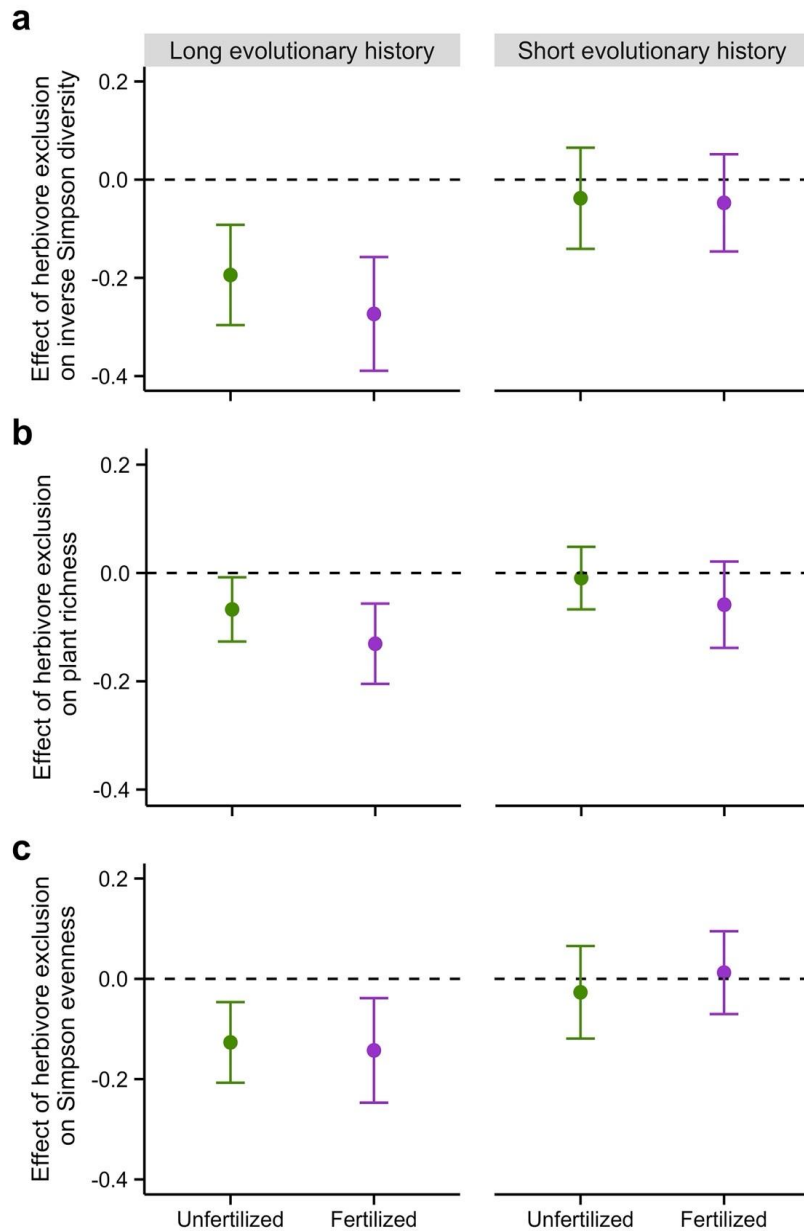


Fig. 2 | Herbivore exclusion effects on plant diversity, richness and evenness. Effect of herbivore exclusion on (a) inverse Simpson's diversity, (b) richness and (c) Simpson's evenness calculated as $LRR = \ln(\text{fenced/unfenced})$ for unfertilized (green) and fertilized (NPK μ) plots (purple) in sites with a long- or short-history of grazing. Points represent the mean effect across all 57 sites with the LRR s 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.

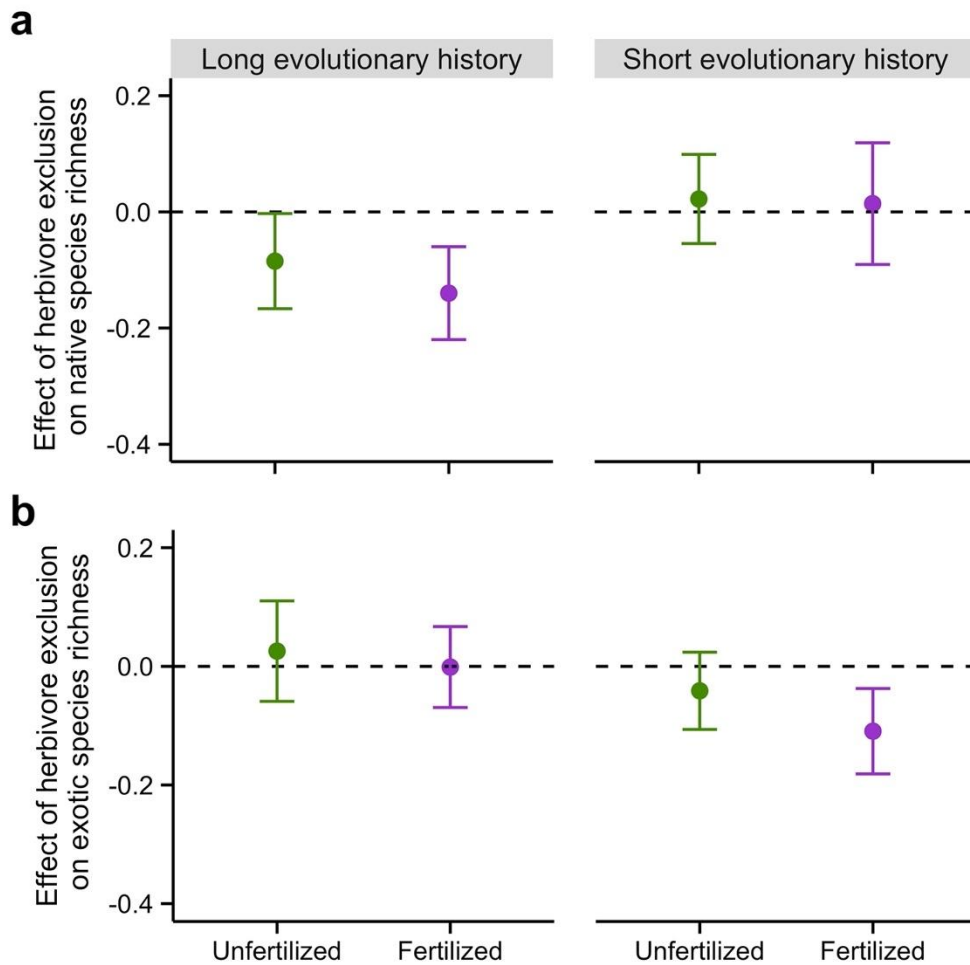


Fig. 3 | Herbivore exclusion effects on native and exotic species richness. Effect of herbivore exclusion on (a) native species richness and (b) exotic species richness calculated as $LRR = \ln(\text{fenced/unfenced})$ for unfertilized (green) and fertilized (NPK μ) plots (purple) in sites with a long- or short-history of grazing. Points represent the mean effect across all 57 sites with the LRR s 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.

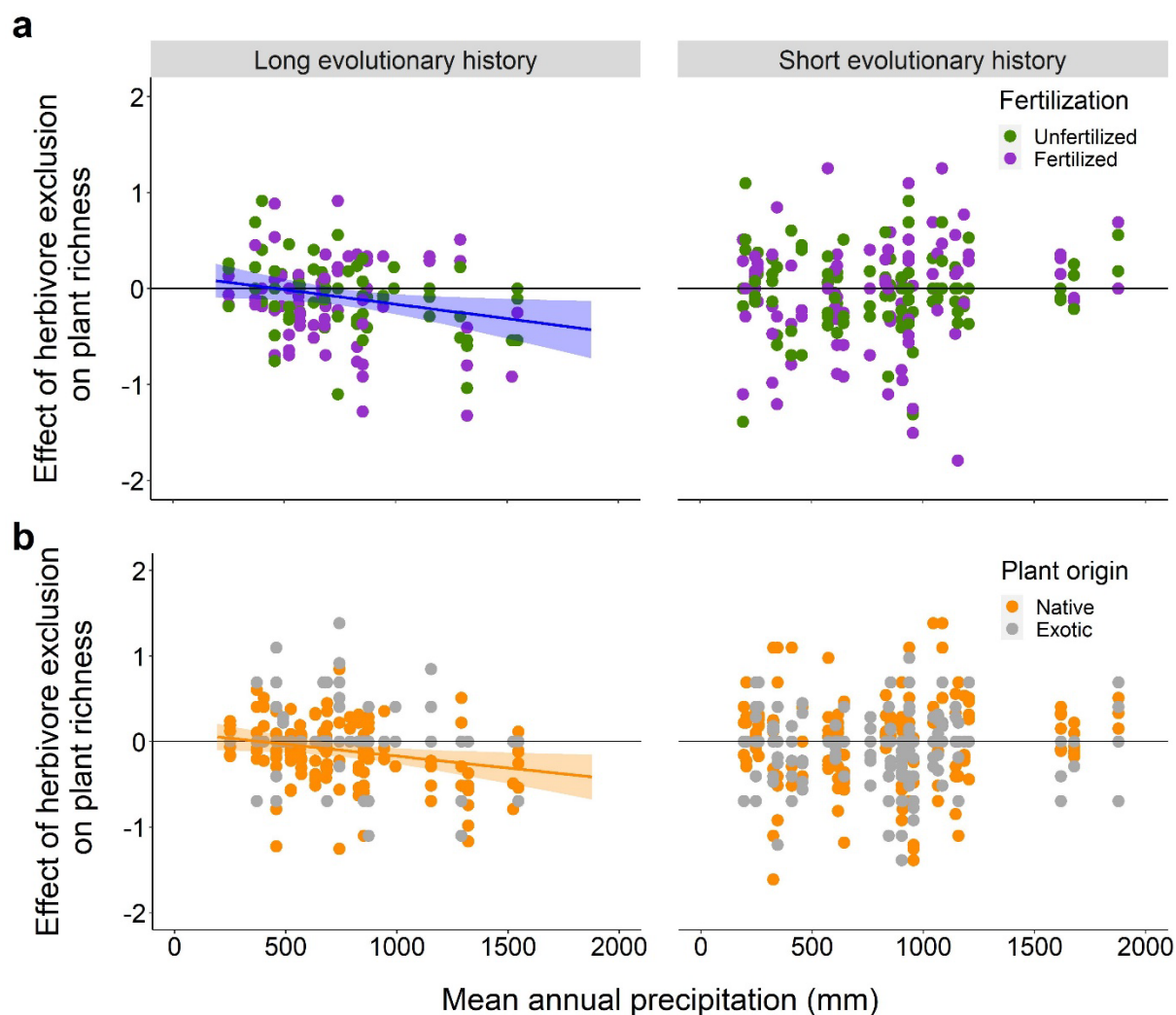


Fig. 4 | Herbivore exclusion effects on plant richness related to rainfall. Relationship between mean annual precipitation and the *LR* of (a) total plant richness and (b) native (orange points) and exotic (grey points) plant richness to herbivore exclusion in sites with a long ($n = 152$ plots from 24 sites) or short ($n = 206$ plots from 33 sites) evolutionary history of grazing. Sites included unfertilized control plots and plots fertilized with NPK μ , 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.

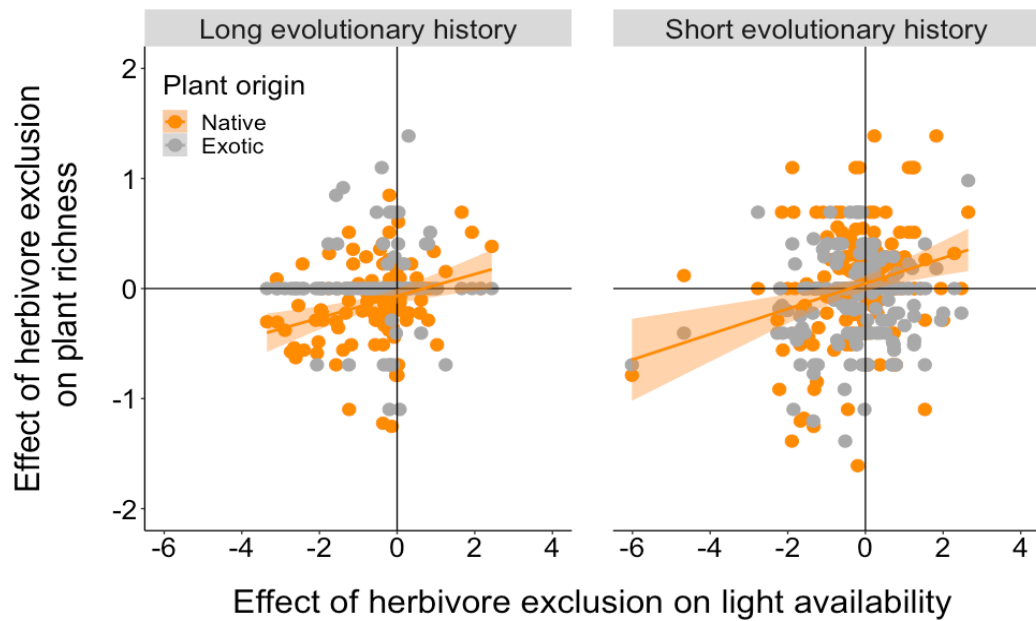


Fig. 5 | Herbivore exclusion effects on plant richness related to changes in light availability.

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 μ . Per plant provenance category, $n = 119$ for long-history sites and $n = 182$ for short-history sites. For native richness, there was a significant relationship in both long- ($P < 0.001$, $R^2 = 0.08$) and short-history sites ($P < 0.001$, $R^2 = 0.06$), so trendlines were produced using `geom_smooth` (*ggplot2* package in R).

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. 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, degrees of freedom, test statistic, and *P*-value for the 2-tailed 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
MAP	-4.49 × 10⁻⁴	2.05 × 10⁻⁴	53	-2.20	0.033
FERT	-0.35	0.19	175	-1.87	0.063
EVO × MAP	5.18 × 10⁻⁴	2.47 × 10⁻⁴	53	2.10	0.041
EVO × FERT	0.25	0.23	175	1.10	0.273
MAP × FERT	3.53 × 10 ⁻⁴	2.25 × 10 ⁻⁴	175	1.57	0.119
EVO × MAP × FERT	-2.48 × 10 ⁻⁴	2.71 × 10 ⁻⁴	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
MAP	-4.34 × 10⁻⁴	1.73 × 10⁻⁴	53	-2.51	0.015
FERT	-0.27	0.16	175	-1.68	0.096
EVO × MAP	4.64 × 10⁻⁴	2.09 × 10⁻⁴	53	2.22	0.031
EVO × FERT	0.09	0.20	175	0.46	0.646
MAP × FERT	2.77 × 10 ⁻⁴	1.98 × 10 ⁻⁴	175	1.40	0.163
EVO × MAP × FERT	-1.14 × 10 ⁻⁴	2.39 × 10 ⁻⁴	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 ⁻⁵	1.63 × 10 ⁻⁴	53	-0.14	0.887
FERT	-0.07	0.18	175	-0.42	0.677
EVO × MAP	6.54 × 10 ⁻⁵	1.96 × 10 ⁻⁴	53	0.33	0.741
EVO × FERT	0.16	0.22	175	0.74	0.463
MAP × FERT	7.54 × 10 ⁻⁵	2.13 × 10 ⁻⁴	175	0.35	0.723
EVO × MAP × FERT	-1.35 × 10 ⁻⁵	2.56 × 10 ⁻⁴	175	-0.53	0.599