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RESEARCH ARTICLE

Realistic species loss has little effect on local resource depletion and competitive pressure in a temperate wet meadow

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Abstract

1. Biodiversity loss has been repeatedly demonstrated to decrease community resistance to colonization, increasing their sensitivity to invasive species. Based on these results, Elton's hypothesis proposed that biodiversity loss reduces the competitive ability of plant communities by emptying ecological niches and, thus, increasing the availability of unused resources. Yet, direct evidence for the effect of diversity loss on resource use and the consequences for the community competitive ability remains scarce, especially under natural conditions.
2. We created a species richness gradient (1–27 species on average) simulating a realistic species loss through the long-term (6 years) removal of rare and subordinate species in a species-rich oligotrophic wet meadow. To assess the effects of species richness on the competitive ability of communities, we transplanted phytometers of two species (the grass *Holcus lanatus* and the forb *Plantago lanceolata*) into plots varying in species richness and into gaps where plant competition was eliminated. We then compared phytometers' performance (relative growth rate) and functional traits responses (vegetative height, leaf area, leaf nitrogen and phosphorus content, specific leaf area and leaf dry matter content). We also tested the effect of targeted species richness on local light interception, soil nutrient content, soil water content, and soil surface temperature and whether they could reliably explain phytometers' performance and trait responses.
3. Both phytometer species showed a much lower relative growth rate in the vegetation than in gaps demonstrating strong responses to plant competition. However, the effects of species richness were surprisingly weak as all plant communities ranging from monocultures of a dominant species to the richest communities (27 species on average) exerted a similar competitive pressure. Surprisingly, almost none of our measures of local resource depletion explained trait or growth rate responses.
4. **Synthesis.** Phytometers effectively quantified competitive pressure exerted by natural plant communities. However, the realistic loss of rare and subordinate

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species had only limited effects on community competitive ability, and these effects were not explained by changes in local resource depletion. This highlights the complexity of diversity–competition relationships and cautions against inferring invasion resistance from species richness alone.

KEYWORDS

community competitive ability, competition-free gaps, determinants of plant community diversity and structure, diversity loss, phytometers, relative growth rate response, removal experiment, resource depletion, trait response

1 | INTRODUCTION

Elton's hypothesis stipulates that communities of high diversity should demonstrate a high resistance to colonization due to strong competitive abilities against newcomers (Elton, 1958). Usually, the competitive ability of diverse communities is expected to stem from the complementarity effect of diversity (Cardinale et al., 2007; Dee et al., 2023). Indeed, a high number of complementary species exploiting resources differently is expected to reduce the availability of empty niches for a newcomer individual (Barry et al., 2019; Levine & HilleRisLambers, 2009; Loreau & Hector, 2001). Consequently, diverse communities should pre-empt most local resources, reducing their availability for new individuals (Craine & Dybzinski, 2013; Frankow-Lindberg, 2012; Lanta & Lepš, 2008). The positive effect of diversity on resource use has been demonstrated experimentally, where an increasing diversity of plant functional groups decreased the light penetration and the soil nitrogen content in a biodiversity experiment (Tilman et al., 1997). More generally, both simulations and most of the grassland biodiversity–ecosystem functioning experiments (BEF experiments hereafter) have found strong support for Elton's hypothesis (see Tilman et al., 2014 and references therein).

Based on these results, it has been proposed that local biodiversity losses could reduce the competitive ability of plant communities by emptying ecological niches and reducing the use of local resources (Barry et al., 2019; Hooper et al., 2005; Reich et al., 2012). However, most BEF experiments have been designed to determine the consequences of random gains or losses of species (Cardinale et al., 2012; Guerrero-Ramírez et al., 2017; Jucker et al., 2014). Yet, species losses are rarely random in nature (Dee et al., 2023; Schmid et al., 2022; van der Plas, 2019), and whether the same results hold under realistic species loss remains largely unknown (but see Lyons & Schwartz, 2001; Zavaleta & Hulvey, 2004). Removal experiments simulating the loss of rare and uncommon species (see Lisner et al., 2023; Lyons & Schwartz, 2001; Sasaki et al., 2017; Smith & Knapp, 2003) are often presented as a good alternative to traditional BEF experiments to simulate a realistic species loss scenario (Wardle, 2016). To our best knowledge, no study has reported the effect of a realistic scenario of plant diversity loss on resource acquisition, connecting this relationship to the community competitive ability.

A common way to evaluate the competitive ability of a community is the use of phytometers (Keddy et al., 2002; Violle et al., 2009). Indeed, it is usually assumed that the performance of standardized individuals (ideally same genotypes of same age) transplanted into different conditions is a good marker of resource availability and can be used to evaluate the intensity of plant–plant competitive interactions (Kikvidze & Armas, 2010). Typically, the intensity of competition should negatively affect the relative growth rate (RGR) of plant individuals due to increasing resource limitations (Peltzer et al., 1998; Schwinning & Weiner, 1998). Moreover, plastic responses of phytometers' traits (i.e. trait changes in response to varying conditions) could also be used as markers of resource limitations (Violle et al., 2009). Indeed, different plastic responses are expected depending on the limiting resource (see Table 1). In highly productive grasslands, light is often considered the most limiting resource (DeMalach et al., 2017; Hautier et al., 2009) while, when nutrient availability is limiting, competitive interactions are expected to occur mainly in the below-ground compartment (Rajaniemi et al., 2003).

TABLE 1 Expected plastic responses (↑ increasing, ↓ decreasing, - unknown) of trait values to different types of resource limitations and temperature.

Trait	Plastic response of trait value to			
	Light limitation	Nutrient limitation	Water limitation	Low temperature
Height	↑ [1, 2]	↓ [1, 2]	↓ [10,11]	↓ [12, 13]
LA	↑ [2]	↓ [7, 8]	↓ [11]	↓ [14]
SLA	↑ [1–6]	↓ [7–9]	↓ [10,12]	-
LDMC	↓ [2]	↑ [9]	↑ [9]	↑ [12, 13]
LNC	↑ [2, 6]	↓ [1, 9]	↓ [11]	↑ [13]
LPC	↑ [6]	↓ [7]	-	-

Note: 1: Lipowsky et al. (2015), 2: Siebenkäs et al. (2015), 3: Burns (2004), 4: Poorter et al. (2009), 5: Violle et al. (2009), 6: Poorter et al. (2019), 7: Sardans et al. (2006), 8: Moeneclaey et al. (2024), 9: Pakeman (2013), 10: Jung et al. (2010), 11: Geange et al. (2017), 12: Kemppinen and Niittynen (2022), 13: Albert et al. (2010), 14: Tang et al. (2024).

Abbreviations: LA, leaf area; LDMC, leaf dry matter content; LNC, leaf nitrogen content; LPC, leaf phosphorus content; SLA, specific leaf area.

Another important resource influencing plant functional traits is water (Craine & Dybzinski, 2013). Finally, not only resources but also temperature can cause microenvironmental heterogeneity leading to plastic responses of phytometers' traits (Westerband et al., 2021).

Here, we simulated a realistic species loss through the long-term removal of rare and subordinate species in a species-rich oligotrophic wet meadow (see Lisner et al., 2023). To investigate whether species loss affected the competitive ability of communities, we transplanted phytometers of two species typical for the vegetation (the grass *Holcus lanatus* and the forb *Plantago lanceolata*) into plots varying in species richness and into competition-free gaps. The competitive ability of plant communities was assessed through its effect on the RGR, a key component of the phytometer performance. Additionally, we combined repeated measures of the canopy light interception, soil nutrient contents, soil water content and ground-level temperature with measures of phytometers' traits that are expected to respond to resource depletion to test mechanistic hypotheses. Overall, we propose an integrated approach to test the causal relationships between a realistic species loss and community competitive abilities.

We asked: (1) What is the effect of species loss on the competitive ability of the community? (2) Are these effects directly related to a change in resource use at the community level?

We expected much lower RGRs in phytometers growing in the vegetation compared to those in competition-free gaps. Moreover, according to Elton's hypothesis, we expected a significant negative relationship between RGR of phytometers and targeted species richness of the plant community. The competitive ability of communities would be directly related to resource depletion resulting in trait responses in phytometers.

2 | MATERIALS AND METHODS

2.1 | Design of long-term removal experiment

The study was conducted in an oligotrophic wet meadow in the South Bohemia region, 10 km southeast of České Budějovice, Czech Republic (48.953 N, 14.593 E, 510 m a.s.l.). The mean annual temperature of this locality is 9°C with the mean annual precipitation 725 mm (data averaged across years 1990–2021 available from: <https://www.meteoblue.com>, accessed on 20 November 2024). It is a very species-rich meadow with over 30 species per 1 m² dominated by *Molinia caerulea*, *Danthonia decumbens* and *Betonica officinalis* (alliance *Molinion caeruleae*). This meadow is nutrient-poor with intermittently wet soils, partially drying out especially during the summer period. This meadow is traditionally extensively managed by mowing once a year at the peak of the vegetation season. We did not need any permission for fieldwork.

We used a long-term diversity removal experiment established in 2016 in the study site which simulates a realistic species loss scenario. In addition to control plots corresponding to the intact

vegetation, four species richness levels were created by the manual removal of rare and subordinate species, keeping the 1, 3, 6 and 12 most abundant species. These five treatments were replicated five times in 1×1 m plots organized in a Latin square (Figure 1). Plots have been maintained twice a year (mid-May and the end of August) by manually removing the above-ground biomass of non-targeted species with the efforts to remove also their roots. More details about the experimental design could be found in Lisner et al. (2023).

When the phytometer experiment started in 2021, all the plots had recovered from the disturbance associated with the initial species removal—as demonstrated by the relatively stable biomass production in all plots (see Lisner et al., 2024)—and the maintenance of the species richness levels required the removal of only a few plant individuals representing a small coverage of the vegetation.

2.2 | The phytometer experiment

We used the grass *Holcus lanatus* and the forb *Plantago lanceolata* as phytometers. Both species are perennial hemicryptophytes, resident and typical for the study site. Both species are typical especially for European temperate meadows and mesic pastures. They are generalists to temperature, moisture and nutrients and resistant to intermediate disturbances (www.pladias.cz, accessed on 12 June 2026; Chytrý et al., 2021). Moreover, they are known to be successful in germinating and establishing in natural communities and are thus ideal model species (Dietrich et al., 2013; Janíková et al., 2024; Tammaru et al., 2021; Wilfahrt et al., 2021). Seeds from a commercial supplier (Planta Naturalis, Czech Republic) were sown on 8 March 2021 into peat jiffy pots (4 cm of diameter) and grown in a climatic room at 20°C under a 12-h:12-h day–night cycle. While seeds of *P. lanceolata* started to germinate on 10th March, *H. lanatus* seeds started to germinate on 14th March but in very low amount; thus, we repeated the sowing of *H. lanatus* on 18th March with more success in germinating seeds on 24th March.

On 14 April 2021, we transplanted four individuals of each species into every plot of the long-term removal experiment. *P. lanceolata* seedlings were 5 weeks old while *H. lanatus* seedlings were 3 weeks old. Hence, all transplanted individuals were approximately the same age and reached a similar development stage in each species. Phytometers were planted with their jiffy pots by coring a hole of the appropriate size, one individual of each species in each of the four inner edges per plot (Figure 1). In addition, we transplanted phytometers into 0.3×0.3 m competition-free gaps artificially created next to each row of the experiment (2 gaps per block), where above- and below-ground parts of the vegetation were removed. In each gap, we planted two phytometers per species (thus, we had also four individuals per species for this type of plot per block; Figure 1).

On 8 June 2021 (1 week before the biomass harvest of the long-term removal experiment), we harvested above-ground biomass of planted phytometers (100% individuals survived) and measured their functional traits after 55 days in the field (spring period hereafter). As most phytometers resprouted from their below-ground

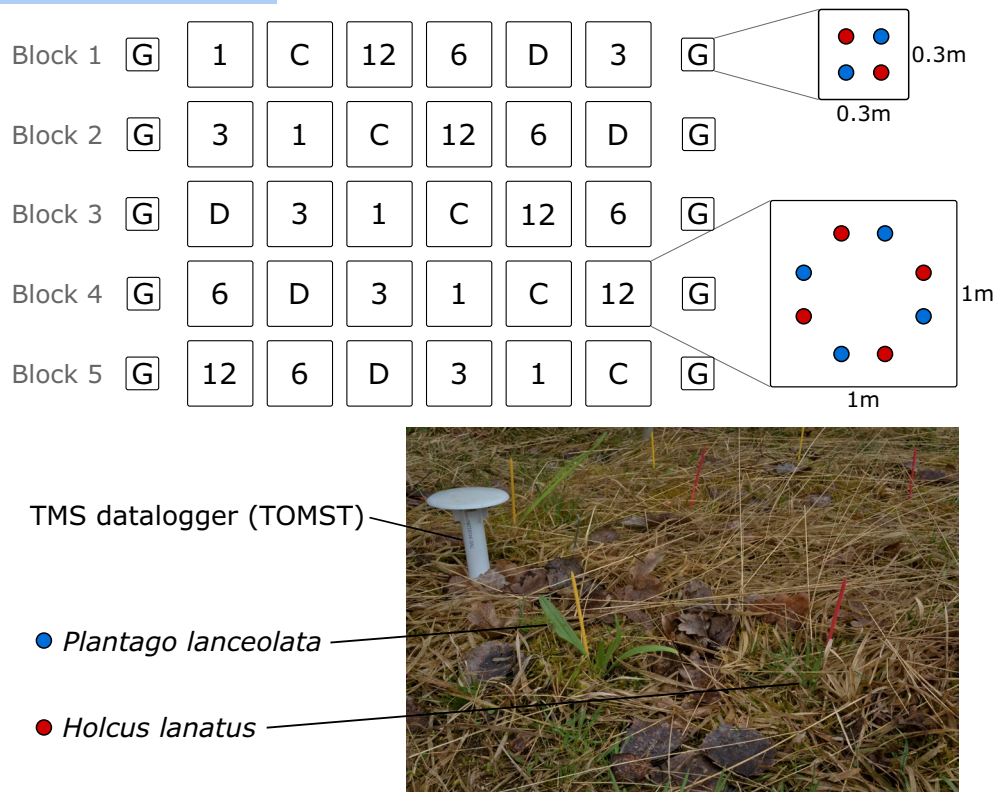


FIGURE 1 Design of the long-term removal experiment with phytometers placement. Species richness plots: 1 (1 species), 3 (3 species), 6 (6 species), 12 (12 species), C (control—27 species on average), D (disturbance plot—not used for phytometer experiment). Competition-free gaps (G). Blue and red dots represent planted phytometers of *Plantago lanceolata* and *Holcus lanatus*, respectively.

parts (92% and 47% individuals of *P. lanceolata* and *H. lanatus*, respectively), we conducted a second harvest on 9 August 2021, after 2 months (62 days) of growth (summer period hereafter). Overall, we specifically followed the species growth patterns during their first year to follow the establishment of new individuals under different species richness contexts.

2.3 | Phytometers' relative growth rates

The relative growth rate of phytometers was calculated as follows:

$$\text{RGR} = \log_{10} \left(\frac{M_t}{M_0} \right) / t$$

where M_0 and M_t are the above-ground initial and final dry mass, respectively, for the spring period ($t=55$) and for the summer period ($t=62$).

To account for the differences in size between individuals at the time of transplantation, we predicted M_0 in spring using non-destructive traits. Calibration curves were produced using a training dataset corresponding to a selection of 30 individuals, representative of all plant sizes at the time of the transplantation. For *H. lanatus*, we used plant height in cm (h), the cumulative length of all leaves in cm (l) and their interaction as predictors of M_0 in mg. A multiple linear regression model based on the selected individuals provided the following estimates:

$$M_0 = 0.08h + 0.08l + 0.05hl$$

For *P. lanceolata*, the measured M_0 was predicted using h , number of leaves (n) and their interaction. Similarly, a multiple linear regression model based on the selected individuals provided the following estimates:

$$M_0 = -0.8h - 4.7n + 2.8hn$$

The predictive models were validated using an independent dataset composed of 15 extra individuals that were not used to estimate the parameters. The high goodness of fit ($r^2=0.94$ and 0.75 for *H. lanatus* and *P. lanceolata*, respectively) confirmed the quality of our models (see Figure S1).

For summer, we used the measured above-ground biomass at the spring harvest as M_0 . Therefore, the value calculated in summer cannot be directly interpreted as a measure of relative growth rate. Nevertheless, we hypothesized that individuals with large above-ground biomass were also larger below-ground, allowing a better resprout after the harvest.

2.4 | Phytometers' traits

During each harvest, we measured six functional traits of phytometers which are expected to respond to light, soil nutrient and water depletion and can be affected by temperature. Vegetative height

was measured as the highest non-stretched leaf. The area of a leaf (LA) was based on scans of two undamaged and fully developed leaves per individual analysed in ImageJ 1.x (Schneider et al., 2012). Specific leaf area (SLA, the ratio between LA and dry mass) and leaf dry matter content (LDMC, the ratio between the dry and water saturated masses) were measured on the same leaves. We also measured the nitrogen and phosphorus contents in leaves (LNC and LPC, respectively). To ensure enough material for chemical analyses, we combined the leaf biomass of the four individuals growing in each plot. Leaf chemical analyses were conducted at the research centre laboratory in Chomutov of the Crop Research Institute, Czech Republic. Total nitrogen [% in dry matter] was determined by dry combustion according to Dumas method (Dumas, 1826), and total phosphorus [% in dry matter] was determined after mineralizing using Mehlich III extractant solution (Mehlich, 1984).

2.5 | Estimates of resource depletion and temperature

We determined soil nutrient content by collecting a soil core of 1.5 cm in diameter and 20 cm deep in each species richness plot at the time of the biomass harvest (i.e. in mid-June 2021). Soil samples were air-dried, sieved through a 2-mm mesh and milled. Samples were then analysed at the research centre laboratory in Chomutov of the Crop Research Institute, Czech Republic, to measure the soil nitrogen and phosphorus contents. The Mehlich III extractant solution (Mehlich, 1984) was used to measure the dissolved soil phosphorus content, while the total soil nitrogen content was determined according to Kjeldahl method (Kjeldahl, 1883).

The light interception by the canopy was calculated from data measured by the SS1 SunScan Canopy Analysis System device (Delta-T Devices Ltd). The device compares the incident to the transmitted photosynthetic active radiation (PAR) at the ground level using 64 sensors embedded in a 1-m long probe. We conducted two orthogonal measurements per each species richness plot and repeated measurements four times in each plot in spring (21 April, 11 May, 26 May and 9 June 2021) and summer (16 June, 29 June, 22 July and 9 August 2021). The light intercepted by the canopy was calculated as follows:

$$1 - \left(\frac{\text{transmitted PAR}}{\text{incident PAR}} \right)$$

The values for each period were averaged for subsequent analyses.

Soil water content and ground-level temperature were measured using TMS dataloggers (TOMST) equipped with temperature and soil moisture sensors. These dataloggers collected data every 15 min; thus, we averaged these data for each species richness plot. The temperature that we used was measured in °C at the level of the soil surface. Although the temperature is not a real resource that can be depleted but rather an environmental variable, we were using it together with the estimates of resource depletion hereafter. Water

content received from dataloggers is not the volumetric water content but signal values which should be calibrated according to the appropriate soil type. For our use, we did not calibrate the data and used only the signal values from the dataloggers because we looked at the relative differences and not at the absolute values.

2.6 | Data analyses

All statistical analyses were conducted on R version 4.3.3 (R Core Team, 2024).

For each species, we calculated a response of RGR and traits to different levels of plant species richness as follows:

$$X_{\text{response}} = \frac{(X_{\text{plot}} - X_{\text{gap}})}{X_{\text{gap}}}$$

where X_{plot} is the mean value of the four individuals collected in each plot varying in species richness (1, 3, 6, 12 and control plots) and X_{gap} is the mean value of the four individuals growing in the competition-free gap in the same block. A response of 0 means no change compared to gaps while a negative, respectively positive, response means a decreased, respectively increased value compared to gap. To test whether RGR and trait responses differed from 0, we used linear mixed-effects models (LMM) in the R package 'lmerTest' (Kuznetsova et al., 2017) with a *t*-test on the intercept (Table S1) and considering the block as a random factor to account for the variation caused by possible heterogeneity in the experimental plots.

We run LMM in the R package 'lme4' (Bates et al., 2015) with type II Wald chi-square test of analysis of variance (ANOVA) from the R package 'car' (Fox & Weisberg, 2019) to test firstly the effect of targeted species richness on RGR and functional traits responses of phytometers and secondly the effect of targeted species richness on measures of resource depletion. We used log-transformed targeted species richness as the expression of diversity, that is, 1, 3, 6 and 12 species for the corresponding removal treatments and 27 species for control plots (i.e. the mean species richness observed in control plots during the monitoring of the vegetation in June 2021). Targeted species richness was treated as a continuous variable. The block identity was considered as a random factor. The analyses were run separately for RGR and each trait response, each phytometer species and each season, as well as for each measure of resource depletion. For data visualization (Figures 2 and 3), we used the R package 'ggplot2' (Wickham, 2016).

To test the direct and indirect effects of targeted species richness on phytometers' responses through the measures of resource depletion, we constructed structural equation models (SEM) using the R package 'piecewiseSEM' (Lefcheck, 2016) for each phytometers' response significantly responding to targeted species richness in the previous LMM analysis. For each species and season, we established a common a priori model where the targeted species richness is hypothesized to affect measures of resource depletion, which in turns, affects a given phytometers' response (Figure S2). These models were constructed based on the results from previous studies (Table 1). Each phytometers' response was analysed

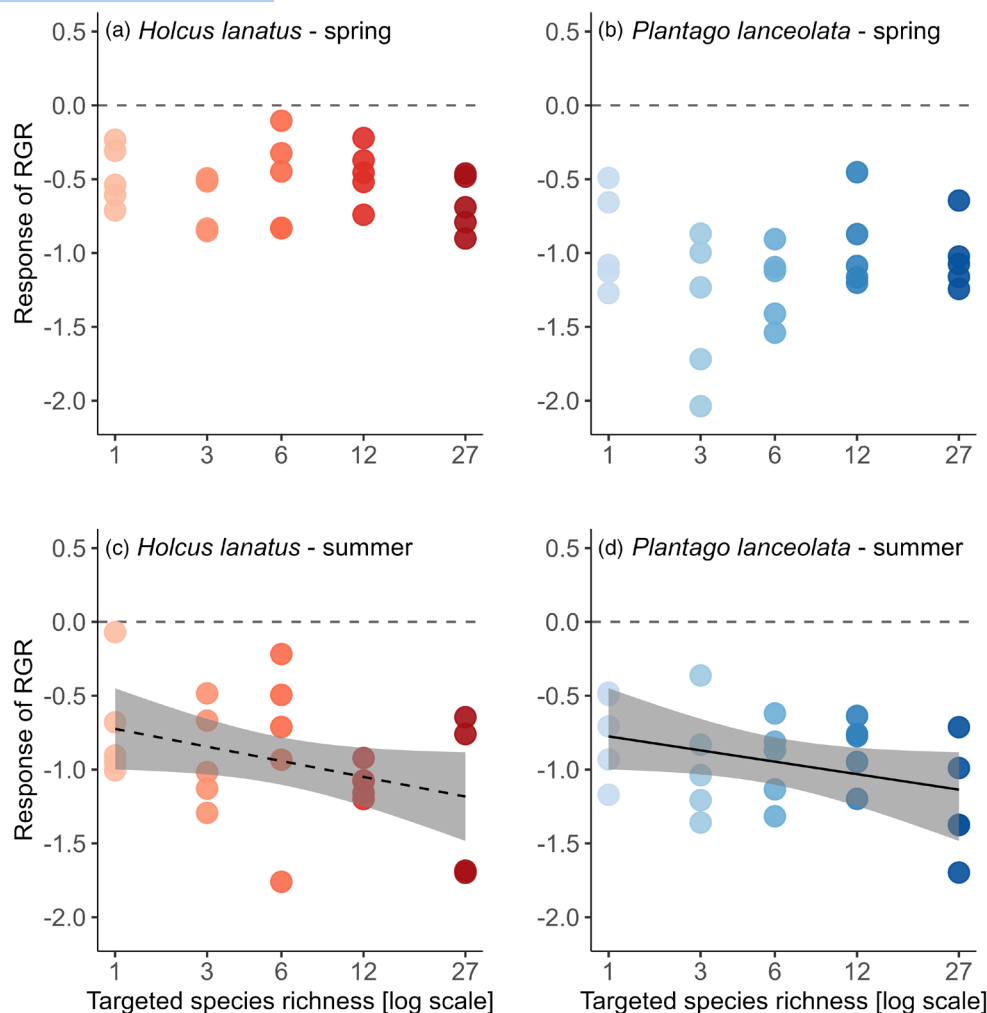


FIGURE 2 The effect of targeted species richness on the response of relative growth rate (RGR) of two phytometer species: *Holcus lanatus* (a, c) and *Plantago lanceolata* (b, d) in spring and summer, respectively. The horizontal grey-dashed line represents a response value of 0 indicating no change compared to gaps while values below or above this line indicate decreased or increased values compared to gaps, respectively. Each point represents the mean value of four individuals growing in each plot ($n=5$ plots for each diversity level). The black solid and dashed regression lines represent the significant and marginally significant relationships, respectively, with shaded areas for confidence intervals.

separately. Individual structural equations (i.e. links) were fitted by a LMM with the block identity as a random factor. The ability of the SEM to represent the data well was tested by Fisher's C statistics.

3 | RESULTS

For both species and in both seasons (spring and summer), RGR values were significantly lower in vegetation than in competition-free gaps (negative RGR responses, Figure 2; Table S1). Nevertheless, we did not find the expected negative relationship between targeted species richness and the RGR response for any of the species in spring (Figure 2a,b; Table 2). However, the latter relationship was significant for *P. lanceolata* (Figure 2d; Table 2) and marginally significant for *H. lanatus* (Figure 2c; Table 2) in summer. Contrary to *P. lanceolata* (only 8% of the individuals died across all treatments), a large proportion of individuals did not resprout in *H. lanatus* (53% of

the individuals died across all treatments). Resprouting of both species in gaps was 100%. Consequently, RGR values were calculated based on surviving individuals only, which can result in an underestimation of the effect of the vegetation on their growth.

Almost all traits responded significantly to plant-plant competition and the direction of responses tended to be consistent in both species and across seasons (Figure 3; Table S1). In *H. lanatus*, all trait responses were significantly different from 0 and individuals growing in the vegetation had lower LA and LNC, and higher SLA and LPC than in gaps (Figure 3a,c; Table S1). Plant height and LDMC had different responses depending on the season: height was higher in the vegetation in spring and lower in summer while the opposite was found for LDMC (Figure 3a,c; Table S1). Similar trends were found in *P. lanceolata*: individuals growing in the vegetation had lower LA and LNC and higher LPC than in gaps for both seasons (Figure 3b,d; Table S1). However, SLA was higher only in spring, and height was lower only in summer. LDMC showed no significant response in this species (Figure 3b,d; Table S1).

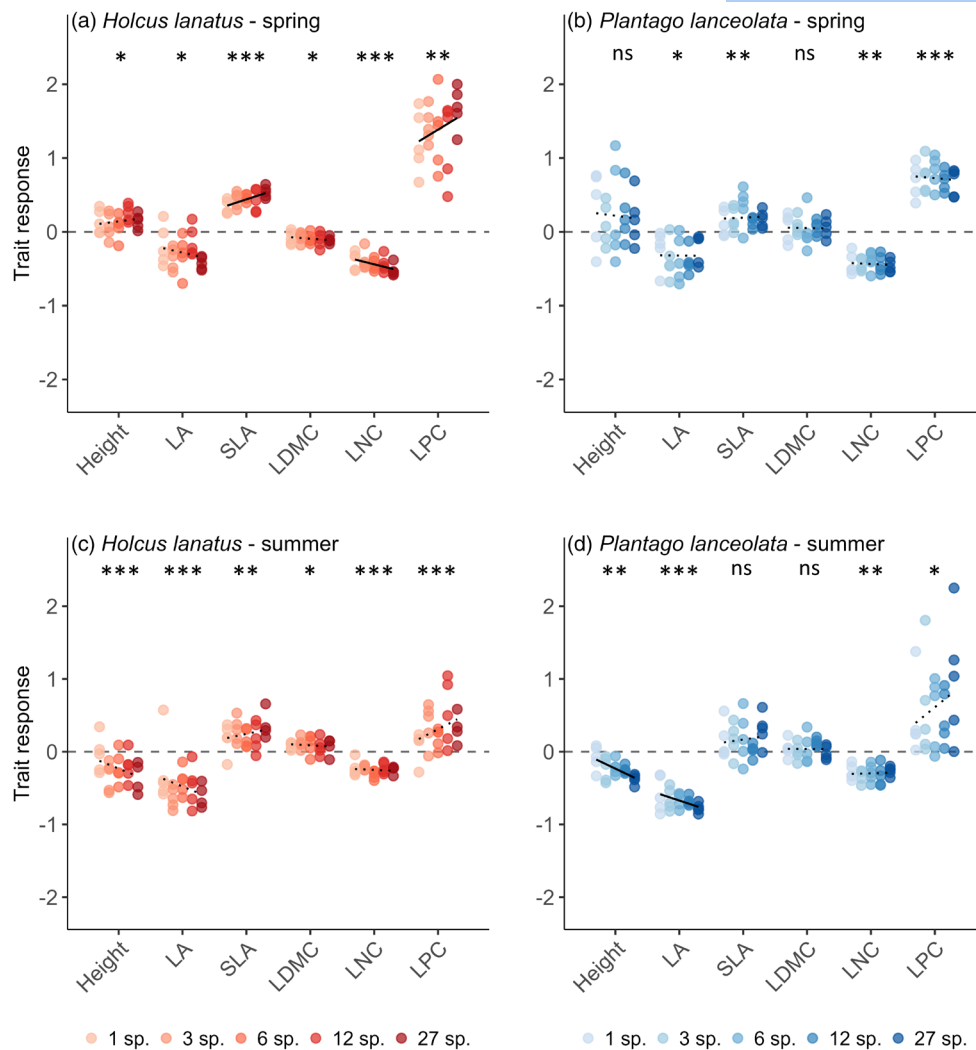


FIGURE 3 Trait responses of *Holcus lanatus* (a, c) and *Plantago lanceolata* (b, d) in spring and summer, respectively, and the effect of targeted species richness on these traits. The horizontal grey-dashed line represents a response value of 0 indicating no change compared to gaps while values below or above this line indicate decreased or increased values compared to gaps, respectively. Symbols above each trait values (ns (non-significant) $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) refer to significance levels of each trait differentiating from 0 (see Table S1 for statistical details). Each point represents the mean value of four individuals growing in a plot ($n = 5$ plots for each species richness level). Black solid and dotted regression lines represent the significant and non-significant relationships to targeted species richness, respectively. LA (leaf area), SLA (specific leaf area), LDMC (leaf dry matter content), LNC (leaf nitrogen content) and LPC (leaf phosphorus content).

Surprisingly, only a few trait responses were affected by the targeted species richness of the plots (Figure 3; Table 2). The targeted species richness had no effect on height, LA and LDMC responses but affected positively SLA and LPC, and negatively LNC in *H. lanatus* in spring (Figure 3a; Table 2). No effect of targeted species richness was found for all trait responses in *P. lanceolata* in spring (Figure 3b; Table 2). We found no effect of targeted species richness on any of the trait responses in *H. lanatus* in summer (Figure 3c; Table 2). Targeted species richness had a negative effect on height and LA in *P. lanceolata* in summer (Figure 3d; Table 2).

Regarding community estimates of resource depletion, only the soil surface temperature in spring and mean soil water content in summer were significantly affected by targeted species richness (Table 3). Targeted species richness had only a weak negative effect on soil N content (marginally significant) which was measured only once, at the

peak of the vegetation (Table 3). All other measures of resource depletion (light interception and soil P) were not affected by targeted species richness in either season (Table 3). SEMs showed that temperature was negatively directly linked with the plot targeted species richness in spring (Figure S2a–c) while the mean water content was negatively directly linked with targeted species richness in summer (Figure S2d–f). SEMs showed that all significant targeted species richness effects on phytometers' responses were direct as the indirect path through resource depletion was never significant (Figure S2).

4 | DISCUSSION

By formally putting together well-established methods that are usually conducted separately, that is, comparing the performance and

TABLE 2 Effect of targeted species richness on relative growth rate (RGR) and functional traits of phytometers *Holcus lanatus* and *Plantago lanceolata* in the spring and summer periods.

	Trait	Spring		Summer	
		χ^2	<i>p</i>	χ^2	<i>p</i>
<i>Holcus lanatus</i>	RGR	0.71	0.398	3.55	0.059
	Height	0.68	0.408	2.50	0.114
	LA	1.13	0.287	1.46	0.227
	SLA	12.52	<0.001	1.08	0.299
	LDMC	2.73	0.098	0.29	0.593
	LNC	9.45	0.002	0.35	0.555
	LPC	4.42	0.036	2.32	0.128
<i>Plantago lanceolata</i>	RGR	0.13	0.714	8.30	0.004
	Height	0.22	0.636	26.11	<0.001
	LA	0.01	0.939	9.46	0.002
	SLA	0.05	0.819	0.55	0.459
	LDMC	0.01	0.904	0.00	0.957
	LNC	0.46	0.498	0.11	0.742
	LPC	0.18	0.673	2.55	0.110

Note: Wald χ^2 test with *df* = 1. Statistically significant results are in bold. Abbreviations: LA, leaf area; LDMC, leaf dry matter content; LNC, leaf nitrogen content; LPC, leaf phosphorus content; SLA, specific leaf area.

TABLE 3 Effect of targeted species richness on resource depletion in the spring and summer periods.

		Water content	Light interception	Temperature
Spring	Estimates	-0.0007	0.0051	-0.6428
	χ^2	1.38	0.51	5.96
	<i>p</i>	0.24	0.474	0.015
Summer	Estimates	-0.0007	-1.2925	-0.4419
	χ^2	5.35	0.6	0.82
	<i>p</i>	0.021	0.437	0.366
		Soil N	Soil P	
	Estimates	-2.5997	-0.0259	
	χ^2	3.29	0.48	
	<i>p</i>	0.07	0.486	

Note: There was only one measurement of soil nutrients (N and P) which is thus common for both seasons. Wald χ^2 test with *df* = 1. Statistically significant results are in bold. Soil N (nitrogen) and P (phosphorus).

traits of phytometers growing in competition-free gaps and in communities varying in species composition and assessing local resource depletion—our study brings valuable insights in our understanding of the relationship between plant diversity and plant competition in a realistic field experiment. Importantly, our integrated approach included measures of local resource depletion, allowing us to test the mechanisms by which natural oligotrophic wet meadows exert competitive pressure on establishing individuals.

As expected, and consistently for both species and seasons, phytometers had a significantly lower RGR when growing in the vegetation than in competition-free gaps, consistent with the idea that competition negatively affects species performance (Aschehoug et al., 2016; Pillay & Ward, 2014; Švamberková & Lepš, 2020) probably due to the reduction of resource availability (Lehsten & Kleyer, 2007; Poorter et al., 2009). Moreover, individuals growing under competition are also expected to change their traits to cope with limiting resources (Violle et al., 2009). Here, phytometers had systematically lower LA and LNC and higher SLA (although not significant in summer for *P. lanceolata*) in vegetation than in competition-free gaps. These trait changes corresponded to expected plastic responses to low nitrogen and water availability or light limitation (see Table 1). Nevertheless, we did not directly compare resources between gaps and the vegetation. Our results from SEMs do not support the hypothesis that this pattern was driven by resource depletion as almost none of the tested phytometers' responses (reflecting gaps as control conditions to estimate phytometers' plastic responses) were directly linked with our measures of resources. Phytometers tended to be taller under competition than in gaps in spring but showed an opposite response in summer. In spring, despite smaller leaves, this higher vegetative height was achieved through an upward orientation of the leaves in response to competition for light (Coomes & Allen, 2007). However, this strategy appeared insufficient in summer when the fast growth of a dense canopy after mowing (see Segrestin et al., 2025) had a stronger effect on the overall plant growth. According to Doležal et al. (2019), biomass in mown plots of the same meadow as in our experiment reached ca. 90% of its June value by August. Nevertheless, all plots were mown at the same time irrespective of the species richness level. Hence, it should not interfere with the experiment species richness gradient.

Contrary to our expectations, LPC was significantly and systematically higher in the vegetation than in gaps for both species and seasons. One possible explanation could be a decrease in soil phosphorus content following the creation of artificial gaps and resulting in a lower availability for the phytometers in competition-free gaps (Lipowsky et al., 2015; Pakeman, 2013; Sardans et al., 2006). Nevertheless, this decrease is less likely as phosphorus is known to be among the most stable elements in the soil (especially when compared to nitrogen, De Sisto et al., 2023; Holford, 1997) and the formation of gaps is usually expected to rather increase nutrient contents through the enhanced decomposition of dead material (Janíková & Lepš, 2023). A more probable explanation could be facilitation effects of other plants on phosphorus availability in the vegetation. Indeed, phytometers could have benefited from the effect of other plant activities changing the chemical properties of the soil (Barry et al., 2019; Michalet & Pugnaire, 2016; Wright et al., 2017). Specifically, plant associations with endophytic fungi (e.g. arbuscular mycorrhizal fungi) are known as one of the main pathways for phosphorus acquisition in grassland communities (Almarío et al., 2017; Richardson et al., 2009). Thus, it can be hypothesized that a larger abundance of plant roots would result in a higher microbial

mobilization which, in turn, can increase the availability of phosphorus for plants (Johri et al., 2015).

Surprisingly, we found only a few significant effects of species removal on the phytometers' RGR and trait responses. In spring, all levels of species richness—from monocultures of the dominant species (i.e. *M. caerulea*) to the richest communities (27 species on average)—triggered similar growth responses. These results challenge common assumptions regarding the importance of diversity for the competitive ability of the vegetation and suggest that a single dominant species can form a strong competitive environment constraining the colonization by new individuals (Cadotte, 2017; Levine et al., 2004; Smith et al., 2004). Our previous study, conducted on the same experiment, showed that *M. caerulea* was able to consistently produce, alone, as much above-ground biomass as the local natural and species-rich community (Lisner et al., 2023), explaining its ability to exert a strong competitive pressure on the phytometers. Another phytometer experiment from the same locality has shown no effect of the *M. caerulea* removal on phytometers despite the fact that removal increased the species richness (Kelemen et al., 2015). It is worth noting that, despite having similar RGR in all diversity levels, *H. lanatus* showed increasing SLA and decreasing LNC with targeted species richness in our experiment. These results suggest that individuals of the latter species experienced a small increase in competitive pressure with increasing species richness, which they overcame through adaptive plastic responses. These relationships between targeted species richness, SLA and LNC are consistent with other studies, which showed similar trends using artificial experiments (Bachmann et al., 2018; Daßler et al., 2008; De Giorgi et al., 2025; Felix et al., 2023; Kostenko et al., 2017; Roscher et al., 2018). However, studies simulating realistic species loss in natural communities are relatively rare. Among the few comparable studies, Lyons and Schwartz (2001) manipulated diversity by removing less abundant species, like in our experiment, and introduced seeds of an exotic grass *Lolium temulentum*. Although they did not compare the species performance in plots or competition-free gaps, they showed a negative effect of diversity on the establishment of the exotic species. Although diversity experiment with randomly assessed communities, Galland et al. (2019) varying functional and phylogenetic diversity, keeping the species richness constant, found weak if any effect of functional diversity increasing the colonization resistance. Overall, we conclude that the consequences of species loss for the competitive ability of plant communities might have been overestimated in previous works, and more studies are needed to evaluate the generalization of our findings for natural communities and explain these contrasting results.

In summer, the common trend in both species towards a lower RGR with an increasing targeted species richness suggested that the small effects detected in spring might accumulate during seasons and result in significant trends in the long term. This was particularly striking in *P. lanceolata* which showed a lower growth rate, height and LA in species-rich communities compared to monocultures. Whether these patterns emerged from stronger competitive interactions in summer or from cumulative effects across both seasons

remains to be tested and requires careful interpretation. Moreover, the mowing of phytometers might have affected their responses to competition calling for more precise measurement in the future. For example, replacing phytometers with new seedlings in summer would allow us to isolate the pure effect of competition in summer. Nevertheless, our previous study, conducted on the same experiment, revealed that species removal delayed the onset of vegetation growth in early spring (both above- and below-ground), while most community properties remained unaffected at the peak of vegetation (Segrestin et al., 2025). Therefore, species loss might benefit phytometers through the release of a temporal niche in the early season while smaller effects are expected in summer (Levine et al., 2024). Overall, we speculate that species loss resulted in a small decrease in the competitive ability of the natural community in the early season, but its effect on phytometers was only apparent after a few months of exposure, especially after the annual cut of the vegetation. Measuring phytometers' response during only one vegetation season (1 year) may appear to be a limitation, as the survival and reproduction of phytometers can be affected by competitive pressure differently in subsequent years compared with the first year. Nevertheless, our approach requires standardized young individuals to be transplanted into the plots and the measurement of initial dry mass for estimation of RGR. Therefore, phytometers should not be regarded as a simulation of the competition experienced by native plants within the community, but rather as a tool for estimating resource availability for plant growth.

Our study revealed the difficulty to connect local measures of resources (light interception, soil nutrient contents and soil water content) or microclimatic conditions (ground-level temperature) to plant performance in the field. In fact, most of our measures did not respond to the removal of species and the only few weak relationships (negative effects of targeted species richness on ground-level temperature in spring and on soil water availability in summer) failed to explain the differences in phytometers' performance and trait responses along the species richness gradient. This is directly in contrast with the common assumption that the diversity-competitive ability relationship should be primarily driven by resource depletion (Davis et al., 2000; Duarte et al., 2021; Hooper & Vitousek, 1998). This result could be due to inaccurate methodological choices or an inappropriate selection of resource measures. From soil nutrients, we measured soil concentrations in N and P—as commonly done in other BEF experiments (Dybzinski et al., 2008; Furey & Tilman, 2021)—which might deviate significantly from their availability for plants. A more detailed evaluation of the nutrient forms (e.g. organic and inorganic) might provide a better understanding of their availability (Andrews et al., 2013; Holford, 1997). Moreover, it can be assumed that, at a given level of nutrient concentration, variations in plant-microbial interactions would significantly affect their availability (Pantigoso et al., 2022). It is also possible that micronutrients which we did not measure can play an important role in species performance (Fay et al., 2015). Because our experiment was conducted on an oligotrophic wet meadow, our results might be limited to this vegetation type. In habitats where the limiting resources are easier

to identify, such as soil moisture in arid or semi-arid grasslands, stronger patterns might be revealed.

5 | CONCLUSION

Our study shows that realistic species loss does not necessarily weaken the competitive ability of natural plant communities, challenging the generality of biodiversity–competition links inferred from random assembly experiments. In species-rich oligotrophic wet meadows, community dominance, seasonal dynamics and biotic interactions appear to outweigh species richness per se in regulating competitive pressure on establishing individuals. Overall, we demonstrated here the great value of phytometer experiments which allowed us to detect small effects of targeted species richness on the competitive ability of the plant community that would not have been shown through standard measures of resource availability. These findings suggest that biodiversity loss may have subtler and more context-dependent consequences for invasion resistance than commonly assumed, emphasizing the need to reassess mechanistic expectations of Elton's hypothesis under realistic, long-term scenarios of species loss.

AUTHOR CONTRIBUTIONS

Aleš Lisner and Jan Lepš conceived the long-term experiment; Jules Segrestin conceived the ideas for the study; Jan Lepš, Jules Segrestin, Aleš Lisner, Marie Konečná and Eva Janíková designed methodology; Eva Janíková, Aleš Lisner, Marie Konečná, Markéta Applová and Jules Segrestin conducted the experiment, collected data and processed the lab data of the experiment; Eva Janíková analysed the data with the help of Jules Segrestin, Jan Lepš and Aleš Lisner; Eva Janíková led the writing of the manuscript, and all authors contributed substantially to revisions and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.0zpc867cd> (Janíková et al., 2026).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Predicted versus observed models of relative growth rate of phytometers.

Figure S1. Predicted against observed above-ground dry mass (DM) of 45 individuals at the time of the first transplantation for both species.

Appendix S2. Differences in phytometers' responses in vegetation with different diversity levels from competition-free gaps.

Table S1. The results of t-tests testing the difference of relative growth rate (RGR) and trait responses of phytometers *Holcus lanatus* and *Plantago lanceolata* in spring and summer period from 0 (i.e. from the intercept) indicating the differences from competition-free gaps.

Appendix S3. The structural equation models representing direct and indirect effects of targeted species richness on phytometers' trait and relative growth rate (RGR) responses.

Figure S2. The structural equation models representing direct and indirect effects of targeted species richness on selected phytometers' trait (SLA (specific leaf area), LNC (leaf nitrogen content), LPC (leaf phosphorus content), LA (leaf area)) and relative growth rate (RGR) responses and on estimates of resource depletion of community (i.e. soil nitrogen (soil N), soil phosphorus (soil P), light interception, water content, temperature) of *Holcus lanatus* in spring (a–c) and *Plantago lanceolata* in summer (d–f).

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