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Size-selective exclusion of mammals and invertebrates differently affects grassland plant communities depending on vegetation type

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Abstract

1. Human-caused loss of vertebrate and invertebrate animals, defaunation, is increasing, and potentially affects plant community structure of diverse grassland ecosystems worldwide.
2. We experimentally simulated defaunation using size-selective fences to progressively exclude large-, medium- and small-sized mammals, and invertebrates from two subalpine vegetation types in the Swiss National Park (SNP): intensively grazed short-grass and moderately grazed tall-grass vegetation. We assessed plant community properties yearly from 2009 to 2013, and examined treatment effects on plant community structure in the two grassland types.
3. In the short-grass vegetation, the exclusion of large mammals increased total plant biomass, while the exclusion of large and medium-sized mammals increased total, grass and forb biomass compared to when all animals had access. These increases became stronger when also invertebrates were excluded. The exclusion of all mammals and invertebrates increased biomass of grasses by 205%, forbs by 100% and total plant biomass by 118% compared to when all animals had access, hence enhancing relative biomass of grasses from 43.6% to 60%, changing plant species composition and lowering richness of forbs by 16%, the number of plant families by 13% and family-level Shannon diversity by 23%. In contrast to these significant community-level responses found in the short-grass vegetation, there was no evidence that the size-selective exclusion of animals altered the plant community structure of the tall-grass vegetation. The contrasting results were due to the difference in plant community composition prior to our experiment, which were related to differences in quantity and quality of forage and in grazing intensities of herbivores between the two grassland types.
4. Synthesis. Our results showed that different-sized animals, in particular large mammals and invertebrates, contributed to maintain the plant community structure in the short-grass vegetation, highlighting the importance of multiple, functionally different animal groups for ecosystem functioning and stability. In contrast to the short-grass vegetation, we could not detect such a top-down control by animals in the tall-grass vegetation. Our results suggest that potential defaunation effects on grassland plant

community structure depend on the degree of grazing pressure release and grassland vegetation type.

KEYWORDS

animal biodiversity loss, bottom-up control, defaunation, grazing effects, herbivore exclusion, herbivores of different sizes, plant diversity, plant-herbivore interactions, insects, top-down effects

1 INTRODUCTION

Grasslands cover 52.5 million km² or 40.5% of the earth's ice-free land and provide important ecosystem functions and services (White, Murray, & Rohweder 2000). They support large populations of vertebrates and invertebrates (Suttie, Reynolds, & Batello 2005) and store up to 30 percent of the terrestrial soil carbon (Frank, Timothy, Mclauchlan, & Risch 2011; White et al., 2000). However, grasslands worldwide are being threatened by a loss of animal biodiversity, which is mainly caused by land-use and climate change (Sala et al., 2000), habitat fragmentation (Collins, Knapp, Briggs, Blair, & Steinauer 1998; Bakker, Ritchie, Olff, Milchunas, & Knops 2006), eutrophication (Borer et al., 2014; Hautier et al., 2014) and pesticide application (Sánchez-Bayo & Wyckhuys, 2019). This loss of animal diversity, defaunation, is size-selective (Dirzo et al., 2014; Smith, Elliott Smith, Lyons, & Payne 2018), with large-bodied animals being at greater risk of extinction compared to smaller-sized ones. Current animal losses involve not only vertebrates (Estes et al., 2011; Svenning, Munk, & Schweiger 2019), but also invertebrates (Dirzo et al., 2014; Risch et al., 2018; Eisenhauer, Bonn, & Guerra 2019; Sánchez-Bayo & Wyckhuys, 2019). The rate of these animal losses is increasing, therefore, it is urgent to assess how and to what extent grassland plant biomass, plant diversity and community composition (hereafter plant community structure) are affected by this continuous body-size downgrading of animal communities (Smith et al., 2018).

Large mammalian herbivores are often thought to be keystone species (Mills, Soulé, & Doak 1993) that can control ecosystem structure and stability via top-down effects, for example, bison in North American tallgrass prairie (Collins et al., 1998; Knapp et al., 1999) or red deer in meadows in the Alps (Catorci et al., 2016). When large herbivores

are excluded or their numbers are reduced, vegetation height and biomass generally increase (Turkington, 2009) and plant community structure changes (Collins et al., 1998; Knapp et al., 1999; Erschbamer, Moser, & Vorhauser 2004; Koerner et al., 2018) as a direct result of decreased grazing pressure (Hobbs, 1996), or indirectly due to a reduction in trampling (Kohler, Gillet, Gobat, & Buttler 2004) or dung and urine inputs (Schütz et al., 2006). Large herbivore removal also intensifies plant competition for light (Milchunas, Sala & Lauenroth 1988; Fahnestock & Knapp, 1994; Bakker et al., 2006; Borer et al., 2014; Burkepile et al., 2017), leading to the suppression of low- or small-growing species, and ultimately, a decrease in the number of plant species (light competition and density hypotheses; see Rajaniemi, 2002; Endara & Coley, 2011). Similarly to their larger counterparts, also medium- or small-sized mammals (e.g. rabbits, hares, mice, voles) can play key roles in grassland ecosystems (e.g. Brown & Heske, 1990; Olofsson, Moen, & Oksanen 2002; Davidson et al., 2010), for example, via selective plant consumption (see Davidson et al., 2010), seed predation (Brown & Heske, 1990), trampling (Pascual, Alberti, Daleo, & Iribarne 2017), and below-ground burrowing (Davidson et al., 2010). Therefore, also the loss of these smaller mammals can have a large impact on plant communities (Andersson & Jonasson, 1986; Weltzin, Archer, & Heitschmidt 1997; Davidson et al., 2010; also see references in Bakker et al., 2006). Finally, also invertebrates play important ecological roles in grassland ecosystems (Curry, 1994; Ibanez, Bison, Lavorel, & Moretti 2013). Similar to mammals they can accelerate nutrient cycling as they produce considerable amounts of frass and drop large amounts of leave fractions to the soil surface rather than consuming them (Curry, 1994). Invertebrates consume a considerable proportion of plant biomass, also in non-outbreak periods (Blumer & Diemer, 1996; Coupe & Cahill Jr, 2003). In Swiss alpine and subalpine grasslands, for example, invertebrate herbivores in general and grasshoppers in particular, removed up to 30% of the aboveground biomass during a growing season (Blumer & Diemer, 1996; Risch, Haynes, Busse, Filli, & Schütz 2013). Invertebrates may encounter less competition when mammalian herbivores are missing due to a reduction in direct disturbance (see Gish, Dafni, & Inbar 2010) and the degree of dietary overlap (Vandegehuchte et al., 2018). As a consequence, invertebrate herbivores can increase their abundance (resource concentration hypothesis, see Rambo & Faeth, 1999; also see case studies by Davidson et al., 2010; Vandegehuchte et al., 2018) and will likely

consume more plant biomass. Hence, size-selective defaunation, will likely change vertebrate-invertebrate-plant interactions dramatically (Rambo & Faeth, 1999).

Recently, researchers started to disentangle the joint and independent effects of multiple vertebrate herbivores on grassland plant communities (e.g. Bakker et al., 2006; Davidson et al., 2010; Liu et al., 2015; Burkepile et al., 2017; Wang et al., 2019). These studies showed that different sized mammals may have additive or synergistic impacts on the development of grassland plant communities (Ritchie & Olff, 1999; Davidson et al., 2010). Additive effects may occur when different herbivore species selectively consume the same plant species or plant species with similar functional traits (Ritchie & Olff, 1999), while synergistic impacts can be found when the presence of a specific herbivore enhances the density or occurrence of another herbivore, which has a similar preference for specific plant species (Davidson et al., 2010). However, to date, how the vertebrates-invertebrates-plants interactions are altered by defaunation is largely unknown (but see Risch et al., 2018; Leverkus & Crawley, 2020).

To disentangle how the size-selective animal loss alters grassland plant community structure, we simulated defaunation, by excluding aboveground consumers according to their body size, with ungulates excluded first, then medium- and small-sized mammals and finally the entire aboveground dwelling invertebrate community (Risch et al., 2013). This is, to our knowledge, the first such experiment in a real-world ecosystem (Risch et al., 2018). We conducted our exclosure experiment over five consecutive growing seasons in subalpine grasslands in the Swiss National Park (SNP) where differences in historic land-use and grazing by large ungulates (Schütz, Risch, Leuzinger, Krüsi, & Achermann 2003; Schütz et al., 2006) resulted in two contrasting vegetation types: 1) resource-limited tall-grass vegetation (roughly 20 cm in height), where plant biomass consumption by herbivores averages roughly 20% of the primary production (Risch et al., 2013), and 2) resource-rich short-grass vegetation (roughly 5 cm in height), where herbivore plant biomass consumption approximates roughly 60% (Risch et al., 2013), with approximately 30% consumed by invertebrates (Risch et al., 2013). The SNP thus provides an ideal setting to investigate whether effects of defaunation vary with grassland vegetation types that differ in soil resources and grazing intensity.

Based on our experiment, we specifically hypothesised to find (1) a significant top-down

effect of large herbivores on plant community structure, (2) a stronger effect when more groups of herbivores of different sizes were removed; in particular, an additive effect of ungulates and invertebrates on the short-grass vegetation where these two groups consume the largest proportion of the available plant biomass (Risch et al., 2013) and therefore are more likely to have a high dietary overlap; and (3) that the size-selective exclusion of herbivores will result in fewer changes in plant community properties in tall-grass compared to the short-grass vegetation due to the large differences in grazing intensity between the two vegetation types.

2 MATERIALS AND METHODS

2.1 Study area

The study was conducted in subalpine grasslands in the Swiss National Park (SNP). The SNP is located in the central Alps, and covers an area of 172 km² ranging from 1350 to 3170 m above sea level (a.s.l.), with mean annual precipitation of 826 ± 112 mm and mean annual temperature of 0.9 ± 0.5°C (mean ± SD, 2009 - 2013; MeteoSchweiz, 2014). Forests (50 km²), alpine grasslands (33 km²) and subalpine grasslands (approximately 3 km²) cover around 50% of the SNP (Risch et al., 2013), the rest is dominated by rock and scree. The underlying bedrock of the subalpine grasslands is dolomite.

Since its foundation in 1914, the SNP has been protected from human disturbances (no livestock grazing, no hunting, no fishing, no camping or no off-trail hiking). The subalpine grasslands are comprised of homogeneous patches (usually >1 ha) of short- and tall-grass vegetation, which developed mainly because of differences in land-use history and grazing by domestic livestock and wild ungulates over several centuries (Schütz et al., 2003, 2006). Soon after banning cattle in 1914, red deer (*Cervus elaphus* L.) re-migrated into the SNP and started to preferentially graze where cattle formerly rested over night and soils became enriched in nutrients, creating what we today call short-grass vegetation (Schütz et al., 2003). Tall-grass vegetation developed where cattle frequently grazed, but not rested before the foundation of the SNP. These areas became depleted of soil nutrients. The vegetation therefore is less nutrient rich, hence, red deer graze these areas with much lower intensity (Schütz et al., 2003).

The aboveground animals found in our study area can be divided into four groups (Risch et al., 2013): (1) large mammalian herbivores (30–150 kg; mainly red deer and chamois *Rupicapra rupicapra*), (2) medium mammals (3–6 kg; e.g. alpine marmot *Marmota marmota* and mountain hare *Lepus timidus*), (3) small mammals (30–100 g; small rodents such as *Clethrionomys* spp., *Microtus* spp. and *Apodemus* spp.), and (4) invertebrates (< 5 g; e.g. grasshoppers, caterpillars and leafhoppers). At present a total of 26 species of large to small wild mammals can be found in the SNP (see Risch et al., 2018), with large mammalian predators (wolf, bear or lynx) being absent or non-resident during our experiment. Reptiles, amphibians and birds are scarce in the subalpine grasslands. See Risch et al. (2018) for a full list of invertebrate species captured in the SNP in summer 2013.

2.2 Experimental design

In early May 2009, we established eighteen fencing setups (referred to as random factor 'Fence' in the Statistical Analysis section) in six subalpine grasslands (random factor 'Grassland') located across the SNP at elevations ranging from 1975 to 2300 m a.s.l. There were two fencing setups per vegetation type (short-grass vs. tall-grass) in each of the three large grasslands, and one fencing setup per vegetation type in each of the three small grasslands. Hence, we established nine fencing setups in short-grass vegetation and nine in tall-grass vegetation in a paired design (Fig. S1). The fencing setups were left in the field for five consecutive growing seasons (May 2009 - October 2013), but to protect them from snow damage and avalanches they were temporarily dismantled in late October every year, and reconstructed in early May of the following year, immediately after snowmelt.

For a detailed fencing protocol see Risch et al. (2013). Briefly, each fencing setup consisted of a 2.1 m tall and 7 × 9 m large main fence and we established five 2 × 3 m plots that simulated the size-selective exclusion treatments (Fig. S1). The 'LMSI' plot was located at least five meters away from the main fence (i.e., control plot; 2 × 3 m) and gave access to large (L), medium (M) and small (S) mammals, and invertebrates (I). The main fence consisted of wooden posts and electrical wires mounted at regular intervals between 0.5 and 2.1 m to keep large mammals out. Note that the bottom wire (0.5 m) was not electrified to allow smaller mammals to enter safely. Within each main fence, we

randomly established four 2×3 m treatment plots one meter from the main fence line to avoid edge effects and separated by one meter walkways: (1) the 'MSI' plot remained unfenced and gave access to medium (M) and small (S) mammals and all aboveground dwelling invertebrates (I); (2) the 'SI' plot was surrounded by an electrical fence with 10×10 cm mesh size and allowed small (S) mammals and invertebrates (I) to enter; (3) the 'I' plot was surrounded by 2×2 cm mesh-sized metal fence, double-folded at the bottom 50 cm, which only allowed invertebrates (I) to enter; and (4) the 'None' plot was surrounded by a 1.5×2 mm mesh-sized mosquito net and covered with a mosquito mesh lined roof to exclude all vertebrate and invertebrate animals (Fig. S1). We built additional six 'micro-climate control' enclosures (one in each of the six main grasslands; see Risch et al., 2013, 2018 for the construction details) with a comparable micro-climate to the 'None' plots, but also a comparable feeding pressure by invertebrates to the 'I' plots. As discussed in detail in Risch et al. (2013, 2018), differences in plant and soil properties between the 'I' and the 'None' treatments were not due to the enclosure design (mesh and roof) of the 'None' enclosure, but a function of animal exclusions.

2.3 Vegetation characteristics

We collected plant data in a total of 90 plots (18×5). We quantified vegetation characteristics yearly at peak biomass (July) in predetermined, randomly assigned 1×1 m subplots within each 2×3 m plot over five consecutive growing seasons (2009 - 2013). We assessed plant community composition by identifying all vascular plants to species level. We estimated percentage cover of each species and assigned each species to one of five plant functional groups: forbs, grasses, sedges, legumes or woody species. We measured aboveground biomass (g/m^2) of each plant functional group (see Table 1) using the canopy intercept method (Frank & McNaughton, 1990). Total aboveground plant biomass (i.e., biomass of live shoots, 'BM.shoots') was defined as the sum of biomass of all five plant functional groups. As woody species were rare and their biomass negligible, we did not further analyse their responses to the exclusion treatments.

For each plot and study year, we calculated different plant diversity measures: overall species richness (total number of plant species), Shannon diversity (exponential of Shannon entropy), plant family richness (number of taxonomic families), family-level

Shannon diversity, and richness of each plant functional group. We also calculated Rao's quadratic entropy (Laliberté, Legendre, & Shipley 2014), which accounts for both species relative abundance and traits, as an overall measure of plant functional diversity (FDq). Species traits that we considered here were taxonomic family, functional group, and ecological indicator values for moisture, pH, nutrients, light and temperature (Landolt et al., 2010; see Table S4). Life span was not included in the trait dataset because over 95% of species recorded in our study are perennials.

We recorded 169 plant species, 113 forbs, 18 grasses, 14 sedges and 8 legumes, belonging to 110 genera and 41 families over the five growing seasons and across the 90 plots in our two vegetation types. When comparing the 'LSMI' plots (control plots) where all aboveground animals were present, we were able to confirm the differences in plant species composition previously described for the two vegetation types (Schütz et al., 2003, 2006). In the tall-grass vegetation, tussocks of the tall-growing evergreen sedge *Carex sempervirens* accounted for $21\% \pm 5.4\%$ of total cover of all species, and "sedges", the most dominant plant functional group, accounted for $26.7\% \pm 5.3\%$ of the total cover; other common species were *Sesleria caerulea* (grass, $7.1\% \pm 3\%$), *Nardus stricta* (grass, $6.6\% \pm 4.3\%$), *Crepis spp.* (forb, $4.2\% \pm 1.4\%$), *Briza media* (grass, $3.3\% \pm 1.1\%$) and *Festuca rubra* (grass, $3.0\% \pm 1.2\%$). Within the short-grass vegetation, the most dominant plant functional group was "grasses" ($26.2\% \pm 3.0\%$ of the total cover). Instead of having a single dominant plant species, there were several dominating species with similar coverages in this vegetation type: *Briza media* (grass, $7.8\% \pm 3.2\%$), *Carex caryophyllea* (sedge, $6.5\% \pm 4\%$), *Crepis spp.* (forb, $5.7\% \pm 1.8\%$), *Cirsium acaule* (forb, $5.6\% \pm 1.3\%$), *Festuca rubra* (grass, $5.4\% \pm 1.3\%$) and *Deschampsia caespitosa* (grass, $5.0\% \pm 3.3\%$). Further details with regard to all the plant species recorded in our study, their relative abundances and traits (including data sources) are provided in Table S4.

2.4 Statistical analyses

We conducted data analyses for the short- and tall-grass vegetation separately. To examine the responses of plant abundance (total plant biomass, biomass and relative biomass of different functional groups, relative cover of key species and plant functional groups) and diversity measures (overall species richness, functional diversity, richness of families and different functional groups, species- and family-level Shannon diversity) over

the course of the study, we used the data of all five growing seasons (2009 - 2013). We used repeated-measures linear mixed effects models (LMMs) with fixed effects 'Treatment', 'Year' and 'Treatment' × 'Year', and 'Fence' within 'Grassland' as a nested random effect. We corrected each model with a time serial correlation structure ('corCAR1', see Pinheiro & Bates, 2000) to account for the temporal autocorrelation of the data. For each model, normality of residuals was examined. When the normality assumption was not met, the dependent variables were transformed (see Table 1). When a treatment effect was significant, *post hoc* tests with adjusted *p* values were performed to obtain all pairwise comparisons for the treatments.

For each diversity measure with a treatment effect, we conducted a LMM analysis of covariance (LMM ANCOVA) to determine whether the treatment effect still existed after controlling for the effect of specific covariates (i.e., biomass or cover of plant species, family or functional group; see Table S2). For this purpose, we added covariates individually to the initial LMMs with other model terms unchanged. If the treatment effect became non-significant and the covariate was significantly related to the diversity measure, this means that the covariate is key in mediating the effects of exclusion treatments on the diversity measure.

For plant community data, we conducted multivariate analyses after transforming the data (cover estimates by species) using the 'Hellinger' transformation (Legendre & Gallagher, 2001). Principle response curves (PRC; with 1999 Monte-Carlo permutations) were used to illustrate the trends of the effect of 'Treatment' on community data (van den Brink & ter Braak, 1999). To determine in which year statistically significant differences occurred between the animal exclusion treatments, we conducted redundancy analyses (RDA) for each year separately, with 'Treatment' as a constrained factor.

To explore key variables mediating the exclusion effects on plant community composition, we only considered community data of 2013, the last growing season of the study that showed the strongest responses. For each continuous explanatory variable, we created a partial redundancy analyses (pRDA) model to compute the partial ('unique') contribution (Legendre & Legendre, 2012) of 'Treatment' to the variance of plant communities in the presence of a specific explanatory variable (covariates). Both the covariates and 'Treatment' were included in the pRDA as the constrained factors.

Monte Carlo permutation tests (n=999) were used to assess the significance of the constrained factor(s) in the RDA and pRDA. The permutation tests were done with 'anova.cca' (Oksanen et al., 2019), the function allows to be used for hierarchical (nested) experimental designs (Lepš & Šmilauer, 2003; Legendre & Legendre, 2012). The results of significance tests for the RDA and pRDA can indicate whether the covariate is key in mediating the effect of the experimental exclosures on plant community composition. As a key mediator, the covariate should not only be significantly related to the variation in plant communities, but also be able to cause the significant 'Treatment' effect detected in the RDA model to be non-significant in the pRDA model. This approach therefore is comparable to the approach we used for our univariate analyses. We also created ordination triplots to visualise our results.

All analyses were performed in R ver. 3.5.2 (R Core Team 2019). The functional diversity index (FDq) was calculated using the package 'FD' (Laliberté et al., 2014). The LMM analyses with tests of significance were performed using package 'nlme' (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team 2019). The *post hoc* tests for the LMM analyses were conducted using package 'multcomp' (Hothorn, Bretz, & Westfall 2008). The (p)RDA analyses with permutation tests were done with the 'vegan' package (Oksanen et al., 2019).

3 RESULTS

3.1 Lack of responses to animal exclusions in tall-grass vegetation

Size-selective exclusion in the tall-grass vegetation did not affect the relative cover of dominant or common individual plant species such as *Carex sempervirens* ($F_{4,32} = 1.805$, $p = 0.152$) or the relative abundance of any plant functional group (Fig. 1, Table S4), thus did not greatly alter the plant community structure (Fig. 1, 2; Fig. 3b, 4b). There were, however, two exceptions from these general patterns: biomass of forbs was higher compared to the control plots when large mammals, all mammals, or all animals were excluded (Fig. 1d) and richness of grasses was lowest when all animals were excluded, but this number only differed from the one measured on plots where all mammals were excluded (Fig. 2f).

3.2 Animal exclusion effects in short-grass vegetation

In contrast to the general lack of responses found in the tall-grass vegetation, the size-selective exclusion had, as expected, a much larger effect in the short-grass vegetation. Yet, the exclusion of large ungulates ('MSI' vs. 'LMSI') only affected total aboveground plant biomass, which increased by roughly 50% in comparison to when no animals were excluded (Fig. 1a). The exclusion of large and medium mammals ('SI' vs. 'LMSI') led to increases in total aboveground plant biomass (Fig. 1a), biomass of grasses (Fig. 1b) and forbs (Fig. 1d), while the exclusion of all mammals ('I' vs. 'LMSI') led to increases in total aboveground plant biomass (Fig. 1a), biomass of grasses (Fig. 1b) and forbs (Fig. 1d), as well as decreases in family-level Shannon diversity (Fig. 2d) and richness of forbs (Fig. 2h). In addition, total aboveground plant biomass (Fig. 1a), biomass of grasses (Fig. 1b) and relative cover of *Festuca rubra* (Fig. 1i) further increased when all mammals and invertebrates were excluded ('None' vs. 'I'), emphasising the important roles of invertebrates in the system.

Our results thus showed that large and medium herbivores (together) as well as invertebrates significantly affected both total aboveground plant biomass (Fig. 1a) and biomass of grasses (Fig. 1b), suggesting a dietary overlap of these herbivore groups. In addition, we found that the relative abundances of specific plant functional groups changed because of disproportional changes in absolute biomass of the functional groups. For example, excluding all animals increased biomass of grasses by 205%, forbs by 100% and total aboveground plant biomass by 118% (Fig. 1a, 1b, 1d), resulting in significant increases in relative biomass of grasses from 43.6% to 60% and relative cover from 26.2% to 39.3% (Fig. 1f, 1j). Consequently, grasses won over forbs when all the animals were excluded. In contrast, the absolute biomass of sedges did not significantly change in response to different animal exclusion treatments (Fig. 1c), but their relative biomass decreased due to the increases in total plant biomass (Fig. 1g). Further, we found that forb richness decreased by 16%, which corresponds to a loss of three plant species (Fig. 2h) when all animals were excluded ('None') compared to the control ('LMSI'). In addition, the number of plant families (i.e., family richness) decreased by 13% (i.e., a loss of two taxonomic families; Fig. 2e) and family-level Shannon diversity by 23% (Fig. 2d) when comparing the 'None' with the 'LMSI' plots.

3.3 Grasses mediate the responses of short-grass vegetation to animal exclusions

Plant community composition of the short-grass vegetation differed significantly among the exclusion treatments in 2011, 2012 and 2013, but not during the first two years of the experiment (2009 & 2010; Table 2). In addition, the PRC (Fig. 3a) and RDA (Table 2) analyses showed that the effects of the treatment became stronger with time of exclusion. For the 2013 plant community data, we observed a distinct difference in short-grass vegetation plant species composition between the treatment where all animals had access and the treatment where all animals were excluded (Fig. 4a). Changes in biomass and relative cover of grasses, biomass of live shoots, richness of forbs and Shannon diversity of families were responsible for this significant treatment effect (Fig. 4a, Table S3). Furthermore, all significant changes we found in short-grass plant diversity (Table 3 and Table S2) were explained by exclusion related changes in biomass and relative cover of grasses. Although not all species of grasses significantly increased their relative abundances when excluding all animals in the short-grass vegetation, the three species with the greatest increase in cover were all grass species: *Trisetum flavescens* (from 0.7% in control to 6.8% in 'None' treatment), *Festuca rubra* (from 5.4% to 11.4%) and *Agrostis capillaris* (from 1.1% to 5.2%; Fig. 3a, Table S4).

4. DISCUSSION

As expected, we found that the size-selective exclusion of animals had less of an effect on the plant community structure in the tall-grass compared to the short-grass vegetation. The fact that almost all significant responses we found to our animal exclusions were from short-, rather than tall-grass vegetation, suggests that defaunation effects on plant communities strongly vary with vegetation type. The contrasting results found were related to differences in historic land-use, which resulted in differences in soil resources and plant community composition. This in turn, leads to differences in the quantity and quality of forage (Schütz et al., 2003, 2006) and therefore grazing intensity. Higher grazing pressure in the short-grass vegetation resulted in this vegetation type being top-down controlled by herbivores, while we did not detect such an effect in the tall-grass vegetation. We discuss the potential mechanisms responsible for the contrasting findings in the two vegetation types in detail below:

4.1 Lack of response in the tall-grass vegetation

The lack of response of the tall-grass vegetation to our animal exclusions can likely be linked to the low grazing pressure of all animal species present in this vegetation type and to the dominant plant species *Carex sempervirens*. This sedge species is slow-growing, has a high physical resistance (tough tissue), slow decomposition rate and low palatability, and therefore persists for hundreds of years (de Witte, Armbruster, Gielly, Taberlet, & Stöcklin 2012; Landolt et al., 2010). Hence, *C. sempervirens* is generally not preferred by most herbivores, but some specialists like sedge-sucking leaf hoppers (Vandegehuchte et al., 2018). In addition, *C. sempervirens* tussocks generally occupy the top-layer of the vegetation, thus are highly competitive in terms of resource acquisition and likely limit the growth of other plant species.

As the tall-grass vegetation in the SNP was reported to be stable over long time periods (Wildi & Schütz, 2000), plant species in this community might respond asynchronously to perturbation such as reduced grazing (Hautier et al., 2014). Hence, increases in the biomass of some plant species might result in declines in biomass of other species when the grazing pressure is reduced. Moreover, all animal groups together only consumed roughly 20% of the primary production (Fig. 1a, see also Risch et al., 2013). Hence, it may not be surprising that the animal exclusions did hardly affect the community structure of this vegetation type.

One of the few responses we found in the tall-grass vegetation as a result of our experimental treatments, was an increase in the biomass of forbs when all mammals or all animals were excluded, likely was mainly related to an increase in leaf size and resource use efficiency, not to an increase in the number of leaves as discussed in detail in Firn, Schütz, Nguyen and Risch (2017). Even so, this 'growth bonus' of forbs did not lead to a change in relative abundance of forbs or any other plant functional group in the tall-grass vegetation. Although we did not find any evidence for the tall-grass vegetation being top-down controlled by animals, it is possible that this vegetation type is bottom-up controlled by limiting soil resources. However, as we did not directly manipulate limiting soil nutrients, this remains to be studied.

4.2 Size-selective exclusion effects on short-grass vegetation

For short-grass vegetation, the decreases in plant diversity and changes in plant community composition, were only found when all mammals or all animals were excluded. This suggests that animal diversity plays an important role in maintaining grassland plant community structure in this vegetation type (also see Wang et al., 2019). This finding agrees with our expectation that the more animal groups are excluded the stronger the response of the vegetation to animal exclusions. The response can be attributed to the fact that both large ungulates and invertebrate herbivores are dominant biomass consumers in this vegetation type (Risch et al., 2013), and that the two groups have the largest dietary overlap on grasses (also see Moran & Hurd, 1997; Suter, Suter, Krüsi, & Schütz 2004). However, our findings were contrary to our prediction that large mammals alone can exert a strong top-down effect on the short-grass plant community. Hence, our findings contrast with results from other experimental studies reporting that large vertebrate herbivores played a predominant top-down control on plant communities (Collins et al., 1998; Bakker et al., 2006; Liu et al., 2015; Burkepile et al., 2017; see also references in Turkington, 2009 and Svenning et al., 2019). Yet, differences in how large mammals affect plant communities can be related to the differences in grazing intensities, population sizes, or even species and foraging behaviour (see Liu et al., 2015) of large herbivores among the studies. For example, Liu et al. (2015) reported that in the high plant diversity grassland they studied, the absence of large herbivores resulted in lower plant richness compared to plots where certain types of livestock (cattle alone, sheep & cattle together, but not sheep alone) were allowed to graze. However, their "moderate" stocking rates resulted in roughly 50% of the plant biomass being consumed, while in our system grazing by large, wild ungulates only amounted to roughly 30% biomass removal in the short-grass vegetation (Risch et al., 2013). Yet, we detected changes in plant species composition and decreases in plant diversity measures when we reduced the grazing pressure by roughly 60% (Risch et al., 2013), i.e., when all vertebrate and invertebrate herbivores were excluded.

When all animals were progressively excluded with our size-selective fences, grazing pressure on grasses continually decreased. This reduction in plant biomass consumption resulted in an increase in relative abundance of grasses, which was a key mechanism mediating the community-level responses of the short-grass vegetation to our herbivore

exclusion experiment. Many grass species have a superior ability to exploit soil nutrients (Risch et al., 2015; Firn et al., 2017), and can increase the number of tillers when the grazing pressure is lowered (i.e., clonal growth ability; Ibanez et al., 2013; Firn et al., 2017). Clonal growth is an important factor influencing plant assembly (Benot et al., 2011), vegetation structure and stability in grassland ecosystems (see Dong et al., 2015). In contrast to grasses, forbs and sedges generally have more chemical (secondary compounds; Whiles & Charlton, 2006) or morphological defences (e.g. spines, trichomes, tough tissues; Schütz et al., 2003; Proctor & Bradshaw, 2013), thus lower potential or maximum growth rates (growth-defence tradeoff hypothesis; Coley, Bryant, & Chapin 1985). Consequently, an uniform roughly 5 cm high plant canopy and a high plant community evenness, i.e., several dominating species with similar abundances prior to our experiment, allowed grasses to quickly obtain a greater relative abundance when herbivores were excluded. With the reductions in grazing pressure, the tall-growing grasses started to occupy the upper canopy and affected microhabitat and microclimatic conditions, for example, leading to an increase in litter layer thickness and a reduction in light availability at ground level (Risch et al., 2013, 2018; also see Fahnestock & Knapp, 1994; Zhu, Zhang, & Liu 2016). These changes in the environmental conditions likely limit biomass production and fitness of slow growers, especially the low-growing and recumbent forbs (Fahnestock & Knapp, 1994), but have little influence on tall-growing grasses themselves as they can easily pierce through the litter layer and are less constrained by light (Zhu et al., 2016). Forbs represent the bulk of plant species diversity in ours and many other grasslands (Whiles & Charlton, 2006; Knapp et al., 1999), but as small- or low-growing species, have lower shade tolerance compared to grasses and therefore are likely to disappear first when herbivore exclusions altered the light environment and photoperiod (Zhu et al., 2016; Firn et al., 2017).

In summary, with the progressive exclusion of herbivores of different body-sizes, grazing pressure continuously decreased, which led to an increase in grass biomass and dominance. During this process, plant-plant interactions changed, which resulted in decreases in forb richness and family-level plant diversity as well as a changed plant species composition in the short-grass vegetation (this study and Firn et al., 2017). We previously demonstrated with our experiment that the size-selective exclusion of animals

(defaunation) affected soil and aboveground invertebrate communities (Vandegehuchte, Raschein, Schütz, Gwiazdowicz, & Risch 2015; Wang, Steiner, Schütz, Vandegehuchte, & Risch 2018) and ecosystem properties (Haynes et al., 2014; Risch et al., 2013, 2018). All these impacts of animal exclusions were particularly strong in the short-grass vegetation, suggesting that the close relationships between above- and belowground communities and their abiotic environment (i.e., ecosystem coupling; see Risch et al., 2018) are controlled by top-down forces in this vegetation type. Especially large ungulates and invertebrates contributed to this top-down control via their preferential feeding on grasses (also see Moran & Hurd, 1997; Suter et al., 2004). Hence, our findings were consistent with Ritchie & Olff (1999) and Bokdam (2001) reporting that co-occurring herbivores with a high dietary overlap had additive effects on suppressing the abundance of plant species they all preferred, which could in turn affect plant-plant interactions and community dynamics. This grazing-driven, grass-mediated maintenance of plant diversity is similar to what has been reported from other grasslands (Bobbink & Willems, 1987; Knapp et al., 1999; Koerner et al., 2018). Unlike studies reporting that abundant smaller mammalian herbivores can exert strong top-down control on plant communities (e.g. Andersson & Jonasson, 1986; Brown & Heske, 1990; also see references in Bakker et al., 2006), there was no evidence that medium and small-sized mammals independently altered the subalpine grassland plant community structure in our study area.

5. Conclusions

Our simulated defaunation showed that plant diversity and community composition of the subalpine short-grass vegetation was maintained by different-sized animals, but this was not the case in the tall-grass vegetation. The contrasting results we found in the tall- and short-grass vegetation were due to differences in plant community composition, which in turn affected the quantity and quality of forage for herbivores (Schütz et al., 2003) and led to large differences in grazing intensity between the two systems. This is similar to what other studies reported, in particular, that herbivore effects on grassland ecosystems depend on initial site conditions (e.g. soil nutrients) and biotic community characteristics of both plants and animals (see Andersson & Jonasson, 1986; Ritchie & Olff, 1999; Erschbamer et al., 2004). Schütz et al. (2006) suggested that the two subalpine

vegetation types currently found in the SNP will likely persist for hundreds of years, if large ungulate communities remain unchanged and no large-scale disturbances occur (land and rock slides, avalanches, etc.), but they did not assess the role of invertebrates in their study. Our study suggests that a removal of large ungulates and medium mammals can increase total plant biomass and biomass of grasses, but will not result in a reduction in plant diversity in the short-term (i.e., five years), unless plant (grass) biomass consumption by invertebrate communities is also greatly suppressed. The SNP is a strictly preserved area and currently does not face a danger of defaunation caused by over-exploitation of animal resources or overuse of insecticides. However, large predators that were previously locally extinct, started to sporadically explore the area and might re-establish in the area in the future. Hence, densities as well as distributions of large and medium herbivores maybe altered, which could affect the dynamics of the SNP subalpine grasslands in the long term.

Overall, we can only speculate on the long-term impacts of size-selective defaunation on the vegetation of our grasslands, as our experimental exclosures were only in place for five growing seasons. However, as the changes in plant community composition became more and more prominent over the duration of our experiment, long-term animal exclusion induced changes in plant community structure might be quite different from our current results. Our results also showed that not only mammals, but also invertebrates contribute to the maintenance of plant community structure in the short-grass vegetation, which adds to the recent literature that highlighted the importance of animal diversity for ecosystem functioning (Soliveres et al., 2016; Risch et al., 2018; Wang et al., 2019).

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AUTHORS' CONTRIBUTIONS

A.C.R and M.S conceived the ideas and designed methodology; A.C.R and M.S collected the data; X.W analysed the data; X.W and A.C.R led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository:

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SUPPORTING INFORMATION

Additional supporting information (Supplementary Figure S1 and Table S1-S4) can be found online in the Supporting Information section.

FIGURE LEGENDS

FIGURE 1. Effects of size-selective animal exclusion (2009 - 2013) on plant biomass (BM; g dry weight m⁻²; a-e), relative biomass (rBM; f-i) and relative cover (rCov; %, j-l) in short- (open circles) and tall-grass (solid circles) vegetation (mean \pm 95% confidence intervals). 'LMSI' = Large, medium, small mammals and invertebrates have access to the plots; 'MSI' = Medium, small mammals and invertebrates have access; 'SI' = Small mammals and invertebrates have access; 'I' = Invertebrates have access; 'None' = No animals have access. *Fest.rubr* = *Festuca rubra*. Different small (short-grass) or capital letters (tall-grass) above error bars indicate significant differences between treatments. Statistical results for the main effects of exclusion treatments are shown in Table 1, the ones for *post hoc* pairwise tests for all treatments are listed in Table S1.

FIGURE 2. Effects of size-selective animal exclusion (2009 - 2013) on richness, Shannon diversity and functional diversity of plant species (a-c), Shannon diversity and richness of plant families (d-e) and richness of different plant functional groups (f-i) in short- (open circles) and tall-grass (solid circles) vegetation (mean \pm 95% confidence intervals). 'LMSI' = Large, medium, small mammals and invertebrates have access to the plots; 'MSI' = Medium, small mammals and invertebrates have access; 'SI' = Small mammals and invertebrates have access; 'I' = Invertebrates have access; 'None' = No animals have access. Different small (short-grass) or capital letters (tall-grass) above error bars indicate significant differences between treatments. Statistical results for the main effects of exclusion treatments are shown in Table 1, the ones for *post hoc* pairwise tests for all treatments are listed in Table S1.

FIGURE 3. Principal response curves (PRCs) showing the effects of 'Treatment' and 'Year' on plant community composition in (a) short- and (b) tall-grass vegetation. The grey zero lines represent the unfenced control where large, medium, small mammals and invertebrates have access ('LMSI'). 'MSI' = Medium, small mammals and invertebrates have access; 'SI' = Small mammals and invertebrates have access; 'I' = Invertebrates have access; 'None' = No animals have access. The left y-axes show the effect size of 'Treatment' on plant communities over the course of the study (2009 - 2013). The right y-axes display species scores. The overall significance tests of the PRC for short-grass

vegetation: $F_{(1, 200)} = 3.45$, $p = 0.006$; and for tall-grass vegetation: $F_{(1, 200)} = 3.24$, $p = 0.072$.

FIGURE 4. Ordination triplots for (a) short- and (b) tall-grass plant communities in the final year of the experiment (2013). The directions and lengths of blue arrows show the strength of the correlations between plant species composition and other plant community properties. The centroids of the different treatments are labeled according to the herbivore groups that have access to the plots. 'LMSI' = Large, medium, small mammals and invertebrates have access to the plots; 'MSI' = Medium, small mammals and invertebrates have access; 'SI' = Small mammals and invertebrates have access; 'I' = Invertebrates have access; 'None' = No animals have access. Grey plus signs (+) represent plant species. For readability, only species with relative cover higher than 3% are labeled with their name abbreviation.

Table 1. Results of linear mixed effects models (LMMs) testing the effects of 'Treatment' (i.e. size-selective animal exclusion), 'Year' and their interaction on plant abundance and diversity measures in short- and tall-grass vegetation. To meet the normality of residuals assumption, data of plant functional diversity (Rao's quadratic entropy) were square-transformed, all biomass data were square-root-transformed, relative biomass of sedges and legumes and relative cover of sedges and *Festuca rubra* were arcsine-square-root-transformed. For richness of legumes, the normality of residuals assumption was not met after data transformation. We therefore only present its results in Fig.2i, but no statistical test in this table. All other data were not transformed. df = the numerator and denominator degrees of freedom. *p*-values in bold are statistically significant at the 0.05 level.

Dependent variables	Short-grass vegetation						Tall-grass vegetation					
	Treatment		Year		Treatment: Year		Treatment		Year		Treatment: Year	
	$F_{(df=4,32)}$	<i>p</i>	$F_{(df=4,160)}$	<i>p</i>	$F_{(df=16,160)}$	<i>p</i>	$F_{(df=4,32)}$	<i>p</i>	$F_{(df=4,160)}$	<i>p</i>	$F_{(df=16,160)}$	<i>p</i>
Richness of species	1.447	0.241	18.156	<.0001	1.450	0.125	0.159	0.958	36.926	<.0001	1.151	0.314
Shannon diversity of species	1.015	0.415	6.585	0.0001	1.114	0.346	0.675	0.615	2.585	0.039	1.401	0.147
Functional diversity of species	2.324	0.078	7.902	<.0001	0.889	0.583	1.855	0.143	2.069	0.087	0.838	0.641
Shannon diversity of families	4.240	0.007	4.888	0.001	1.547	0.089	1.505	0.224	1.862	0.120	0.875	0.599
Richness of families	2.746	0.045	2.249	0.066	1.024	0.435	1.208	0.327	11.267	<.0001	0.760	0.729
Richness of grasses	1.275	0.300	22.536	<.0001	0.783	0.704	3.357	0.021	17.080	<.0001	1.860	0.028

Dependent variables	Short-grass vegetation						Tall-grass vegetation					
	Treatment		Year		Treatment: Year		Treatment		Year		Treatment: Year	
	$F_{(df=4,32)}$	p	$F_{(df=4,160)}$	p	$F_{(df=16,160)}$	p	$F_{(df=4,32)}$	p	$F_{(df=4,160)}$	p	$F_{(df=16,160)}$	p
Richness of sedges	0.471	0.757	5.032	0.0008	0.737	0.754	0.300	0.876	9.046	<.0001	0.978	0.483
Richness of forbs	3.145	0.027	5.560	0.0003	1.243	0.241	0.582	0.678	22.673	<.0001	0.931	0.536
Richness of legumes	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Biomass of shoots	13.241	<.0001	11.448	<.0001	0.591	0.888	2.516	0.061	16.487	<.0001	0.364	0.989
Biomass of grasses	9.946	<.0001	7.309	<.0001	0.661	0.828	1.692	0.176	17.270	<.0001	1.037	0.421
Biomass of sedges	1.564	0.208	9.249	<.0001	0.730	0.760	1.002	0.421	13.365	<.0001	0.626	0.860
Biomass of forbs	6.061	0.001	9.243	<.0001	0.416	0.977	4.138	0.008	16.902	<.0001	0.474	0.957
Biomass of legumes	1.804	0.153	1.971	0.101	0.877	0.597	0.395	0.811	4.193	0.003	1.552	0.088
Relative biomass of grasses	3.216	0.025	1.678	0.158	0.846	0.632	1.127	0.361	6.950	<.0001	0.977	0.484
Relative biomass of sedges	3.162	0.027	11.051	<.0001	0.471	0.958	1.503	0.225	10.854	<.0001	0.955	0.509
Relative biomass of forbs	0.295	0.879	8.439	<.0001	0.884	0.588	1.198	0.331	8.970	<.0001	0.708	0.783

Dependent variables	Short-grass vegetation						Tall-grass vegetation					
	Treatment		Year		Treatment: Year		Treatment		Year		Treatment: Year	
	$F_{(df=4,32)}$	p	$F_{(df=4,160)}$	p	$F_{(df=16,160)}$	p	$F_{(df=4,32)}$	p	$F_{(df=4,160)}$	p	$F_{(df=16,160)}$	p
Relative biomass of legumes	0.338	0.850	1.162	0.330	0.796	0.688	0.235	0.917	1.092	0.363	1.357	0.170
Relative cover of grasses	2.785	0.043	1.881	0.116	1.412	0.142	0.913	0.469	7.179	<.0001	1.174	0.294
Relative cover of sedges	2.017	0.116	5.093	0.0007	0.580	0.9	1.124	0.363	1.98	0.10	1.12	0.341
Relative cover of <i>Festuca rubra</i>	3.772	0.013	4.170	0.003	0.646	0.843	1.455	0.239	5.833	0.0002	0.755	0.733

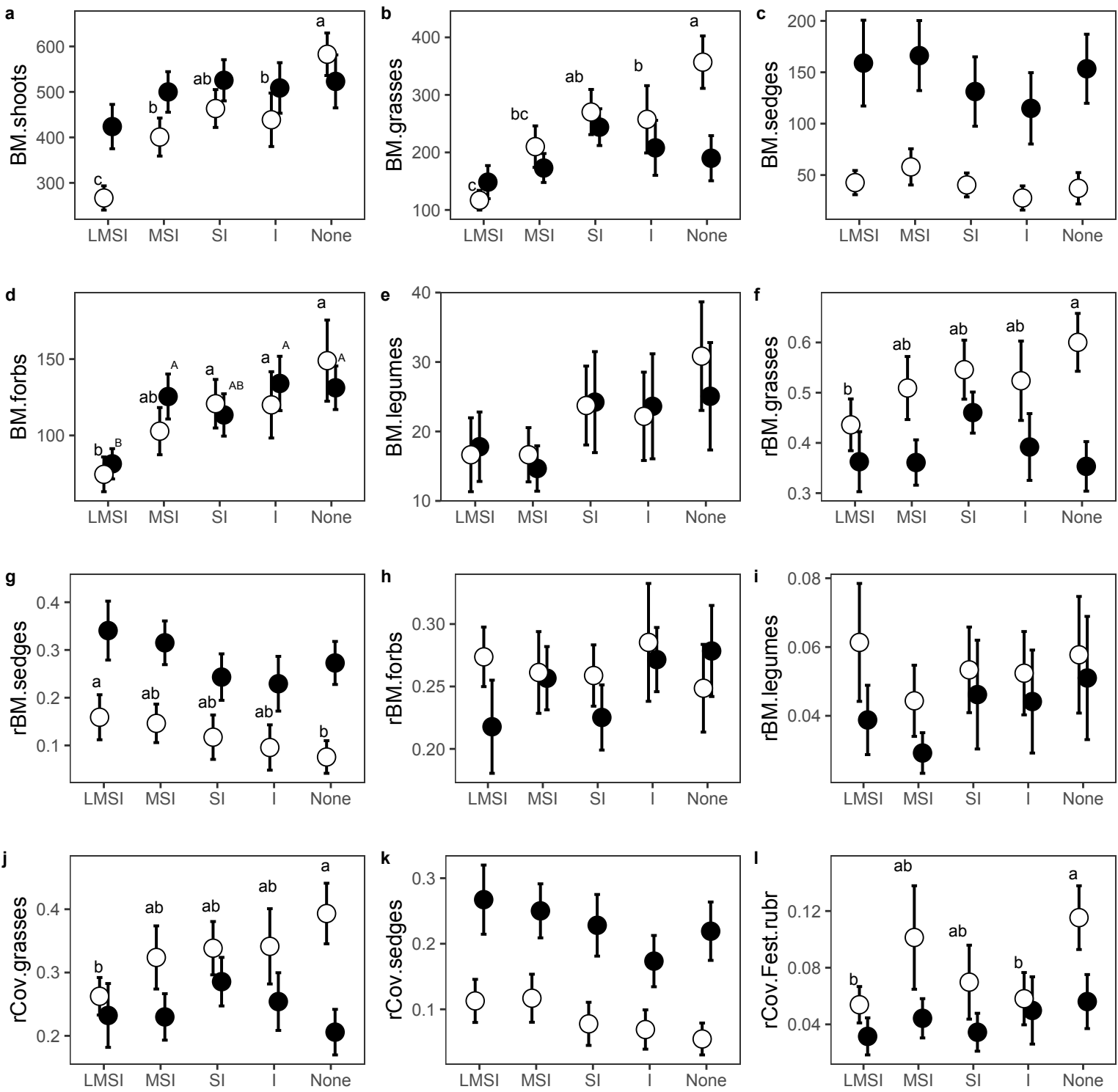
Table 2. Results of the redundancy analyses (RDA) testing the overall effect of size-selective animal exclusion on plant community composition in short- and tall-grass vegetation over the course of the study (2009 - 2013), for each year separately. The results of significance tests were based on 999 permutations.

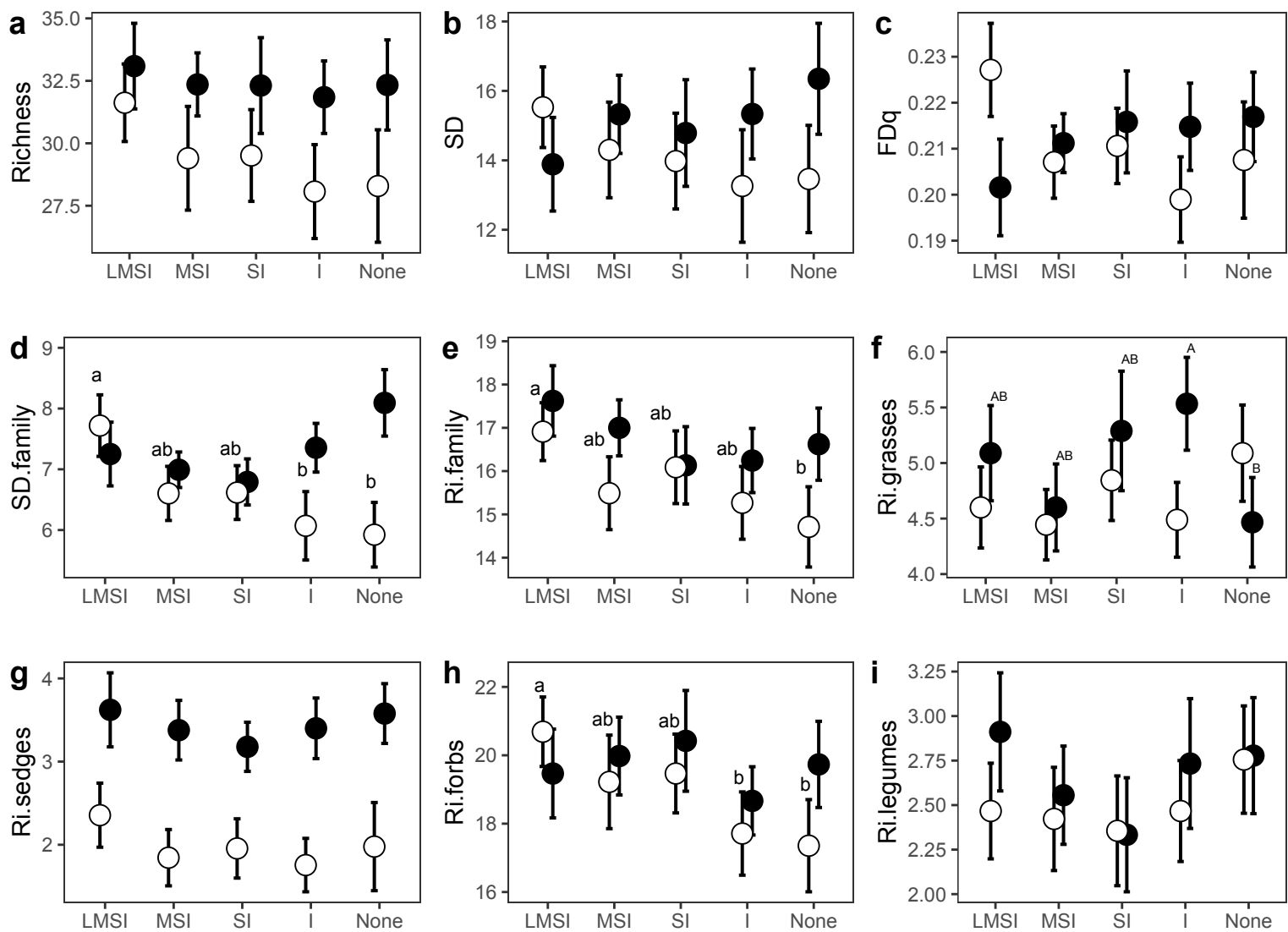
Year	Short-grass			Tall-grass		
	Df	F	Pr(>F)	Df	F	Pr(>F)
2013	4	0.660	0.001 ***	4	0.609	0.22
2012	4	0.559	0.014 *	4	0.672	0.068 .
2011	4	0.531	0.027 *	4	0.621	0.205
2010	4	0.461	0.074 .	4	0.631	0.179
2009	4	0.537	0.094 .	4	0.594	0.383

Table 3. Summary table showing covariates that are responsible for the significant effects of size-selective animal exclusion on richness of forbs, richness and Shannon diversity of plant families in short-grass vegetation, marked with '✓'. See Table S2 for the original table of results.

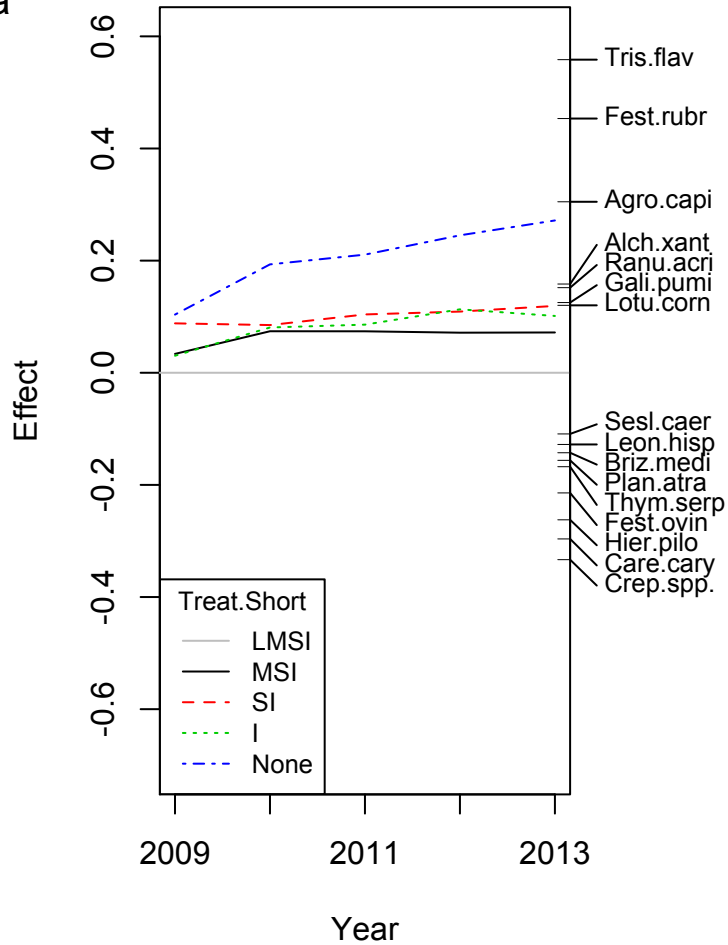
Covariates	Dependent variables		
	Richness of forbs	Richness of families	Shannon diversity of families
Biomass of shoots		✓	
Biomass of grasses	✓	✓	✓
Biomass of sedges			
Biomass of forbs			
Biomass of legumes			
Relative biomass of grasses		✓	
Relative biomass of sedges			
Relative biomass of forbs			
Relative biomass of legumes			
Relative cover of grasses	✓	✓	✓

Covariates	Dependent variables		
	Richness of forbs	Richness of families	Shannon diversity of families
Relative cover of sedges			
Relative cover of <i>Festuca rubra</i>			





a



b

