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Original Research Article

Six-year removal of co-dominant grasses alleviated competitive pressure on subdominant grasses but dominant shrub removal had neutral effects in a subalpine ecosystem

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ABSTRACT

The 'stress-gradient hypothesis' predicts increasing facilitative interactions with increasing environmental stress, but it remains unclear if the prevailing type of interaction (i.e. facilitative or competitive) between dominant and subordinate plant species occurring in harsh environments is dependent on the plant functional type. In addition, most plant-species removal experiments in grasslands are short-term (1–2 years), which may imprecisely reflect transient effects arising from methodological limitations. We conducted a dominant species removal experiment in a subalpine ecosystem, containing a mosaic of grass-dominated and shrub-dominated community patches, both of which are common in the subalpine zone of the Qinghai-Tibetan Plateau. We examined the direction and magnitude of the effects of three co-dominant grass and a dominant shrub species on subordinate species richness and biomass over a 6-year period. Removal of the dominant grass species alleviated their competitive pressure on subdominant grasses, which resulted in similar total and grass biomass detected in the final year of the study. By contrast, shrub removal showed no effects on its subordinate species biomass. Furthermore, neither the removal of the dominant shrubs nor the grasses altered their respective subordinate species richness. Thus, in subalpine ecosystems that experience harsh environmental conditions, our results showed that the direction of interactive effects of dominant plant species on subordinate species may be dependent on the plant functional type and are not necessarily facilitative. Furthermore, we showed that longer-term plant-removal experiment observations may be required to better determine the effects of species removal for this subalpine and other montane ecosystem(s).

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1. Introduction

Environmental changes such as drought, warming, nitrogen deposition or land management practices (e.g. fire-suppression and grazing intensity changes) are increasingly common in grassland ecosystems globally (e.g. Shaw et al., 2002; Menge and Field, 2007; Wardle et al., 2011). These changes can strongly influence plant-plant interactions that can, in turn, lead to detrimental shifts in the vegetation community composition (Eldridge et al., 2011; Loranty and Goetz, 2012; Naito and Cairns, 2011; Ratajczak et al., 2012). Grassland vegetation communities are typically dominated by a few plant species, typically grasses (Grime, 1998; Gibson, 2009), which are considered to be superior competitors compared with other co-occurring forb species (Collins and Glenn, 1990; Miles and Knops, 2009). As such, dominant grass species are expected to drive plant community dynamics via competitive interactions (in benign environmental conditions) (e.g. Bertness and Callaway, 1994; Grime, 1998; Smith and Knapp, 2003; Smith et al., 2020). However, it is unclear if competitive or facilitative interactions prevail in driving vegetation dynamics in ecosystems that contain dominant plants from different plant functional groups and typically experience stressful environmental conditions. This is because contrasting plant functional/physiological trait(s) may confer different types of plant-plant interaction with respect to the environmental stress factor(s) the community experiences, and field experiments examining interactions in stressful environments have produced inconsistent findings (Olofsson et al., 1999; Pugnaire et al., 2015; Michalet et al., 2015). The relatively short duration of many of these studies may further confound the findings due to the possible detection of ‘transient’ effects (e.g. unintentional short-term effects arising from possible methodological limitations associated with plant removals or inter-annual environmental variability) (He and Bertness, 2014; Pugnaire et al., 2015). Thus, it is valuable to accurately determine the predominant type of plant-plant interaction occurring in grasslands experiencing stressful abiotic environments over a longer period of time, which can in turn, inform their management and restoration.

The expected net effect of a dominant species on neighboring plants is likely to be influenced by their respective functional/physiological trait(s) with respect to the environmental stress factor(s) the community experiences (Schöb et al., 2013; Soliveres, 2014; Soliveres et al., 2015). For example, in dry alpine regions, net facilitation on neighboring plants is expected to be detected for drought-tolerant shrubs whose canopy-form (e.g. shelter from wind, high ultraviolet irradiation, and/or improve organic matter accumulation; Padilla and Pugnaire, 2006) and resource acquisition strategies (e.g. deeper roots that help transport water from lower to upper soil layers via the hydraulic lift process; Horton and Hart, 1998; Prieto et al., 2011) benefit neighboring understory plant species more than the detriments arising from the shrubs’ competitive abilities. Conversely, in arid grasslands, a hypothetical dominant grass species that is relatively fast-growing, shallow-rooted and less water-use efficient is expected to be highly competitive (e.g. spatial, water, and light availability). However, facilitative effects have been hypothesized to increase with increasing environmental stress (e.g. Callaway et al., 2002; Maestre et al., 2009), with many studies supporting the ‘stress-gradient hypothesis’ (Aksenova et al., 1998; Lortie and Callaway. 2006). On the other hand, there are also studies showing the importance of competitive interactions in harsh conditions (e.g. del Moral, 1983; del Moral, 1985; Olofsson et al., 1999). As such, in ecosystems containing several dominant plant species from contrasting plant functional groups, more experimental tests are required to examine if facilitative effects generally prevail under harsh environmental conditions as hypothesized by the stress-gradient hypothesis (Bertness and Callaway, 1994).

Plant-plant interactions are frequently examined by comparing the performance of a target plant with and without a neighboring plant (Aarssen and Epp, 1990; Diaz-Sierra et al., 2017; Dıaz et al., 2003). At present, there is an increasing trend towards examining the interactions between a target plant species and its community using a similar plant removal approach (Michalet et al., 2015) to determine how the target species influence co-occurring species, plant diversity (Brooker, 2006; Xu et al., 2010), community dynamics, and ecosystem functioning (Grime, 1998; Mariotte, 2014; Huang et al., 2020). However, the use of species removal experiments to determine how plant species influence/interact with its community in cold and stressful environments can be difficult. This is because ecosystem processes (e.g. soil microbial activity, decomposition, nutrient cycling, plant growth rates) in cold and stressful environments tend to be relatively slow (e.g. Atkin et al., 1996; Körner, 2003; Bradford et al., 2016; Wang et al., 2016), and many experimental removal studies were conducted over a relatively short period of time (1–2 years) (e.g. Smith and Knapp, 2003; Xu et al., 2010; Michalet et al., 2015). Thus, it is unclear if previous results reflected transient effects (e.g. short-term local microclimatic disturbances, soil legacy effects from slowly decomposing roots of clipped plants, and/or climatic fluctuations between years) or indicated the definite interactive effect(s) of the removed species (Lepš, 2014; Kardol et al., 2018;). Furthermore, plant removal studies that examined plant interactions over sufficiently long time periods are rare, and may be essential to be able to detect interactive effects accurately (Adler et al., 2010; Lepš, 2014).

A subalpine ecosystem consisting of a mosaic of vegetation patches dominated by either the shrub *Potentilla fruticosa* or co-dominated by three grasses *Roegneria nutans, Elymus nutans* and *Leymus secalinus* is common within the subalpine zone of the Qinghai-Tibetan Plateau (Michalet et al., 2015). A previous study in this study area showed that the removal of a dominant shrub species led to a decrease in the biomass of subdominant grass species, but no net effect on forbs (Xu et al., 2010), while a separate study at the same site showed that the removal of dominant grass species had no net effect on the species richness of forbs (Michalet et al., 2015). However, considering observations by both these studies were done over a relatively short period of time (Xu et al., 2010; Michalet et al., 2015), it remains unclear if the plant community responses (to consistent shoot removal of target species) detected in these studies are indeed due to the removed species. This is because clonal regeneration of clipped species from unremoved belowground plant organs (Qian et al., 2017; Ott et al., 2019) or transient effects may persist (Kardol et al., 2018) as a result of relatively slow ecosystem processes in cold environments (Atkin et al., 1996; Körner, 2003; Germino, 2014; Bradford et al., 2016; Wang et al., 2016).

To better determine if the net effect by dominant species on the subordinate herbaceous species is dependent on the contrasting plant functional traits of the dominant species in a cold and harsh environment, we conducted a longer-term removal experiment over 6 growing seasons within a subalpine ecosystem. Our study site is a subalpine meadow grass-shrubland ecosystem, located at an altitude of 2900m, consisting of a mosaic of meadow grass- and shrub-dominated patches. We hypothesized that: (1) the plant functional type of the dominant species is important to consider under stressful environmental conditions, because the dominant grass species are expected to be superior competitors (Collins and Glenn, 1990; Miles and Knops, 2009), and the removal of the dominant grasses, due to competitive release, will lead to an increase in the richness and biomass of herbaceous subordinate species at the end of the study; while (2) the dominant shrub *P. fruticosa* is expected to function as a facilitative 'nurse' species, because its shrub growth form may alter the microclimate of the herbaceous understory layer to facilitate co-occurring herbaceous species in this climatically harsh subalpine environment, and shrub removal may lead to decreases in both the herbaceous understory richness and biomass. Furthermore, (3) the net direction and magnitude of interaction measured between the first and final year of the study will not be consistent due to transient effects arising from the possible methodological limitations associated with plant (aboveground) removals.

2. Materials and methods

2.1. Study site

This experiment was conducted at the Alpine Meadow and Wetland Ecosystem Research Station of Lanzhou University (34°44′N, 102°53′E), located on the eastern Qinghai-Tibetan Plateau in Hezuo, China, at an altitude of 2900 m. The study site has been fenced since 2007 and is used as winter grazing pastures. The site has an annual grazing intensity of 8–10 Tibetan sheep per ha for 20 days in the winter, and such grazing regime is typical for the regional area (Michalet et al., 2015). The growing season is short with 54 frost-free days annually, and temperature varies from −8.3 °C in December and 11.9 °C in July (Institute of Hezuo Meteorology, China). Mean annual precipitation is 558 mm with 85% of the precipitation occurring during the growing season and with an annual potential evapotranspiration of 1222 mm (Institute of Hezuo Meteorology, China).

The soil type is classified as subalpine meadow soil according to the Chinese soil classification system (Gong, 1999), with a mean soil organic carbon content of 34.9 ± 0.6 g kg^{-1} dry soil and soil total nitrogen of 3.3 ± 0.8 g kg^{-1} dry soil. The mean soil pH is 7.2 ± 0.1.

The study site has a subalpine ecosystem consisting of a mosaic of two subalpine vegetation patch types: subalpine meadow grass and shrub patches. The subalpine meadow grass patch typically contains three co-dominant grass species, *Roegneria nutans, Elymus nutans,* and *Leymus secalinus*, which combine to contribute 45 ± 5% of the total plant cover (Li et al., 2018). The shrub patch is dominated by the only shrub species occurring at our study site, *Potentilla fruticosa*, which accounts for 59 ± 11% of the total plant cover (Xu et al., 2010).

2.2. Experimental design and plot selection

Twenty-four 60 cm × 60 cm plots were established in total within the study site containing a mosaic of subalpine grass- and shrub-dominant meadow patches in 2012. Twelve plots were selected in patches with the dominant shrub present (hereafter also referred to as 'shrub plots') to test for the effects of the dominant shrub species on co-occurring subordinate plant species, while another 12 plots were selected within patches containing all 3 co-dominant grass species (hereafter also referred to as 'grass plots') to test their effects on the subordinate species. All plots were also selected to contain other plant species from the four plant functional groups of legumes, grasses, sedges and forbs. Plots were permanently marked, and all plots were at least 1.5 m apart from one another, and situated within a 1-ha area.

Each removal treatment was randomly assigned to 6 of the 12 plots for each vegetation patch-type, with the remaining 6 plots as control. For both the grass and shrub removal plots, we clipped the 3 co-dominant grasses or the shrub to ground level using a similar approach utilized by Michalet et al. (2015) and Elumeeva et al. (2017). Briefly, all removal treatments were repeated every two weeks between early June and late July each year.

2.3. Vegetation sampling

Species richness of all vascular plants for each 50 cm × 50 cm plot was recorded at the peak of every growing season in early-August (prior to winter grazing). Each plot area was subdivided into four equal sized subplots (25 cm × 25 cm). Aboveground biomass was estimated by clipping one of the four subplots to ground level each year, in early-August, from 2012 to 2017, except for 2016. The clipped vegetation from each plot was further sorted into legumes, grasses, sedges or forbs, and then weighed to obtain biomass data for each plant functional group. All clipped samples were dried for 48 h at 75 °C prior to weighing.

We choose to keep plots relatively small to capture the proximate area that the dominant shrub and grasses are likely to influence their subordinate species. Although we clipped a relatively large proportion within each plot to estimate the plant biomass of each plot, we do not think that this impacted our results. This is because the site is grazed by sheep during winter, and the clipped and unclipped areas of our plots visually appear similar post-winter grazing.

2.4. Statistical analyses

For each patch type, we assessed the effects of dominant species removal treatments (i.e. removal, non-removal), and the possible interaction with temporal effects (i.e. inter-annual environmental variability), on the following response variables: (i) total aboveground biomass and (ii) total species richness of the herbaceous layer, and the respective herbaceous plant functional groups' aboveground biomass [(iii) grass, (iv) sedge, (v) legume, and (vi) forb biomass] and species richness [(vii) grass (dominant + subordinate species), (viii) subordinate grass, (ix) sedge, (x) legume, and (xi) forb species richness]. We used a repeated measures analysis of variance (ANOVA) test ($\alpha = 0.05$) to analyze each response variable, and performed *post-hoc* Tukey HSD tests ($\alpha = 0.05$) if significant differences were detected. For each statistical analysis, we visually assessed data for normality and heterogeneity of variance, and applied appropriate transformations when necessary. All statistical analyses were performed in SPSS 20.0 (IBM Corp., New York, USA).

Shrub data were not included in the calculations or analyses for two reasons: (1) the community responses of the herbaceous layer were of interest in our study; and, (2) comparisons involving the dominant shrub and herbaceous plants are partial because many herbaceous plants are generally smaller (relative to *P. fruticosa*) and are limited spatially within the herbaceous layer stratum, whereas the dominant shrub is less palatable to large herbivore grazers (Elkington and Woodell, 1963) and is intrinsically taller/larger. That is, the shrubs' upper canopy position, woody growth and low palatability allow the accumulation of a large proportion of its woody biomass in the vertical shrub layer stratum over a longer period of time.

3. Results

3.1. Co-dominant grass species removal

Removal of co-dominant grasses significantly reduced grass (dominant + subordinate species) biomass (~21% of total) and total aboveground biomass in the initial four sampling years, but not in the final year (Fig. 1A; Table 1). By contrast, the removal of the co-dominant grasses had little or no effect on the biomass of legumes and sedges (Fig. 1A; Table 1). In addition, within each given year, there was no significant difference in the biomass of forbs between the removal treatments (Fig. 1A; Table 1).

Forbs comprised between 67 and 83% of the total species richness (6–26 total species per plot), and this functional group represented the largest proportion of plant species in the grass plots of this subalpine ecosystem (Fig. 2A; Table 1). Within each given year, the removal of co-dominant grasses had little or no effect on the total, legume or forb species richness between the removal treatments (Fig. 2A; Table 1). Further, the subordinate grass species richness was generally similar between removal treatments in most years, except in 2015 (Fig. 2B; Table 1).

3.2. Dominant shrub species removal

Only forb biomass varied significantly among years, but the total plot biomass and the biomass of each plant functional group were, respectively, similar between the shrub removal treatments within each given year (Fig. 1B; Table 1).

The total species richness of each shrub plot ranged between 6 and 26, and the forb functional group also represented the largest proportion of plant species in the shrub patches (ranging between 50 and 87%) of this subalpine ecosystem (Fig. 2C; Table 1). The total biomass and the biomass of grass and forbs varied significantly among years, but not the biomass of sedges or legumes (Fig. 2C; Table 1). However, within each given year, total species richness and the species richness of each plant functional group were respectively similar between both shrub removal treatments (Fig. 2C; Table 1).

4. Discussion

In this study, we found some indication of competitive effects by co-dominant grasses as hypothesized. However, the competitive effects of the co-dominant grasses were limited only to the subordinate grass species (i.e. marginal aboveground biomass compensation by subordinate grass species). This suggests that plant functional type is important to consider, and that facilitative effects are not necessarily predominant under harsh abiotic environments (e.g. del Moral, 1983; del Moral, 1985; Olofsson et al., 1999). In addition, these competitive effects were only detected in the 6th and final year of the study, which could support our hypothesis that observations of transient effects may diminish over a longer term, and suggest that observations over a longer period may be required to verify effects. Contrary to our hypothesis, the dominant shrub species showed little or no effect on the herbaceous understory layer (i.e. neutral effects). Findings from our longer-term study were in contrast with two shorter-term studies in the same study area that detected either neutral (i.e. Michalet et al., 2015) or facilitative effects (Xu et al., 2010), and studies elsewhere detecting facilitative effects in ecosystems experiencing harsh abiotic environments (e.g. Callaway et al., 2002; Soliveres et al., 2015). Furthermore, the absence of a strong response from subordinate species to dominant grass or shrub species removal, in terms of species richness, may also suggest that factors other than plant-plant interactions could be important drivers of plant community structure in this subalpine

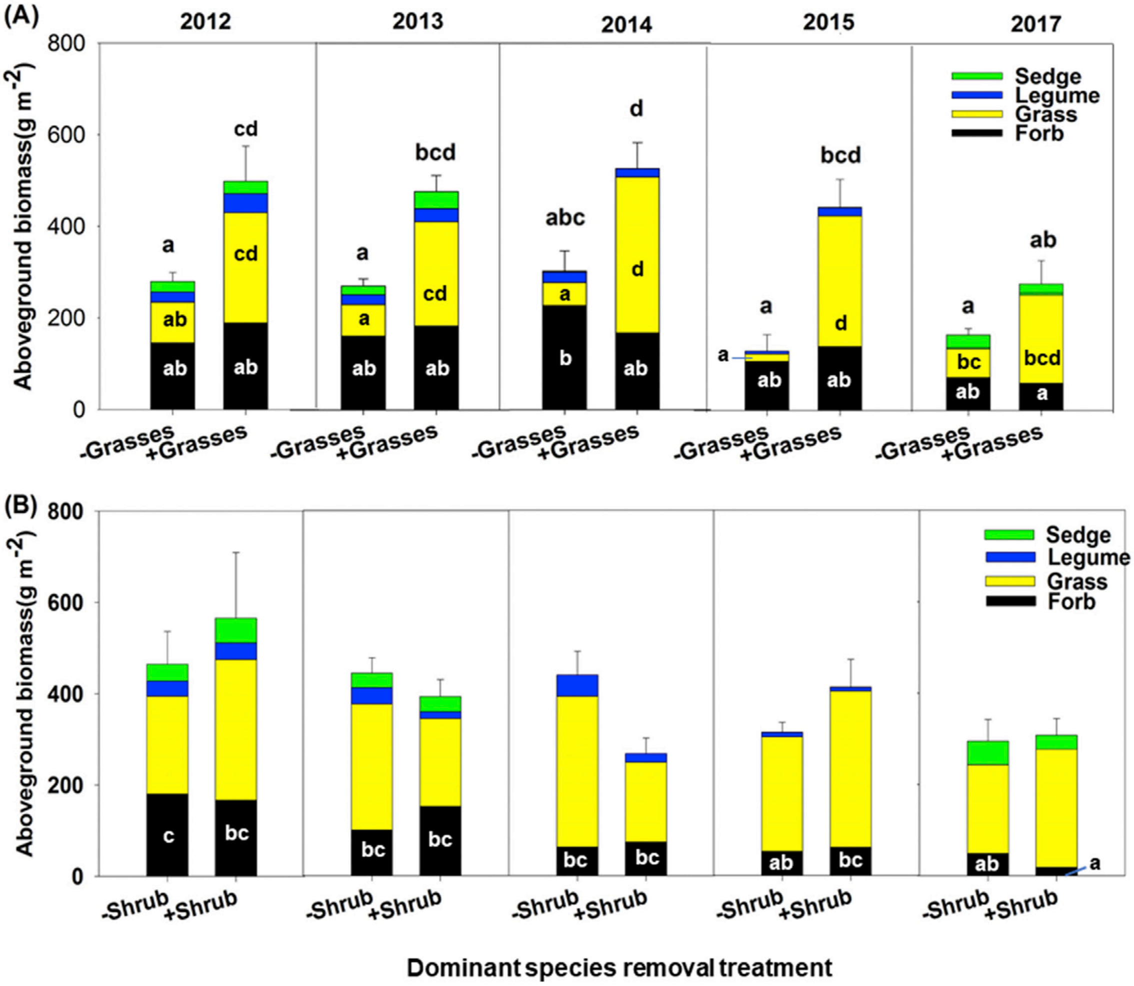

Fig. 1. Effects of (A) co-dominant grass or (B) dominant shrub species removals on the aboveground (dry) biomass of the four plant functional groups of the herbaceous layer from 2012 to 2017, except for 2016. The grasses removed (-Grasses) were *Roegneria nutans, Elymus nutans, Leymus secalinus*, and the shrub removed (-Shrub) was *Potentilla fruticosa*. Bars represent means and error bars represent ±1 standard error. Different letters above each bar indicate significant differences among total aboveground biomass, while different letters within the respective plant functional group-sections indicate differences within the same functional group (*post-hoc* Tukey HSD test following significant repeated measures analysis of variance test on $ln(x+1)$ transformed biomass values; $p \leq 0.05$; Table 1); letters not shown when no significant differences was detected.

ecosystem (Mitchell et al., 2009). Together, our results showed that the plant functional types of the dominant plant species as well as transient effects are important to consider to some extent. However, other factors such as grazing intensity, growing season length, rainfall and temperature may also be important at influencing plant community dynamics in this subalpine and other montane ecosystem(s) (Mitchell et al., 2009). Thus, we recommend future studies to explore the importance of these factors (and their interactions) in combination with species removal in montane environments.

4.1. Biomass responses of the herbaceous layer

We found that the competitive effects of the co-dominant grass species were limited to subordinate species that were functionally similar (i.e. grasses) and likely to occupy the same niche (McKane et al., 2002). This is because the removal of co-dominant grass species showed marginal biomass compensation by subordinate grass species in the 6th year, but had little or no effect on both the biomass and plant species richness of all other plant functional groups examined. In addition, the similar overall aboveground biomass between plot removal treatments in the final year may also suggest that the subordinate grass species may play a functionally equivalent role as the co-dominant grass species to maintain the ecosystem function of biomass production (Smith and Knapp, 2003). Thus, highlighting the importance of functional redundancy of less dominant species in ecosystem functionality (Fetzer et al., 2015) and biodiversity conservation (Wohl et al., 2004). Nonetheless, because biomass compensation was only observed in the 6th and final year, we recommend that future studies should examine

Table 1

Test statistics of repeated measures analysis of variance tests on plant aboveground (dry) biomass (g m^{-2}) and plot (50 cm × 50 cm) species richness of all species or respective plant functional groups of the herbaceous layer in response to co-dominant grass or dominant shrub species removals between 2012 and 2017. Biomass data were analyzed using *ln*(x+1) transformed values. *F*- and *p*-values in bold indicate significant differences ($p \leq 0.05$; $n = 6$). ns = not significant ($p > 0.05$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, / no data.

Variables	Co-dominant grass species removal			Dominant shrub species removal		
	Removal Treatment (T)	Year (Y)	Y*T	Removal Treatment (T)	Year (Y)	Y*T
	F-, *p*-values			*F*-, *p*-values		
Biomass						
Total biomass (g m^{-2})	**19.64*** **	4.68ns	**16.62** **	0.01ns	**6.84* **	0.01ns
Grass (dominant + subordinate) biomass (g m^{-2})	**21.80** **	0.52ns	**27.10** **	0.01ns	0.01ns	0.12ns
Sedge biomass (g m^{-2})	0.91ns	0.02ns	**9.53* **	0.002ns	0.85ns	0.79ns
Legume biomass (g m^{-2})	0.03ns	**8.43* **	1.72ns	0.74ns	**17.53** **	0.11ns
Forb biomass (g m^{-2})	0.18ns	**16.84** **	1.81ns	0.09ns	**38.35*** **	0.48ns
Species richness						
Total species richness (plot^{-1})	0.06ns	0.25 ns	0.77ns	1.44ns	2.46ns	0.04ns
Grass (dominant + subordinate) species richness (plot^{-1})	**11.49** **	**6.01* **	0.79ns	0.10ns	**21.28*** **	1.14ns
Subordinate grass species richness only (plot^{-1})	**9.11* **	**18.76** **	0.07ns	/	/	/
Sedge species richness (plot^{-1})	0.09ns	0.75ns	**14.03** **	0.08ns	**36.80*** **	0.01ns
Legume species richness (plot^{-1})	3.67ns	**7.90* **	0.16ns	0.07ns	1.78ns	0.52ns
Forb species richness (plot^{-1})	2.05ns	2.43ns	0.04ns	0.95ns	4.35ns	0.04ns

removal effects over a longer period of time to validate that observations were not due to transient effects associated with inter-annual environmental variability.

On the other hand, shrub removals had little or no net effect on the total and respective plant functional group biomass of the herbaceous layer in our study, which is in contrast to a number of other studies that show either a nurse/facilitative or competitive effect on understory species in ecosystems exposed to harsh climatic effects (e.g. Bråthen and Lortie, 2016; Pajunen et al., 2011; Xu et al., 2010). However, our results are in line with a six-year shrub-removal experiment in the Alaskan tussock tundra (Bret-Harte et al., 2008). We speculate that many herbaceous understory species in the shrub plots, as well as in our study system, are well-adapted to the harsh conditions of the subalpine environment (Sun et al., 2014). Moreover, resource and vertical spatial partitioning may also occur among the shrub and other understory species (McKane et al., 2002; Hu et al., 2013). Thus, based on our results, the shrub *P. fruticosa* appeared to have little or no influence on herbaceous forage vegetation production in our study system. Nevertheless, because *P. fruticosa* has a widespread distribution across the northern hemisphere, future studies should examine how the effects of climate (e.g. climate warming) may promote shrub expansion into higher altitude ecosystems (Myers-Smith et al., 2011; Eldridge et al., 2011), which in turn has negative impacts on forage production (Pajunen et al., 2011).

4.2. *Transient belowground effects of remnant roots*

The initially lower total and grass aboveground biomass in the co-dominant grass removal plots (2012 - 2015) followed by a shift to similar levels of biomass as the control plots in the final year (2017) could indicate transient effects. The transient effects may have resulted from the short-term recurrence of plant clonal regeneration from remnant belowground organs (Ott et al., 2019) and/or generation of soil legacies through the decomposition of remnant roots of the clipped grasses (Lepš, 2014; Kardol et al., 2018). For example, the unremoved remnant roots could continue to allow dominant grass species to exert their effects on the community via spatial exclusion, maintenance of interactions with subordinate species via clones (Ott et al., 2019), and/or suppressing establishment of new species (Lepš, 2014; Kardol et al., 2018). Although the lack of biomass compensation by subordinate grasses during the initial years could also be, in part, explained by the slower, conservative growth traits of subalpine plants that may constrain their growth responses to the increase in available resources (Bret-Harte et al., 2004), we consider this to be less likely. This is because the dead standing aboveground biomass/vegetation is entirely consumed by sheep during the winter (i.e. unclipped areas of non-removal plots visually similar to clipped subplots), but the vegetation appears to recover to similar functional proportions in the following season.

However, we did not measure root biomass nor root competition in this study, and are unable to ascertain if the subordinate species may have been suppressed by the negative effects from the remnant roots of dominant plant species, or if non-detection of effects were due to conservative growth traits of subalpine plants (Atkin et al., 1996; Körner, 2003; Germino, 2014). Moreover, plant removal studies in this subalpine study system are limited and previous shorter-term experiments

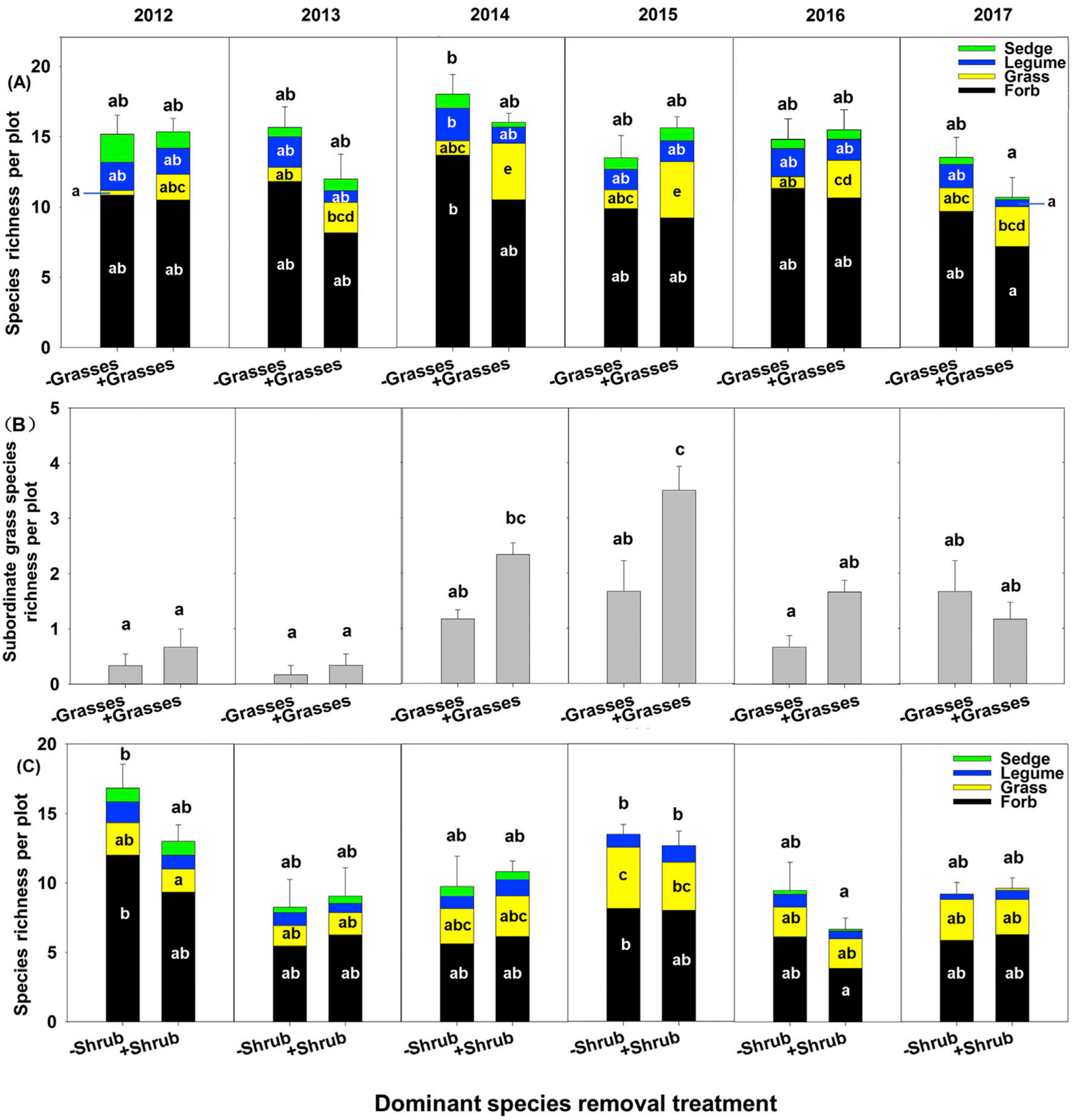

Fig. 2. Effects of (A) co-dominant grass species removals on the plot (50 cm × 50 cm) species richness of four different plant functional groups and (B) subordinate grasses, or the effects of the (C) dominant shrub species removals on the species richness of the herbaceous layer from 2012 to 2017. The grasses removed (-Grasses) were *Roegneria nutans*, *Elymus nutans*, *Leymus secalinus*, and the shrub removed (-Shrub) was *Potentilla fruticosa*. Bars represent means and error bars represent ±1 standard error. Different letters above each bar indicate significant differences among plot total species richness, while different letters within the respective plant functional group-sections indicate differences within the same functional group (*post-hoc* Tukey HSD test following significant repeated measures analysis of variance test; $p \leq 0.05$; Table 1); letters not shown when no significant differences was detected.

have yielded inconsistent results (Xu et al., 2010; Michalet et al., 2015). As such, little is known about the intensity and relative importance of belowground interactions in this subalpine meadow ecosystem. Therefore, future studies should involve more greenhouse or mesocosm studies that allow the examination of both aboveground and belowground plant interactions.

4.3. Seed propagation strategies and grazing intensity may explain the lack of species richness responses

The lack of responses in terms of subordinate species richness, particularly forbs (Li et al., 2019; Siebert et al., 2019), could be due to the seed propagation strategy of many plant species in this subalpine meadow ecosystem. Many non-graminoid species within this subalpine vegetation mosaic are long-lived, slow-growing perennials that likely rely on seed reproduction (cf. clonal reproduction) (Weppler et al., 2006). This is because seeds provide the advantage of being able to remain dormant and persist during extended periods of unfavorable environmental conditions or climatic variability common to montane ecosystems (Bewley, 1997; Baskin and Baskin, 1998). As such, changes in species richness after plant removals may only be detected when new individuals are episodically recruited in response to (a series of) specific environmental cue(s) that occasionally occur (e.g. shorter cold growing seasons; Körner, 2003; Mondoni et al., 2012). As such, species richness responses of the plant community to dominant plant species removal in this study system may require longer-term observations (i.e. >6 years).

Conversely, the current grazing intensity of the study area – while considered typical (Michalet et al., 2015) – may also work to obscure the plant species richness response to dominant species removals. This is because hand-clipped and unclipped areas of our plots tended to appear similar post-winter grazing, which suggests that the current level of sheep herbivory may inhibit seedling establishment of new plant species. However, because grazing only took place in our sites during winter, do note that the effects of herbivory on seedling establishment in our study is likely to affect seeds that germinate in fall, and not those in spring. Nevertheless, grazing is clearly an important factor, and should be considered in future plant-plant interaction studies (Graff et al., 2007; Le Bagousse-Pinguet et al., 2012).

5. Conclusions

After six years of dominant grass species removal in a subalpine ecosystem, our results showed significant biomass compensation of subordinate grass species in response to dominant grass species removals only in the final (i.e. 6th) year. In contrast, shrub removals had little or no effect on subordinate species within the herbaceous layer, which indicated that the direction of interactive effects of dominant plant species on subordinate species may be dependent on the plant functional type. Furthermore, the competitive effects of dominant grass species detected in this subalpine ecosystem suggested that plant-plant interactions are not necessarily facilitative in cold, stressful environments. Taken together, our results highlight the importance of considering plant functional groups as well as conducting longer-term observations of plant-removal experiments to better understand plant-plant interactions in this subalpine and other montane ecosystem(s).

Author contributions

WL conceptualized this study, led field surveys and wrote the first draft. JK, GKP, RS, LJ and HZ interpreted the results and revised the manuscript for readability. XY and HD collected and analyzed data. All authors contributed to this work and approved the final submission. The authors declared no competing interests.

Declaration of competing interest

The authors declared no competing interests

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References

Aarssen, L.W., Epp, G.A., 1990. Neighbour manipulations in natural vegetation a review. J. Veg. Sci. 1, 13–30.
Adler, P.B., Ellner, S.P., Levine, J.M., 2010. Coexistence of perennial plants: an embarrassment of niches. Ecol. Lett. 13, 1019–1029.
Aksenova, A.A., Onipchenko, V.G., Blinnikov, M.S., 1998. Plant interactions in alpine tundra: 13 years of experimental removal of dominant species. Écoscience 5, 258–270.
Atkin, O.K., Botman, B., Lambers, H., 1996. The causes of inherently slow growth in alpine plants: an analysis based on the underlying carbon economies of alpine and lowland *Poa* species. Funct. Ecol. 10, 698–707.
Baskin, C.C., Baskin, J.M., 1998. Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination. Elsevier.
Bertness, M.D., Callaway, R., 1994. Positive interactions in communities. Trends Ecol. Evol. 9, 191–193.
Bewley, J.D., 1997. Seed germination and dormancy. Plant Cell 9, 1055.
Bradford, M.A., Berg, B., Maynard, D.S., Wieder, W.R., Wood, S.A., 2016. Understanding the dominant controls on litter decomposition. J. Ecol. 104, 229–238.
Bråthen, K.A., Lortie, C., 2016. A portfolio effect of shrub canopy height on species richness in both stressful and competitive environments. Funct. Ecol. 30, 60–69.
Bret-Harte, M.S., García, E.A., Sacré, V.M., Whorley, J.R., Wagner, J.L., Lippert, S.C., Chapin, F.S., 2004. Plant and soil responses to neighbour removal and fertilization in Alaskan tussock tundra. J. Ecol. 92, 635–647.
Bret-Harte, M.S., Mack, M.C., Goldsmith, G.R., Sloan, D.B., DeMarco, J., Shaver, G.R., Ray, P.M., Biesinger, Z., Chapin, F.S., 2008. Plant functional types do not predict biomass responses to removal and fertilization in Alaskan tussock tundra. J. Ecol. 96, 713–726.
Brooker, R.W., 2006. Plant–plant interactions and environmental change. New Phytol. 171, 271–284.
Callaway, R.M., Brooker, R., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R., Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E.T., 2002. Positive interactions among alpine plants increase with stress. Nature 417, 844–848.

Collins, S.L., Glenn, S.M., 1990. A hierarchical analysis of species' abundance patterns in grassland vegetation. Am. Nat. 135, 633–648.

del Moral, R., 1983. Competition as a control mechanism in subalpine meadows. Am. J. Bot. 72, 232–245.

del Moral, R., 1985. Competitive effects on the structure of subalpine meadow communities. Can. J. Bot. 63, 1444–1452.

Diaz-Sierra, R., Verwijmeren, M., Rietkerk, M., de Dios, V.R., Baudena, M., 2017. A new family of standardized and symmetric indices for measuring the intensity and importance of plant neighbour effects. Methods Ecol. Evol. 8, 580–591.

Dıaz, S., Symstad, A.J., Stuart Chapin, F., Wardle, D.A., Huenneke, L.F., 2003. Functional diversity revealed by removal experiments. Trends Ecol. Evol. 18, 140–146.

Eldridge, D.J., Bowker, M.A., Maestre, F.T., Roger, E., Reynolds, J.F., Whitford, W.G., 2011. Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. Ecol. Lett. 14, 709–722.

Elkington, T.T., Woodell, S.R.J., 1963. *Potentilla fruticosa* L. J. Ecol. 51, 769–781.

Elumeeva, T.G., Onipchenko, V.G., Werger, M.J.A., 2017. No other species can replace them: evidence for the key role of dominants in an alpine *Festuca varia* grassland. J. Veg. Sci. 28, 674–683.

Fetzer, I., Johst, K., Schäwe, R., Banitz, T., Harms, H., Chatzinotas, A., 2015. The extent of functional redundancy changes as species' roles shift in different environments. Proc. Natl. Acad. Sci. Unit. States Am. 112, 14888–14893.

Germino, M.J., 2014. Plants in alpine environments. In: Monson, R. (Ed.), Ecology and the Environment. Springer, New York, NY.

Gibson, D.J., 2009. Grasses and Grassland Ecology 1–2. Oxford University Press.

Gong, Z.T., 1999. Chinese Soil Taxonomy: Theories Methods and Applications. Science Press, Beijing.

Graff, P., Aguiar, M.R., Chaneton, E.J., 2007. Shifts in positive and negative plant interactions along a grazing intensity gradient. Ecology 88, 188–199.

Grime, J.P., 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. J. Ecol. 86, 902–910.

He, Q., Bertness, M.D., 2014. Extreme stresses, niches, and positive species interactions along stress gradients. Ecology 95, 1437–1443.

Horton, J.L., Hart, S.C., 1998. Hydraulic lift: a potentially important ecosystem process. Trends Ecol. Evol. 13, 232–235.

Hu, J., Hopping, K.A., Bump, J.K., Kang, S., Klein, J.A., 2013. Climate change and water use partitioning by different plant functional groups in a grassland on the Tibetan Plateau. PLoS ONE 8, e75503.

Huang, M., Liu, X., Zhou, S., 2020. Asynchrony among species and functional groups and temporal stability under perturbations: patterns and consequences. J. Ecol. In press.

Kardol, P., Fanin, N., Wardle, D.A., 2018. Long-term effects of species loss on community properties across contrasting ecosystems. Nature 557, 710–713.

Körner, C., 2003. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems., second ed. Springer, Berlin.

Li, W.J., Zhang, R.L., Liu, S.S., Li, W.X., Li, J.H., Zhou, H.K., Knops, J.M.H., 2018. Effect of loss of plant functional group and simulated nitrogen deposition on subalpine ecosystem properties on the Tibetan Plateau. Sci. Total Environ. 631–632, 289–297.

Le Bagousse-Pinguet, Y., Gross, E.M., Straile, D., 2012. Release from competition and protection determine the outcome of plant interactions along a grazing gradient. Oikos 121, 95–101.

Lepš, J., 2014. Scale-and time-dependent effects of fertilization, mowing and dominant removal on a grassland community during a 15-year experiment. J. Appl. Ecol. 51, 978–987.

Li, W.J., Liu, S.S., Li, J.H., Zhang, R.L., Rang, K.Z.C., Zhou, H.K., Yao, B.Q., Wang, J.F., 2019. Plant traits response to grazing exclusion by fencing assessed via multiple classification approach: a case from a subalpine meadow. Pol. J. Ecol. 67, 33–52.

Loranty, M.M., Goetz, S.J., 2012. Shrub expansion and climate feedbacks in Arctic tundra. Environ. Res. Lett. 7, 011005.

Lortie, C.J., Callaway, R.M., 2006. Re-analysis of meta-analysis: support for the stress-gradient hypothesis. J. Ecol. 94, 7–16.

Maestre, F.T., Callaway, R.M., Valladares, F., Lortie, C.J., 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J. Ecol. 97, 199–205.

Mariotte, P., 2014. Do subordinate species punch above their weight? Evidence from above- and below-ground. New Phytol. 203, 16–21.

McKane, R.B., Johnson, L.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E., Kielland, K., Kwiatkowski, B.L., Laundre, J.A., Murray, G., 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415, 68–71.

Menge, D.N., Field, C.B., 2007. Simulated global changes alter phosphorus demand in annual grassland. Global Change Biol. 13, 2582–2591.

Michalet, R., Chen, S.Y, An, L.Z, Wang, X.T, et al., 2015. Communities: are they groups of hidden interactions? J. Veg. Sci. 26, 207–218.

Miles, E.K., Knops, J.M., 2009. Shifting dominance from native C_4 to non-native C_3 grasses: relationships to community diversity. Oikos 118, 1844–1853.

Mitchell, M.G., Cahill Jr., J.F., Hik, D.S., 2009. Plant interactions are unimportant in a subarctic-alpine plant community. Ecology 90, 2360–2367.

Mondoni, A., Rossi, G., Orsenigo, S., Probert, R.J., 2012. Climate warming could shift the timing of seed germination in alpine plants. Ann. Bot. 110, 155–164.

Myers-Smith, I.H., Forbes, B.C., Wilmking, M., Hallinger, M., et al., 2011. Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. Environ. Res. Lett. 6, 045509.

Naito, A.T., Cairns, D.M., 2011. Patterns and processes of global shrub expansion. Prog. Phys. Geogr. 35, 423–442.

Olofsson, J., Moen, J., Oksanen, L., 1999. On the balance between positive and negative plant interactions in harsh environments. Oikos 86, 539–543.

Ott, J.P., Klimešová, J., Hartnett, D.C., 2019. The ecology and significance of below-ground bud banks in plants. Ann. Bot. 123, 1099–1118.

Padilla, F.M., Pugnaire, F.I., 2006. The role of nurse plants in the restoration of degraded environments. Front. Ecol. Environ. 4, 196–202.

Pajunen, A.M., Oksanen, J., Virtanen, R., 2011. Impact of shrub canopies on understorey vegetation in western Eurasian tundra. J. Veg. Sci. 22, 837–846.

Prieto, I., Padilla, F.M., Armas, C., Pugnaire, F.I., 2011. The role of hydraulic lift on seedling establishment under a nurse plant species in a semi-arid environment. Perspect. Plant Ecol. Evol. Sys. 13, 181–187.

Pugnaire, F.I., Zhang, L., Li, R., Luo, T., 2015. No evidence of facilitation collapse in the Tibetan plateau. J. Veg. Sci. 26, 233–242.

Qian, J., Wang, Z., Liu, Z., Busso, C.A., 2017. Belowground bud bank responses to grazing intensity in the inner-Mongolia steppe, China. Land Degrad. Dev. 28, 822–832.

Ratajczak, Z., Nippert, J.B., Collins, S.L., 2012. Woody encroachment decreases diversity across North American grasslands and savannas. Ecology 93, 697–703.

Schöb, C., Armas, C., Guler, M., Prieto, I., Pugnaire, F., 2013. Variability in functional traits mediates plant interactions along stress gradients. J. Ecol. 101, 753–762.

Shaw, M.R., Zavaleta, E.S., Chiariello, N.R., Cleland, E.E., Mooney, H.A., Field, C.B., 2002. Grassland responses to global environmental changes suppressed by elevated CO_2. Science 298, 1987–1990.

Siebert, F., Dreber, N., 2019. Forb ecology research in dry African savannas: knowledge, gaps, and future perspectives. Ecol. Evol. 9, 7875–7891.

Smith, M.D., Knapp, A.K., 2003. Dominant species maintain ecosystem function with non-random species loss. Ecol. Lett. 6, 509–517.

Smith, M.D., Koerner, S.E., Knapp, A.K., Avolio, M.L., Chaves, F.A., Denton, E.M., Hoover, D.L., 2020. Mass ratio effects underlie ecosystem responses to environmental change. Journal of Ecology 108 (3), 855–864.

Soliveres, S., 2014. Functional traits determine plant co-occurrence more than environment or evolutionary relatedness in global drylands. Perspect. Plant Ecol. Evol. Sys. 16, 164–173.

Soliveres, S., Smit, C., Maestre, F.T., 2015. Moving forward on facilitation research: response to changing environments and effects on the diversity, functioning and evolution of plant communities. Biol. Rev. 90, 297–313.

Sun, H., Niu, Y., Chen, Y.S., Song, B., Liu, C.Q., Peng, D.L., Chen, J.G., Yang, Y., 2014. Survival and reproduction of plant species in the Qinghai-Tibet Plateau. J. Sys. Evol. 52, 378–396.

Wang, P., Heijmans, M.M., Mommer, L., van Ruijven, J., Maximov, T.C., Berendse, F., 2016. Belowground plant biomass allocation in tundra ecosystems and its relationship with temperature. Environ. Res. Lett. 11, 055003.

Wardle, D.A., Bardgett, R.D., Callaway, R.M., Van der Putten, W.H., 2011. Terrestrial ecosystem responses to species gains and losses. Science 332, 1273–1277.

Weppler, T., Stoll, P., Stöcklin, J., 2006. The relative importance of sexual and clonal reproduction for population growth in the long-lived alpine plant *Geum reptans*. J. Ecol. 94, 869–879.

Wohl, D.L., Arora, S., Gladstone, J.R., 2004. Functional redundancy supports biodiversity and ecosystem function in a closed and constant environment. Ecology 85, 1534–1540.

Xu, J., Michalet, R., Zhang, J.L., Wang, G., Chu, C.J., Xiao, S., 2010. Assessing facilitative responses to a nurse shrub at the community level: the example of *Potentilla fruticosa* in a sub-alpine grassland of northwest China. Plant Biol. 12, 780–787.