Original article

On the relevance of facilitation in alpine meadow communities: An experimental assessment with multiple species differing in their ecological optimum

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ARTICLE INFO

ABSTRACT

It has been recently shown that co-occurring species with different stress tolerance and ecological optima may show differential responses to the same neighbors in a given community. Despite this, most facilitation studies have evaluated a given plant-plant or plant-group of plants interactions. We conducted a removal experiment in an alpine meadow of the Qing-Hai Tibet Plateau to test two hypotheses: (i) facilitation will be the dominant plant-plant interaction in this stressful environment; and (ii) the magnitude of positive interactions among species will differ, with those species closer to their ecological optimum (i.e. those species more abundant in the community) showing competitive or neutral interactions. The experiment was conducted using seven co-occurring species differing in their relative abundances within the community. Neighbors positively affected the growth in biomass and height of all the studied species. Thus, and as predicted by our first hypothesis, facilitation was the dominant interaction within the studied community. According to our second hypothesis, biomass growth responses to neighbor removal were species-specific, with the dominating species showing a weak facilitative response. Our results contribute to advance our knowledge on the interplay of facilitation and competition in multi-species communities, and can be used to refine current conceptual models regarding the outcome of plant-plant interactions and abiotic stress.

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1. Introduction

Over the past decades, a large number of empirical and theoretical studies have shown that plant-plant interactions, either positive (facilitation) or negative (competition), are crucial determinants of the composition and dynamics of plant communities, thus affecting ecosystem structure and functioning (Callaway, 1995; Bruno et al., 2003; Cheng et al., 2006; Kikvidze et al., 2006). It has been also empirically shown that both facilitation and competition act simultaneously (Holzapfel and Mahall, 1999; Maestre et al., 2003), and that facilitation is more likely to occur in physically stressful environments than in physically favorable environments (Callaway, 1995; Flores and Jurado,

2003). Environments where facilitation has been widely documented include salt marshes (Bertness and Ewanchuk, 2002), alpine areas (Choler et al., 2001; Callaway et al., 2002; Klanderud and Totland, 2005), arid environments (Callaway, 1995; Maestre et al., 2003) and sub-alpine meadows (Kikvidze et al., 2001, 2006).

Although it is well known that species differ widely in their physiological and ecological optimum, most facilitation studies have evaluated a given plant-plant or plant-group of plants interactions (for reviews see Callaway, 1995; Flores and Jurado, 2003; Maestre et al., 2005). As recently shown by Liancourt et al. (2005), the response of a given species to its neighbors is strongly related to both its tolerance to stress, and to its competitive response. For example, Choler et al. (2001) showed that the type (competition or facilitation) and magnitude of interspecific interactions were dependent on the distributional optimum of the target species, with negative interactions in the most favorable part of the niche and positive interactions in its most constrained part (Choler et al., 2001). Thus, co-occurring species with different stress tolerance and ecological optimum may show differential responses to the same neighbors in a given community. This issue, often overlooked, must be considered when extrapolating and interpreting the results of studies using a single pair of interacting species (Maestre et al., 2006).

The use of multiple, co-occurring, species when evaluating plant responses to neighbors is highly relevant to assessing the importance and prevalence of facilitation in a given community, and to accurately test theories predicting how the net outcome of plant-plant interactions will change with abiotic stress (for a discussion of this issue see Lortie and Callaway, 2006; Maestre et al., 2006). In this article we report the results of a removal experiment conducted in an alpine meadow of the Tibet Plateau, to evaluate the effects of neighbors on seven co-occurring species differing in their competitive ability and ecological optimum. To our knowledge, few studies have evaluated plant-plant interactions in alpine meadows previously (Egerton and Wilson, 1993; Wilson, 1993; Zhang and Welker, 1996; Dong et al., 2005; Song et al., 2006). We tested two hypotheses: (i) facilitation will be the dominant plant-plant interaction in the studied community (Bertness and Callaway, 1994); and (ii) the magnitude of positive interactions among species will differ, with those species closer to their ecological optimum (i.e. those species more abundant in the community) showing competitive or neutral interactions (Choler et al., 2001).

2. Materials and methods

2.1. Study area

Our study was conducted at the Maqu alpine meadow (33°58′N, 101°53′E; 3500 m a.s.l.; 5° slope), in the eastern part of the Qing-Hai Tibet Plateau, China. Its average temperature is approximately 1.2 °C, with January and July averages of −10 °C and 11.7 °C respectively, and the average annual precipitation is 620 mm (averages for the past 35 years from Maqu Weather Station, Luo et al., 2006). It is classified as alpine meadow soil (Gong et al., 1999). The vegetation is a species-rich

(40–50 species/0.25 m²) alpine meadow, dominated by sedges such as *Scirpus pumilus* Vahl, *Kobresia capillifolia* (Decne.) C.B. Clarke and *Kobresia macrantha* Boeck (Wang et al., 2005, 2006). Plant growth is usually nutrient limited in this ecosystem, with major growth responses occurring in August (Song et al., 2006; Xu et al., 2006).

2.2. Experimental design and measurements

To test our hypotheses, we carried out a removal experiment during the growing season of 2006 in a south-facing slope protected from grazing by a fence. Seven co-occurring target species were chosen for our experiment: the forbs *Halenia corniculata* (L.) Cornaz and *Thermopsis lanceolata* R. Brown, the grasses *Elymus nutans* Griseb and *Deschampsia cespitosa* (L.) Beauv, and the sedges *Scirpus pumilus* Vahl, *Kobresia capillifolia* (Decne.) C.B. Clarke and *Kobresia macrantha* Boeck. Based on their abundance in the study area, and following the logic of Liancourt et al. (2005), the sedges (*S. pumilus*, *K. capillifolia* and *K. macrantha*) should be closer to their ecological optimum than the rest of the studied species (Table 1).

Plant-plant interactions were assessed by the removal of aboveground biomass of all neighboring plants within approximately 15 cm of a target individual, and by comparing target plant performance to that of paired controls in which neighbors were left intact (Kikvidze et al., 2001, 2006; Liancourt et al., 2005). At the beginning of the growing season (June), 9–11 paired individuals, with the same shoot size and number of leaves, were selected for each of the seven target species. These pairs were located as close as possible to each other, to minimize differences in microclimate, but far apart enough so they were unlikely to influence each other (distance between paired individuals ranging from 35 to 60 cm). After the selection of pairs, one of the individuals was randomly selected for neighbor removal. At the end of the experiment (September), we harvested the above-ground biomass of the control and experimental plants and dried them at 80 °C until constant weight. Before that, we measured the height of all the paired plants. In addition, we collected soil cores (0–10 cm depth, 4 cm diameter) from paired plants (with and

Table 1 – Density of the studied species in the alpine meadow studied and in a subalpine meadow (Hezuo; 34°55′N, 102°53′E; 2900 m a.s.l.) located 196 km away from the study site

Species	Alpine meadow	Subalpine meadow
Halenia corniculata	16.28 ± 3.83	14.88 ± 0.69
Thermopsis lanceolata	3.30 ± 0.36	2.89 ± 0.63
Elymus nutans	22.30 ± 1.42	19.13 ± 1.81
Desohampsia caespitose	8.81 ± 2.42	6.91 ± 0.99
Scirpus pumilus	55.23 ± 11.67	31.64 ± 6.91
Kobresia capillifolia	31.10 ± 3.55	15.09 ± 1.49
Kobresia macrantha	172.89 ± 29.89	32.36 ± 7.20

At each site, 50 cm × 50 cm sampling quadrats were randomly arranged in August, during the peak of the growing season, and the number of ramets of every species in each quadrat was counted. Data represent means ± SE ($n = 20$ for alpine meadow and $n = 15$ for subalpine meadow).

without neighbors) three times during the growing season (mid July, mid August and early September) to determine soil moisture. Ten different pairs were randomly selected in each sampling date. However, and to minimize the influence of soil sampling on plant performance, we excluded those paired plants sampled before. The soil water content of these samples was determined gravimetrically.

2.3.   *Statistical analyses*

From biomass and height data we calculated the relative neighbor effect (RNE) as follows: $RNE = (X_t - X_c)/X$, where X_t and X_c are target biomass or height on the absence and presence of neighbors, respectively, and X is either X_t (when $X_t > X_c$) or X_c (when $X_c > X_t$). To facilitate interpretation of data, RNE values were multiplied by -1 (Callaway et al., 2002). RNE ranges from -1 to $+1$, with negative values indicating competition and positive values facilitation.

To test whether RNE values of biomass ($RNE_{biomass}$) and height (RNE_{height}) differed from 0 (no effect of neighbors on plant performance), *t*-tests were used. To evaluate differences among the target species in the effect of neighbors, a one-way ANOVA with species as a fixed factor was used. RNE values obtained for biomass and height followed a normal distribution, but the former did not meet the homogeneity of variances assumption. Therefore, Dunnett's T3 test, which is robust to this deviation (Day and Quinn, 1989), was used for post-hoc analyses of these data. Post-hoc analyses of height data were conducted with the Tukey's HSD test. We evaluated the relationship between RNE (biomass and height) and species abundance using linear regressions. RNE and abundance data were in all cases $\log_{10}$-transformed prior to these analyses to meet their assumptions. Soil moisture data were analyzed with a split-plot ANOVA, with sampling date as between-plot fixed factor, treatment (control vs. neighbor removal) as within plot fixed factor and block (each block was formed by a given pair sampled) as a random factor nested within sampling date (Quinn and Keough, 2002). These data were $\log_{10}$-transformed prior to ANOVA to meet the assumptions of the analysis. All the statistical analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). *P* values were not adjusted for multiple testing as this approach is considered overly conservative (Gotelli and Ellison, 2004).

3.   Results

Neighbors had a positive effect on the biomass and height growth of all the species evaluated (Fig. 1). The magnitude of this effect, however, differed among the target species. For biomass data, the sedges (*S. pumilus*, *K. capillifolia* and *K. macrantha*) showed a weaker positive response to neighbors than the other species (Fig. 1). The magnitude of the effect of neighbors on height was, for most of the evaluated species, much lower than that on biomass. This effect was similar among all the target species except for *S. pumilus*, which showed a significantly lower response to neighbors than that observed for *K. macrantha*. Species density and $RNE_{biomass}$ showed a negative relationship, but the relationship between
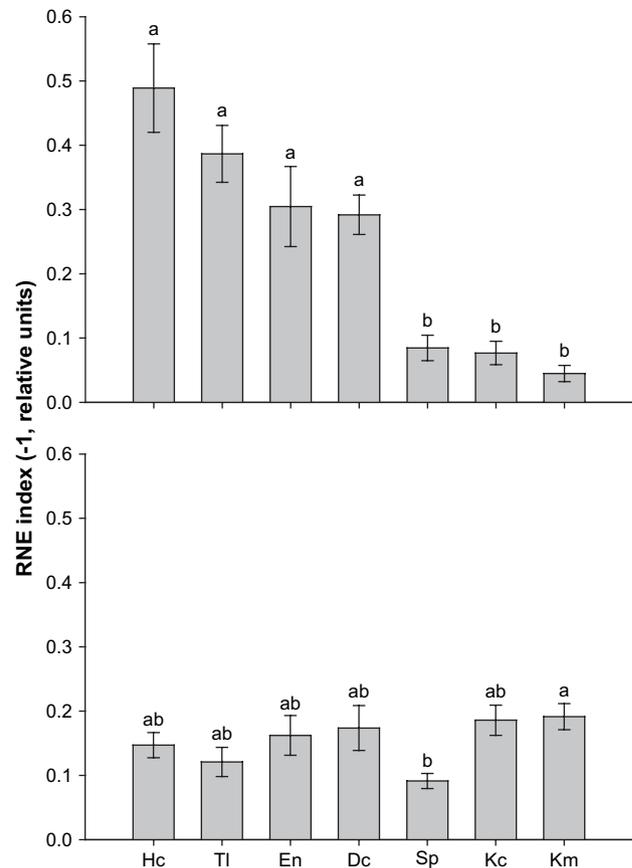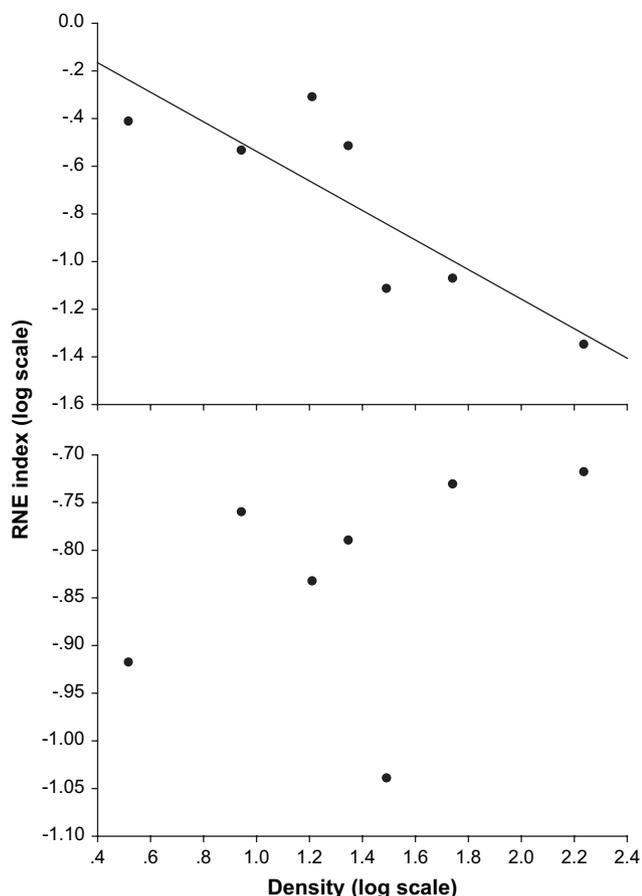

Fig. 1 – Results of the relative neighbor index (RNE) for biomass growth (upper panel) and height (lower panel) of the target species. All the species had average values significantly different from 0 (for growth, *t*-test, P < 0.007 in all cases; for height, *t*-test, P < 0.002 in all cases). Data represent means ± 1 SE (*n* = 9–11). Shared lowercase letters indicate no significant differences among species (ANOVA, P > 0.05). Hc = *Halenia corniculata*, Tl = *Thermposis lanceolata*, En = *Elymus nutans*, Dc = *Deschampsia cespitosa*, Sp = *Scirpus pumilus*, Kc = *Kobresia capillifolia*, and Km = *Kobresia macrantha*. See Appendix 1 for raw biomass data.

the former and RNE_{height} was not significant (Fig. 2). Neighbors had a positive effect on soil moisture throughout the growing season, albeit the magnitude of this effect differed among sampling periods (Fig. 3; Table 2).

4.   Discussion

As predicted by our first hypothesis, neighbors positively affected the growth of all the studied species. These results agree with previous studies conducted in subalpine meadows of the Caucasus Mountains (Callaway et al., 2002; Kikvidze et al., 2001, 2006). Interestingly, they do not fully match the results obtained by Song et al. (2006) in anotheralpine meadow of the Tibetan Plateau. In that study, conducted with four

Fig. 2 – The relationships of the relative neighbor index (RNE) with density for biomass growth (upper panel) and height (lower panel) of the target species. For biomass, $r = -0.8389$, $P = 0.0183$; for height, $r = 0.3986$, $P = 0.3758$. Significant relationships ($P < 0.05$) are denoted with a bold line.

species, two species showed positive biomass responses to neighbor removal (*Kobresia humilis* and *Stipa aliena*), while the remaining two showed no significant responses to this treatment (*Saussurea superba* and *E. nutans*). Our study site differed from that of Song et al. (2006) in important aspects, including average temperature (1.2 vs. 1.7 °C), average rainfall (620 vs. 560 mm) and the identity of the species dominating the meadow (*K. macrantha* vs. *K. humilis*). Given the strong influence of climate and the identity of the interacting plants on the response of a target species to neighbors (Maestre and Cortina, 2004; Liancourt et al., 2005; Maestre et al., 2005), these discrepancies are not fully surprising. According to our second hypothesis, growth responses to neighbor removal were species-specific. Sedges, which dominate the alpine meadow and are considered to be much closer to their ecological optimum, showed a weak facilitative response, while the magnitude of such a response was much higher in non-dominating species (Fig. 1). Furthermore, we found a clear negative relationship between the magnitude of the net biomass response of a given species to neighbors and its density
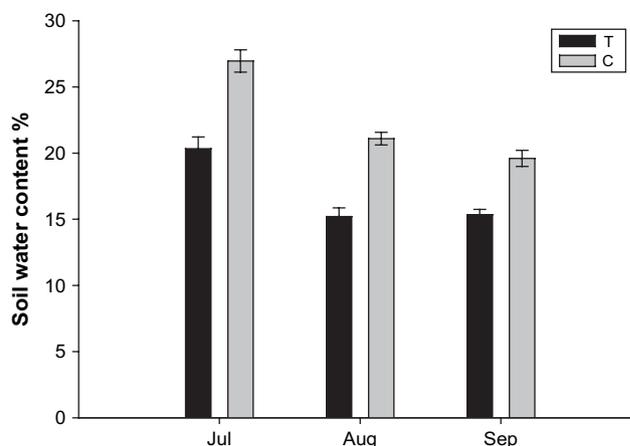
Fig. 3 – Soil water content in the treatment (T) and control (C) plots during the growing season. Data represent means ± SE ($n = 10$).

(Fig. 2). This is consistent with the results of Liancourt et al. (2005) and Song et al. (2006).

The magnitude of the positive effect of neighbors detected on biomass was much higher than that detected using height as the estimator of plant performance. This was likely due to aboveground competition by light, which has been empirically demonstrated in alpine meadows of the Tibetan Plateau (Qiu and Du, 2004; Song et al., 2006; Wu et al., 2006). Therefore, and as found in other environments (Armas and Pugnaire, 2005; Holzapfel and Mahall, 1999; Maestre et al., 2003), our results and those of previous studies (Song et al., 2006) suggest that both facilitative and competitive interactions are operating in alpine meadows of the Tibetan Plateau, and that the net outcome of a given plant-plant interaction in this environment fluctuates with small changes in abiotic stress conditions.

Our experimental design and measurements do not allow us to fully elucidate the mechanisms underlying the responses observed, but some clues can be obtained from them. Soil moisture was higher in the presence of neighbors throughout the growing season (Fig. 3; Table 2), despite the increased water uptake in this treatment (over 90% of roots in alpine meadows of the Tibetan Plateau are concentrated in the upper 15 cm of soil; Cao et al., 2004). Therefore, it is likely that the shade from neighbors decreased soil water evaporation and improved the overall soil moisture status, as found in other stressful environments (Domingo et al., 1999; Maestre et al., 2003; Barberá et al., 2006). During the growing season (from June to September), temperature fluctuates substantially in our study area, with differences from day to night ranging from 15 to 20 °C (data from Maqu Weather Station). In addition, our study site is also prone to heavy winds as well as hailstones during this period (Chengjin Chu, personal observation). The amelioration of negative effects on plant growth of low temperatures, strong winds and hailstones by neighbors is well known in alpine environments (e.g. Choler et al., 2001; Callaway et al., 2002; Cavieres et al., 2006). Therefore, target plants growing in the presence of neighbors would

Table 2 – Results of a three-way split-plot ANOVA showing the effects of sampling period and treatment (neighbor removal) on the soil water content					
Source of variation	SS	df	MS	F	P
Sampling period	0.202	2	0.101	24.30	**< 0.001**
Block (Sampling period)	0.112	27	0.004		
Treatment	0.235	1	0.235	476.85	**< 0.001**
Sampling period × Treatment	0.004	2	0.002	3.87	**0.035**
Block (Sampling period) × Treatment	0.013	27	0.0005		
P values below 0.05 are in bold.					

have benefited from improved microclimatic conditions, and this could also explain the responses observed.

Our results showed that facilitation was a significant ecological process in the alpine meadow explored, and that different species had different responses to the removal of neighbors. The fact that facilitation dominated over competition suggests that this interaction could be critical in determining the structure and composition of these species-rich ecosystems. However, as we only evaluated this interaction in seven of the 40–50 species that can be typically found in alpine meadows such as studied, this affirmation should be taken with some caution. Our results contribute to advance our knowledge on the interplay between facilitation and competition in multi-species communities, and can be used to refine current conceptual models regarding the outcome of plant-plant interactions and abiotic stress, and to establish appropriate conservation and management strategies in alpine meadows. Further research will be required to explore in more depth the mechanisms underlying the results observed, and to quantify the relative importance of facilitation against other abiotic and biotic factors as a driver of ecosystem structure and functioning in alpine meadows.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.actao.2007.10.002.

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