Net outcome of competition and facilitation in a wet meadow changes with plant's life stage and community productivity

Poměr vlivu kompetice a facilitace na vlhké louce se mění během ontogeneze rostliny a v závislosti na produktivitě prostředí

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Positive and negative plant-plant interactions generally co-occur in communities but their relative importance should depend on site productivity; the importance of facilitation is expected to increase and that of competition to decrease with the adversity of the environment. Moreover the effect of surrounding vegetation on an individual's performance can vary depending on the individual's life stage and on the variables used to characterize an individual's performance. To test these theories, we established a transplant experiment in a wet meadow in order to assess the effects of surrounding vegetation on individual plants under varying environmental conditions and changes in these effects during an individual's development within one growing season. We asked whether (i) the net effects of plant interactions differ with differences in productivity and disturbance, and (ii) the net effects of interactions differ according to life stage, species and the performance measure used. We utilized a long-term experiment with three treatments (application of fertilizer, mowing and removal of the dominant species) in a full factorial design, yielding eight combinations, with three replicate plots per combination. In each plot four individuals of three species (*Lysimachia vulgaris*, *Prunella vulgaris* and *Plantago lanceolata*) were transplanted, two into gaps and two into intact vegetation. Survival (alive/dead) of each individual was recorded twice during the season. The presence of flowers and above- and below-ground biomass were recorded at the end of the transplant experiment. The survival of transplants early in the season was higher when growing among vegetation, indicating that at an early stage in its life the net

effect of the surrounding vegetation was positive. At later stages, competition became more important and had a negative effect on biomass production and plant reproduction. This negative effect was more pronounced in fertilized plots while the effect of mowing and removal of dominant species on plant interactions was generally negligible. Our results indicate, particularly under more productive conditions, the importance of changes in the net outcome of plant interactions during different life stages, highlighting the dynamic nature of positive and negative interactions within a community.

K e y w o r d s : above-ground biomass, disturbance, fertilization, *Molinia* removal, mowing, neighbour-effect, ontogenetic shift, plant-plant interactions, root-shoot ratio, survival

Introduction

Competition between plants is proposed as the dominant interaction between individuals in productive habitats (Grime 1973). Whereas some authors suggest that the intensity of competition increases with increasing productivity or decreasing stress (Grime 1973), others suggest that the intensity of competition does not change along productivity gradients (Tilman 1987). Discrepancies in existing empirical evidence may to a large extent be due to the use of different methods to quantify the effect of competition (Grace 1993). Nevertheless, both theoretical considerations and empirical data suggest that the relative importance of competition for light increases with productivity, while competition for nutrients decreases (Tilman 1987, Wilson & Tilman 1993, Lepš 1999). Competition for light is size-asymmetric, which implies that with increasing productivity we can expect an increase in competitive effect of tall over small individuals.

In addition to competition, positive interactions among plants (i.e. facilitation) are also important in shaping plant communities (Michalet et al. 2006, Le Bagousse-Pinguet et al. 2012, 2014a, b, Kelemen et al. 2015). Facilitation is considered to occur more frequently in plant communities subjected to high stress and/or disturbance (Bertness & Callaway 1994, Brooker & Callaghan 1998). However, recent studies indicate that facilitation may also be important in communities characterized as of intermediate productivity (Maestre et al. 2005, Michalet et al. 2006, Gross et al. 2010, Holmgren & Scheffer 2010, Malkinson & Tielbörger 2010, Le Bagousse-Pinguet et al. 2014b), particularly those subject to severe herbivory (Smit et al. 2009). For example, Holmgren & Scheffer (2010) propose that facilitation has an important role in moderate conditions, particularly when sensitive life stages benefit from the surrounding vegetation improving the environmental conditions.

While the relative importance of positive and negative interactions between plants should depend on site productivity, the evaluation of these effects is also affected by the variables used to characterize an individual's performance. For example the effect of biotic interactions on survival can differ from their effect on growth (Brooker et al. 2008, Maestre et al. 2009). Facilitative and competitive interactions should co-occur in various types of environments, but affect individuals differently depending on their life stage and size (Grubb 1977, Liancourt et al. 2005, Lamb & Cahill 2006, Schiffers & Tielbörger 2006). Seedling establishment might be facilitated by surrounding vegetation (Ryser 1993, Le Roux et al. 2013) but competition should prevail during the adult stage (Eckstein 2005, Liancourt et al. 2005, Le Roux et al. 2013). Consequently, we can expect changes in the net outcome of competitive and facilitative interactions during the life

span of an individual. It remains unclear, though, how this net outcome changes in different types of environment and how it affects various aspects of performance of an individual. Differences in the net outcome of plant interactions, particularly in terms of survival and growth, may help us arrive at an understanding of the mechanisms causing the ontogenetic shifts in plant interactions (Eckstein 2005, Schiffers & Tielbörger 2006). They have been mostly documented in stressful habitats (e.g. Armas & Pugnaire 2009, Soliveres et al. 2010, le Roux et al. 2013); for example, Armas & Pugnaire (2009) document an ontogenetic shift in the interaction between two dominant shrubs in semiarid shrub land. Whereas mature shrubs facilitated the establishment of seedlings of both species, the relationship turned to asymmetric competition in the later life stages. Nevertheless, ontogenetic shifts may also play an important role in driving plant communities in benign environments (Holmgren & Scheffer 2010, McIntire & Fajardo 2013).

The main objective of this study is to assess the differences in the net outcome of plant interactions in a wet oligotrophic meadow subjected to combinations of experimental treatments (fertilizer, mowing and removing the dominant plant species). We determined whether the changes in net effect of plant interactions depended on the type of treatment, the life stage of the individuals and the performance measure used. Thus we aimed to answer two main questions: (i) To what extent does the net effect of plant interactions differ under different levels of productivity and disturbance in a wet meadow? (ii) Does the net effect of interactions differ for different life stages, different species and different performance measures? We carried out a transplant experiment, in a long-term experimental set up in a wet meadow, where application of fertilizer (for manipulating productivity), mowing (the disturbance factor) and the removal of the dominant species (for manipulating species composition) were used in a full factorial design.

Materials and methods

Study site and experimental set-up

The long-term experiment was established in 1994 close to the village of Ohrazení in southern Bohemia, Czech Republic (48°57'11.3"N, 14°35'34.0"E, at 510 m a.s.l.). The mean annual temperature recorded at the nearby České Budějovice meteorological station (400 m a.s.l.) is 7.8 °C and mean annual precipitation 620 mm. The long-term experiment was established in a wet meadow, where the soil nutrient levels are relatively low (total nitrogen 6–8 g/kg dry soil weight, total phosphorus 400–500 mg/kg dry soil weight, C/N ratio 16 to 20; for details, see also Lepš 1999, 2014).

The meadow was traditionally mown (once or twice a year) until the late 1980s. At the start of the long-term experiment the plant community was characterized as *Molinion* with some elements of *Violion caninae*. The dominant grasses were *Molinia caerulea* (L.) Moench., *Nardus stricta* L., *Festuca rubra* L. and *Holcus lanatus* L. This species-rich meadow hosted several species of sedges and many herbaceous plants. In the original community, the species richness often exceeded 30 species of vascular plants per m^2.

The long-term experiment combined mowing, application of fertilizer and removal of the dominant species (*Molinia caerulea*) in a full factorial design. Three replicates of each of the eight treatment combinations were established (24 plots in total). Each replicate consisted of a 4 m^2 quadrat, located in a 4 × 6 quadrat lattice, with treatment combinations

regularly alternating. At the beginning of the long-term experiment, the application of fertilizer consisted of 65 g/m^2 of commercial NPK fertilizer: 12% N (nitrate and ammonium), 19% P (as P$_2$O$_5$) and 19% K (as K$_2$O). The fertilizer was applied in autumn (50 g/m^2) and spring (15 g/m^2). From 1997 onwards the full dosage of fertilizer was applied in spring.

The central 1 m^2 of each quadrat is regularly monitored for changes in species composition (results are reported in Lepš 1999, 2014). The mown plots were mown only after harvesting the experimental plants (see below), so that there was no mowing during the course of the transplant experiment. In 2000, six years after the start of the long-term experiment, the plots already differed considerably in their vegetation (see Electronic Appendix 1 for a detailed description of differences in vegetation). The differences in species composition were mostly associated with the fertilizer treatment, followed by mowing and removal of *Molinia*. The plots treated with fertilizer were dominated by tall species (Lepš 1999), often by competitive grasses (*Holcus lanatus*, *Festuca rubra*). Small species of vascular plants and mosses were mostly confined to unfertilized, mown plots, which were the most species rich. Vegetation biomass, which is available only for mown plots, was significantly higher for those treated with fertilizer (~460 g.m^{-2}, dry weight) than the unfertilized plots (~240 g.m^{-2}), with no effect of *Molinia* removal (Electronic Appendix 1). For a detailed description, graphic plan of the long-term experiment and photographs of vegetation in individual treatment combinations see appendices of Lepš (2014).

Transplantation of target species

Three target species were selected for transplantation. All three were perennial plants, common in the community studied. *Lysimachia vulgaris* L. is a rhizomatous plant, which is very effective at clonal spreading by forming underground stolons; the stems (50–150 cm) are erect. *Lysimachia vulgaris* is typical of wetlands and wet meadows and it was the most abundant of the three species studied in the meadow where the experiment was established. *Prunella vulgaris* L. has creeping, self-rooting stems, and grows to a height of 5–30 cm. It is a common species in mesophilous grasslands, woodland edges and moist areas. *Plantago lanceolata* L. is a rosette-forming plant with leafless flower stems (10–40 cm), common in several types of grassland and disturbed areas.

Seeds of these target species were first germinated, then put into starter "Jiffy" pots (peat soil pellets) and pre-grown and finally specimens of a similar size were selected (*P. lanceolata* about 4 cm long leaves, *P. vulgaris* and *L. vulgaris* about 1.5 cm long shoots) and planted in mid-April 2000. Four individuals of each of the three target species were planted in each 2 × 2 m plot (outside of the central 1 m^2, which was reserved for regular monitoring of species composition). Two individuals of each of the target species were planted in the surrounding vegetation and two in 15 cm diameter gaps. Gaps were created by hand-weeding without digging into the soil. No trenching was applied in the gaps, so the transplants in the gaps are likely to have experienced some below-ground competition. Thus, 12 individuals were planted in each plot (3 target species × 2 levels of surrounding vegetation × 2 replicates) resulting in a total of 288 target individuals (12 individuals per plot × 24 plots). Transplants that died immediately were replaced at the end of the original transplantation on 25 April (T0) in order to have all transplants established by

the first measurement. Survival (alive/dead) was recorded on 25 May (T1) and finally on 1 July (T2), when all the live target individuals were harvested. On this date, presence/absence of fertile shoots was also recorded for *P. lanceolata*, the only target species reaching the flowering stage. Above- and below-ground biomass of the individuals was also determined (dried at 80 °C for 48 hours).

Data analysis

The survival of the individuals in the different treatments was analysed using generalized linear mixed models (GLMMs). As all outcomes fall between 0 and 1 the errors will be binomial and as such a logit link was used to limit the outcomes to between these values. Restricted maximum likelihood method (REML) was used to parameterize the models. We fitted two separate models, one for studying plant survival from the beginning of the experiment to 25 May (first sampling period, i.e. from T0 to T1) and a second for the survival of plant individuals between 25 May and the end of the experiment (second sampling period, i.e. from T1 to T2). Two models were used in order to reduce model complexity, which made it impossible to fit a full model using a repeated measurement ANOVA design (due to convergence failures), which would ideally be a more appropriate way to test such data. The survival of individuals was used as a response variable in both models. Target species (*L. vulgaris*, *P. lanceolata*, *P. vulgaris*), surrounding vegetation (gap or amongst vegetation), fertilizer application (unfertilized or fertilized), mowing (unmown or mown) and *Molinia caerulea* removal (not removed or removed) were used as fixed factors. Because of the hierarchical structure of the data and to get rid of random variation due to plot identity, plot identity was used as a random factor. It is not possible to obtain reliable P-values for such models using a test based on mean squares and the residual degrees of freedom for the terms given the problems of estimating residual degrees of freedom (Bolker et al. 2009). Moreover we still experienced model convergence failure due to the complexity of the full model. Thus, acknowledging that a full model approach would be better for analysing our experimental data and that the pitfalls of step-wise model selection have been discussed (Whittingham et al. 2006) we opted for a forward selection of the terms in our models. Our aim was to reduce model complexity and obtain reliable P-values for the terms by comparing models, following Bates et al. (2014). Thus the selection of significant terms was carried out by comparing models using likelihood ratio test (LRT) values. As P-values obtained using a LRT comparison assuming a chi-square distribution are unreliable for small sample sizes (Sørensen 2008, Bolker et al. 2009), we used a parametric bootstrap to obtain a reference distribution for the LRT values (i.e. we used 1000 simulation). In this approach P-values are calculated as the fraction of simulated LRT-values that is larger or equal to the observed LRT value (see Halekoh & Højsgaard 2014). Using this procedure significant main effects were added first and proper interaction terms later.

The above-ground biomass and root-shoot ratio was analysed using mixed effects nested ANOVA, after log-transformation of variables. Only two of the three species transplanted were included in this analysis, because the number of individuals of *L. vulgaris* surviving (only 17 out of the 96) was too low for the analysis. Species (*P. lanceolata*, *P. vulgaris*), surrounding vegetation (gap or amongst vegetation), fertilizer application (unfertilized or fertilized), mowing (unmown or mown) and *Molinia*

caerulea removal (not removed or removed) were used as fixed factors. Plot effect was used as a random factor, nested in the full cross combination of types of management (fertilization × mowing × dominant removal).

The analysis of the flowering success of *P. lanceolata* (the only species that flowered) was also studied using generalized linear mixed models (GLMMs) and a binomial distribution (logit link function). Presence/absence of fertile shoots was used as the response variable and surrounding vegetation (gap or amongst vegetation), fertilizer application (unfertilized or fertilized), mowing (unmown or mown) and *Molinia caerulea* removal (not removed or removed) were used as fixed factors, whereas the plot effect was used as a random factor. Model selection was carried out using forward selection of significant terms by parametric bootstrap with 1000 simulations.

The generalized linear mixed models (GLMMs) of the survival of the species and the flowering success of *P. lanceolata* were produced using the glmer function from lme4 package (Bates et al. 2014) for R software version 3.1.1 (R Core Team 2014). Parametric bootstrap model comparisons were carried out using the PBmodcomp function in the pbkrtest package (Halekoh & Højsgaard 2014). The mixed effects nested ANOVA of the log-transformed biomass and root-shoot ratio was done using STATISTICA 10.0 (StatSoft Inc., Tulsa).

Results

Survival

The model selection procedure (Electronic Appendix 2) indicates that during the first period, the survival of transplants differed significantly for the different species and was significantly higher amongst vegetation than in gaps (Table 1, Fig. 1). The greatest difference in survival amongst vegetation and gaps was recorded for *L. vulgaris* (Fig. 1). Nevertheless, the interaction term between species and surrounding vegetation was not significant, the mortality of all the species was higher in gaps than amongst vegetation during the first period (Electronic Appendices 2 & 3, Fig. 1). In the second period, survival differed only among species (Table 1, Electronic Appendix 2). Thus, in both periods species differed in their survival, with the lowest survival recorded for *L. vulgaris*, especially in the first period, and the highest for *P. lanceolata* and *P. vulgaris* irrespective of when sampled (Fig. 1, Electronic Appendix 3). No other factor had a significant effect on survival (see stepwise selection results on Electronic Appendix 2).

Above-ground biomass, root shoot ratio and flowering success

Both the final above-ground biomass and the root-shoot ratio differed among the target species and were affected by surrounding vegetation (Table 2, Electronic Appendices 4 & 5). The magnitude of the effect of surrounding vegetation depended on the target species, which is indicated by the significant interaction term between species and surrounding vegetation (Table 2). The above-ground biomass recorded for both species was greater (Fig. 2, Electronic Appendix 6) and root-shoot ratio lower in gaps than when growing amongst vegetation (Fig. 3, Electronic Appendix 6). The differences in above-ground biomass and root-shoot ratio values between plants growing in gaps and amongst

Table 1. – Significant effect terms of the GLMMs of the survival of the plants (for full results see the Electronic Appendix 2). nDF – numerator degree of freedom; SS – sum of squares; MC – model comparison; T0-T1 – first period sampled; T1-T2 – second period sampled. P-values were evaluated using parametric bootstrap model comparisons.

	nDf	SS	F	MC P-value
T0-T1 model:				
Species	2	5.32	2.66	< 0.001
Surrounding	1	0.67	0.67	< 0.001
T1-T2 model:				
Species	2	7.56	3.78	< 0.001
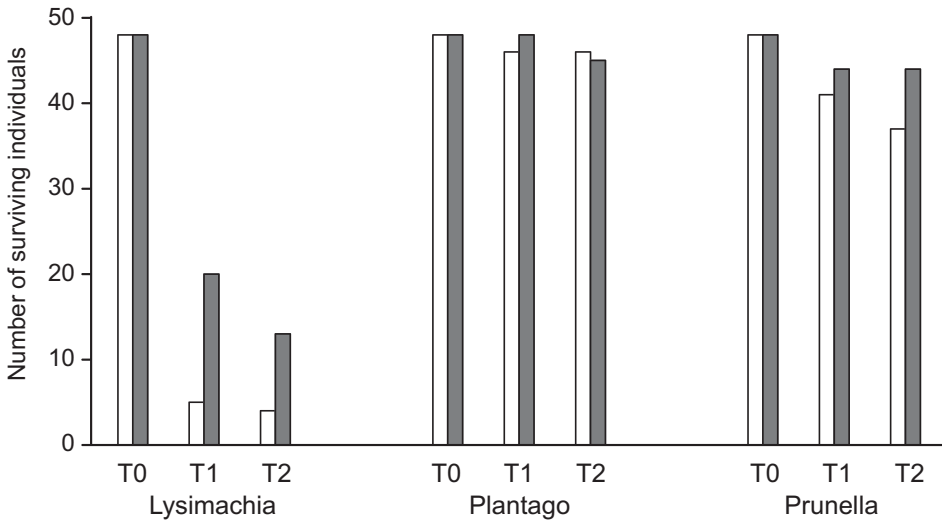
Fig. 1. – Number of individuals of transplanted species that survived in gaps and amongst vegetation. Empty bars – gaps; grey bars – amongst vegetation; T0 – start of experiment; T1 – time of first sample; T2 – time of second sample; Lysimachia – *Lysimachia vulgaris*; Plantago – *Plantago lanceolata*; Prunella – *Prunella vulgaris*.

Table 2. – Significant effect terms of the mixed effect nested ANOVA for above-ground biomass and root-shoot ratio (for full results see Electronic Appendices 4 & 5). nDF – Numerator degree of freedom; dDF – denominator degree of freedom; SS – sum of squares; Surrounding – surrounding vegetation.

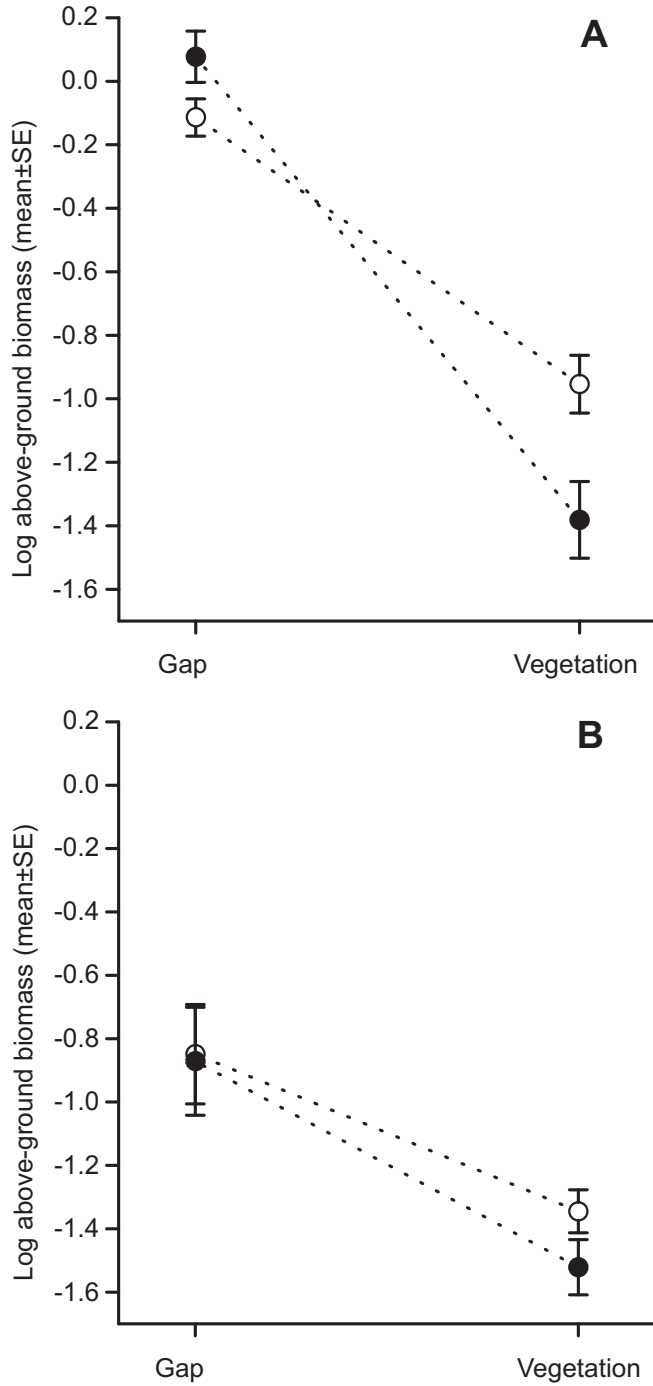|	nDF	dDF	SS	F	P
Above-ground biomass:					
Species	1	16.46	11.69	58.64	< 0.001
Surrounding	1	16.20	28.14	97.59	< 0.001
Species × Surrounding	1	13.06	3.46	11.14	< 0.01
Fertilization × Surrounding	1	16.06	1.90	6.57	< 0.05
Root-shoot ratio:					
Species	1	16.43	2.15	30.21	< 0.001
Surrounding	1	16.63	3.35	79.19	< 0.001
Species × Surrounding	1	13.13	0.51	10.18	< 0.01
Fertilization × Surrounding	1	16.81	0.28	6.71	< 0.05

Fig. 2. – Above-ground biomass of individual transplant (log; [g]) of *Plantago lanceolata* (A) and *Prunella vulgaris* (B) planted in gaps and amongst vegetation (gap, vegetation). Empty symbols – fertilizer not applied; full symbols – fertilizer applied. The non-parallel dotted lines connecting the averages signify interaction.
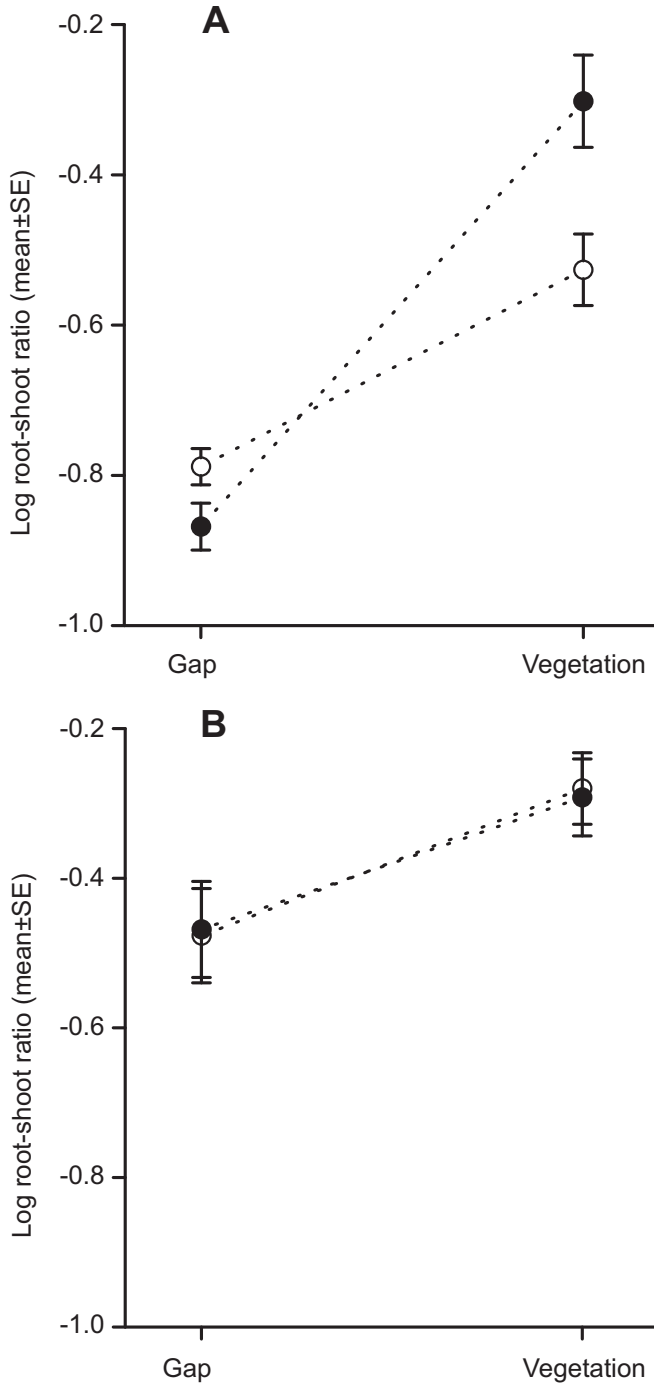
Fig. 3. – Root-shoot ratios (log) of *Plantago lanceolata* (A) and *Prunella vulgaris* (B) planted in gaps and amongst vegetation (gap, vegetation). Empty symbols – fertilizer not applied; full symbols – fertilizer applied. The non-parallel dotted lines connecting the averages signify interaction.

vegetation (indicating proportional differences because of the log transformation) were higher for *P. lanceolata* than *P. vulgaris* (Figs 2 & 3).

Significant interaction terms between the application of fertilizer and growing amongst vegetation were detected both for above-ground biomass and root-shoot ratio (Table 2). For individuals growing amongst vegetation, the above-ground biomass was lower in fertilized than in unfertilized plots, whereas individuals growing in gaps had a higher biomass in fertilized plots (Fig. 2), indicating an increased effect of competition on plant growth in fertilized plots. The root-shoot ratio was greater for individuals growing amongst vegetation in fertilized than in unfertilized plots, which also indicates greater competition for light in more productive conditions (Fig. 3).

Plantago lancelata was the only species that flowered and almost exclusively only when growing in gaps. Growing amongst vegetation was the only variable to have a significant effect on flowering (Electronic Appendices 7 & 8).

Discussion

Our results indicate the co-occurrence and interplay of positive and negative interactions among plants in a species-rich wet meadow. The effect of these processes changed with life stage and environmental productivity, confirming the existing, although untested hypotheses (see Eckstein 2005). The net effect of neighbouring vegetation was positive for the survival of young plants (facilitation), but negative (competition) for biomass and flowering towards the end of the experiment. This shift in the net effect of plant interactions was increased by the application of fertilizer. Altogether, our results indicate that both competition and facilitation occur in a benign habitat, with positive and negative interactions affecting different plant life stages and different components of plant fitness. The results highlight the dynamic nature of positive and negative interactions within a community.

Survival of young plants (in the first period) was higher when growing amongst vegetation than in the gaps, indicating the occurrence of facilitation in the environment studied. While facilitation is often assumed to increase with abiotic stress and/or disturbance (Bertness & Callaway 1994, Brooker & Callaghan 1998) it has also recently been suggested to occur in benign environments (e.g. Gross et al. 2010, Holmgren & Scheffer 2010) including temperate deciduous or riparian forests (Gómez-Aparicio et al. 2005). Facilitation may occur in benign environments when positive effects of surrounding vegetation against desiccation outweigh the direct negative effect of shading (Holmgren et al. 1997, Kikvidze et al. 2006). It is long recognized as an important process affecting survival and establishment of early life stages (e.g. Grubb 1977, le Roux et al. 2013); in fact, shading by surrounding vegetation can protect young individuals (Semschenko et al. 2012, McIntire & Fajardo 2013). Our experimental site is a wet meadow; nevertheless, the relatively thin upper soil layer can desiccate, particularly when not protected by vegetation. This desiccation can be fatal for young seedlings, all the roots of which are in the upper soil layer.

Facilitation effect on early life stages was most evident for *L. vulgaris,* for which the percentage survival was lower than that recorded for *P. lanceolata* and *P. vulgaris* in all the treatments. High mortality of young individuals of *L. vulgaris* concurs with Lamb & Cahill (2006) finding that plants with an effective clonal spreading ability often suffer high juvenile mortality. *Lysimachia vulgaris* spreads at our study site very effectively by

relatively long (tens of cm) underground stolons and in this way it is able to colonize dense vegetation and achieve a relatively high abundance. *Prunella vulgaris* spreads vegetatively by above-ground stolons, which are rather short (in our locality ~5 cm; Macek & Lepš 2003), and lateral vegetative spread of *Plantago* is negligible (this species is thus dependent on seedling regeneration). Similar to our study, Ryser (1993) also reports the high survival of *P. lanceolata* seedlings.

Above-ground biomass of target individuals was lower and the root-shoot ratio higher in fertilized plots when growing amongst vegetation indicating that there is an increase in competition when fertilizer is applied, as suggested by Grime (1973). Above-ground competition has been shown to be either more important than below-ground competition in productive grasslands (Wilson & Tilman 1991) or to be equally important (Twolan-Strutt & Keddy 1996). The decrease in the above-ground biomass of target individuals growing amongst vegetation in plots treated with fertilizer indicates the occurrence of competition for light. Application of fertilizer both results in an increase in total biomass and canopy height of the vegetation (Lepš 1999) and leads to increased size-asymmetric competition for light (Hautier et al. 2009). The transplants were generally smaller than the established vegetation and the selected species are not strong competitors for light. *Plantago lanceolata* and *P. vulgaris* are low growing species, that quickly disappear from plots treated with fertilizer (Lepš 1999); *L. vulgaris* is able to grow tall (and so compete successfully with the surrounding vegetation), but it never reached this size in this experiment. Consequently, it is likely that asymmetric competition for light in the fertilized plots affected all these species. The root-shoot ratio was mostly affected by changes in above-ground biomass, likely because roots did not have enough time to grow during the three-month experiment (see Electronic Appendix 6). *Plantago lanceolata* flowered almost exclusively in gaps, suggesting that competition can also decrease flowering success. The flowering success of plant species strongly depends on the size of the individuals (Eckstein 2005, le Roux et al. 2013). Competitive suppression of growth clearly results in a decrease in the probability of flowering (see also Lamb & Cahill 2006).

The competitive response (i.e. differences in above-ground biomass between plants in gaps and in vegetation) of *P. lanceolata* was greater than the competitive response of *P. vulgaris* (see Fig. 2). This is likely because *P. lanceolata* is better able to utilize the gaps due to its higher relative growth rate (RGR); the mean RGR of young plants of *P. lanceolata* is 1.4 week^{-1} and for *P. vulgaris* is 0.86 week^{-1} (see Grime & Hunt 1975). Consequently, *P. lanceolata* is able to reach the reproductive stage in three months and complete its whole life cycle in gaps. *Prunella vulgaris* is also suppressed when growing amongst vegetation, but is not able to grow so fast in gaps.

Mowing and *Molinia caerulea* removal did not affect the outcome of plant interactions. As the once a year mowing was carried out after the end of the transplant experiment (in late June or early July in every year; Lepš 1999), it is likely that mowing did not strongly affect the vegetation structure (i.e. canopy height and biomass) during the three-month experiment. *Molinia* removal started in 1994 and the spaces previously occupied by *Molinia* were already filled by other species at the time of the transplant experiment and the total biomass was also similar in removed and unremoved plots (Lepš 1999, Electronic Appendix 1), which probably had a similar effect on the transplants as *Molinia*. A one season experiment in the same locality (Macek & Lepš 2003) demonstrated that the response of *Prunella* to competition from *Molinia caerulea* was very similar to the

response to competition from two other tussock graminoids (*Nardus stricta* and *Juncus effusus*). However, a two-year study (Chaloupecká & Lepš 2004) of *Lychnis flos-cuculi* and *Myosotis nemorosa* transplanted into these three tussock graminoids revealed differences in clonal growth and investment in reproductive effort among individuals. It is likely, therefore, that in the early life stages investigated here, the surrounding biomass was the most important factor, and this was increased by the application of fertilizer (Lepš 1999, 2014).

A positive effect of the surrounding vegetation (i.e. facilitation) on the early stages and a shift to competition for light, decreasing the growth of aboveground biomass was recorded. Nevertheless, a negative effect of surrounding vegetation on seedling germination is reported for the area studied (Kotorová & Lepš 1999). This suggests that surrounding vegetation first decreases the germination by shading and mechanically preventing seeds from reaching the soil surface, then facilitates establishment by preventing desiccation and finally the competition for light results in a decrease in the rate of growth. This highlights the co-occurrence of facilitation and competition in the same community and the many different mechanisms that can result in a combination of positive or negative effects of surrounding vegetation on the target individual.

The stress experienced by young individuals can be greater than that experienced by mature individuals (Eckstein 2005, Liancourt et al. 2005). Hence, young individuals may have narrower niches than mature individuals (Young et al. 2005). If the surrounding vegetation improves the abiotic environment, this improved environment can overlap with the niche requirements of young individuals allowing their establishment (Choler et al. 2001, McIntire & Fajardo 2013); accordingly the better survival of young transplants is probably due to a decreased risk of desiccation when growing amongst vegetation (Ryser 1993). However, once the roots grow slightly deeper the very thin layer of soil prone to desiccation no longer has a negative effect and the protective effect of surrounding vegetation becomes negligible compared to the negative effect of competition (see Liancourt et al. 2005). This possibly accounts for the shift from a positive effect of the surrounding vegetation on survival of the early stages to a negative effect of competition on the later stages of development. The prevalence of competitive effects is also supported by the results of the long-term experiment in which the application of fertilizer decreased species diversity (Lepš 1999, 2014). Furthermore, a stronger shift in plant interactions was recorded in fertilizer treatments due to an increase in the intensity of competition for light.

Our results highlight the important role of both competition and facilitation in a benign habitat and indicate shifts in the net outcome of plant interactions during plant development. Productivity altered the intensity of the shift in plant interactions by increasing the effect of competition on the mature stage. Our short-term experiment only allowed the testing of the effect of plant interactions in the early establishment and growth of the individuals, even if one of the three species was able to reach the flowering stage. However this study shows that assessing the net outcome of species interactions needs to consider different aspects of plant fitness at different stages in the life of plants and that, although competition is generally prevalent in benign environments, even in this environment some critical stages in plant development might benefit from the positive effects of the surrounding vegetation.

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Acknowledgements

Souhrn

Kompetice a facilitace se obvykle vyskytují v rostlinných společenstvech společně; předpokládá se, že negativní vliv převládá v produktivním prostředí a pozitivní vliv v méně příznivých podmínkách. Vliv okolní vegetace na jedince se navíc může lišit v závislosti na jeho ontogenetickém stádiu a výsledek je také ovlivněn tím, jak tento vliv měříme. V této studii jsme využili dlouhodobý terénní pokus na vlhké, druhově bohaté louce, kde kombinujeme ve faktoriálním uspořádání kosení, hnojení a odstranění dominanty (*Molinia caerulea*). Každá kombinace je ve třech opakováních, celkem tedy 24 ploch. Do každé plochy jsme šest let po zahájení dlouhodobého experimentu vysadili mladé rostliny a sledovali vliv okolní vegetace na jejich vývoj během jedné vegetační sezóny. Zjišťovali jsme, zda se výsledný efekt interakcí rostlin (i) liší v závislosti na produktivitě prostředí a intenzitě disturbance a (ii) mění během ontogeneze vysazených individuí podle jejich druhové příslušnosti, či podle toho, jak tento vliv měříme. Do každé pokusné plochy jsme vysadili čtyři jedince každého z tří druhů, *Lysimachia vulgaris*, *Prunella vulgaris* a *Plantago lanceolata*, dva do umělých mezer zbavených vegetace a dva do nenarušené vegetace. Přežívání každého jedince bylo zaznamenáno ve dvou časových intervalech. Na konci experimentu jsme zaznamenali, zda rostlina vykvetla, a stanovili jsme její nadzemní a podzemní biomasu. Přežívání mladých jedinců v prvním časovém intervalu bylo vyšší ve vegetaci, což naznačuje, že okolní vegetace měla pozitivní vliv na raná vývojová stádia rostlin. Později však převládla kompetice, což mělo negativní vliv jak na produkci biomasy, tak na reprodukci. Tento negativní efekt byl výraznější v hnojených plochách, vliv kosení a odstranění dominanty na interakce rostlin byl zanedbatelný. Naše výsledky tedy naznačují možnost ontogenetického posunu v rostlinných interakcích, zvláště v produktivnějším prostředí. To ukazuje na dynamické změny rovnováhy mezi pozitivním a negativním vlivem, který může mít okolní vegetace na jedince ve společenstvu.

References

Armas C. & Pugnaire F. I. (2009): Ontogenetic shifts in interactions of two dominant shrub species in a semi-arid coastal sand dune system. – J. Veg. Sci. 20: 535–546.

Bates D., Maechler M., Bolker B. & Walker S. (2014): lme4: linear mixed-effects models using eigen and S4. R package version 1.1-7. – URL: http://CRAN.R-project.org/package=lme4.

Bertness M. D. & Callaway R. (1994): Positive interactions in communities. – Trends Ecol. Evol. 9: 191–193.

Bolker B. M., Brooks M. E., Clark C. J., Geange S. W., Poulsen J. R., Stevens M. H. H. & White J. S. S. (2009): Generalized linear mixed models: a practical guide for ecology and evolution. – Trends Ecol. Evol. 24: 127–135.

Brooker R. W. & Callaghan T. V. (1998): The balance between positive and negative plant interactions and its relationship to environmetnal gradients: a model. – Oikos 81: 196–207.

Brooker R. W., Maestre F. T., Callaway R. M., Lortie C. L., Cavieres L. A., Kunstler G., Liancourt P., Tielbörger K., Travis J. M. J., Anthelme F., Armas C., Coll L., Corcket E., Delzon S., Forey E., Kikvidze Z., Olofsson J., Pugnaire F., Quiroz C. L., Saccone P., Schiffers K., Seifan M., Touzard B. & Michalet R. (2008): Facilitation in plant communities: the past, the present, and the future. – J. Ecol. 96: 18–34.

Chaloupecká E. & Lepš J. (2004): Equivalence of competitor effects and tradeoff between vegetative multiplication and generative reproduction: case study with *Lychnis flos-cuculi* and *Myosotis nemorosa*. – Flora 199: 157–167.

Choler P., Michalet R. & Callaway R. M. (2001): Facilitation and competition on gradients in alpine plant communities. – Ecology 82: 3295–3308.

Eckstein R. L. (2005): Differential effects of interspecific interactions and water availability on survival, growth and fecundity of three congeneric grassland herbs. – New Phytol. 166: 525–536.

Gómez-Aparicio L., Gómez J. M. & Zamora R. (2005): Microhabitats shift rank in suitability for seedling establishment depending on habitat type and climate. – J. Ecol. 93: 1194–1202.

Grace J. B. (1993): The effects of habitat productivity on competition intensity. – Trends Ecol. Evol. 8: 229–230.

Grime J. P. (1973): Competitive exclusion in herbaceous vegetation. – Nature 242: 344–347.

Grime J. P. & Hunt R. (1975): Relative growth rate: its range and adaptive significance in a local flora. – J. Ecol. 63: 393–422.

Gross N., Liancourt P., Choler P., Suding K. N. & Lavorel S. (2010): Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions. – Persp. Plant. Ecol. Evol. Syst. 12: 9–19.

Grubb P. J. (1977): The maintenance of species richness in plant communities: the importance of the regeneration niche. – Biol. Rev. 52: 107–145.

Halekoh U. & Højsgaard S. (2014): A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models: the R package pbkrtest. – J. Stat. Softw. 59: 1–30.

Hautier Y., Niklaus P. A. & Hector A. (2009): Competition for light causes plant biodiversity loss after eutrophication. – Science 324: 636–638.

Holmgren M. & Scheffer M. (2010): Strong facilitation in mild environments: the stress gradient hypothesis revisited. – J. Ecol. 98: 1269–1275.

Holmgren M., Scheffer M. & Huston M. A. (1997): The interplay of facilitation and competition in plant communities. – Ecology 78: 1966–1975.

Kelemen A., Török P., Valkó O., Deák B., Tóth K. & Tóthmérész B. (2015): Both facilitation and limiting similarity shape the species coexistence in dry alkali grasslands. – Ecol. Complex. 21: 34–38.

Kikvidze Z., Khetsuriani L., Kikodze D. & Callaway R. M. (2006): Seasonal shifts in competition and facilitation in subalpine plant communities of the central Caucasus. – J. Veg. Sci. 17: 77–82.

Kotorová I. & Lepš J. (1999): Comparative ecology of seedling recruitment in an oligotrophic wet meadow. – J. Veg. Sci. 10: 175–186.

Lamb E. G. & Cahill J. F. (2006): Consequence of differing competitive abilities between juvenile and adult plants. – Oikos 112: 502–512.

Le Bagousse-Pinguet Y., Liancourt P., Gross N. & Straile D. (2012): Indirect facilitation promotes macrophyte survival and growth in freshwater ecosystems threatened by eutrophication. – J. Ecol. 100: 530–538.

Le Bagousse-Pinguet Y., Maalouf J. P., Touzard B., Touzard B. & Michalet R. (2014a): Importance, but not intensity of plant interactions relates to species diversity under the interplay of stress and disturbance. – Oikos 123: 777–785.

Le Bagousse-Pinguet Y., Xiao S., Brooker R. W., Gross N., Liancourt P., Straile D. & Michalet R. (2014b): Facilitation displaces hot-spots of diversity and allows communities to persist in heavily stressed and disturbed environments. – J. Veg. Sci. 25: 66–76.

Le Roux P. C., Shaw J. D. & Chown S. L. (2013): Ontogenetic shifts in plant interactions vary with environmental severity and affect population structure. – New Phytol. 200: 241–250.

Lepš J. (1999): Nutrient status, disturbance and competition: an experimental test of relationships in a wet meadow. – J. Veg. Sci. 10: 219–230.

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.

Liancourt P., Callaway M. & Michalet R. (2005): Stress tolerance and competitive-response ability determine the outcome of biotic interactions. – Ecology 86: 1611–1618.

Macek P. & Lepš J. (2003): The effect of environmental heterogeneity on clonal behaviour of *Prunella vulgaris* L. – Plant Ecol. 168: 31–43.

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.

Maestre F. T., Valladares F. & Reynolds J. F. (2005): Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. – J. Ecol. 93: 748–757.

Malkinson D. & Tielbörger K. (2010): What does the stress-gradient hypothesis predict? Resolving the discrepancies. – Oikos 119: 1546–1552.

McIntire E. J. B. & Fajardo A. (2013): Facilitation as a ubiquitous driver of biodiversity. – New Phytol. 201: 403–416.

Michalet R., Brooker R. W., Cavieres L. A., Kikvidze Z., Lortie C. J., Pugnaire F. I., Valiente-Banuet A. & Callaway R. M. (2006): Do biotic interactions shape both sides of the humpedback model of species richness in plant communities? – Ecol. Lett. 9: 767–773.

R Core Team (2014): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org.

Ryser P. (1993): Influences of neighbouring plants on seedling establishment in limestone grassland. – J. Veg. Sci. 4: 195–202.

Schiffers K. & Tielbörger K. (2006): Ontogenetic shifts in interactions among annual plants. – J. Ecol. 94: 336–341.

Semchenko M., Lepik M., Götzenberger L. & Zobel K. (2012): Positive effect of shade on plant growth: amelioration of stress or active regulation of growth rate? – J. Ecol. 100: 459–466.

Smit C., Rietkerk M. & Wassen M. J. (2009): Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. – J. Ecol. 97: 1215–1219.

Soliveres S., DeSoto L., Maestre F. T. & Olano J. M. (2010): Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. – Persp. Plant. Ecol. Evol. Syst. 12: 227–234.

Sørensen H. (2008): Small sample distribution of the likelihood ratio test in the random effects model. – J. Stat. Plan. Infer. 138: 1605–1614.

Tilman D. (1987): On the meaning of competition and the mechanisms of competitive superiority. – Funct. Ecol. 1: 304–315.

Twolan-Strutt L. & Keddy P. (1996): Above- and belowground competition intensity in two contrasting wetland plant communities. – Ecology 77: 259–270.

Whittingham M. J., Stephens P. A., Bradbury R. B. & Freckleton R. P. (2006): Why do we still use stepwise modelling in ecology and behaviour? – J. Anim. Ecol. 75: 1182–1189.

Wilson S. D. & Tilman D. (1991): Components of plant competition along an experimental gradient of nitrogen availability. – Ecology 72: 1050–1065.

Wilson S. D. & Tilman D. (1993): Plant competition and resource availability in response to disturbance and fertilization. – Ecology 74: 599–611.

Young T. P., Petersen D. A. & Clary J. J. (2005): The ecology of restoration: historical links, emerging issues and unexplored realms. – Ecol. Lett. 8: 662–673.