Research

Experimental reduction of pollinator visitation modifies plant–plant interactions for pollination

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The strength of interactions between plants for pollination depends on the abundance of plants and pollinators in the community. The abundance of pollinators may influence plant associations and densities at which individual fitness is maximized. Reduced pollinator visitation may therefore affect the way plant species interact for pollination. We experimentally reduced pollinator visitation to six pollinator-dependent species (three from an alpine and three from a lowland community in Norway) to study how interactions for pollination were modified by reduced pollinator availability. We related flower visitation, pollen limitation and seed set to density of conspecifics and pollinator-sharing heterospecifics inside 30 dome-shaped cages partially covered with fishnet (experimental plots) and in 30 control plots. We expected to find stronger interactions between plants in experimental compared to controls plots. The experiment modified plant–plant interactions for pollination in all the six species; although for two of them neighbourhood interactions did not affect seed set. The pollen limitation and seed set data showed that reduction of pollinator visits most frequently resulted in novel and/or stronger interactions between plants in the experimental plots that did not occur in the controls. Although the responses were species-specific, there was a tendency for increasing facilitative interactions with conspecific neighbours in experimental plots where pollinator availability was reduced. Heterospecifics only influenced pollination and fecundity in species from the alpine community and in the experimental plots, where they competed with the focal species for pollination. The patterns observed for visitation rates differed from those for fecundity, with more significant interactions between plants in the controls in both communities. This study warns against the exclusive use of visitation data to interpret plant–plant interactions for pollination, and helps to understand how plant aggregations may buffer or intensify the effects of a pollinator loss on plant fitness.

The densities of co-flowering con- and hetero-specifics in the neighbourhood of an individual plant may affect the frequency of pollinators it receives and its fecundity. The relationship of an individual plant with its neighbours can be positive (facilitation), if the neighbours increase pollinator visitation and/or pollen availability for the focal individual (Johnson et al. 2003, Moeller 2004); or negative (competition), if the neighbours decrease pollinator visitation (Chittka and Schürkens 2001, Mustajärvi et al. 2001, Brown et al. 2002) and/or hinder proper pollen transfer (Caruso and Alfaro 2000, Jakobsson et al. 2007, Morales and Traveset 2008, Runquist and Stanton 2013). However, because the strength of an interaction is the result of the costs and benefits involved in the association, the net outcome of the interaction may vary along a continuum from positive to negative (Bronstein 1994). Within this continuum, the local abundances of the involved partners can influence the outcome

of the interactions. Rathcke (1983) proposed a model for the effects of both con- and hetero-specific neighbors on the pollination of focal plants. This density-dependent model predicted that increased flower density would have a positive effect on per-flower visitation rate at low densities and a negative effect at high densities, when visitation declines as the pool of pollinators is saturated. Thus, a facilitative interaction between plants for pollination at low abundance can become competitive when abundance increases (Rathcke 1983). Such a hump-shaped relationship between the densities of neighbours and the pollination success of plants has been found in several empirical studies (Sabat and Ackerman 1996, Steven et al. 2003, Brys et al. 2008).

Similar effects to those created by variations in plant densities are expected when the pollinator activity varies, because pollinators are a resource for plants and a change in pollinator abundance implies that the pollinator-to-flower

ratio is modified and that resource availability for plants changes. This is of particular relevance in the context of the current pollinator decline (Biesmeijer et al. 2006, Goulson et al. 2008). Thus, a pollinator decline may not exclusively cause an overall reduction in visitation frequencies to plants, with the consequent reduction in species' fecundity (Lundgren et al. 2013) and abundance (Biesmeijer et al. 2006). Besides, reduced pollinator availability may affect the way neighbours interact for pollination, by influencing the relative attractiveness of focal plants and by modifying plant associations and densities at which individual fitness is maximized. Based on competition theory (Alley 1982), competition between co-flowering neighbours for pollination should be more intense when pollinator abundance is low. However, situations of high stress level, as those in which the resource (pollinators, in this case) is limiting, may also increase the relative frequency offacilitative relationships between plants (stress-gradient hypothesis; Bertness and Callaway 1994). Therefore, interactions (both positive and negative) between plants for pollination may be more frequent (and more intense) when pollinator activity is reduced. Despite this general prediction, the effect of reduced pollinator visitation on interacting plant species may be species-specific. For instance, highly visited plant species might be more susceptible to a sudden imbalance in the pollinator-to-flower ratio than plant species that commonly face a shortage of pollinator visits, because the latter often have a breeding system that do not require outcross pollen for seed production (Motten 1986, Barrett 1996) or only require a small amount of pollen to set seeds (Molano-Flores et al. 1999).

We established an experiment that partially excluded pollinators in two communities in southern Norway (one alpine and one lowland community). In a previous study on the lowland community, we used data on 15 species to assess how a reduction in pollinator visitation affected the reproduction of plants, showing a positive relationship between the species' degree of pollinator dependence and their vulnerability to a pollinator loss (Lundgren et al. 2013). In the present study, we examine how a reduction in pollinator availability affected the number, direction and intensity of interactions (measured as the regression slope of the relationship) between plants for pollination. For that, we recorded visitation rates, pollen limitation and seed set for six plant species (five species for pollen limitation data) and related them to conspecific and pollinator-sharing heterospecific densities inside thirty 2 × 2 m dome-shaped cages partially covered with fishnet (experimental plots) and in 30 control plots. Our specific hypotheses were: 1) the interactions between neighbours for pollination vary with conspecific and heterospecific flower densities; 2) the reduction of pollinator availability increases the relative frequency and/or the intensity of interactions between plants for pollination (both positive and negative); 3) the interactions between plants for pollinator attraction are reflected, at least partially, in their magnitude of pollen limitation and their seed set; and 4) the effects of experimental reduction in pollinator availability are species-specific depending on factors such as their degree of pollinator dependence and their natural visitation rates and pollen limitation levels.

Material and methods

Study areas

We conducted our study in two plant communities in south Norway. We selected these communities because they differed considerably in climate conditions, and composition and density of pollinators. One community is located within a species-rich semi-natural meadow, at 300 m altitude at Ryghsetra (59°44′03″N, 10°02′48″E), Buskerud county, ca 2.5 km southeast of Mjøndalen. The blooming season in this meadow starts in early-mid May and terminates in mid-late August, and during this period ca 55 species bloom. The average number of pollinator visits per hour per plot under natural conditions was 64.7 (for all the plant species in the community). The flower visitor assemblage of this community during the study year consisted of 72.7% bumblebees, 11% muscoid flies, 5.5% solitary bees, 4.7% hover flies, 2.4% ants, 1.6% butterflies, 0.9% honeybees, 0.5% beetles, 0.5% beeflies and 0.04% wasps.

The other community is located on a southwest exposed slope at ca 1500 m altitude on Sandalsnuten, Finse, in the northern part of Hardangervidda (60°36′36″ N, 7°31′12″E), in the alpine region of southwest Norway. The blooming season in this community starts in late June and terminates in late August, and during this period ca 25 species bloom. The average number of pollinator visits per hour per plot under natural conditions, was 47.9 (for all the plant species in the community). The flower visitor assemblage of this community in the study year consisted of 85.8% muscoid flies, 7.9% butterflies, 3.6% bumblebees and 2.5% hover flies.

Study species

To study the effects of the floral neighbourhood on pollinator visits, pollen limitation and seed set, we selected three perennial herb species, *Centaurea jacea, Galium mollugo* and *Leucanthemum vulgare,* at Ryghsetra, and three, *Astragalus alpinus, Bartsia alpina* and *Parnassia palustris,* at Finse. We selected these species because they are typical abundant species in temperate and alpine meadows, and therefore they may representative of these types of communities. All of them are entomophilous and highly dependent on pollinators for reproduction (DPD; Table 1). Three of the species are mainly pollinated by bumblebees, *Astragalus alpinus, Bartsia alpina* and *Centaurea jacea*, although with different degrees of specialization (Lázaro et al. 2008; PG, Table 1); whereas the other three, *Galium mollugo, Leucanthemum vulgare* and *Parnassia palustris*, are mainly pollinated by flies. In addition, these six study species occurred in diverse floral contexts (i.e. co-flowered with several plant species) and were relatively abundant in their communities (i.e. occurred in ≥ 50% of the plots), which allowed us to obtain adequate sample sizes to perform statistical analyses. Visitation rates to all these species were successfully reduced by our experiment. Table 1 shows relevant characteristics of these species, including family, flowering length, main pollinator, pollen limitation under natural pollination, degree of pollinator dependence (i.e. capability of autonomous selfing) and abundance.

Table 1. Characteristics of the study plant species. PL is the pollen limitation index that ranges from 0 (no pollen limitation) to 1 (total pollen limitation) and is based on supplemental hand-pollination experiments conducted on 15-30 individuals for each species outside the experimental plots (PL = 1- (seed set control plants/seed set supplemental hand-pollinated plants; unpubl. data). DPD is the degree of pollinator dependence that ranges from 0 (total independence of pollinators) to 1 (total dependence of pollinators) and is based on bagging and hand-pollination experiments conducted on 15 individuals for each species on the study communities (DPD = 1- (seed set bagged plants/seed set supplemental hand-pollinated plants; Lázaro et al. unpubl.). Flow. length is the number of days the species flowered the study year. Main poll. is the pollinator that conducted most of the visits to the focal species: BB, bumblebees; MF, muscoid flies. PG: pollination generalization, calculated using Simpson's (1949) diversity index with data from 2006; see Lázaro et al. 2008 for details. Ab. (%): abundance, i.e. percentage of plots the species was present in. Red. (%): Percentage of reduction in visitation rate obtained with the experiment, calculated as 1 –e/c, where e is the average visitation rate in the Experimental plots and c is the average visitation rate in the Control plots. N_{vis}: sample size for the analyses of visitation rate, i.e. number of observation periods recorded for the control (C) and the experimental (E) plots; ; N_{PL}: sample size for the analyses of pollen limitation, i.e. number of control (C) and experimental (E) plots in which the hand-pollination experiment was performed. N_{SS}: sample size for the analyses of seed set, i.e. number of individuals recorded for the control (C) and the experimental (E) plots. NA: Non-available data.

Community	Species	Family	PL	DPD	Flow. length	Main poll.	PG	Ab. (%)	Red. (%)	N_{vis} C	N_{vis} E	N_{PL} C	N_{PL} E	N_{SS} C	N_{SS} E
Ryghsetra	*Centaurea jacea*	Asteraceae	0.05	0.79	15	BB	1.23	50.0	42.4	30	33	19	16	58	54
	Galium mollugo	Rubiaceae	0.24	0.94	27	MF	1.81	88.3	19.6	47	46	26	26	110	114
	Leucanthemum vulgare	Asteraceae	0.15	0.76	32	MF	2.26	66.7	68.0	29	30	28	23	71	59
Finse	*Astragalus alpinus*	Fabaceae	0.42	0.95	27	BB	2.93	51.7	14.7	42	40	11	11	42	40
	Bartsia alpina	Scrophulariaceae	0.16	0.99	26	BB	2.00	68.3	47.6	42	44	23	19	65	57
	Parnassia palustris	Celastraceae	0.04	0.77	16	MF	1.20	76.0	33.4	46	36	NA	NA	72	65

Experimental manipulations to reduce the number of flower visitors

In 2006, we placed 30 pairs of permanent plots (2 × 2 m) at the beginning of the field season in each study area. An inner square of 1 × 1 m of each plot was marked to observe flower visitation. At Ryghsetra, where the meadow was large and relatively homogeneous, we placed the pairs in a systematic way, along two lines separated by ca 3 m. At Finse, however, where vegetation was heterogeneous across the whole area, we placed the pairs randomly within suitable sub-areas in the community, with a minimum separation among pairs of ca 3 m. Separation between plots within pairs was ca 2 m. We randomly selected one plot of each pair for experimental reduction in flower visitation (experimental plots hereafter), whereas the other plot was left open for natural pollination (control plots hereafter). To obtain a reduction in the number of flower visits, we constructed dome-shaped cages by bending two 4 m long PVC-tubes diagonally and covered the domes with two layers of fishnet, each with a mesh width of 1.05 × 1.05 cm. The fishnet was totally transparent, so flowers were fully visible from outside the net. In order to allow flower visitors inside cages to exit easily, we left a 10-cm opening between the mesh and the ground, and a 0.5 × 0.5 m opening in the top of the dome.

In a pilot-study conducted in 2005 at Ryghsetra, with the same experimental set up, we checked potential physical side-effects of the cages, such as changes in herbivory, seed predation, photosyntetically active radiation and wind speed. No significant side-effects of the cages were found (all differences between controls and experimental plots had p > 0.05), although there was a marginally non-significant reduction in wind speed inside the cages (19.4%; p = 0.066). We do not expect this small reduction in wind speed to have effects on the results because our focal species are all entomophilous, and the reduction in wind speed inside the cages did not appear to modify the behavior of insects.

Data collection: insect visitation, flower abundance and seed set

The pollinator visitation data presented here are based on 247 20-min observations periods conducted from 15 June to 19 July 2006 at Ryghsetra; and on 267 20-min observations periods conducted from 1 July to 5 August 2006 at Finse. These periods covered the flowering episode for the six study species this year. The observations were conducted every day when weather conditions allowed pollinator activity, between 09.00 and 18.00 hours. Experimental and control plots within a pair were observed simultaneously or immediately after each other. The order of observation of plots was random, but we did not observe the same plot more than once in a day. During each observation period we noted the number of pollinator visits to flowers or inflorescences (depending on species) of any species occurring in the inner 1 × 1 m square. A visit was defined to have occurred when there was contact between the visitor's body and the reproductive organs (stigma or anther) of the flower. After each observation period, we counted the number of open flowers (or inflorescences) of all entomophilous species inside the 2 × 2 m observed plot and the inner 1 × 1 m square. Table 1 shows the data on number of observation periods per study species.

We obtained the visitation rate per flower (or inflorescence) to each study plant species in each 20-min observation period, by dividing the total number of flower visits to one plant species by the number of open flowers or inflorescences of this plant species in the 1 × 1 m inner square. To characterize the floral neighbourhood, we used the data on the number of open flowers (or inflorescences) in the 2 × 2 m plots.

During the flowering period of the study species, we haphazardly selected and marked (with a small piece of drinking straw) one flower on 1–3 individuals (depending on the availability) per study plant species in each plot where they occurred. We also measured the height and

counted the number of flowers of each marked individual. We collected the fruits of the marked flowers when fruits were dry and immediately before dispersal, between 20 June and 27 July 2006 at Ryghsetra, and between 20 and 27 July at Finse. Fruits were stored in paper bags until they were dissected in the laboratory to count undeveloped, aborted (seeds that did not have the size and the colour of developed seeds) and fully developed seeds. Seed set was obtained by dividing the number of fully developed seeds by the total number of ovules (sum of undeveloped, fully developed and aborted seeds). In *Centaurea jacea* and *Leucanthemum vulgare,* Asteraceae-species with single-seed fruits, the average seed set was calculated for each infructescence to obtain a single measure of seed set per individual plant. Table 1 presents the number of individuals used for each study species.

Pollen limitation

In spring 2006, we performed supplemental hand-pollination experiments to determine the effect of neighbors on the reproduction of the focal species through pollination. Hand-pollination experiments were conducted in each plot where the study species occurred (both experimental and control plots). For this, we selected two similar-sized individuals (other than those used for measures of seed set) per study plant species in each plot. One individual of each pair was haphazardly assigned to receive supplemental hand-pollination (S) and the other to receive only natural pollination (N). Pollen supplementation was conducted by smoothly depositing pollen from at least 5 individuals situated across the meadow onto stigmas with a small brush, every second day until all flowers of each individual had been supplementary pollinated at least twice. We also measured the height and counted the number of flowers of each individual in this experiment.

We collected all the fruits of each individual when dry and immediately before dispersal, and stored them in paper bags. Seed set was obtained as explained above. With these data, we calculated an index of pollen limitation per species and plot as: $PL = 1 - (N/S)$, where N is the seed set of natural pollinated plants and S is the seed set of supplementary hand-pollinated plants (Larson and Barrett 2000). Negative values, which indicate a higher seed set in open-pollinated than pollen-supplemented plants, were set to zero (Young and Young 1992, Larson and Barrett 2000, Jakobsson et al. 2009) because large negative values may be caused by negative experimental side-effects, and small negative values may occur due to chance; when pollen limitation does not actually occur. The use of negative values in the analyses did not change the main results.

The supplemental pollination experiment was conducted in experimental and control plots for all the study species except *Parnassia*. In this species logistical problems during field work only allowed us to obtain a pollen limitation index under natural pollination, calculated using 15 pairs of plants outside the plots. Table 1 shows indices of pollen limitation under natural pollination for each study species, and sample sizes for the analyses of pollen limitation.

Neighbourhood variables

To describe the floral neighbourhood of each study species in each observation period we used: 1) 'conspecific density', i.e. the number of flowers of the focal species in the 2×2 m plots; and 2) 'heterospecific density', i.e. the number of flowers of the pollinator-sharing species in the 2×2 m plots, excluding the number of flowers of the focal species. To determine which plant species shared their main pollinators in the study communities, we grouped the plant species according to the relative abundance (the proportion of the total) of the different insect groups as their pollinators. For that, we used the cluster analyses (PRIMER package; Clarke and Gorley 2001) reported in Lázaro et al. (2009). These cluster analyses are based in a similarity coefficient (S) which measures how similar any pair of samples are in terms of the biological communities they contain (in this case, how similar any pair of plant species are in terms of their pollinator assemblage).

For the analyses of pollen limitation and seed set we calculated conspecific and heterospecific density per plot and study species by averaging conspecific and heterospecific density across all days the focal species flowered.

Statistical analyses

Our experiment effectively reduced visitation rates by ca 50% in total when the whole communities were considered (see Table 1 and Supplementary Material Appendix 1 for reduction for each study species). These reductions occurred without modifying the behaviour or the composition of visitors to the study species (except for *Centaurea* and *Astragalus* that showed a very small difference in the composition of visitors inside and outside the experimental plots (Supplementary material Appendix 1 Table A1).

To determine whether our experiment modified pollination interactions between the focal species and their conspecific and heterospecific neighbourhood, we performed general additive mixed models (GAMM, Zuur et al. 2009) on visitation rate and seed set, and general additive models (GAM, Zuur et al. 2009) on pollen limitation. The analyses were conducted in R (function gamm/gam; library mgcv). We used additive models because they are non-parametric and allow the exploration of both linear and non-linear responses, and the expected responses to plant density are not necessarily linear (Rathcke 1983). The focal species did not always occur in both plots within a 'pair' and therefore, the pair of plots could not be included as a random factor in the models for visitation rate and seed set. Instead, we included the individual plots (nested within the particular treatment) as a random factor in these models. This should not affect the results because with plot location we ensured a homogeneous distribution of both plots types through the communities. In the models of pollen limitation there was no need to include this random factor since we only had one measure of pollen limitation per plot. The experimental treatment was entered as a fixed factor and conspecific and heterospecific density as continuous predictor variables (these variables were log-transformed for a better

Table 2. General additive models relating conspecific and heterospecific densities to visitation rate, pollen limitation and seed set inside and outside the experimental plots (E: experimental plots; C: controls) for each of the study plant species. When a variable was significant F-values and their significance (coded: ***$0 \geq 0.001$; **$0.001 \geq 0.01$; *$0.01 \geq 0.05$; •$0.05 \geq 0.1$) are given. Ns: non-significant relationship; (c): consistent relationship across treatments. (f) indicates variable log-transformation (except for conspecifics in *Astragalus* seed set model and heterospecifics in *Parnassia* visitation model). R^2, sample size (n) and other significant variables included in the models (i.e. plot type – experimental or control plot- and plant size) are also indicated for each model.

Model	Neighborhood variable	Visitation rate E	Visitation rate C	Pollen limitation E	Pollen limitation C	Seed set E	Seed set C
(a) *Centaurea jacea*	conspecific density (f)	5.41**	ns	ns	ns	ns	ns
	heterospecific density	ns	ns	ns	ns	ns	ns
		$R^2 = 0.31$; n = 63; plot type***		$R^2 = 0.10$; n = 35; plant size•		$R^2 = 0.03$; n = 102; plant size*	
(b) *Galium mollugo*	conspecific density	ns	4.19*	6.74**	6.11*	5.05*	ns
	heterospecific density (f)	3.89	13.75***	ns	ns	ns	ns
		$R^2 = 0.18$; n = 93		$R^2 = 0.25$; n = 52		$R^2 = 0.08$; n = 227; (f)plant size**	
(c) *Leucanthemum vulgare*	conspecific density (f)	ns	8.25***	ns	4.68*	ns	2.88•
	heterospecific density	ns	ns	ns	ns	ns	ns
		$R^2 = 0.07$; n = 59; plot type*		$R^2 = 012$; n = 51		$R^2 = 012$; n = 138; Exclosure**; plant size*	
(d) *Astragalus alpinus*	conspecific density (f)		(c) 7.09**	21.36***	8.13*	3.97*	ns
	heterospecific density (f)		(c) 5.01*	22.24***	4.85*	3.75*	ns
		$R^2 = 0.02$; n = 82		$R^2 = 0.62$; n = 21; plant size*		$R^2 = 0.16$; n = 72; plant size*	
(e) *Bartsia alpina*	conspecific density	ns	ns	ns	ns	ns	ns
	heterospecific density	ns	7.41**	4.40*	ns	ns	ns
		$R^2 = 0.02$; n = 86		$R^2 = 0.08$; n = 42		$R^2 = 0.00$; n = 129	
(f) *Parnassia palustris*	conspecific density (f)	ns	5.91**			14.20***	ns
	heterospecific density (f)	ns	3.08•			3.26*	3.63•
		$R^2 = 0.13$; n = 81				$R^2 = 0.14$; n = 156; plant size*	

fit of some of the models; Table 2). We also included the size of the plant in all the analyses, since it could affect visitation and fecundity. We used plant height to characterize plant size for all species except *Astragalus,* because study plants of this species had from one to three similar-height branches and therefore the number of flowers was a better indicator of plant size than plant height in this population. Since visitation rate also can vary across dates we checked for potential presence of temporal auto-correlations in our data sets. For that, we averaged the residuals of each model by day and calculated, using the autocorrelation function (Zuur et al. 2009), whether any temporal correlation existed between these averaged residuals. We found no signal of temporal autocorrelation and therefore sampling date was not included in the models. Due to the nature of the data, we used gamma distributions and log-link functions for the analyses of visitation rates (except for *Centaurea* where we used a normal distribution) and pollen limitation, and binomial distribution with logit link for the analyses of seed set. The observation periods were the sampling units for the analyses of visitation rates, plots for pollen limitation and individual plants for seed set. Therefore, we averaged the values of predictor variables within plots to obtain a single measure of the predictor variables per plot for the analyses of pollen limitation and seed set. Since we were specifically interested in the interactions between the treatment factor and the continuous variables, we first tested the significance of these interactions. Once we obtained this model, we checked whether the model improved (i.e. AIC values decreased; Zuur et al. 2009) when the significant interactions were substituted by the predictor variables alone and therefore the relationships could be considered consistent across experimental treatments. Analyses were conducted separately for each of the six focal species, since previous analyses showed significant triple interactions between species, experimental treatment and the other predictor variables. However, to check for the existence of any global pattern within communities, we also performed global analyses for each community. These global models included the focal species as an additional random factor.

Results

Effect of the experiment on plant–plant interactions for pollination

The experimental reduction in pollinator visitation modified plant–plant interactions for pollination in all the six study species (Table 2), although for two of them we did not find any effect of neighbours on seed set. The models for each species explained a very variable percentage of the variance in visitation rates and pollen limitation, and a low to moderate percentage of the variance in seed set (Table 2). As expected, the responses to the experiment were species-specific:

Centaurea jacea
Visitation rate in *Centaurea* increased with increasing conspecific density, but only significantly in the experimental

plots. Increased visitation rate with conspecifics did not translate into lower pollen limitation or higher seed set, and these variables only decreased and increased respectively with increasing plant size (Table 2a, Fig. 1).

Galium mollugo
Per flower visitation rate in *Galium* was negatively related to conspecific density and positively related to heterospecific density, but these effects were only significant in the control plots. Only the interactions with conspecifics had an effect on reproduction, decreasing more strongly pollen limitation and increasing seed set as the density of conspecifics in the experimental plots increased (Table 2b, Fig. 2).

Leucanthemum vulgare
Visitation rate in *Leucanthemum* increased with increasing conspecific density, but this relationship was only significant in the control plots. Such increased visitation translated into a reduction in pollen limitation and consequent increase in seed set at intermediate conspecific densities in the control plots. Plants with intermediate size showed the highest seed set (Table 2c, Fig. 3).

Astragalus alpinus
Visitation rate in *Astragalus* was positively related to conspecific density and negatively related to heterospecific density, consistently across treatments. Pollen limitation increased with increasing conspecific density in the experimental plots, and decreased with increasing conspecific density in the control plots. Seed set showed a hump-shaped relationship with conspecific density in the experimental plots. Decreased visitation with heterospecific density translated into increased pollen limitation in both plot types (although the relationship was stronger in the experimental plots) and decreased seed set in the experimental plots. Pollen limitation decreased and seed set increased as plant size increased (Table 2d, Fig. 4).

Bartsia alpina
Per flower visitation rate in *Bartsia* decreased as hetero-specific density increased, although the relationship was only significant in the control plots. Pollen limitation increased with increasing heterospecific density in the experimental
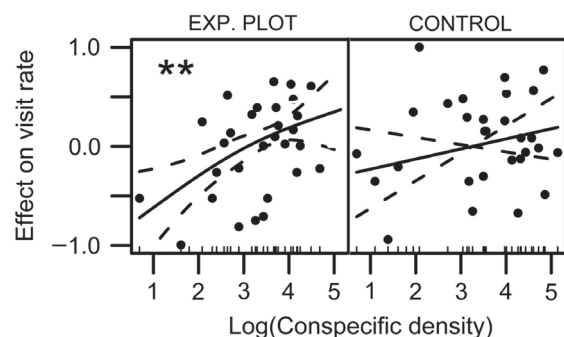

Figure 1. Relationships between conspecific density and visitation rate for *Centaurea jacea* in control (CONTROL) and experimental plots (EXP. PLOT). Dots represent the partial residuals of the model and dotted lines the 95% confidence intervals. **p-value: $0.001 \geq 0.01$.

(a) Visitation rate

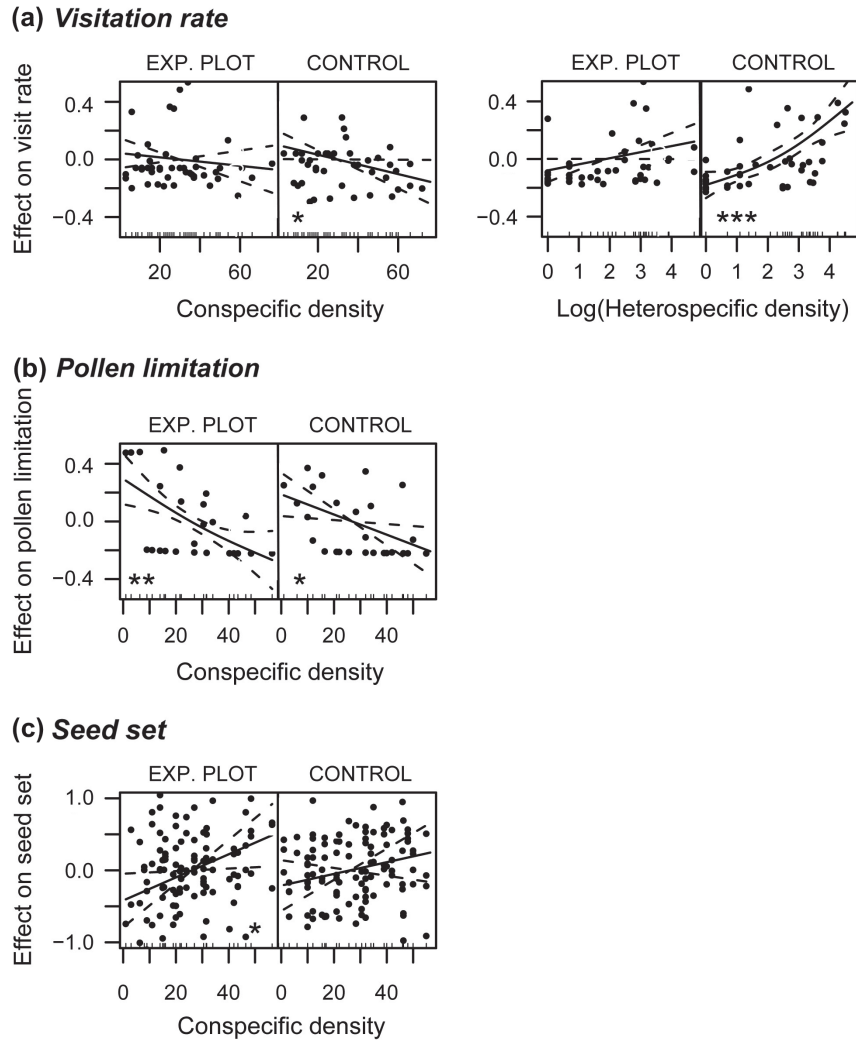**(b) Pollen limitation**

(c) Seed set

Figure 2. Relationships between conspecific and heterospecific density and (a) visitation rate; and between conspecific density and (b) pollen limitation and (c) seed set for *Galium mollugo* in control (CONTROL) and experimental plots (EXP. PLOT). Dots represent the partial residuals of the model and dotted lines the 95% confidence intervals. p-values: ***$0 \geq 0.001$; **$0.001 \geq 0.01$; *$0.01 \geq 0.05$.

plots, while tended to decrease as heterospecific density increased in the control plots (marginally significant relationship). Seed set was not affected by any of the variables included in the model (Table 2e, Fig. 5).

Parnassia palustris
Visitation rate in *Parnassia* decreased quadratically as conspecific and heterospecific densities increased, but only in the control plots. Seed set increased as conspecific density increased, whereas it decreased with increasing heterospecific density, but only significantly in the experimental plots. Plant size was positively related to seed set (Table 2f, Fig. 6).

In summary, the results for pollen limitation and seed set were consistent for all species and five of the six species showed more significant and stronger interactions between the focal species and the neighbourhood inside the experimental plots than outside. On the contrary, more significant relationships were detected in the controls for visitation rates, although the direction of relationships was maintained across treatments. There were facilitative interactions with conspecifics for pollination in half of the study species (seen either in visitation or pollen limitation/seed set data), but only in the experimental plots. In two of the other species a hump-shaped relationship between conspecific density and seed set appeared, increasing seed set with conspecifics at low conspecific densities and decreasing at high densities. Heterospecific density only influenced pollen limitation and seed set in the species at Finse and only in the experimental plots, where they competed with the focal species. These general trends were confirmed by the global analyses conducted for each community, where plant species was included as a random factor. The global analyses for pollen limitation and seed set showed a negative relationship between pollen limitation and conspecific density (at Ryghsetra) and positive relationships between seed set and conspecific density (at both Ryghsetra and Finse) in the experimental plots (Table 3), indicating facilitation of conspecifics in the experimental plots in both communities. The global analysis for Finse also showed a

(a) Visitation rate
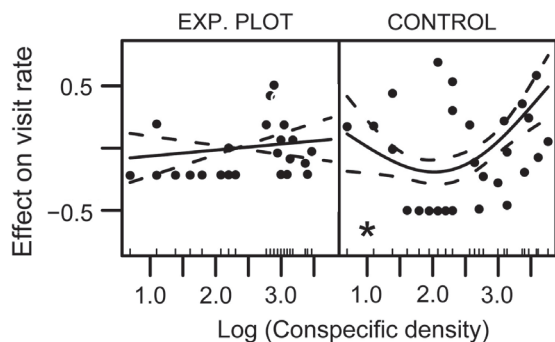
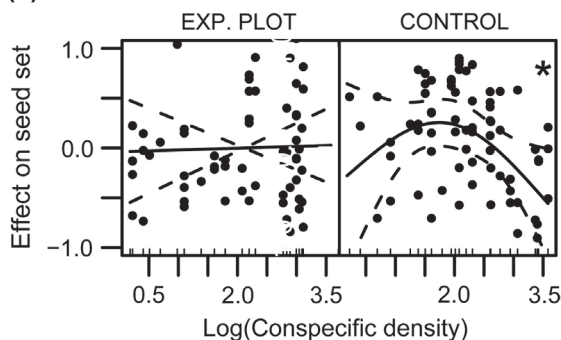## (b) Pollen limitation
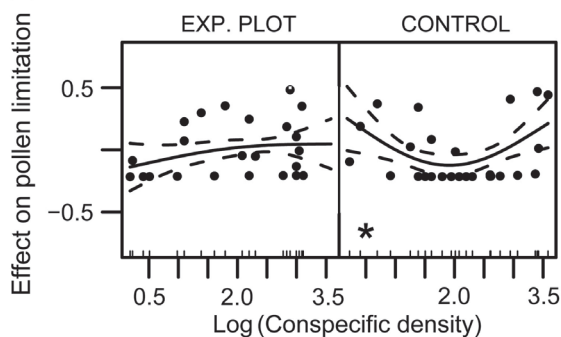
(c) Seed set

Figure 3. Relationships between conspecific density and (a) visitation rate, (b) pollen limitation and (c) seed set for *Leucanthemum vulgare* in control (CONTROL) and experimental plots (EXP. PLOT). Dots represent the partial residuals of the model and dotted lines the 95% confidence intervals. *p-value: $0.01 \geq 0.05$.

positive relationship between pollen limitation and heterospecific density, and a marginally significant negative relationship between seed set and heterospecific density in the experimental plots (Table 3b), indicating competition with heterospecifics in the experimental plots in this community. Interactions for visitation rate were stronger in the control plots in the global analyses for both communities (Table 3).

Discussion

We have experimentally tested, for the first time to our knowledge, the effect of a pollinator reduction on neighbouring

interactions for pollination. Our experiment influenced how plants interacted for pollination in complex ways. The results on pollen limitation and seed set showed that reducing insect visitation most commonly increased the number and intensity of interactions between plants for pollination. Although the responses were highly species-specific, there was a tendency for increasing facilitative interactions with conspecific neighbours in the plots where the availability of pollinators was experimentally reduced. Heterospecifics influenced pollination and fecundity only in species at Finse and in the experimental plots, where they competed with the focal species. The results for visitation rates contradicted the general tendency of the other results, with mostly significant interactions between plants in the controls that could be either competitive or facilitative depending on the species.

Effect of the experiment on plant–plant interactions for pollination

Our experimental reduction in the availability of pollinators affected the interactions for pollination with neighbours in all the study species. We predicted higher number of, and more intense, plant–plant interactions inside the experimental plots, where the availability of pollinators was reduced. Neighbours had an effect on pollen limitation in five of the study species. For four of these five species, we detected more and stronger relationships in the experimental plots than in the controls when we analyzed pollen limitation and seed set data. This supports the hypothesis that neighbours have a stronger effect on the fitness of individual plants when the pollinators are scarce. We believe that the reason why the other species (*Leucanthemum*) showed stronger relationships inside the control plots is that visitation of this species was too reduced by the experiment (nearly 70%; Table 1) to show any pattern with density inside the experimental plots. Although the responses were highly species-specific, there was a tendency (found for half of the species) for increasing facilitative interactions with conspecifics when pollinator visitation was experimentally reduced; and this result was further corroborated by the global analyses per community. Pollination success and conspecific density are often positively related (Kunin 1997, Bosch and Waser 2001), which is attributed to increased pollinator attraction (Kunin 1997, Bosch and Waser 2001) and/or quantity or quality of pollen deposited on stigmas (Kunin 1997). Moreover, an increase in the relative frequency of positive relationships between plants under high stress levels is predicted by the stress-gradient hypothesis (Bertness and Callaway 1994). This hypothesis is based on the idea that in these situations of stress, slight differences in local conditions created by neighbours may favour growth and survival, increasing facilitative relationships between plants. This hypothesis has not been applied to the pollination context before; however, it might apply, because the pollinators are a resource for the plants and plants may be under stress when pollinator availability is reduced. Thus, when pollinator availability is reduced, local increases in conspecific density may enhance patch attraction and favour proper pollen transfer, increasing pollination success in plants occurring
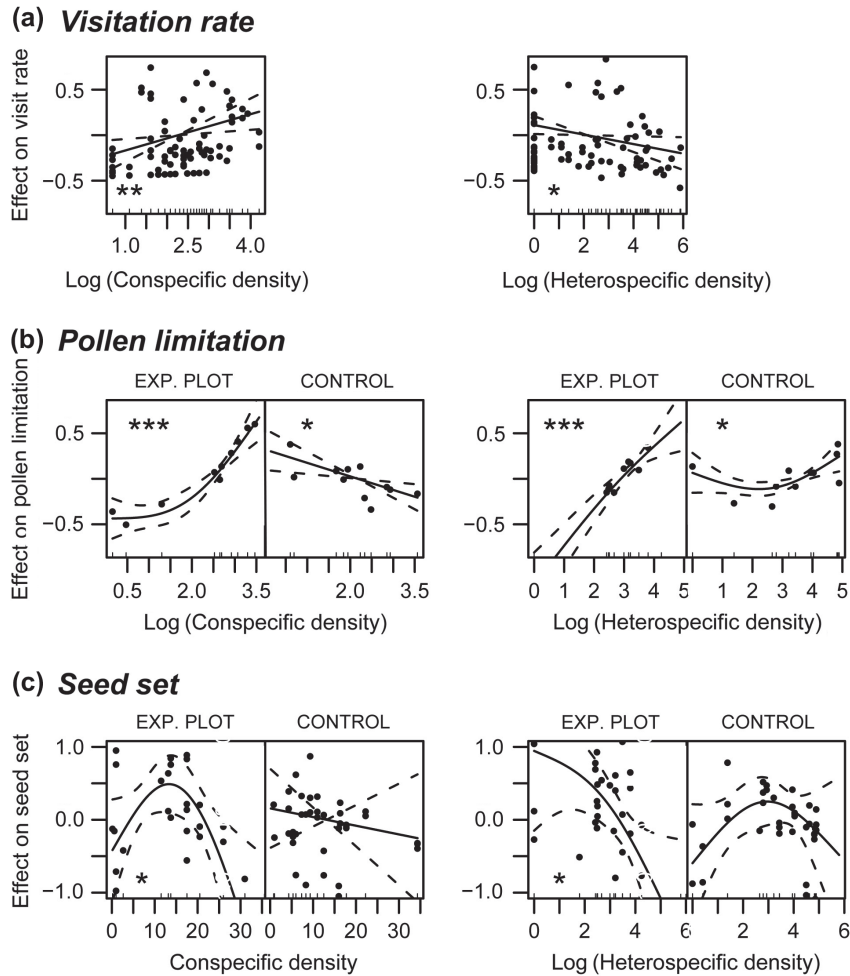
Figure 4. Relationships between conspecific and heterospecific density and (a) visitation rate (consistent relationship across experimental treatments), and (b) pollen limitation and (c) seed set for *Astragalus alpinus* in control (CONTROL) and experimental plots (EXP. PLOT). Dots represent the partial residuals of the model and dotted lines the 95% confidence intervals. p-values: ***$0 \geq 0.001$; **$0.001 \geq 0.01$; *$0.01 \geq 0.05$.

in dense conspecific patches. Although the effect of conspecifics was facilitative for half of the study species, we also found hump-shaped relationships between conspecific density and seed set in two other study species (*Astragalus* and *Leucanthemum*). In these species seed set increased with conspecifics at low conspecific densities and decreased at high densities, as predicted by Rathcke (1983) and found in other studies (Sabat and Ackerman 1996, Steven et al. 2003, Brys et al. 2008).

The density of pollinator-sharing heterospecifics may also impact pollination of focal plants (Johnson et al. 2003, Chittka and Schürkens 2001) by modifying pollinator visitation frequencies (Brown et al. 2002, Moeller 2004) and/or proper pollen transfer (Caruso and Alfaro 2000, Jakobsson et al. 2007). In our study, heterospecifics influenced pollination and fecundity only in the species from Finse and in the experimental plots where they competed with the focal species. Novel competitive interactions were expected in the experimental plots based on competition theory (Alley 1982) which predicts that a resource reduction (pollinators in this case) will increase competitive interactions between plants. The fact that competition with

heterospecifics only appeared in species from Finse is perhaps related to pollinator scarcity in this alpine community. Future studies will have to confirm whether the increase in inter-specific competition when pollinators are reduced occurs more often in alpine (or pollinator-poor) communities than in lowland (or pollinator-rich) communities.

The results for visitation rate were opposite to our expectations and to the general tendency of the other results since many of the significant relationships between the flowering neighbourhood and visitation rate in control plots disappeared in experimental plots, although in all the cases the direction of the relationships was similar across treatments. In visitation rate models, only *Centaurea*, the species with the highest visitation rate (average of 4.8 visits/flower per 20 min observation period in the controls vs 0.1–1.3 visits/flower per 20 min observation period for the other species), showed a significant relationship between conspecific density and visitation in experimental plots that was not present in the controls. It could be that visitation rate of the other species in the experimental plots is too low to give sufficient statistical power to test the relationships among neighbours for pollinator attraction. In addition, it

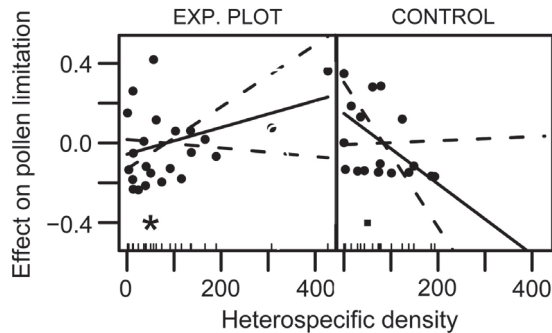1045
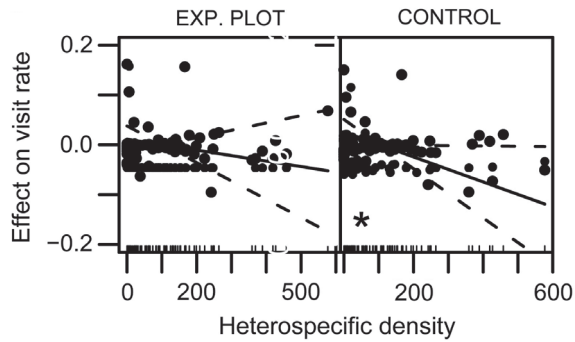
(a) Visitation rate

(b) Pollen limitation

Figure 5. Relationships between heterospecific density and (a) visitation rate and (b) pollen limitation for *Bartsia alpina* in control (CONTROL) and experimental plots (EXP. PLOT). Dots represent the partial residuals of the model and dotted lines the 95% confidence intervals. p-values: **$0.001 \geq 0.01$; *$0.01 \geq 0.05$.

could be that reduced visitation imposed by the experiment limits the range of variation of the response variable (visitation rate), thereby diminishing the strength of the response and flattening the relationships.

Although our experiment did not significantly modify pollinator composition or important components of pollinator behaviour such as number of contacted flowers or visit duration, it may still have its limitations. Pollinators that enter the experimental plots have previously foraged outside the exclosures, where pollinator visitation was not reduced. Therefore, our experiment represents a reduction of pollinator availability from a plant perspective, but only a local reduction of competitors from the pollinator perspective. Many of the insect behavioural choices may have been developed outside the exclosures, based on their experiences there. However, we would expect that a global reduction in pollinators (opposite to our local experimental reduction) would accentuate the effects found here.

Concordance between response variables

There was a great consistency between the results of pollen limitation and seed set models conducted per species, which suggests that seed set actually reflects neighbouring interactions for pollination and not interactions for abiotic resources (Weiner 1982, Mustajärvi et al. 2001) in the study species. Indeed, only the seed set of one species (*Bartsia*) was not influenced by the flowering neighbourhood, despite effects of co-flowering neighbours on pollen limitation; but this may be because slight effects of neighbours on pollen limitation in this species were offset by stronger effects on resource availability that were independent of
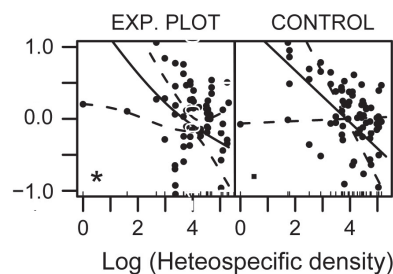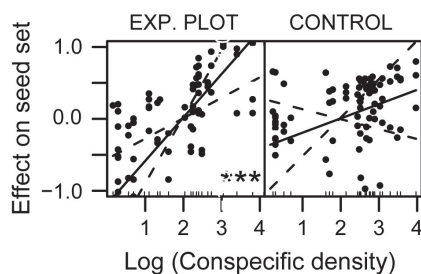
(a) Visitation rate
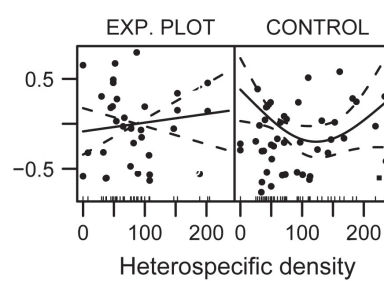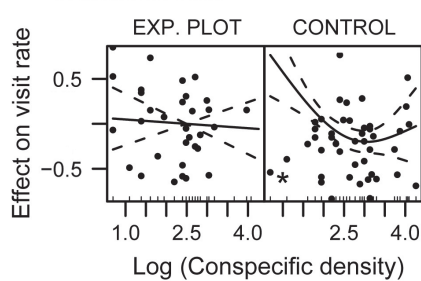
(b) Seed set

Figure 6. Relationships between conspecific and heterospecific density and (a) visitation rate and (b) seed set for *Parnassia palustris* in control (CONTROL) and experimental plots (EXP. PLOT). Dots represent the partial residuals of the model and dotted lines the 95% confidence intervals. p-values: ***$0 \geq 0.001$; **$0.001 \geq 0.01$; *$0.01 \geq 0.05$;. $0.05 \geq 0.1$.

Table 3. General additive mixed models relating conspecific and heterospecific densities to visitation rate, pollen limitation and seed set inside and outside the experimental plots (E: experimental plots; C: controls) for the study communities (including species as a random factor). When a variable was significant F-values and their significance (coded: ***$0 \geq 0.001$; **$0.001 \geq 0.01$; *$0.01 \geq 0.05$; •$0.05 \geq 0.1$) are given. Ns: non-significant relationship; R^2, sample size (n) and other significant variables included in the models (i.e. plot type – experimental or control plot- and plant size) are also indicated for each model. All the significant relationships were linear (edf = 1.00): (+) indicates a positive relationship between variables; (−) indicates a negative relationship.

Model	Neighborhood variable	Visitation rate		Pollen limitation		Seed set	
		E	C	E	C	E	C
(a) Ryghsetra	Conspecific density	ns	(+) 2.87•	(−) 3.73*	ns	(+) 4.27*	ns
	Heterospecific density	ns	ns	ns	ns	ns	ns
		$R^2 = 0.03$; n = 215; plot type**		$R^2 = 0.05$; n = 138; plant size•		$R^2 = 0.05$; n = 467; plant size***	
(b) Finse	Conspecific density	ns	ns	ns	ns	(+) 5.57*	ns
	Heterospecific density	ns	(−) 4.85*	(+) 4.49*	ns	(−) 2.99•	ns
		$R^2 = 0.02$; n = 249		$R^2 = 0.03$; n = 63		$R^2 = 0.07$; n = 353; plant size*	

neighbours. Conflicting results for pollen limitation and seed set were only detected in the global analyses for Finse that showed a positive relationship between conspecifics and seed set that was not detected in the pollen limitation model (Table 3). This shed doubts to whether facilitative interactions with conspecifics in this community were related to pollination or abiotic resources. However, likely such facilitative effect of conspecifics on seed set was also related to pollination, because separate models for each species indicated interactions related to pollination; and because the global analysis for pollen limitation at Finse only included two species (pollen limitation data for *Parnassia* was not available), which could have affected the detection of such a relationship.

The results obtained with visitation data, however, were not always consistent with the results obtained with pollen limitation data. First, we detected significant relationships with neighbours for a couple of species that were not present when pollen limitation was studied. Strong effects of the neighbourhood on visitation can be diluted in fecundity if species can compensate via autogamy (Karoly 1992, Kalisz et al. 2004), if there is a conflict between the quantity and the quality of visits (Caruso and Alfaro 2000, Jakobsson et al. 2007), or if the species receive more visits than those required to fertilize the ovules (Molano-Flores et al. 1999). In case of *Centaurea*, the positive effect of conspecifics on visitation rate disappear in pollen limitation models probably because this species is by far the most visited species both inside and outside the experimental plots and shows one of the lowest pollen limitation indices in natural conditions (Table 1). In case of *Galium*, the positive effect of heterospecifics on visit rates is probably counterbalanced by negative effects of heterospecific pollen transfer (Caruso and Alfaro 2000, Jakobsson et al. 2007). More interestingly, part of the results for visitation and pollen limitation/seed set data for the other three species (conspecifics in *Astragalus* and *Galium* and *Leucanthemum*) seem contradictory (i.e, while one model indicates facilitation, the other competition with conspecifics), which could be due to a differential effect of conspecifics on the quality and quantity of pollinator visits (Feinsinger 1987). Because pollinator visitation can be very stochastic, only visitation data of frequently visited and highly specialized plant species

may be reliable to predict effects on reproduction; whereas pollinator visitation may be difficult to relate to plant fecundity in more generalist plants, as these three study species (Table 1). Our study therefore highlights that visitation patterns are not always reflected in the consequent plant fecundity, and warn against the exclusive use of visitation data to interpret plant–plant interactions for pollination.

Conclusions

We reduced insect visitation without modifying the behaviour of individual pollinators, to test the effects on plant–plant interactions for pollination. Our experiment influenced interactions between plants for pollination in all the study species in complex species-specific ways. Results on pollen limitation and seed set showed that reduced pollinator availability mostly increased the number and intensity of plant–plant interactions for pollination, as expected. There was a tendency for increasing facilitative interactions with conspecifics and competitive interactions with heterospecifics, although the effect of heterospecifics was limited to the species from the alpine community. The lack of correspondence between the results on visitation rate and seed set was remarkable and suggests that studies that rely on visitation data should be cautiously interpreted. Future work, both correlative and experimental, would be needed to corroborate our results, to identify general patterns in the structure of interactions at the community level, and to help to predict how pollinator loss may influence plant–plant interactions for pollination.

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Supplementary material (available online as Appendix oik-01268 at <www.oikosjournal.org/readers/appendix>). Appendix 1.