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Combining observational and experimental methods in plant–plant interaction research

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Background: Neighbour-removal experiments (NRE) and spatial pattern analyses (SPA) are commonly used methods to investigate plant–plant interactions. Although they address the same issue, they measure different aspects of plant interactions: experiments indicate contemporary processes, whilst observations of spatial patterns integrate the results of interactions that have prevailed in the past.
Aim: The aim of this study was to propose a new conceptual approach that takes into account the chronological order between processes and the arising patterns i.e., the time lag between neighbour effects quantified with NRE and SPA, to detect shifts in the balance of plant interactions due to current environmental change.
Methods: This conceptual approach was applied to alpine snowbeds. Data from NRE were used to calculate the importance index of current neighbour interactions. Spatial patterns were quantified using variance ratio statistics and were assumed to reflect historic interactions.
Results: The results of the two approaches showed a consistent difference in the prevailing type of plant interactions and suggested a shift towards competition in recent times.
Conclusions: The simultaneous application of NRE and SPA allows the detection of a recent shift in the balance of plant interactions and provides a deeper and more accurate insight into the temporal dynamics of plant communities that could not be gained using one method alone.

Keywords: alpine; climate change; competition; conceptual model; facilitation; neighbour-removal experiment; snowbed; spatial pattern analysis; stress-gradient hypothesis; Swiss Alps

Introduction

The importance of species interactions for the dynamics, structure and composition of plant communities is well recognised (Brooker and Callaghan 1998; Grime 2001). A number of studies have shown that net interactions between plant species vary over space and time due to shifts from inter-specific facilitation to competition or vice versa (Callaway 2007; Antonsson et al. 2009; le Roux and McGeoch 2010; Armas et al. 2011). These findings are conceptualised in the stress-gradient hypothesis, which predicts shifts in the balance of positive and negative interactions along gradients of environmental stress (Bertness and Callaway 1994; Brooker and Callaghan 1998). Indeed, abiotic environmental conditions appear to be important determinants of the variation in the importance and frequency of competitive and facilitative interactions (Holmgren et al. 1997; Choler et al. 2001; Pugnaire and Luque 2001; Callaway et al. 2002; Brooker 2006). To study shifts in the balance between competition and facilitation due to changing environmental conditions, ecologists usually measure plant interactions at two different points in time or, more often, they use a space-for-time approach and look at plant interactions along naturally occurring environmental gradients.

Common field methods used to measure plant interactions include experimental neighbour-removals or observational small-scale spatial pattern analysis (SPA) (e.g. Kikvidze et al. 2011). Although neighbour-removal experiments (NRE) and spatial pattern analysis address the same issue, they measure different aspects of plant interactions. NREs usually compare the biomass, reproductive output or survival of individuals within intact neighbouring vegetation (control) with those of the target individuals whose neighbours (e.g. dominant species or nurse plants) have totally or partially been removed. The difference between the control and target individuals is assumed to estimate the direct effects of the neighbouring plants on the target plants (Kikvidze et al. 2006; Kikvidze and Armas 2010), even though they are difficult to separate from potential impacts of perturbation caused by the removal treatment (Nagy and Grabherr 2009, 249). Recent examples for NRE include Callaway et al. (2002), Maestre et al. (2003), Klanderud (2005), Schiffers and Tielbörger (2006), and Weigelt et al. (2007). By comparison, observational studies using SPA detect non-random distributions of species using null model analysis (Gotelli 2000). Detecting plant interactions from SPA is not as straightforward because, apart from plant interactions, environmental heterogeneity

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or limited dispersal may also generate non-random spatial patterns (Seabloom et al. 2005). Nevertheless, spatial patterns at a very small spatial scale, including only the nearest neighbours, can provide indications for the prevailing type of inter-specific plant interactions whilst dispersal and environmental heterogeneity should generate distribution patterns at larger spatial scales (Silander and Pacala 1985; Purves and Law 2002; Reitalu et al. 2008). Recent examples of SPA to infer plant–plant interactions include Seabloom et al. (2005), Dullinger et al. (2007), Pottier et al. (2007), Reitalu et al. (2008), le Roux and McGeoch (2008) and McIntire and Fajardo (2009).

Since both methods measure plant–plant interactions, ecologists have often found similar results when applying both methods in a single study (Choler et al. 2001; Olofsson 2004; Tirado and Pugnaire 2005; Kikvidze et al. 2005a,b, 2011; Michalet et al. 2011); however, as pointed out by Michalet (2006), for studies performed in semi-arid environments, experimental and observational studies on plant–plant interactions do not necessarily coincide. Indeed, the two methodological approaches do not deal with exactly the same aspect of plant–plant interactions (Nagy and Grabherr 2009, 246–249). Usually performed over a time period of one to a few growing seasons, NREs show the contemporary direct effect of neighbouring plants on the target plant. In contrast, SPAs indicate the long-term effect of the prevailing plant interactions accumulated over time and, accordingly, they provide information on the plant interactions that have occurred in the past (as recognised earlier by Campell et al. 1991; Silvertown et al. 1994). Here we propose a new conceptual model that is largely based on the stress-gradient hypothesis (Bertness and Callaway 1994; Brooker and Callaghan 1998) and which describes the time lag between the process of plant interactions and

the spatial distribution of species as a result of these interactions. In addition, we show how this concept can be applied in practice to detect shifts in the predominating interaction type as a result of ongoing environmental change.

The conceptual model

The time lag between NRE and SPA is demonstrated using two hypothetical examples (Figure 1). The first example (Figure 1, upper part) starts with a neutral co-occurrence of two species (t0). Due to environmental change (e.g. ongoing climate change), both species show improved growth, which will likely result in (more) competitive interactions between the two species (t1), where the competitively superior species (circle) will suppress the inferior species (star). At this stage (t2), NRE will indicate competition whereas SPA will not detect this early stage of competition and one may deduce neutral co-occurrence or even facilitation. Intensified and/or long-lasting competition due to additional amelioration of the growth conditions can end up in local competitive exclusion of the inferior species (t3) and, finally, in spatial segregation of the two species (t4). At this stage, SPA will be able to detect segregation between species and we may therefore deduce the presence of competition. Segregation may eventually weaken a direct negative neighbour effect and NRE thus may detect only weak competition. It should be noted that 'segregation' here only refers to the exclusion from the immediate neighbourhood and thus the 'inferior' species may survive in other localities of the same community.

The second example (Figure 1, lower part) depicts a situation where two neutrally co-occurring species experience increased levels of disturbance such as herbivory (t0). Increased herbivory will reduce the performance of
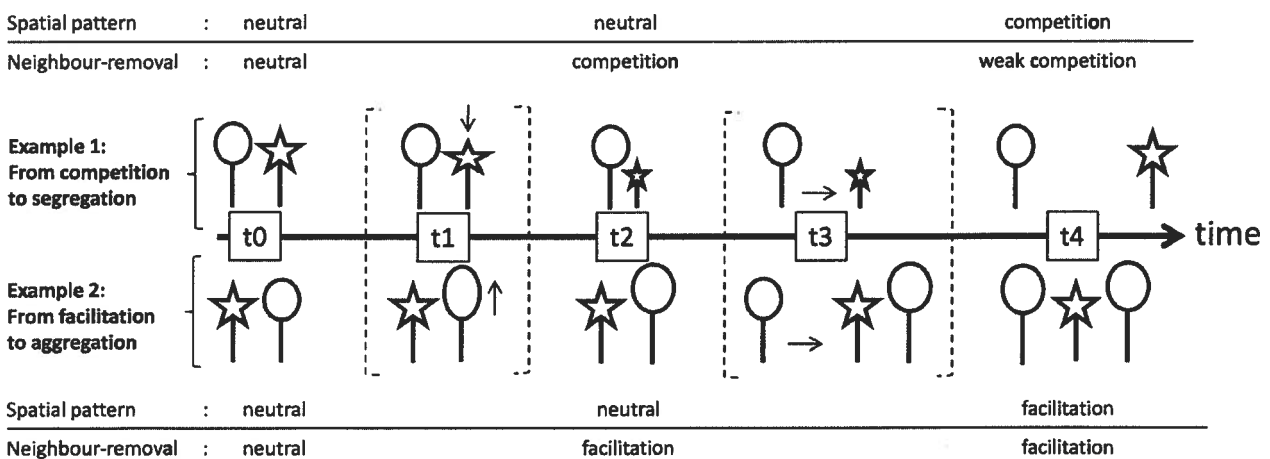

Figure 1.   The generation of spatial patterns by plant interactions through time with indications of the expected results from spatial pattern analyses and neighbour-removal experiments respectively. The upper part of the graph shows the segregation between two species due to increasing competition. The lower part shows the aggregation between two species due to facilitative effects. See text for a more detailed description of the examples. Decreased size of symbols indicates poorer performance whereas increased size of symbols indicates improved performance. In the upper part of the figure the circle-plant represents the superior competitor, whereas the star-plant represents the inferior competitor. In the lower part of the figure, the star-plant represents the unpalatable benefactor species whereas the circle-plant represents the palatable beneficiary species.

palatable species unless they are protected by unpalatable benefactor species (e.g. due to spines or toxicity). Consequently, palatable species (circle) growing next to unpalatable species (star) grow better relative to palatable species without protection (t1). The positive effect of the benefactor species on palatable beneficiary species at this early stage of facilitation (t2) can be detected by NRE but not by SPA. If herbivory increases and/or lasts for a longer time, palatable plants will more frequently or even exclusively co-occur with unpalatable benefactor species. In other words, the occurrence of palatable species will be increasingly clumped around the benefactor species (t3). This can finally end up in (obligate) associations with the benefactor species (t4). At this late stage of the interaction, facilitation can be observed with SPA and NRE even though spatial associations may also turn into asymmetric competition between benefactor and beneficiary species (Olofsson et al. 1999), which would be indicated by observed facilitation in SPA and observed competition in NRE.

In both examples, there is a time of transition (t2) where NRE and SPA indicate diverging types of plant–plant interactions. The time lag between the NRE-indicated and SPA-indicated state will certainly depend on the rate of species turnover and longevity of species in a system, but it will also depend on the intensity of the competitive or facilitative effects and the rate of environmental changes. The conceptual model shown in Figure 2 illustrates the different dynamics of a shifting balance in immediate neighbour effects and consecutive changes of small-scale spatial patterns in a plant community under changing environmental conditions. This divergence between NRE

and SPA can provide insight into the ongoing transition of the prevailing type of plant interactions and can help predict the shift of spatial patterns that will follow the shift in neighbour effects.

The example of alpine snowbeds

Alpine snowbeds provide an excellent system to illustrate and apply the conceptual model because plant interactions in this habitat have been characterised by a sensitive balance of competition for resources during the short time available for growth and facilitation against herbivory by means of associational resistance (Schöb et al. 2010), where growth in a mixture of different species protects from herbivores due to visual or olfactory complexity (Callaway 2007). In addition, snowbeds show severe growth conditions with a very short growing season, intermittent water-logging, and often rather poor nutrient availability (Vonlanthen et al. 2006; but see Björk and Molau 2007) in combination with low temperatures and relatively high herbivory pressure (Tomaselli 1991; Razzhivin 1994; Björk and Molau 2007; Schöb et al. 2009, 2010). However, due to a warming climate, growth conditions in snowbeds in the Alps are changing: the growing season is becoming longer and warmer (Laternser and Schneebeli 2003; Raible et al. 2006). These changes can positively affect resource conditions, for example, due to increased microbial activity and nutrient cycling (Seastedt et al. 2001; Körner 2003) but probably do not influence herbivory pressure in our study system, unless increased temperature or increased plant growth would affect the abundance of the main herbivores (O'Connor et al. 2011). With ongoing
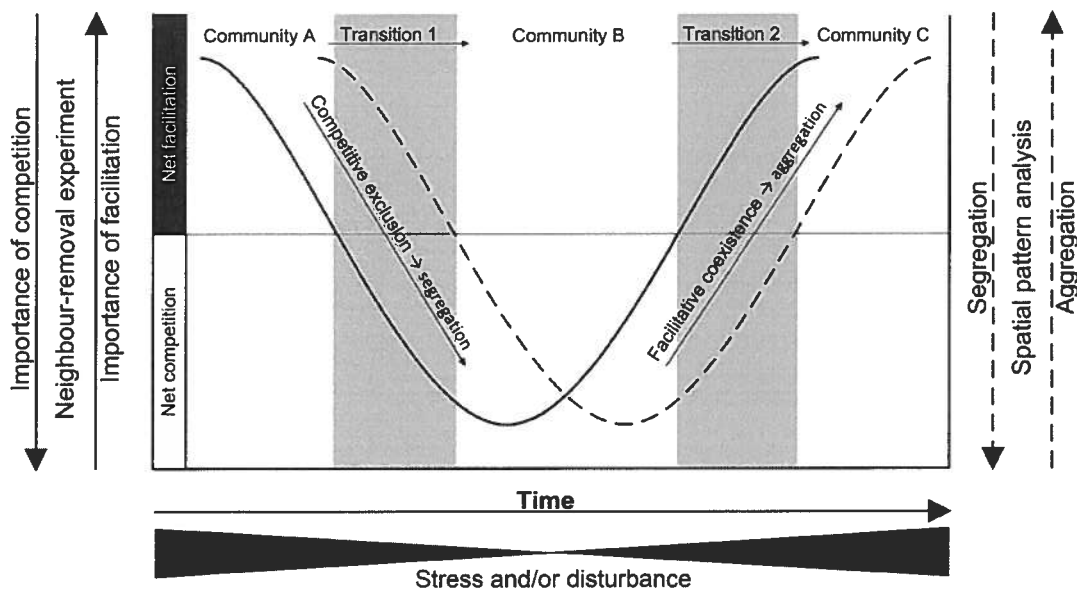

Figure 2.　Conceptual model showing the different dynamics of plant interactions revealed by neighbour-removal experiments and spatial patterns analyses in a plant community under changing environmental conditions. In a stable plant community (communities A, B & C) neighbour-removal experiments (———) and spatial pattern analysis (- - - -) show the same net interaction type; however, during the transition from one community to another the results of experiments and spatial pattern analysis diverge in such a way that the results of the spatial pattern analysis follow the results of the neighbour-removal experiment. The figure design was inspired by Bertness and Callaway (1994).

climate change, snowbeds may therefore experience a significant shift in the balance of plant interactions (Schöb et al. 2010; Hülber et al. 2011). Based on our conceptual model, we hypothesise that the combination of NRE and SPA will show divergent results if this balance of competition and facilitation is currently shifting towards a more important role of competition due to the likely effects of climate warming on soil resources.

Data of plant–plant interactions using NRE and SPA were derived from studies by Schöb et al. (2008, 2010). These studies were conducted in alpine snowbeds in the Swiss Alps (2400 m a.s.l.; 46°25' N, 7°37' E). A detailed description of the study site and methods applied is given in Appendix 1. Forty blocks in 24 spatially separated snowbeds were studied along a snowmelt gradient of 21 days and a mean temperature gradient during the growing season of 1.5 °C. Main herbivores were sheep and caterpillars of *Zygaena* sp. Apart from herbivory, we are not aware of any other major disturbances in these snowbeds. Both NRE and SPA were carried out in 480 circular plots of a radius of 5 cm. The NRE was made by removing all above-ground biomass surrounding half of the target individuals of six species that represented different distribution types of species in snowbeds described by Schöb et al. (2008, 2009). We assumed that the results obtained for those species apply to most of the frequent species occurring in the snowbed community under study and therefore allowed generalisations beyond the target species. Type and strength of plant interactions based on NRE were calculated using the interaction importance index I_{imp} (Seifan et al. 2010) based on above-ground biomass ($I_{imp} = \{b_{control} - b_{removal}\}/\{[|b_{control} - b_{removal}|]+[|b_{removal} - b_{max}|]\}$ where $b_{control}$ is biomass in the control plot, $b_{removal}$ is biomass in the removal plot and b_{max} is the maximum biomass observed for a species either in a control or removal plot). We selected I_{imp} as a measure of plant interactions for NRE to maximise comparability with plant interactions derived from SPA because spatial patterns indicate the consequences of plant interactions and assess the overall importance rather than the direct intensity of interactions (McIntire and Fajardo 2009; Kikvidze et al. 2011).

Spatial patterns were determined based on the co-occurrence of vascular plant species within the same circular plots of 5 cm radius prior to the experiment. Spatial patterns at this scale that only include the nearest neighbours can provide evidence for long-term effects of species interactions because spatial patterns caused by environmental heterogeneity are mostly found at larger spatial scales (Silander and Pacala 1985; Purves and Law 2002; Reitalu et al. 2008). Furthermore, local seed dispersal did not significantly affect the inter-specific spatial patterns observed. We consequently interpreted aggregations of species (i.e. species coexist more frequently than expected by chance) within a single snowmelt date as a result of facilitation, whereas species segregations (i.e. species co-exist less frequently than expected by chance) were considered a result of competition. Nevertheless, we cannot exclude the possibility that potential microscale heterogeneity also

contributed to the observed spatial patterns. Plant interactions based on SPA were assessed by using (1) the variance ratio ($RV = V_{obs}/V_{exp}$ where V_{obs} is the observed variance of species richness per plot within a single snowmelt date and V_{exp} is the variance expected under a random distribution of species [Schluter 1984; Gotelli 2000]) and (2) the C-score that quantifies the mean number of checkerboard combinations per species pair (Stone and Roberts 1990). The RV and C-score have been used in different types of communities to describe spatial relationships (Gotelli 2000) and can indicate random co-occurrence or alternatively aggregation or segregation of species.

The NRE in snowbeds indicated significantly negative I_{imp} ($F = 9.0$, $P = 0.003$) suggesting that competition prevailed during the experimental time frame. I_{imp} was negative throughout the whole snowmelt gradient and showed no consistent change with changing snowmelt dates (Figure 3) but it was not significantly different from 0 for individual snowmelt dates. Similarly, all target species had a negative, albeit non-significant, I_{imp} at the species level (data not shown). These results indicate overall significant but rather weak effects of competition. On the contrary, SPA indicated prevailing aggregation of species in general along the whole snowmelt gradient based on RV (Figure 3) and C-score (Table 1), with the exception of snowmelt date 16 June for RV. Again, we found no consistent change in RV (Figure 3) and C-score (Table 1) along the snowmelt gradient. Therefore, SPA generally indicated facilitation in snowbeds. The comparison between the results of the NRE and the SPA however showed a consistent shift of the interaction balance towards competition measured by I_{imp} compared to RV (Figure 3).
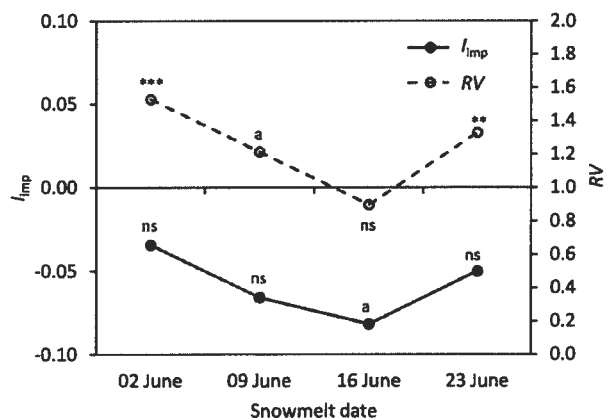

Figure 3. Plant–plant interactions in snowbeds measured with neighbour-removal experiments and spatial pattern analysis. Displayed is the mean interaction importance I_{imp} where $I_{imp} > 0$ indicates facilitation, $I_{imp} < 0$ indicates competition, and RV where $RV > 1$ indicates facilitation and $RV < 1$ indicates competition. For each snowmelt date for I_{imp} $n = 60$ pairs of individuals (10 pairs for each species and snowmelt date), for RV $n = 120$ plots. Statistical significant difference from neutral co-occurrence is indicated by *** ($P < 0.001$), ** ($P < 0.01$), a ($P < 0.1$), ns ($P > 0.1$). Difference of I_{imp} from 0 was tested with linear mixed model F-tests and difference of RV from RV_{null} (≈ 1) was tested with randomisation tests.

Table 1. *C*-score analyses to detect non-random spatial patterns of species distribution in snowbeds. Observed *C*-scores that are lower than null model *C*-scores indicate spatial aggregation of species.

Snowmelt date	$C\text{-score}_{obs}$	$C\text{-score}_{null}$	P-value
02 June 2003	0.56	0.68	<0.001
09 June 2003	0.62	0.71	<0.001
16 June 2003	0.61	0.68	<0.001
23 June 2003	0.51	0.65	<0.001

Based on our conceptual model, we interpret this divergence between NRE and SPA as the currently ongoing shift in the balance of plant interactions from historic facilitation, indicated by SPA, to competition at present, indicated by NRE. Indeed, snowbed habitats are among the alpine habitats that have experienced the strongest environmental changes to date (see Björk and Molau 2007 for review), especially in the European Alps (Theurillat and Guisan 2001). Even though we are aware of the possibility that nitrogen deposition (Klanderud 2010) or extreme events (Zimmermann et al. 2009) could have contributed to the observed shift, we suspect that the consequences of climate warming, i.e., improved resource conditions and an increased number of potential species competing for these resources (Virtanen et al. 2003; Björk and Molau 2007), could be responsible for the observed shift in the balance of interactions towards competition. Accordingly, we see the recent shift in the balance of competition and facilitation in snowbeds mostly as the result of an increasing intensity of competition. Facilitative effects in snowbeds were shown to be a result of associational resistance against herbivores (Schöb et al. 2010). Since we are not aware of any significant changes in herbivory pressure by the main herbivores (i.e., density of caterpillars of *Zygaena* sp. and sheep) during the last decades in the study area (unlike some other alpine areas in the Alps that have experienced significant changes in land use over the past decades), we consider changes in the intensity of facilitation rather unlikely.

Species composition of alpine snowbeds is highly dependent on growing season temperature and date of snowmelt (Tomaselli 1991; Razzhivin 1994; Schöb et al. 2009) i.e., factors that are changing in the Alps due to climate warming. Temperature has increased by 0.57 °C on average over the last three decades (Rebetez and Reinhard 2008) and growing season length has increased by about six days (Menzel and Fabian 1999); however, absolute changes in snow duration are highly dependent on altitude and microtopography (Laternser and Schneebeli 2003).

Higher temperatures, but also less water-logged conditions due to higher temperatures and potentially less summer precipitation (Raible et al. 2006), may significantly affect the plant–plant interaction balance. Both higher temperature and less water-logging can promote soil microbial activity (Seastedt et al. 2001) and increase the rate of decomposition and nitrogen mineralisation (Margesin et al. 2009). Consequently, improved nitrogen conditions could

have increased productivity and boosted competition for other nutrients, water, light or space. This would be in line with other alpine studies that have shown increasing competitive interactions with increasing temperatures along altitudinal gradients (Callaway et al. 2002; Kikvidze et al. 2005b, 2011) in response to nutrient amendment (Eskelinen 2008) or due to simulated climate change (Klanderud 2005; Klanderud and Totland 2007).

Changes in the date of snowmelt seem not to be directly related to the balance of competition and facilitation in snowbeds (Figure 3; Schöb et al. 2010). Nevertheless, the date of snowmelt is an important environmental filter for species and strongly affects species composition in snowbeds (Schöb et al. 2009). An altered snowmelt regime can therefore induce a change in species composition that in turn can be accompanied by changes in competition or facilitation of co-occurring species (Elmedorf and Moore 2007; Nagy and Grabherr 2009; Hülber et al. 2011). In the Alps an invasion of grassland species into snowbeds has already been documented (Grabherr et al. 1995; Grabherr 2003; Schöb et al. 2009). This change in species composition along the whole snowmelt gradient in snowbeds could have resulted in an immigration of more competitive species than typical snowbed species, thereby increasing the role of competition.

Even though we believe that the hypothesised time lag between NRE and SPA in changing environments is responsible for the differences in the observed type of plant–plant interactions by the two methods, other sources of variation are also possible. For example ontogenetic shifts in the direction of plant interactions may, under certain circumstances, result in similar differences between NRE and SPA (Miriti 2006). Importance of facilitation for seedling establishment may result in species aggregations whereas competition for resources at the adult stage of plants would show prevailing competition in a NRE; however, several studies from alpine tundra, including snowbeds, found an important role of competition for seedling establishment (Eskelinen and Virtanen 2005; Gough 2006; Dullinger and Hülber 2011). Therefore, we consider that ontogenetic shifts are rather unlikely drivers of the observed differences between the results of NRE and SPA in our study.

Conclusion

Based on the results from our example of alpine snowbeds we recommend combining experimental and observational approaches to study plant–plant interactions as an ongoing process (by NRE) and as a consequence of historic interactions (by SPA). When explicitly taking into account the time lag between the processes and the patterns generated by historic processes (e.g. Farrer et al. 2010), the state of a community undergoing a transition is possible. If changing environmental conditions are known, combining experimental and observational studies is crucial for identifying potential temporal changes of processes (Dunne et al. 2004). For snowbeds in the Swiss Alps, the divergence between NRE and SPA reported in our case study suggests

that climate warming would increase the role of competition, which would finally lead to inter-specific segregation of species and may potentially even result in the exclusion of competitively inferior species from snowbeds. On the other hand, competitive species would profit from these changes and most probably dominate the vegetation of this habitat in the future. We would therefore argue that the combination of the methods could potentially allow more accurate predictions of changes in species compositions caused by environmental changes as potential changes in the prevailing type of plant–plant interactions can be detected and taken into account. Obviously, such a strategy of research is particularly relevant to studies of the effects of environmental changes, such as land use or climate change, on community composition. It might also be useful for detecting short-term variability of environmental conditions or ontogenetic shifts in the importance of plant interactions that can affect the results of experiments.

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Notes on contributors

Christian Schöb is a post-doctoral fellow with research interests in alpine ecology and biodiversity, and a main focus on plant–plant interactions and the mechanisms of plant species coexistence.

Peter M. Kammer is a professor; in addition to teaching courses on general biology and the ecology of biocoenoses, his research interests focus on the ecology of alpine plant communities and temperate grasslands.

Zaal Kikvidze is a professor; his research centres on community ecology and biogeography, mainly on the importance of plant–plant interactions to biodiversity and distributions of species at various spatial scales.

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Appendix 1. Methods for the neighbour-removal experiment and spatial pattern analysis in alpine snowbeds in the Swiss Alps

Study site

The study area consisted of 24 spatially separated snowbeds each with an area of approximately 50 m² and with a mean distance of 119 m between them. All snowbeds were within a narrow area of approximately 0.3 km² situated in the western part of the Central Alps at the Gemmipass, Leukerbad, Switzerland (2400 m a.s.l., 46°25' N, 7°37' E). We located snowbeds in hollows where the prevailing north-westerly winds cause an accumulation of snow during winter. The main growing period lasted between July and September with average temperatures between 6 and 9 °C (Döbeli 2000). The deep gleyed Brown Earths or gleyed Rendzinas were mesic and very homogeneous among snowbeds (Vonlanthen et al. 2006; CS, personal observations). Apart from herbivory (mainly by sheep and caterpillars of *Zygaena* sp.) we could not observe any other disturbance such as wintertime activities of animals or periglacial processes (Figure A1). The vegetation of all snowbeds belonged to the class of *Salicetea herbaceae* snowbed communities (Ellenberg 1996) and was composed of a dense carpet of tiny plants (mean canopy height approximately 5 cm) and many vascular plant species with a distribution restricted to snowbed habitats. The dominant species were *Alchemilla pentaphyllea* L., *Gnaphalium supinum* L. and *Salix herbacea* L. In a previous study at the same study site, we found 70 vascular plant species with an average relative cover of 72%, furthermore 15% cover of bryophytes, 3% of lichens and 10% was bare ground (Schöb et al. 2009). Most of the species inhabiting the studied snowbeds showed prevailing clonal reproduction (CS, personal observation).

Data sampling

We studied spatial patterns (SPA) and performed neighbour-removal experiments (NRE) in 480 circular plots with a radius of 5 cm. They were unevenly distributed over 40 blocks (approximately 1–2 m²) placed along a snowmelt gradient within 24 snowbeds. Each snowbed consisted of blocks with either one or two different snowmelt dates and up to three blocks with the same snowmelt date. Average distance of blocks within a snowbed was 3.1 m. There were six blocks with snowmelt date 2 June 2003, 13 blocks with 9 June 2003, 13 blocks with 16 June 2003 and eight blocks with 23 June 2003. The following years after the setup of the experiment, the snowmelt timing was generally later but the chronological order of the blocks becoming snow free was constant over the years (Schöb et al. 2009).

Figure A1.   Typical snowbed at the study site in the Gemmipass region, Swiss Alps. Note the high homogeneity of the [topography] within the snowbed forming a very uniform and dense carpet of tiny vascular plants. Only the bottoms of the depressions were investigated.

For the NRE (Schöb et al. 2010), we selected six target species representing the major types of species distribution in snowbeds described by Schöb et al. (2008, 2009). We selected *Alchemilla pentaphyllea* L. representing the group of dominant species being frequent along the whole snowmelt gradient but most abundant in undisturbed late melting sites (Schöb et al. 2008), *Cardamine alpina* WILLD. and *Veronica alpina* L. as representatives of subordinate snowbed species increasing in their frequency and abundance with later snowmelt, *Poa alpina* L. as a ubiquitous species with an indifferent distribution pattern along the snowmelt gradient, and *Ligusticum mutellina* CRANTZ and *Polygonum viviparum* L. representing grassland species decreasing in frequency and abundance with later snowmelt. With this selection we assumed that the results obtained will apply to most of the frequent species occurring in the snowbed community studied and therefore allow for generalisations beyond the target species. We chose target individuals that were well established but had no flowers at the time of selection. Furthermore, we selected distinct individuals to minimise the effects of clonal connections; however, we were not able to absolutely exclude the possibility of below-ground connections for the rhizomatous species. For each species we chose 10 pairs of individuals for each snowmelt date, resulting in 80 target individuals for each species and 120 target individuals for each snowmelt date. Individuals of each pair were chosen to be as similar as possible with respect to leaf number and leaf length or shoot height. The two individuals of each pair were located at an average distance of 30 cm within the same block i.e., far enough from each other to prevent physical connections between them but close enough to minimise the effect of micro-environmental differences on individual performance. We manipulated plant–plant interactions by randomly applying a neighbour-removal treatment to one individual of each pair removing all above-ground biomass of the neighbouring vegetation around the target individual. The area clipped was 5 cm in radius around the target individual and sparse regrowth of plants was cut back twice during each growing season. Because plants in snowbeds are very small, 5 cm is a sufficient clearance to prevent shading and above-ground contact of the target plant shoots with those of neighbouring plants. After two years, the above-ground parts of all target plants that survived were harvested before dissemination of seeds, and total above-ground biomass was determined after drying at 70 °C for 72 h. Finally, above-ground mass of each individual with a removal treatment was compared to the mass of the respective control individual. With

this approach we could determine the effect of diffuse interactions (*sensu* Wilson and Keddy 1986).

The data for SPA (Schöb et al. 2008) were collected at the onset of the neighbour-removal experiment in July and August 2005 by sampling presence/absence of all vascular plant species within each plot. Spatial patterns at the plot scale of 10 cm diameter, including nearest neighbours of plants, can provide indications for long-term effects of species interactions, although they may also arise from local dispersal and environmental heterogeneity (Seabloom et al. 2005). Reproduction by seeds, however, was shown to be rare in snowbeds (Kudo 1991; Dullinger and Hülber 2011; CS, personal observation). Furthermore, local seed dispersal would result in a clumped occurrence of individuals of the same species and consequently segregation between different species. Therefore, under conditions of prevailing aggregation of species (as it was found in our study), local dispersal could only have lowered the level of aggregation but could not have significantly changed inter-specific spatial patterns. The relationship between spatial patterns and environmental heterogeneity is particularly dependent on the spatial scale of the patterns considered (Reitalu et al. 2008). Whereas aggregations and segregations of species at large spatial scales are mostly caused by environmental heterogeneity, at a very small spatial scale of just a few centimeters in grasslands, aggregations and segregations of species can be related to the prevailing type of inter-specific interaction i.e., facilitation or competition (Silander and Pacala 1985; Purves and Law 2002; Reitalu et al. 2008). Consequently, the spatial patterns observed in this study can reasonably be considered the result of plant interactions (Schöb et al. 2008). This is further supported by the fact that the snowbeds studied showed hardly any small-scale topographic variability (Figure A1), and soil pH and soil temperature was shown to be unrelated to spatial patterns at the study site (Schöb et al. 2008). Nevertheless, we cannot exclude the possibility that potential heterogeneity caused by other environmental factors such as soil nutrient concentrations or disturbances could have contributed to the observed spatial patterns.

Analyses of plant interactions

The NRE was analysed by calculating the interaction importance index I_{imp} (Seifan et al. 2010) based on above-ground biomass, according to the following formula:

$$I_{imp} = \begin{cases} \frac{N_{imp}}{|N_{imp}|+|E_{imp}|} & |N_{imp}| + |E_{imp}| > 0 \\ 0 & |N_{imp}| + |E_{imp}| = 0 \end{cases}$$

where N_{imp} refers to the contribution of biotic interactions to plant performance (N_{imp} = biomass$_{control}$ − biomass$_{removal}$) and E_{imp} refers to the environmental effects on plant performance (E_{imp} = biomass$_{removal}$ − biomass$_{max}$). Biomass$_{max}$ is the maximum biomass observed for the species in the study system, either in a removal or control plot. I_{imp} was determined for each pair of individuals of each species and averaged for each species and snowmelt date. Statistical significance of differences in I_{imp} from 0 for all species pooled and for each species or snowmelt date separately was tested with linear mixed models using the R package 'nlme' (Pinheiro et al. 2011) and ANOVA *F*-Tests. Mixed models were selected to take the nested structure of the data into account (plots nested within snowbeds nested within species).

Spatial patterns were analysed using a randomisation technique based on the variance ratio $RV = V_{obs}/V_{exp}$ where V_{obs} is the observed variance of species richness per plot within a single snowmelt date and V_{exp} is the variance expected under a random distribution of species (Schluter 1984; Gotelli 2000). The null model assumes that plant species are randomly distributed over the plots of the same snowmelt date. RV has been used in different types of communities to describe spatial relationships (Schluter

1984; Palmer 1987; Gotelli 2000; Wilson et al. 2000). A value of $RV = 1$ suggests a random distribution of plants. Values of $RV < 1$ indicate a lower observed variance than expected under the null model and suggest a co-occurrence of species less frequently than would be expected if plants were randomly distributed i.e., segregation. In other words, segregation means lower species richness per plot than expected by a random species distribution. Conversely, $RV > 1$ indicates an aggregation of species i.e., higher species richness than expected by a random species distribution. As a second measure for quantifying spatial patterns, we calculated *C*-score values for each snowmelt date quantifying the mean number of checkerboard combinations per species pair (Stone and Roberts 1990). *C*-scores were calculated using the 'C.score' function in the R package 'bipartite' (Dormann et al. 2008). Significantly lower *C*-score values than a *C*-score of a random distribution of species indicates spatial aggregation, whereas higher values indicate species segregation (Stone and Roberts 1990). Statistical significance of the observed indices was tested with null model analysis. To test for a significant difference between RV and RV_{null}, we performed 1000 randomisations and determined the significance level from the number of randomised RV equal or more extreme than the observed RV (Wilson 1987). To generate randomised species assemblages, the presence/absence of each species was reshuffled at random in order that the occurrence of one species in any of the plots did not depend on the occurrence of another species in the same plot. The number of species and the overall frequency for each species were both held to those found in the original field data (Gotelli 2000). Statistical significance of *C*-scores was tested using null models with fixed row and column totals with the 'oecosimu' function of the 'vegan' package (Oksanen et al. 2011) in R (R Development Core Team 2011). For both indices of spatial patterns, aggregations of species within a single snowmelt date were interpreted as the result of facilitation, whereas segregation was considered the result of competition.

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