Where do seedlings go? A spatio-temporal analysis of seedling mortality in a semi-arid gypsophyte

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Studies of seedling population dynamics often focus on survival because it provides an integrated measure of seedling performance. However, this approach involves a substantial loss of information because survival is the net result of a wide range of mechanisms. The present study overcomes these shortcomings by investigating spatial and temporal patterns in the causes of plant mortality in a population of *Helianthemum squamatum* seedlings. We use new point pattern analyses based on K functions combined with a new null model ("independent labeling"). A total of 871 seedlings of *H. squamatum* were mapped and regularly monitored over an 18-month period. More than 60% of seedlings died during this period. Causes of mortality were spatially structured, and these structures shifted through time. Small differences in either the time of emergence or the environment surrounding *H. squamatum* seedlings had profound influences on their fate. Seedlings emerging late in the season under the canopy of adult plants died from drought more often than expected, whereas those emerging earlier in the same microsite survived more than expected. The identity of neighbors also affected the spatio-temporal dynamics of mortality causes. Our results show that seedling-adult interactions cannot be easily predicted from simple models, and that the time of seedling emergence, its age and the identity of its neighbors determine the sign and the spatial scale of these interactions. The new methods introduced in this article open an avenue for the detailed analyses of the spatio-temporal dynamics of plant mortality and can help to disentangle the complexity of biotic interactions along environmental severity gradients.

The "stress gradient hypothesis" (SGH) predicts that the relative importance of facilitation and competition should vary inversely along gradients of abiotic stress, with facilitation increasing in importance over competition as abiotic stress increases (Bertness and Callaway 1994). Most research testing this hypothesis has been conducted on seedlings of long-lived perennial species (Maestre et al. 2003, Maestre and Cortina 2004, Gómez-Aparicio et al. 2005). This is not surprising because the fate of seedlings will determine the structure and dynamics of most plant populations (Harper 1977, Kitajima and Fenner 2001), and because interactions with other plants are critical for seedling establishment, particularly in stressful habitats such as arid and semi-arid environments (Maestre et al. 2003, Gómez-Aparicio et al. 2005).

Although some field experiments suggest that the SGH is applicable under a range of environments and sources of stress, there is a growing number of studies that do not support its predictions (see Brooker et al. 2008 for a recent review). Consequently, a profound revision of the SGH in semi-arid environments has been suggested (Maestre et al. 2005, 2006). These discrepancies might stem from focusing

on particular aspects of the recruitment process, while neglecting other aspects (Escudero et al. 2005, Schiffers and Tielborger 2006). In addition, most studies do not make repeated measures of responses to neighboring individuals. This precludes the detection of changes in the outcome of interactions throughout the life history of seedlings (but see Escudero et al. 2005, Schiffers and Tielborger 2006).

Survival has been the measure of choice for ecologists studying seedling recruitment and plant-plant interactions (Escudero et al. 1999, 2000, Maestre et al. 2003, 2005). Although it provides an integrated estimate of seedling performance, the analysis of survival alone can lead to confusing results when there are multiple mortality causes. For instance, in Mediterranean environments the most relevant causes of plant deaths are summer drought (Herrera et al. 1994, Rey et al. 2004) and herbivory (Baraza et al. 2006). If we focus on survival when evaluating either seedling recruitment or biotic interactions, we will fail to resolve circumstances, moments and places where nurse plants facilitate against herbivory from those where they facilitate against drought. Indeed, in studies where the spatial pattern of live seedlings has been described and

related to nurse plants it is not possible to infer which factors are responsible for such structure (Tirado and Puignaire 2003). Furthermore, some authors suggest that the canopy of determined shrubs constitutes a ''safe site'' for seedlings (Lookingbill and Zavala 2000). However, the patterns evaluated in these studies only reflect a net demographic result; they do not inform whether the nurse effect relates to grazing or drought avoidance or to differential seed dispersal or emergence. Understanding where, when and why seedlings die can provide key insights into the mechanisms controlling the balance of facilitation and competition in terrestrial plant communities. This knowledge can also help to resolve current uncertainties surrounding the SGH, which are related to spatial and temporal shifts of net plant-plant interactions in response to small-scale environmental changes and ontogenetic shifts (Brooker et al. 2008). Such shifts could be understood through a detailed spatio-temporal examination of the incidence of death causes because a microsite minimizing the likelihood of mortality from abiotic stress in a given moment may no be effective in avoiding grazing pressure (Crain 2007).

To our knowledge, no previous study has evaluated the spatio-temporal dynamics of seedling mortality (as opposed to seedling survival). We aimed to do so in a natural population of *Helianthemum squamatum,* a small endemic shrub from the Iberian Peninsula. We considered the following hypotheses: 1) seedling fate is dependent on the place and date of emergence (Escudero et al. 2000); 2) the incidence of mortality promoted by both herbivory and drought shows marked spatial and temporal patterns; 3) the direction of the effects of other plants on the mortality of *H. squamatum* seedlings is species-specific (Choler et al. 2001, Wang et al. 2008), and 4) the direction of these effects shifts with changes in environmental conditions (e.g. competitive interactions could predominate in the benign, early months of the growing season, whereas facilitative interactions could predominate later on as more xeric conditions prevail; Schiffers and Tielborger 2006, Kikvidze et al. 2006. To evaluate these hypotheses, we developed a set of novel spatial point pattern analyses based on the well-known Ripley's K function (Haase 1995). Point pattern analyses based on this function are commonly used to infer competitive and facilitative interactions among woody species (Tirado and Pugnaire 2003, Escudero et al. 2005, Fajardo et al. 2008). However, two main concerns arise from the current use of these techniques by ecologists. First, null models for most multivariate point pattern analyses are very restrictive regarding the type of questions they can help to solve, as they can not test whether positive or negative associations between two patterns are mediated by a third pattern. Second, the common use of the bivariate K function (Goreaud and Pelissier 2003) shows whether two spatial patterns are associated or not, but can not indicate whether a negative association is due to both patterns being segregated from each other or to just one of them showing a strong spatial aggregation. However, this kind of information is useful for revealing the mechanisms determining the spatio-temporal patterns of seedling mortality and its causes. To move beyond these limitations, we use some neglected algorithms based on differences between univariate and bivariate K functions (Dixon 2002). We also propose a new null model, called ''independent labeling'', that allows testing whether the association of two spatial patterns is mediated by a third one. With this new set of analyses we can evaluate if the patterns of seedling mortality are mediated by the adult plants of the community, and study the temporal dynamics of these interactions.

Methods

Study area

The study site was located in Chinchón, near Madrid (Spain, 40°11′N, 3°35′W, 550 m a.s.l.). The climate is semi-arid, with an average annual rainfall of 374 mm and a pronounced summer drought. Mean daily maximum and minimum temperatures in January and July are 9.6°C and 0.6°C, and 32.7°C and 9.6°C, respectively. Soils are calcic gypsisols developed over gypsum parental rocks, and have gypsum contents above 50%. Perennial plant cover is below 20%, and is dominated by isolated individuals of gypsum specialists (*Lepidium subulatum, Helianthemum squamatum, Teucrium pumilum, Herniaria fruticosa, Thymus lacaitae* and *Koeleria castellana*) surrounded by biological soil crusts dominated by lichens (Martínez et al. 2006). Rabbit *Oryctolagus cuniculus* is the most common vertebrate herbivore, as indicated by the abundance of its faeces and by the high number of warren entrances near the study plot.

Field survey

A rectangular plot (7 × 6 m) was laid out on a representative and uniform part of the study area. The longer side was parallel to the maximum slope (<5%). The coordinates of the centroid of all the perennial plants were recorded with a measuring tape. Each emerging seedling of *H. squamatum* was tagged and mapped, and its growth and survival was monitored for 18 months. Monitoring started in March 2000. Monthly censuses were conducted between March and June 2000. Additional surveys were conducted in September 2000, after the summer drought (the period of maximum seedling mortality), and in June 2001. Annual rainfall during the study period was 408 mm (2000) and 280 mm (2001).

Emerging seedlings were assigned to four cohorts (March, April, May and June), depending on the emerging date, and their fate was monitored in June 2000, September 2000 and June 2001. In each survey, dead seedlings were classified as dead because of herbivory or drought. They were assigned to the first group if they died after their hypocotyls were cut or eaten, after half of their leaves were eaten, or if they disappeared between consecutive censuses. The remaining dead seedlings were assigned to the second group (Escudero et al. 1999). The location of each seedling in relation to adult plants (i.e. growing under the canopy of an adult plant or in open space) was also recorded.

Analyses of seedling fates

To test our first hypothesis, we built contingency tables summarizing the frequency of seedling fates (dead because

of drought or herbivory and alive) in relation to census date, cohort and emerging microsite (under the canopy of adult plants or outside). We analyzed these tables using log-linear models. These models fit the expected cell counts to the marginal sums of the contingency table (see Agresti [1996] or Gotelli and Ellison [2004] for more details of the technique). In all the analyses, we fitted a model of independence between seedling fate and the other predictors (census date, cohort and emerging microsite). Deviations from expected frequencies were displayed and explored with mosaic plots (Friendly 1994), as recommended by Gotelli and Ellison (2004). In these mosaic plots, each cell of the contingency table is represented by a rectangle (or "tile") whose area is proportional to the cell frequency (Friendly 1994). Deviations from the expected cell frequencies are represented by different outlines and shadings, depending on the values of the standardized residuals. Filled cells are those with residuals significantly different from standard normal under the fitted model (in our case, the model of independence between predictors), and indicate that seedling fate is not independent from the predictors employed.

Multivariate point pattern analyses

We consider the studied plant community as a multivariate point pattern composed of different types of individuals (e.g. dead seedlings, live seedlings, adult plants, etc). The analysis of such data relies upon: 1) the computation of some functional summary of the observed pattern, and 2) the construction of some confidence limits with Monte Carlo simulation from a given null model. At present, there are only two relevant null models available to contrast multivariate patterns (Goreaud and Pelissier 2003, Wiegand and Moloney 2004): independence and random labeling. The first null model assumes that different processes generate the patterns of the populations studied. Departure from independence indicates that the processes display either attraction or repulsion. The usual Monte Carlo simulation consists in randomly shifting the different patterns ("toroidal shift", Dixon 2002). This model should be used, for example, if we were studying whether the emergence of seedlings is facilitated or inhibited by adults. Alternatively, if we aim to study the spatial structures of seedling fates (alive, dead because of herbivory or drought), we should use the null model of random labeling (Dixon 2002). It assumes that the different patterns are the result of some event affecting randomly the individuals of a population (e.g. mortality randomly affecting the individual seedlings). Departure from random labeling indicates that the process assigning labels to the points does not act randomly (e.g. the incidence of mortality is more intense in some areas). The usual Monte Carlo simulation consists in randomly permuting the labels over the points (Dixon 2002).

However, testing whether the spatial pattern of seedling mortality is related to that of adult plants cannot rely on any of the above mentioned null models. Albeit the spatial patterns of adult plants and those of dead seedlings are generated independently, their relationships will be conditioned by the dependence of the fate "dead" on the locations of the emerging seedlings. To handle these situations, we propose a new null model called "independent labeling". It assumes that the process assigning labels to a population of points (e.g. seedling mortality) acts independently of the locations of individuals of other independent population (e.g. the adult plants). In this case, any of the common bivariate functions (e.g. K, pair correlation or nearest-neighbor distribution; Dixon 2002) would be an appropriate summary statistic; the Monte Carlo simulation consists in randomly permuting the labels of the "labeled" population while keeping the locations of the "independent" population fixed (for example, permuting the labels "alive" and "dead" over the global pattern of seedling points, keeping the locations and labels of the adults fixed). Departure from the null model indicates that the process assigning labels is conditioned on the locations of individuals of the independent population. This method is related to the flexible neutral landscape approach developed by Lancaster and Downes (2004) and Lancaster (2006).

On the other hand, most ecological studies testing the association between two spatial patterns have used the raw bivariate K- or L-functions, something that ignores interesting features of the K function (Wiegand and Moloney 2004). An important property of this function is that, under the null model of random labeling, every individual pattern would be a random thinning of the corresponding bivariate pattern, and, therefore, $K_1(t) = K_2(t) = K_{12}(t) = \pi t^2$ (Dixon 2002). This leads to the development of other functions that provide interesting ecological information. For example, the difference in the intensity of aggregation of the two point patterns, $K_1(t)–K_2(t)$, which has an expected value of 0 for all t distances under random labeling, has considerable potential in recruitment studies. Other relevant functions are $K_1(t)–K_{12}(t)$, and the complementary $K_2(t)–K_{12}(t)$, which have also an expected value of 0 under random labeling. They evaluate whether one type of point tends to be surrounded by other points of the same type. For example, $K_1(t)–K_{12}(t) > 0$ indicates that, on average, type 1 points are surrounded with more type 1 points than expected under random labeling (Dixon 2002).

In our analyses, the function $\lambda K(t)$ is the expected number of neighboring seedlings within a distance t of a randomly chosen seedling, where λ is the intensity (number of plants per unit area) and $K(t)$ is the univariate K function. Edge effects were accounted for by using the local weighting method of Ripley (Haase 1995). The function $\lambda_2 K_{12}(t)$ is the expected number of a second type of point within a distance t of a randomly chosen point of type 1, where $K_{12}(t)$ is the bivariate K function. Reciprocally, $\lambda_1 K_{21}(t)$ is the expected number of type 1 points within a distance t of an arbitrary type 2 point. Since $K_{12}(t) = K_{21}(t)$, and the edge effects influence each function in a non-symmetrical way, the weighted mean estimator K_{12}^* is usually preferred (Dixon 2002), and so we use it in this study.

To test our second hypothesis (i.e. whether the incidence of mortality and its causes are spatially structured), we measured the difference in the intensity of clustering (i.e. if the dead seedlings were more or less clustered than the alive ones) with the difference $K_1(t)–K_2(t)$. With the same objective, we tested whether points of each pattern were

surrounded by points of the same type with more or less frequency than expected using the differences $K_1(t)–K^*_{12}(t)$ and $K_2(t)–K^*_{12}(t)$. We repeated the abovementioned test for the three censuses to evaluate whether the spatial pattern of mortality and its causes change through time.

To test our third hypothesis (i.e. the specificity of the effects exerted by adult plants on seedling fate), we computed the bivariate K^*_{12} function from the patterns of adult individuals of each species and those of dead (or alive) seedlings. We also computed the bivariate K^*_{12} function from the patterns of adult individuals and those of mortality causes (i.e. dead by herbivory or by drought), to test whether the species-specific effect is mediated by the cause of death. In both cases, we relied on the independent labeling null model and, therefore, the Monte Carlo simulation consisted in an independent labeling of seedling fates while keeping fixed the pattern of adult plants. To facilitate the visualization and interpretation of the results, we used the L function ($L^*_{12}[t] = \{K^*_{12}[t]/\pi\}^{1/2} − t$), a linearised form of the K function (Dixon 2002).

Finally, to evaluate our fourth hypothesis (i.e. whether these effects are permanent or shift through time), we repeated the independent labeling tests for the three censuses.

In all the tests conducted, we used 95% confidence envelopes. They were built from the 25th highest and 25th lowest values of the summary function computed on 999 simulations of the selected null model. Statistical and spatial pattern analyses were conducted using the R software (R Development Core Team 2007). A set of original R functions developed to test and plot differences of K functions, and to test bivariate patterns against the null model of independent labeling have been included in the R package ecespa (freely available at <http://cran.r-project.org/web/packages/ecespa>).

Results

Seedling fates

A total of 871 *H. squamatum* seedlings emerged during winter and spring 2000. Seedling mortality peaked during summer 2000, being drought the most important cause of mortality (Fig. 1). However, there were significant differences in fate among censuses ($\chi^2 = 157.677$, DF = 4, p < 0.0001, Fig. 2A). There were many more cases of death, both from drought and herbivory, and less survival than expected in September. In parallel, the frequency of mortality because of drought was significantly lower than expected in the first June census. Seedling fate was also dependent on the cohort considered, with more survival than expected for the March cohort and less for the others ($\chi^2 = 21.5579$, DF = 3, p = 0.0008, Fig. 2B). Throughout all the censuses, the survival of the first cohort was significantly higher under the canopy of adult plants than in open spaces (Fig. 3), albeit mortality driven by drought decreased significantly in the later microsite (Fig. 3A, B). The rest of cohorts survived less than expected under perennial canopies. Mortality from herbivory did not show a consistent pattern, and varied among cohorts and censuses.
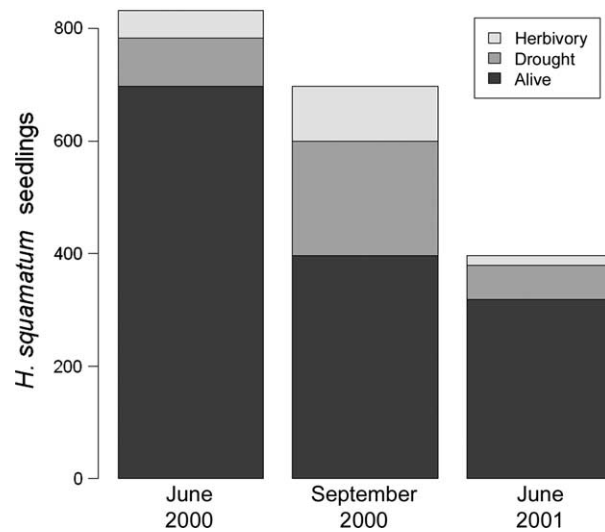

Figure 1. Fate of the population of *Helianthemum squamatum* seedlings in each of the three censuses in which mortality cases were recorded. Each color represents the number of seedlings that survived, died by herbivory or died from drought.

Multivariate point pattern analyses

Seedling mortality was significantly more aggregated than survival in September 2000 (Fig. 4B). In June and September 2000, dead seedlings were significantly surrounded with more dead seedlings than expected, but at different scales (from 0 to 20 and 70 to 90 cm in June, Fig. 4G; from 0 to 150 cm in September, Fig. 4H). Alive seedlings were significantly surrounded with other alive seedlings in all censuses (Fig. 4D, E, F).

The spatial incidence of mortality causes varied remarkably trough time. In June 2000, the pattern of herbivory deaths was significantly more clustered than that of drought deaths (Fig. 5A), whereas the opposite pattern was found in September 2000 (Fig. 5B). In June 2000, the incidences of both mortality causes were intensely segregated from each other (Fig. 5D, G), but in September of the same year seedlings dead from drought were surrounded by seedlings dead from drought more frequently than expected by chance (Fig. 5E, H). In June 2001, their incidences were not different from random (Fig. 5C, F, I).

The spatial association of dead and alive seedlings with the adult individuals of the community varied with the identity of the plant species considered, and shifted through time (Fig. 6 and 7, Table 1). Alive seedlings showed a positive association with adult *H. squamatum* individuals from 70 cm onwards in June 2000, which become negative in September 2000 (from 40 to 120 cm), and not significant in June 2001 (Fig. 6A, E, I). In June and September 2000, mortality from drought was positively associated with adults (from 10 cm onwards, and from 40 to 120 cm, respectively, Fig. 6C, G). Conversely, the spatial pattern of herbivory deaths was negatively associated with adult *H. squamatum* individuals at the same scales (Fig. 6D, H). The spatial association of alive seedlings with *Lepidium subulatum* adults was negative and positive in June and September 2000, respectively (Fig. 7A, E). In these surveys, seedlings dead because of drought were negatively associated
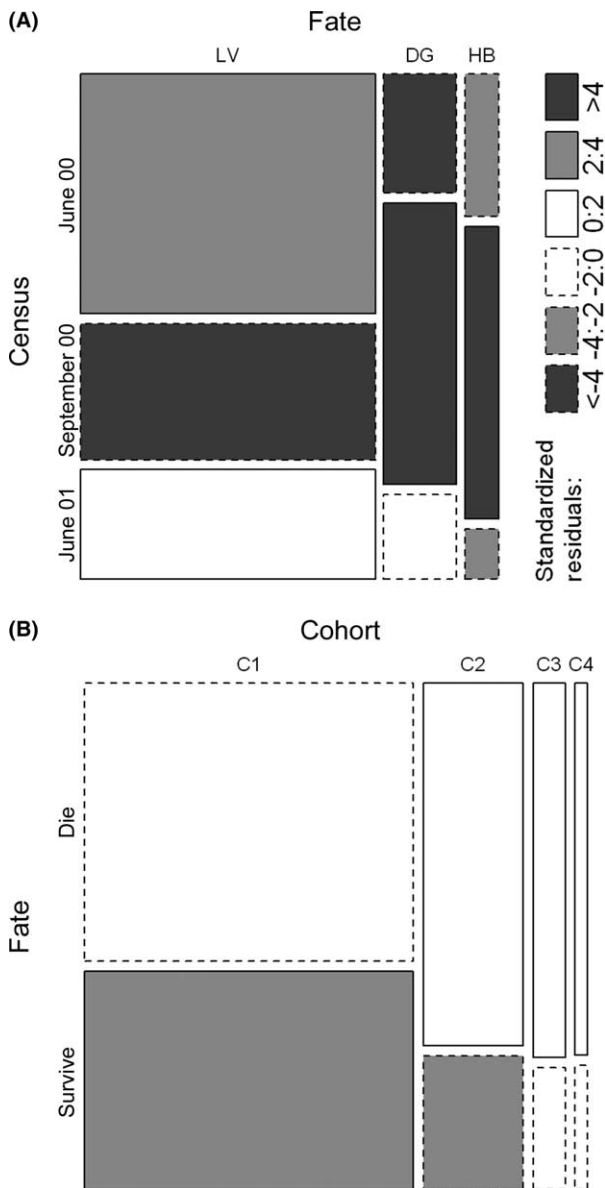
Figure 2. Mosaic plots of seedling fate frequencies by census and cohort. The area of each tile is proportional to the cell frequency of the corresponding contingency table. Solid and dashed outlines indicate respectively positive and negative deviations from the expected frequencies (assuming that seedling fate is independent of the other predictors). The shading of each tile is proportional to the standardized residual from the fitted model (values indicated in the legend). Dark- and light-filled tiles indicate significant deviations from the expected cell frequencies (at $\alpha = 0.05$ and $\alpha = 0.0001$, respectively). (A): Fate differences between censuses (June 00 = June 2000, September 00 = September 2000, June 01 = June 2001; LV: alive, DG: died because of drought, HB: died because of herbivory). (B): Fate differences between the four cohorts in the study (C1: 1st cohort, March 2000, C2: 2nd cohort, April 2000, C3: 3rd cohort, May 2000, C4: 4th cohort, June 2000).

with *L. subulatum* (Fig. 7C, G), while those dead because of herbivory showed a positive association (Fig. 7D, H). The association of herbivory and drought deaths with *L. subulatum* occurred at the same spatial scales (from 10 to 140 cm in June 2000 and from 40 to 50 cm in September 2000; Fig. 7C, G, D, H). The spatio-temporal relationships
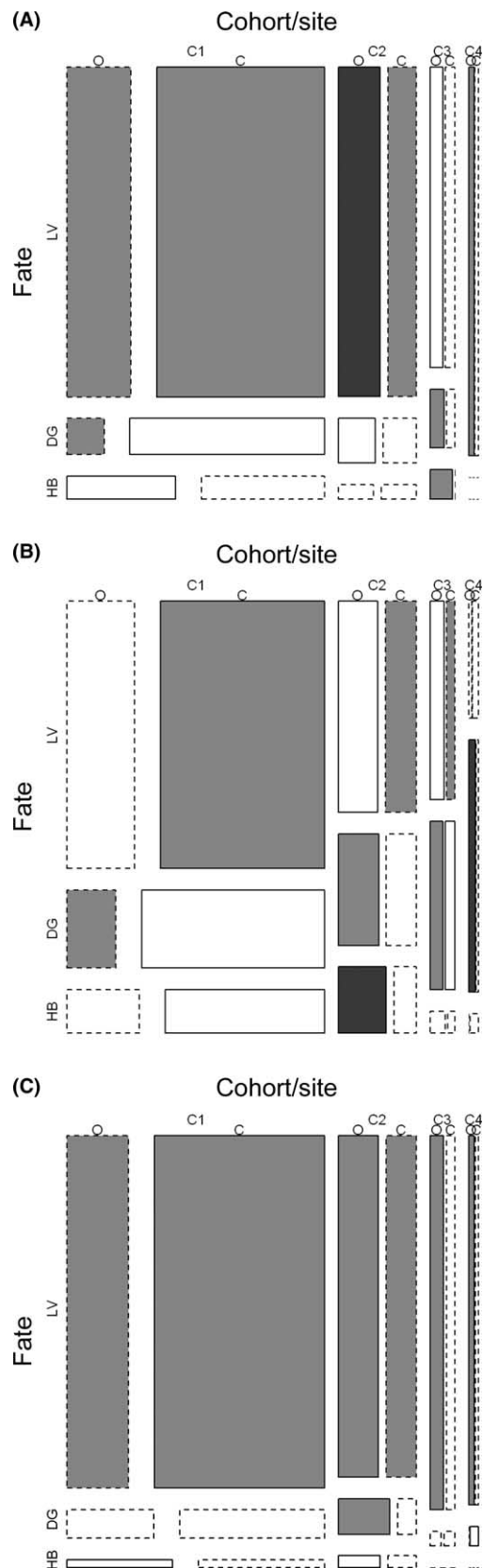

Figure 3. Mosaic plots of seedling fate frequencies between different cohorts and sites in the June 2000 (A), September 2000 (B) and June 2001 (C) censuses. O: open areas, and C: under the canopy of an adult plant. Rest of legend and abbreviations as in Fig. 2.
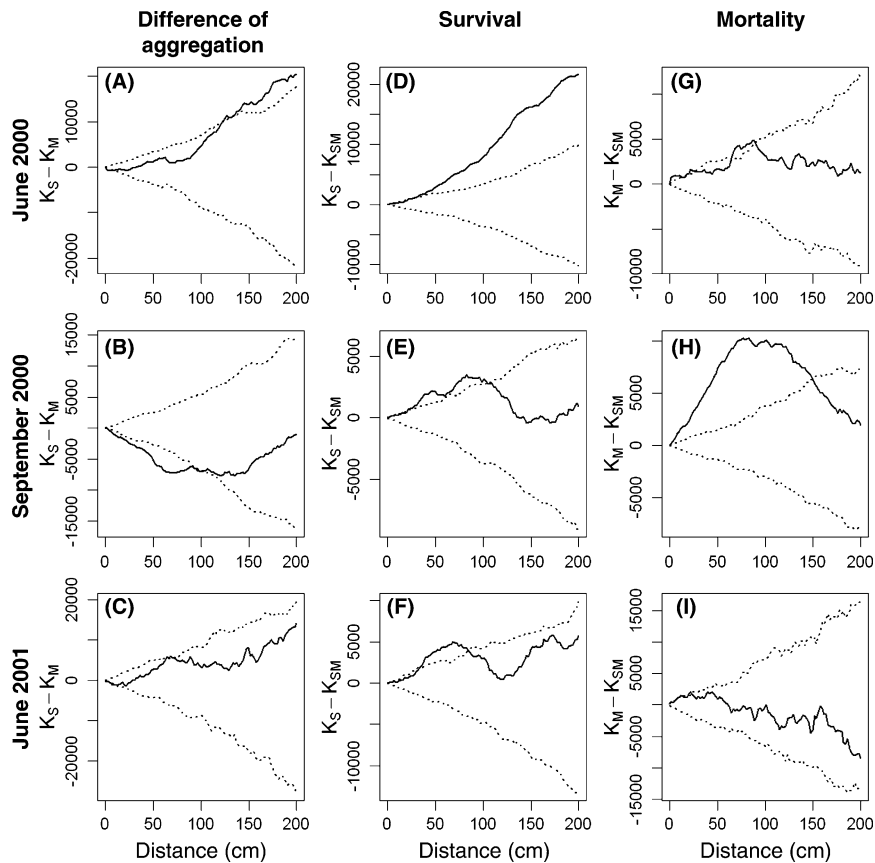
Figure 4. Differences between the spatial pattern of survival and mortality of *Helianthemum squamatum* seedlings in each of the three censuses where mortality causes were recorded. (A–C): difference of aggregation between survival and mortality. (D–I): evaluation of the tendency of alive (D–F) or dead (G–I) seedlings to be surrounded with other seedlings of the same type. K_S: univariate K function for the survival pattern, K_M: univariate K function for the mortality pattern, and K_{SM}: bivariate K function for the bivariate pattern of survival and mortality. The solid line represents the observed difference and the dashed lines the 2.5 and 97.5% percentiles of the distribution of the difference computed on 999 random assignments of the labels "alive" and "dead" over the population of remaining seedlings (see text). In all cases, the expected difference under the null hypothesis of random labeling is 0. Significant departures from 0 occur at scales where the observed difference exceeds the simulated envelopes. In panels D to I, positive departures indicate that seedlings experiencing the corresponding fate (survival or mortality) are surrounded with seedlings experiencing the same fate more frequently than expected by chance.

of seedling fates with other species of the community are summarized in Table 1. Although the direction of these associations changed through time, most shifts were species-specific, hindering the recognition of a general pattern. Most species showed a positive and/or neutral association with seedling survival (Table 1). However, even when this association was positive, some species were also positively associated with seedlings dying from drought (e.g. *Koeleria castellana* and *Thymus lacaitae*, Table 1) or with seedlings dying from herbivory (e.g. *Herniaria fruticosa*, Table 1). In June 2001, none of the species showed a significant association with any of the mortality causes.

Discussion

The incidence of mortality causes is structured temporally and spatially

There were sharp differences in the fate of each seedling cohort. The first cohort experienced lower mortality than the latest (Fig. 2B). This finding was related to a size-

dependent mortality during the summer drought in a previous study (Escudero et al. 1999). Larger seedlings are likely to have higher water requirements in arid environments (Goldberg and Novoplansky 1997), and consequently will have a lower chance of survival. However, this size-dependent relation is modulated for each cohort by local conditions. For instance, seedlings from late cohorts emerging under perennial canopies tend to die from drought at a higher rate than expected, whereas those from the earliest cohort had a higher survival in this microsite. These results emphasize the relevance of size for seedling survival. Small seedlings – from last cohorts – are not able to cope with water stress under plant canopies; on the contrary, larger seedlings – from early cohorts – benefit from the higher resources available under plant canopies while withstanding higher competition for water in this microsite during summer drought (Maestre et al. 2003, Maestre and Cortina 2004, Pugnaire et al. 2004).

Substantial efforts have been devoted to describe how shrub-created islands of fertility drive ecosystem structure and functioning in arid and semi-arid environments (Aguiar and Sala 1999, Reynolds et al. 1999). These fertile patches
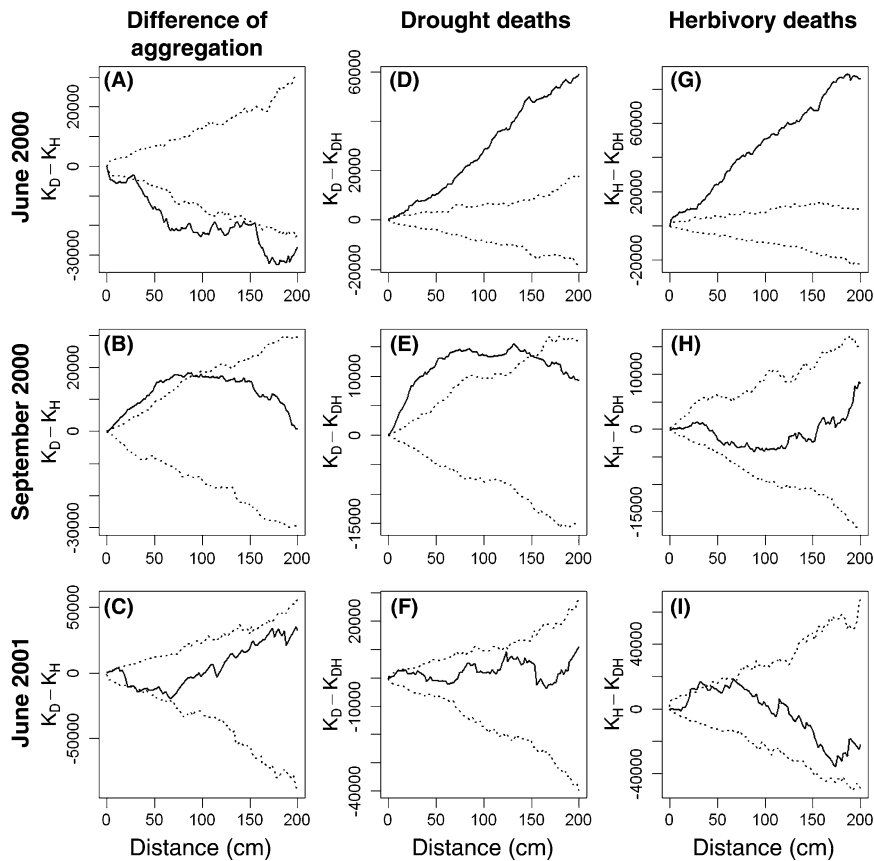
Figure 5. Differences between the spatial pattern of *Helianthemum squamatum* seedlings dead from drought and from herbivory in each of the three censuses where mortality causes were recorded. (A–C): difference of aggregation between drought and herbivory deaths. (D–I): evaluation of the tendency of seedlings dead from drought (D–F) or from herbivory (G–I) to be surrounded with seedlings dead from the same cause. K_D: univariate K function for the pattern of seedlings dead from drought, K_H: univariate K function for the pattern of seedlings dead from herbivory, and K_{DH}: bivariate K function for the bivariate patterns of drought and herbivory. The solid line represents the observed difference and the dashed lines the 2.5 and 97.5% percentiles of the distribution of the difference computed on 999 random assignments of the labels "drought" and "herbivory" over the population of dead seedlings in each census (see text). In all cases, the expected difference under the null hypothesis of random labeling is 0. Significant departures from 0 occur at scales where the observed difference exceeds the simulated envelopes. In panels D to I, positive departures indicate that seedlings experiencing the corresponding fate (drought or herbivory) are surrounded with seedlings experiencing the same fate more frequently than expected by

are points of high biological activity where facilitation is often the dominant interaction between plant species (Flores and Jurado 2003, Callaway 2007, Brooker et al. 2008). However, most of the studies reporting facilitative interactions in these environments have not considered potential ontogenetic effects on the outcome of these interactions (Callaway 2007). Some studies have shown that shrubs may facilitate the establishment of seedlings, but later compete with them as they grow and become adults (Walker et al. 2001, Miriti 2006). Our results agree with them, as we found that microsites initially effective in retaining *H. squamatum* seeds and promoting their germination, such as shrub canopies (Caballero et al. 2008), may not necessarily be effective for seedling establishment. Additionally, we found that shifts between facilitation and competition may abruptly change in relation to the emergence date: a favorable location for an emerging seedling may quickly become an adverse one only some weeks later (Fig. 3). To our knowledge, this result has not been previously described in either Mediterranean or semi-arid environments.

Seedlings dead because of drought were also intensely segregated from those dead because of herbivory, revealing a differential incidence of both mortality causes in space. The feeding behavior of rabbits, the main herbivore in our study system, can also affect seedling fates. Rabbits usually feed in groups and show a high spatio-temporal variability in their use of habitat (Angulo 2003). This may explain why seedlings dead because of herbivory were highly aggregated in the first census, but not in the others (Fig. 5).

Species-specific effects on mortality causes

According to the SGH (Bertness and Callaway 1994, Callaway and Walker 1997), we should expect facilitation against drought being the prevalent interaction in the studied community. Therefore, we should have detected positive spatial association between the pattern of alive seedlings and that of adult plants. Escudero et al. (2005) fitted generalized linear models (GLM) to data on *H. squamatum* seedling survival, and found that only some species of the community (*Teucrium pumilum, Koeleria*
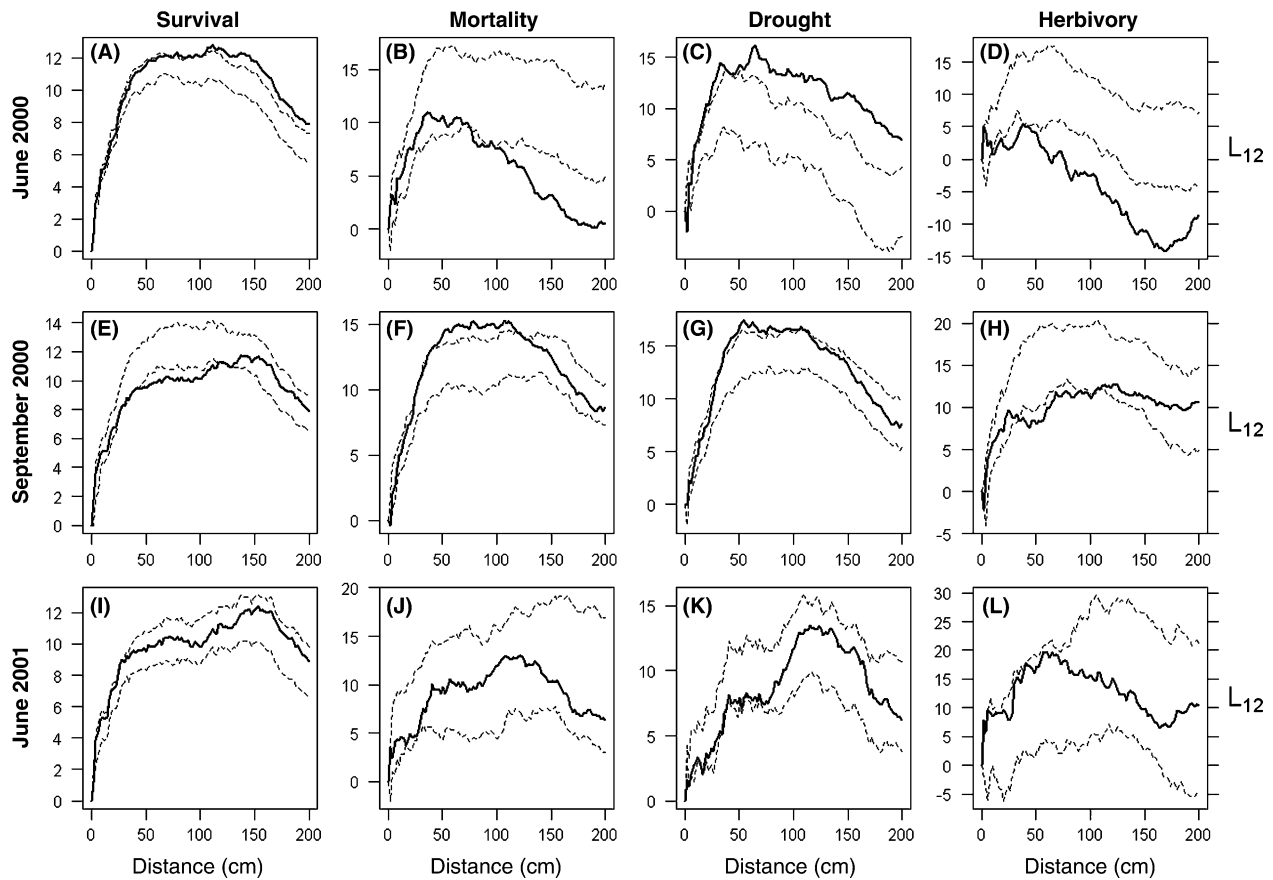
Figure 6. Temporal shifts of the spatial relationship between adult *Helianthemum squamatum* plants and survival, mortality and mortality causes (drought and herbivory) of *H. squamatum* seedlings. The solid line represents the observed bivariate L_{12} function. The dashed lines show the 2.5 and 97.5% percentiles of the distribution of the L_{12} function computed on the fixed pattern of adult plants and 999 random assignments of the appropriate labels ("alive" and "dead" over the population of remaining seedlings in the first and second columns; "drought" and "herbivory" over the population of dead seedlings in the third and fourth columns). Significant departures from the expected values under the hypothesis of independent labeling occur at scales where the observed L_{12} function exceeds the simulated envelopes.

castellana and adult *H. squamatum*) exerted a positive effect on this variable. Difficulties for incorporating spatial dependencies into GLMs limit the amount of information that they can provide when using spatially explicit data. In contrast, with our approach we were able to detect spatial associations between *H. squamatum* seedlings and the entire set of nurse species evaluated, albeit these effects were detectable at different moments depending on the species considered (Fig. 6A, 7E; Table 1). Our results support the idea that the magnitude of biotic interactions in harsh environments is species-specific (Choler et al. 2001, Wang et al. 2008), as we had hypothesized, and indicate that the positive association between adult plants and seedling survival is not consistent through time.

We hypothesized that the direction of the effects of other plants on the mortality of *H. squamatum* seedlings would shift in response to changing environmental conditions. However, the direction of the detected shifts could not be related to temporal changes in the overall environmental stress or consumer pressure. In relation to water stress, some recent studies have shown that interactions between plants switch seasonally from competition to facilitation as conditions change from wet to xeric (Sthultz et al. 2007 and references therein). In this study, we found that the

effects of adult plants over seedling mortality from drought did not shift as expected following the seasonal increase in xeric conditions from June to September 2000, but were mostly concerned with the shift of seedling growth stage from first year (2000) to second year (2001). In fact, only *L. subulatum* and *H. fruticosa* showed a facilitative effect against drought during the most xeric period (September 2000; Fig. 7C, G; Table 1). On the other hand, the pressure of herbivores followed the same seasonal pattern than drought (Fig. 1). Therefore, we should have detected a prevalence of negative associations between adult plants and seedling mortality from herbivory in September. This was true, however, for only two species (*H. squamatum* and *K. castellana*). Our results suggest that the proposed hypothesis of shifting effects following the seasonal environmental variation may depend on the specific nature of the adult plant-seedling relationship.

If we had not analyzed the different mortality causes, we could have attributed the absence of positive plant-seedling interactions in some censuses to the net effect of some concomitant processes, such us herbivory masking a "true" facilitative effect against drought. Our results show, however, that the occasional absence of significant nurse effects on seedling survival (or mortality) was not the result of
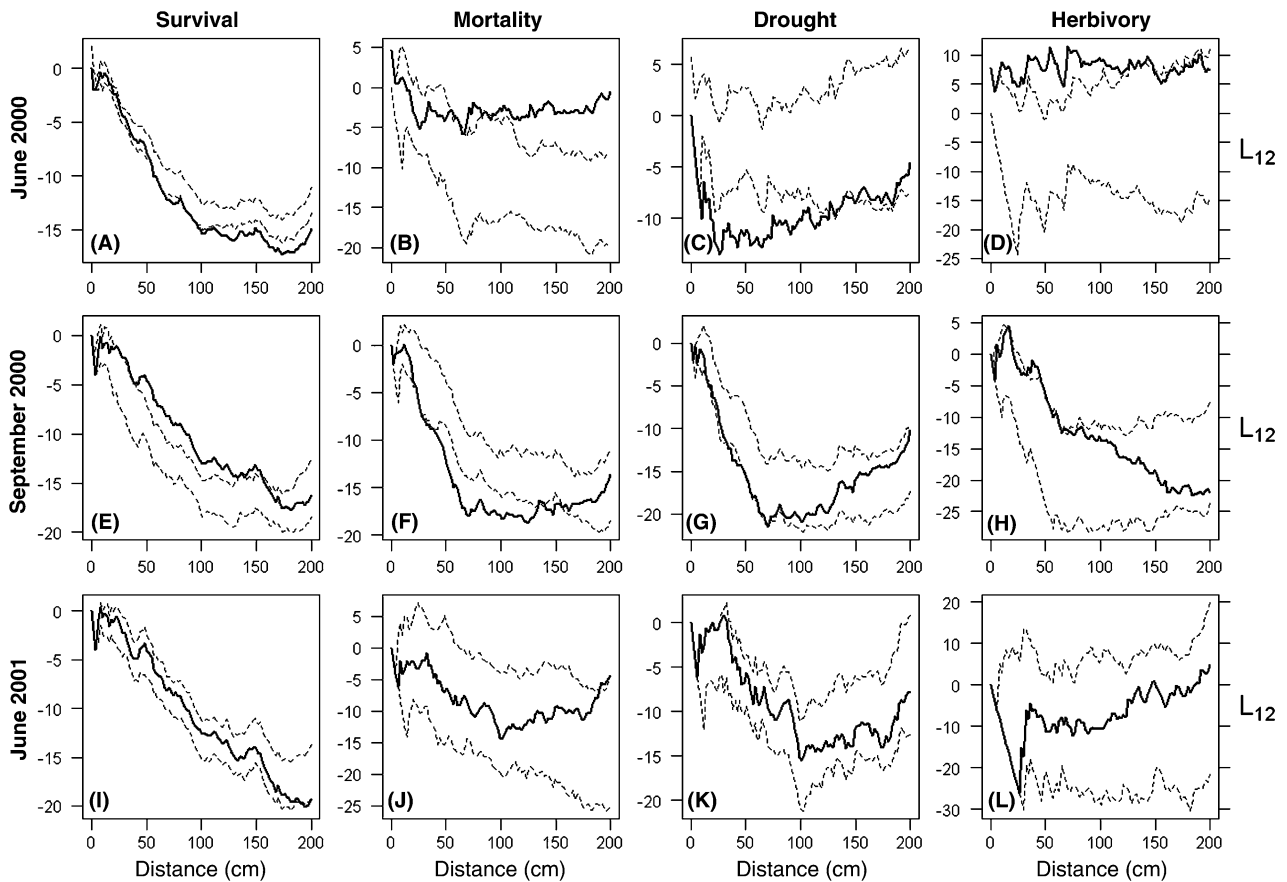
Figure 7. Temporal shifts of the spatial relationship between adult *Lepidium subulatum* plants and the survival, mortality and mortality causes (drought and herbivory) of *Helianthemum squamatum* seedlings. The solid line represents the observed bivariate L_{12} function. The dashed lines show the 2.5 and 97.5% percentiles of the distribution of the L_{12} function computed on the fixed pattern of *L. subulatum* plants and 999 random assignments of the appropriate labels ("alive" and "dead" over the population of remaining seedlings in the first and second columns; "drought" and "herbivory" over the population of dead seedlings in the third and fourth columns). Significant departures from the expected values under the hypothesis of independent labeling occur at scales where the observed L_{12} function exceeds the simulated envelopes.

opposite effects on seedling mortality from drought and from herbivory. Instead we found that even when some nurse plants showed a positive effect on seedling survival, they were also associated with some cause of seedling mortality. The detailed spatial analysis of mortality causes shed light on the mechanism involved in the facilitation against herbivory. For instance, the spatial pattern of deaths by herbivory was negatively associated with adult plants of

Table 1. Temporal shifts in the spatial association between different species of the studied community and the survival, mortality and mortality causes (herbivory and drought) of *Helianthemum squamatum* seedlings. The tabulated results are based on the observed bivariate L_{12} functions and the 2.5 and 97.5% percentiles of the distribution of the L_{12} function computed on the fixed pattern of adult plants and 999 random assignments of the appropriate labels ("alive" and "dead" over the population of remaining seedlings in the first and second columns; "drought" and "herbivory" over the population of dead seedlings in the third and fourth columns). + =positive association (significant positive departures from the expected values under the hypothesis of independent labeling at some of the considered distances), − =negative association (significant negative departures from the expected values under the hypothesis of independent labeling at some of the considered distances), and n.s. =non-significant association.

Species	Census date	Survival	Mortality	Drought	Herbivory
Koeleria castellana	June 2000	+	−	+	−
	September 2000	+	−	+	−
	June 2001	n.s.	n.s.	n.s.	n.s.
Thymus lacaitae	June 2000	+	−	+	−
	September 2000	n.s.	n.s.	n.s.	n.s.
	June 2001	n.s.	n.s.	n.s.	n.s.
Herniaria fruticosa	June 2000	n.s.	n.s.	n.s.	n.s.
	September 2000	+	−	−	+
	June 2001	+	−	n.s.	n.s.
Teucrium pumilum	June 2000	+	−	n.s.	n.s.
	September 2000	n.s.	n.s.	n.s.	n.s.
	June 2001	+	−	n.s.	n.s.

H. squamatum, K. castellana and *T. lacaitae* (Fig. 6D, H; Table 1). Since these species lack thorns or other deterrent structures, they do not act as biotic refuges (in the sense of Baraza et al. 2006). Therefore, their hypothetical facilitative effect against herbivory could be attributed to associational avoidance by herbivores (Milchunas and Noy-Meir 2002), i.e. herbivores may ignore *H. squamatum* seedlings growing in a matrix of other, unpalatable species. Our results suggest that the matrix of "unpalatable plants" could include the presence of drying seedlings, as the spatial pattern of drought is positively associated with these three species (Table 1) and at the same spatial scales (Fig. 6C, G, for *H. squamatum*). This suggestion is reinforced by the observation that the incidence of herbivory is positively associated with the spatial pattern of *H. fruticosa* and *L. subulatum* at the same scales where both species are negatively associated with deaths from drought (Table 1, and Fig. 7 for the case of *L. subulatum*). This suggests that rabbits would eat healthy (juicy) seedlings rather than weak (drying) ones. In fact, in the absence of water, rabbits need to forage green food to survive (Hayward 1961).

Concluding remarks

Our results, based on a new set of spatial point pattern analyses, show that the processes affecting the mortality of *H. squamatum* seedlings are spatially structured, and that these structures shift with time. They also demonstrate how small differences in either the time of emergence or the environment surrounding *H. squamatum* seedlings have profound influences on their fate. From a statistical perspective, focusing on survival, but ignoring where and why seedlings died would have had severely biased the interpretation of their relationships with the mature plants by forcing the study to consider only very simple hypotheses (i.e. the independence hypothesis, Goreaud and Pelissier 2003). These hypotheses are also an ecological simplification because they summarize and lose insight into the net effects of differential ecological processes (i.e. dispersal, germination, competition, herbivory, etc), which are responsible of the observed pattern of alive seedlings. Paying closer attention to differential mortality causes clarifies the relationships of dependence between seedlings and mature plants, allowing a more realistic interpretation of the supposed facilitative/competitive effects upon which conceptual models like the SGH are based. The new methods introduced in this article open an avenue for the detailed analyses of the spatio-temporal dynamics of plant mortality. Our results contribute to improve our understanding on the role of positive and negative interactions as a structuring force of natural communities, and on the consequences that changes in these interactions across environmental gradients have for the spatio-temporal dynamics of plant populations.

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