NET INTERACTIONS IN AN ANNUAL PLANT COMMUNITY
IN THE NEGEV DESERT, ISRAEL

by

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Abstract

In most plant communities, neighbours are likely to interact in at least two ways – negatively through competition and positively by facilitation. However, typically only the end product of these interactions is measured thereby detecting only the outcome, not the interactions themselves. This thesis focuses on understanding *net interactions* within an annual plant community in the Negev Desert, Israel by measuring the effects of spatial pattern, seed density, plant density, and specific species effects at different life-stages (by successive measurements) and levels of abiotic stress. I first tested the assumption that seeds in seed banks are generally clumped. Using geostatistics, a consistent clump size of 85cm^2 was detected across time, stress level, and seed size. There was however variation in the amount of seed present on the dune over time. Subsequently, I experimentally manipulated both the fine scale spatial pattern of patches of seed and the local density of seeds in small patches. Increasing local seed density generally had negative effects on measures of plant growth, whereas patches of seed with adjacent planted patches had increased performance. These results suggest that there is interference between seeds and plants within patches but positive interactions between the patches. The more general effects of density dependence were also tested at the seed and emergent plant levels. Emergence of seedlings was negatively affected by increases in seed density which also supports the interpretation that seed-seed interference may be occurring in this plant community. Mean plant size was negatively affected by increases in plant density, but survival was unaffected. Finally, I tested the prediction that a larger annual, *Erodium laciniatum*, acts as a benefactor species to nearby smaller annuals. *Erodium* was either added to or removed from patches of seed or vegetation. In both experiments, *Erodium* acted as a benefactor by increasing performance of neighbours (i.e., aboveground biomass or survival). Hence, competition and facilitation both play important roles in this plant community and their relative importance is influenced by life stage but not by level of abiotic stress. These studies are the first to demonstrate facilitation similar to shrub-understorey systems but at a much finer spatial scale.

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General Introduction

Context

There is general agreement among plant ecologists that competition plays a major role in determining community structure (Keddy 1989, Crawley 1990, Grace and Tilman 1990, Goldberg and Barton 1992, Goldberg 1996). However, in most plant communities, neighbours are likely to interact in at least two ways – negatively through competition and positively by facilitation (Callaway 1995, 1997). As field ecologists, we typically measure only the end product of these interactions (usually some measure of aboveground biomass or fitness) and thereby only measure the net effect of the positive and negative interactions (Connolly et al. 2001). If the effects of competition are greater than facilitation, as might be expected in productive environments, then we attribute decreased growth in plants to the effects of competition. However, those same competitive effects may have been moderated by concurrent facilitation effects. As we move into more unproductive environments such as deserts (and abiotically harsh ones in general), the importance, or very presence, of competition has been questioned (Grime 1977, 1979, Fowler 1986). However, facilitation has been clearly demonstrated in these environments (Callaway 1995), but the study of its relative importance in structuring communities has remained largely neglected (Hunter and Aarssen 1988, Bertness and Callaway 1994, Callaway 1995). Further, the interaction of, and relative importance of, competition and facilitation may vary with life stage (Walker and Vitousek 1991, Kellman and Kading 1992, Bertness and Shumway 1993, Bertness and Callaway 1994, Chapin et al. 1994, Berkowitz et al. 1995, Callaway and Walker 1997). Hence, research involving explicit tests for facilitation and its interaction with competition is fundamental to developing our understanding of processes that shape community structure and towards a general theory of plant interactions.

The majority of work on positive interactions between plants has been in harsh environments, with emphasis mainly on a nurse-plant shelter effect between shrubs and understorey herbaceous plants (Callaway 1995, 1998). Yet, many areas of positive plant interactions remain uninvestigated. My thesis tests for interactions among annual plants in the Negev desert, Israel, but focuses predominantly on positive interactions. The primary goals are to identify (i) how the density of seeds, juveniles, and adults influence plant interactions in the field, and (ii) how certain species of annuals aggregate and function with other annual plants in environments having different levels of stress. Spatiotemporal patterns in the field are identified, and manipulative experiments are used to test predictions concerning the influence of facilitation on community structure.

Literature review

Plant ecologists have devoted an enormous research effort to the study of competition (Goldberg and Barton 1992). Nonetheless, facilitation or positive plant interactions also comprise an extensive body of literature (DeAngelis et al. 1986, Hunter and Aarssen 1988, Bertness and Callaway 1994, Callaway 1995, Callaway and Walker 1997, Callaway 1998). Facilitation appears to occur primarily under abiotically stressed conditions, however its relative importance on community structure has received little attention. Although studies of positive interactions date back to 1909 (Callaway 1995), it is only in this last 5-10 years that there has been major research interest in facilitation outside the context of plant succession. Thus, this subject is only in the infancy of its theoretical development. Here, I will briefly review the pertinent literature and establish the context of my thesis with the ultimate goal being to promote the establishment of a general theory of plant interactions that integrates both positive and negative plant processes.

Bertness and Callaway (1994) predict that facilitation should be especially important in structuring communities in abiotically harsh environments or communities with high consumer pressure. This hypothesis is derived from the trend that primary productivity has been shown to generally decrease with increasing abiotic stress and Grime's resource based hypothesis (1977,1979) which suggests that the intensity of competition increases with primary productivity. In the event that the intensity of competition is in fact lower in abiotically stressed environments, the importance or intensity of facilitation will be greater and can function to ameliorate the harsh conditions imposed upon the plant by such habitats. Again, it is important to emphasize that both processes may be evident and important but simply that the relative value of each may shift along abiotic gradients. Indeed, there has been some support of a shift towards more positive effects with increased abiotic stress (Bertness and Shumway 1993, Bertness and Callaway 1994, Bertness and Yeh 1994, Callaway 1994, Chapin et al. 1994, Greenlee and Callaway 1996).

<u>Evidence for facilitation</u>
The majority of facilitation studies have focused on nurse-plant effects (Callaway 1995) where an adult of one species (shrub) shelters the juveniles of another species (Niering et al. 1963, Turner et al. 1966, Steenberg and Lowe 1969, Turner et al. 1969, Steenberg and Lowe 1977). This effect has been clearly demonstrated in a variety of harsh habitats including deserts (McAuliffe 1986, 1988, Arriaga et al. 1993, Barnes and Archer 1996, Pugnaire et al. 1996a, Pugnaire et al. 1996b, Pugnaire and Luque 2001) and salt marshes (Bertness and Shumway

1993, Bertness and Yeh 1994, Callaway 1994). To the best of my knowledge, no studies have attempted to identify nurse-plant effects among species having similar phenologies or life-histories. In order to understand the role of facilitation in structuring communities, it is necessary to also consider species other than those that live primarily as understorey plants.

The specific mechanisms that underlie shelter effects are rarely identified (Bertness and Yeh 1994 are notable exceptions, Pugnaire 2001), and the long-term community-level consequences of nurse-plant effects also remain unaddressed. While it is interesting that shrubs facilitate the growth of juvenile plants of other species, it is conceivable that facilitation could function in other capacities such as between annuals and at smaller scales. Hence, to more effectively further our understanding of facilitation, the breadth and scale of research on positive effects needs to be broadened.

Life-stage dependency

The net effect of all plant interactions both positive and negative is typically the value recorded at the end of the growing season or some other prescribed period of growth (Gibson et al. 1999, Cousens 2000, Connolly et al. 2001). In the event that this product is negative, then we conclude that competition is the key process that determined the community structure. However, temporal variation in the strength of plant interactions has been commonly detected (Goldberg and Barton 1992 , Goldberg et al. 2001), and the relative importance of competition and facilitation may vary with life-stage (Walker and Vitousek 1991, Kellman and Kading 1992, Bertness and Shumway 1993, Bertness and Callaway 1994, Chapin et al. 1994, Berkowitz et al. 1995, Callaway and Walker 1997). For instance, facilitation has been found to occur primarily at early life stages of the beneficiaries (McAuliffe 1986, Barnes and Archer 1996), and older plants tend to be more effective benefactors (Kellman and Kading 1992, Pugnaire and Haase 1996). Year-to-year variation in the balance of positive and negative processes has also been detected (Morris and Wood 1989, Del Moral and Bliss 1993, Kadmon 1993, Tielborger and Kadmon 1997). However, life stage effects on positive plant interactions are generally not investigated (Callaway 1995, 1997).

The seed, recruitment, and seedling stages are the most likely stages where positive interactions (facilitation) may be more prominent than negative interactions (competition). This may occur in most habitats - not just higher stress habitats - as seeds require safe sites for germination (Harper 1977). The community-level consequences of seed dispersal, fine scale variation in density and spatial pattern of the seed bank, patch effects, and safe site

characteristics have not been addressed in the context of general plant interactions, although the processes themselves have been individually studied (Inouye 1980, Green 1983, Geritz et al. 1984, Fowler 1988, Chambers and MacMahon 1994, Schupp 1995, Stoll and Prati 2001). Patterns of juvenile recruitment may similarly be a major determinant of community structure in harsh environments; the impact of plant interactions, especially facilitation, acting at this life stage, may thus ultimately influence final community composition. For example, Bertness and Yeh (1994) showed that the adult marsh elder, *Iva fructescens,* altered soil characteristics that facilitated the establishment of conspecific seedlings. Both positive and negative effects were shown to interact in determining recruitment in this study; however, the resulting community structure was not directly linked to either of the interactions. Tracking the balance and impacts of positive and negative interactions with life stage along gradients in plant density or abiotic stress remains largely unexplored.

Density and facilitation

The negative effects of density on plants have been extensively studied (Goldberg and Barton 1992, Murray 1994, Crawley 1997, Goldberg et al. 1999, Goldberg et al. 2001, White 2001). The density of seeds, juveniles, and adult plants also likely influences facilitation. To date, few studies have identified clear evidence for positively density dependent seed germination which could be interpreted as facilitation (Palmblad 1968, Linhart 1976, Waite and Hutchings 1978, Smith 1983, Adler et al. 1993, McMurray and Jenkins 1997). It is possible that mass germination of seeds could increase the probability of individual survival by reducing abiotic stress, penetrating the soil more effectively (Linhart 1976), or through higher tolerance for salinity (Waite and Hutchings 1978). Similarly, high neighbour densities in some situations may serve as a defense effect or ameliorate harsh environmental conditions (Walker 1994, Kikvidze 1996, Kikvidze and Nakhutsrishvili 1998).

Facilitation and water levels

Facilitative interactions can occur through increased water availability for one species because of the presence of another (Callaway 1995, 1998, Pugnaire 2001). There is a general phenomenon of increased survival and growth of understorey plants because shade from nurse-plants reduces water stress in harsh environments (Callaway 1995, Pugnaire and Haase 1996, Pugnaire et al. 1996b, Holmgren et al. 1997, Pugnaire 2001). The facilitative effects include lower transpiration demands (Maranga 1984), increased soil water availability by reduced temperature (Turner et al. 1966), hydraulic-lift (Richards and Caldwell 1987, Williams et al. 1993), and increased water holding capacity (Pugnaire 2001). Aguiar and Sala (1994)

experimentally demonstrated facilitation by a reduction in evapotranspiration of grasses under a shrub canopy, which lead to increased recruitment. Interestingly however, competition for soil moisture prevailed with increased density of grasses near the shrub, which generated a net negative effect (Aguiar and Sala 1994). Hence, facilitation can function by reducing the water stress of harsh environments but studies should also be able to detect density and temporal effects on plant interactions as the net effect of positive and negative processes varies under different conditions.

Conclusions

Current studies of facilitation suggest that life stage, plant density, and abiotic stress impact the prevalence and intensity of positive interactions. However, research thus far is equivocal in some respects (e.g. restricted to very specific systems) and lacks an integration of plant interactions in a more general sense. Consequently, because both facilitation and competition interact in the field, it is necessary to design experiments that test for both, and to test for changes over time and across stress gradients. Furthermore, the species tested should include similar phenologies and life-history characteristics beginning at seed level processes. Coupling long term studies that track interaction transitions with short term mechanistic ones will help clarify and develop a general theory of plant interactions.

Definition of facilitation

During this past 5-10 years, plant ecologists have focussed more research effort on interactions among plants besides competition – namely facilitation. The majority of papers that specifically address facilitation simply conceptualize it as synonymous with positive interactions (Hunter and Aarssen 1988, Bertness and Callaway 1994, Callaway 1995, 1998), as any interaction between plants (intra-specific or inter-specific) that positively impacts at least one of the individuals. To demonstrate facilitation in the field, one needs to show that some measure of plant performance (such as germination rates and establishment, growth rate, aboveground biomass, or seed output) is increased due to the proximity of neighbours.

General site description

The study site is a semi-stabilized sand dune dominated by winter annuals and sparsely distributed shrubs, the *Artemesia monosperma* and *Stipagrostis scoparia* associations at Bir Asluj in the Holot Mashabim Nature Reserve (31°00.212'N, 34°44.474E) in the central Negev desert, Israel. There are typically about 20 species of annual plants in this community (area of dune about 1ha). Common species in this community include *Ammochloa palaestina, Cutandia*

dichotoma, and *Bromus fasciculatus* (all Poaceae), *Erodium laciniatum* (Geraniaceae), *Paronychia arabica* (Caryophyllaceae) *Filago desertorum* (Asteraceae), *Trifolium tomentosum* (Fabaceae), *Matthiola livida* and *Erucaria pinnata* (both Brassicaceae; see Appendix A for full species lists and basic characteristics and Appendix B for photographs). The leguminous species (Fabaceae) include *Hippocrepis multisiliquosa*, *Trifolium tomentosum*, and *Trigonella arabica*.

Rainfall occurs primarily in winter months (December to April, Appendix C) and has a 30 year average of 110 mm per year (Goldberg et al. 2001). The three years represented in this thesis were very different in the total amount and frequency of rainfall they received during the growing season. In 1998, there were 27 rainfall events totalling 96.82mm; in 1999, there were 10 rainfall events totalling 39.6mm, and in 2000, 14 rainfall events totalling 35.7mm (data provided by the Blaustein Meteorological Unit). Hence, the years sampled included one relatively wet year (96.82mm) and two dry years (1/3 the MAP).

The field experiments reported in this thesis were performed on the north slope of the dune with prevailing winds from the north-west (see photo C in Appendix B). The bottom of the dune is typically less stressed (lower temperatures and higher soil moisture) than the top of the dune (Lortie and Turkington, <u>unpublished</u> data). The growth of natural vegetation and transplants confirm that the top of the dune is less suitable for plant growth (lower productivity) than the bottom (Chapters 3 and 5). Nutrient analyses for the soil of the north face of the dune are reported in Appendix D.

Overview of thesis

The chapters in this thesis have been grouped into the following three sections: seed level effects, density effects, and specific species effects. All five of the experimental chapters specifically address the main themes reviewed - namely that interactions involve an interplay between competition and facilitation, and that life-stage, density, and level of abiotic stress influence these processes. The studies described in Section A measure the fine scale spatial pattern of the seed bank and test the effects of changes in local seed density and spatial arrangement of patches of seed. Section B is a single chapter that describes a multi-year experiment that tests the effects of seed and plant density on subsequent plant community structure. The final section, Section C, tests the effects of addition and removal of a larger annual species, *Erodium laciniatum*, on neighbouring plant species.

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Section A: Preface for seed level effects

Variation in either the fine scale spatial pattern of seeds or the density of seeds may influence subsequent plant community structure. By ignoring the underlying variation in the seed bank (both in space and time), plant ecologists are likely to miss processes that influence germination and emergence of seedlings. For instance, seeds may be clumped at fine scales and processes such as interference between established plants (or seeds) may be dependent on the initial degree of spatial aggregation of the seeds. Conversely, facilitation may result if the spatial association of particular species or the combined effect of small annual plants germinating in clumps reduces abiotic stress. Hence, this section addresses the following two questions: (i) what is the underlying spatial pattern of the seed bank, and (ii) how does it affect the emergent plant community?

Chapter 1 is a survey that tests for the small-scale horizontal spatial pattern of a seed bank both over time and along a natural stress gradient from the top to the bottom of a sand dune. To the best of my knowledge, only four other papers have measured the fine scale spatial structure of a seed bank. However, previous studies have not looked at how spatial pattern might change with level of stress and with time and have not explicitly measured the fine scale spatial distribution of the seeds with sufficient rigor. Geostatistics were used to quantify the spatial pattern of the seed bank. Differences in the spatial pattern of large and small seeds were also tested, and relationships between the density of seeds and the density of plants that germinated were investigated.

In chapter 2, the local density of seeds and the spatial pattern of patches of seed was manipulated to experimentally determine if these two factors influence the subsequent plant community at the patch level and at larger spatial scales. This study is a direct extension of the previous chapter - the first study measured natural variation in the field, while the latter tested the consequences of changes in that variation. These studies specifically tested whether local seed density affected performance at the patch and plot level, whether the spatial arrangement of small patches of seed affects a target patch, and finally whether the effects of these two factors interact. This is a significant contribution to the literature in that there are some studies that test the effects of seed density on performance but do so at much larger spatial scales (typically at meters rather than centimeters) and only one study that has tested the effect of both density and spatial pattern on competitive hierarchies between four plant species. In chapter 2,

the entire seed bank for an annual plant community was used to test a spatial scale that approximated the fine scale spatial pattern of seeds in the field.

Chapter One: The small scale spatiotemporal pattern of an annual seed bank in the Negev Desert, Israel.

Abstract

Seed banks are generally considered clumped, but few studies document the small-scale horizontal spatial pattern of seeds. We measured the horizontal spatial pattern of a seed bank for an annual plant community using geostatistics to test whether the seed bank changes over time or along a natural stress gradient. We also tested for differences between large and small seeds. Seed was collected at three different times and at two locations (high and low stress) on a semi-stabilized sand dune using contiguous 3cm cores. Using semivariograms to measure the degree of aggregation, we detected a clump size of 85cm^2 that was consistent across time, stress level, and seed size. This consistency could be extremely important if small-scale clumping of seed banks generates specific associations between species of seeds and the emergent plants. Further work with seed bank-based communities would strongly benefit from measures of spatial pattern and species associations of seeds.

Keywords: seed bank, annual plants, stress, spatial pattern, geostatistics, semivariograms, seed size, desert, temporal, associations.

Introduction

Seed banks have both a spatial and temporal structure (Henderson *et al.*, 1988; Pake & Venable, 1996; Bliss & Zedler, 1998; Crawford & Young, 1998; Mitchell *et al.*, 1998; Arroyo *et al.*, 1999). They are in a state of constant flux -- seed rain adds seed to the seed bank, while death, decay, granivory, germination, and dispersal remove seed from the seed bank (Harper, 1977). This constant flux may give an impression of disorder, yet it is highly likely that subtle changes in this structure influence the emergent plant community. The least studied aspect of seed banks is the spatial and temporal structure. It is a common conception that seeds are clumped in seed banks but there are few studies that actually document this and most describe vertical structure rather than horizontal structure (Bigwood & Inouye, 1988; Baskin & Baskin, 1998). The majority of studies that directly investigate the properties of seed banks do so at a large scale such as the mean number of seeds per sample in various habitats (Henderson *et al.*, 1988; Crawford & Young, 1998; Jutila, 1998a, b; Mitchell *et al.*, 1998). In an exhaustive review of the seed bank literature (Baskin & Baskin, 1998), 70 of 78 papers on persistent soil seed banks measured seed densities as the number of seeds per m^2 of surface area sampled. Although many papers refer to spatial patterns of the seed bank (Schenkeveld & Verkaar, 1984; Henderson *et al.*, 1988; Perez *et al.*, 1998; Arroyo *et al.*, 1999), very few document the small-scale horizontal spatial pattern of the seeds (Kellman, 1978; Thompson, 1986; Elberling, 2000; Houle *et al.*, 2001).

A more common approach to seed bank analysis is to test for similarities in species composition between the seed bank and the growing vegetation (Schenkeveld & Verkaar, 1984; Bigwood & Inouye, 1988; Crawford & Young, 1998; Jutila, 1998a, b; Maranon, 1998; Mitchell *et al.*, 1998; Perez *et al.*, 1998; Arroyo *et al.*, 1999). The more innovative studies have investigated how seed bank composition changes with respect to gradients in the field such as successional status (Mitchell *et al.*, 1998), grazed versus ungrazed (Jutila, 1998a, b; Chang *et al.*, 2001), relation to nearby vegetation type such as shrubs (Schenkeveld & Verkaar, 1984; Crawford & Young, 1998; Davies & Waite, 1998), disturbance gradients (Thompson *et al.*, 1998; Valbuena & Trabaud, 2001), and altitude and topography (Houle, 1998; Peco *et al.*, 1998). These studies are innovative because they concurrently focus on both the spatial and temporal dynamics of seed banks. Nonetheless, the measures used to date are not small-scale and do not explicitly measure the spatial pattern of the seeds along the gradients tested. It is highly likely that the small-scale horizontal spatial structure of seed banks changes across environmental gradients and over time. The subsequent plant community may in turn be influenced by the underlying spatial pattern of seeds within seed banks.

In this study, we investigate the small-scale horizontal spatial structure of a desert annual seed bank. The system is a semi-stabilized sand dune in the Negev desert, Israel, where the plant community is almost entirely annual and seed bank based and where the dynamic properties of the seed bank may be of particular importance to the emergent plant community. We restricted the focus of this study to primarily measuring the spatial structure of the seed bank to determine how dynamic it really is. Of secondary importance, the connection between vegetation and seed density is also tested. To test for small-scale changes in the properties of the seed bank, we addressed the following questions:

1. What is the spatial pattern of the seeds in the seed bank?
2. Do large and small seeds have the same spatial pattern?
3. Does the spatial pattern of the seed bank change over time?
4. How does the spatial pattern of the seed bank change along a natural stress gradient in the field?
5. Does total seed density change over time or along the stress gradient?
6. Is there a relationship between the seed density and the density of plants that germinate from a sample of the seed bank?

Methods

Study site:

The study site is a semi-stabilized sand dune dominated by winter annuals and sparsely distributed shrubs, the *Artemesia monosperma* and *Stipagrostis scoparia* associations (Orshan & Zohary, 1963) at Bir Asluj in the Holot Mashabim Nature Reserve (31°00.212'N, 34°44.474E) in the central Negev desert, Israel. There are typically about 20 species of annual plants in this community (area of dune about 1ha) (Dyer *et al.*, 2001; Goldberg *et al.*, 2001). Rainfall occurs primarily in winter months (December to April) and has a 30 year average of 110 mm per year (Goldberg *et al.*, 2001).

Seed sampling:

We used small plastic film canisters, 3cm in diameter and cut to a depth of 2cm, to collect the seed and sand. A depth of 2cm samples at least 99% of all the seed in the sand in this type of system (Venable, 1989). We sampled contiguously on two sets of three 7.5m transects collecting a total of 250 samples per transect. The transects were positioned at the top (higher stress) and the bottom (lower stress) of the dune and seed was sampled three times spanning two growing seasons: at the end of the 1998 growing season (April 28th, 1998), following secondary dispersal and just prior to germination of the subsequent growing season (October

4th, 1998), and following germination in the subsequent growing season (November 11th, 1998). To separate seed from the sand, we used 2000um and 500um sized sieves to sort the seeds into large (>2000um) and small (500-2000um) seeds. The seed mass from each core was weighed for each size class.

Vegetation sampling:
We also recorded the composition of the vegetation within 5cm of the same transects that we used to collect the seed. To measure the composition, we used a 15cm diameter ring placed contiguously on each transect (50 times). We recorded abundance by species (frequency) for each ring drop at the end of the first growing season on April 28th, 1998.

Germination of seed cores:
The small and large seed from each core was sieved and weighed. All seed was planted into styrofoam planting flats in a well-ventilated greenhouse at the Desert Research Institute, Sede Boker Campus (25 km SE of the study site) on November 15th, 1998. Each flat was 0.61m x 1.51m and had 250 circular 5cm diameter holes which were 5cm deep and 1cm apart. We filled each hole to 2/3 from the top with seedless sand and planted each core to a randomly assigned hole within the tray. The seeds were planted by placing them just below the lip of each hole and lightly sprinkling with sand. The cores were watered daily starting on November 19th, 1998 to correspond with natural field conditions for that growing season. Germination was recorded daily for two weeks or until no new seedlings emerged. Due to the very small size of the seedlings, identification to species was not possible. We recorded total plant density emerging from each core.

Analyses
Seed spatial pattern:
Using the seed mass collected in each core along each transect, we used geostatistics to determine the scale and intensity of spatial aggregation (Rossi *et al.*, 1992). Semivariograms are the most effective approach to detect both scale and intensity of spatial pattern along transects (Rossi *et al.*, 1992; Robertson & Gross, 1994) and these were plotted using the S-Plus 4.0 spatial statistics module (Lucent, 1997). Their interpretation is explained in several papers (Rossi *et al.*, 1992; Robertson & Gross, 1994) and an example of their use in the plant ecology literature is the analysis of the spatial pattern of soil nutrients (Jackson & Caldwell, 1993; Schlesinger, 1996). To calculate the semivariograms we used all 250 samples for each transect but restricted the maximum distance to 50 samples apart as a conservative estimate of the

possible range of spatial dependence (Journel & Huijbregts, 1978). Each semivariogram lag class had at least 250 pairs (used 250 different combinations of pairs of samples for each distance apart). There was no obvious anisotropy in the data (directionality or skewness in seed mass along transects) so no transformations of the data were necessary (Aubry & Debouzie, 2001). The best model was fit to each semivariogram by minimizing the residual sum of squares between the theoretical model (line of best fit) and the semivariogram (Isaaks & Srivastava, 1989; Kaluzny *et al.*, 1998). The fit was always less than an objective value of 0.0001 where a fit of 0 would be perfect (Kaluzny *et al.*, 1998). Other spatial analyses such as blocking of variation techniques were also tested (Ludwig & Reynolds, 1988; Dale, 1999) and were consistent with the geostatistical results so only the semivariograms are presented here.

Ecological properties of the seed bank:

Using the seed mass of each core, we also compared survey time, transect position, and large and small seed sizes to test for differences in the seed bank distribution with repeated measure ANOVAs. All assumptions were satisified.

Relation to vegetation:

Using ANOVA (JMP 4), we tested for differences in plant density and composition between the upper and lower transects on the dune. Diversity and evenness were calculated using Module #8 in the Ecological Methodology program (Krebs & Kenney, 1998). We then used regression analysis to test if there was a relationship between the density of the vegetation and the subsequent mass of seeds in the seed bank, i.e., does plant density predict seed mass? To determine the reciprocal influence that the seed bank might have on the emergent vegetation (i.e., does seed bank density predict emergent plant density?), using regression analysis we tested if the density of the seedlings germinated in the greenhouse could be predicted by the mass of seed planted in each core.

Results

Spatiotemporal pattern of seeds – spatial trends:

Semivariograms for both large and small seeds generally showed strong spatial autocorrelation at distances up to 12 lags (Fig.1.1 the distance at which the semivariogram reaches an asymptote, and Table 1.1 the 'range' values of fitted models that gave the best objective fit). Up to and including the asymptote, the samples are spatially related (clumped), and beyond this range the samples are spatially independent (known as the sill) (Rossi *et al.*, 1992). One lag distance apart is the same as one core which was 3cm in diameter or $7.1cm^2$ (circular cores). A

lag distance of 12 is equivalent to a clump size of 84.8cm^2. Surprisingly, this clump size was consistent between seed sizes and positions on the dune. The intensity of clumping was also consistent across all factors with smaller clump sizes being more intensely aggregated (Table 1.1, generally a spherical model gave best fit – most points clustered near origin and greater slope of line at smaller lag distances). The degree of spatial dependence for each semivariogram (i.e., how much of the variation in the data is explained by the semivariogram) was calculated from the y-intercept or nugget value (C_o) and the sill (C) using (Rossi *et al.*, 1992): $(C-C_o)/C$. The spatial dependence varied considerably from only weakly spatially dependent, i.e., 20% to strongly dependent 72.7% (Table 1.1). In general, smaller seeds were more strongly spatially dependent (more clumped) (Table 1.1).

Spatiotemporal pattern of seeds –temporal trends:

The clump size of seeds was consistent across time (asymptote generally close to 12) but the strength of spatial dependence differed between surveys (Table 1.1 and Fig. 1.1 different relative position of curves). Although the semivariograms for the second survey had the smallest nuggets (y-intercepts), the relatively flat shape of their curves decreased spatial dependence (Fig. 1.1); the seeds were the least clumped on survey 2 and generally the most clumped on survey 1.

Ecological properties of the seed bank:

There was significantly more large seed mass than small seed mass at all 3 survey times and at both positions (top and bottom) on the dune (Table 1.2 with additional contrast analyses, p<0.0001) and significantly more total seed on the lower transects at all times (Table 1.2 with additional contrast analyses p<0.0001, Fig 1.2). However, both seed sizes functioned in the same way with respect to spatial and temporal changes so only total seed mass is depicted here (Fig. 1.2). There was significantly more seed mass at primary dispersal relative to the other two survey times (for lower dune), while the lowest seed mass was just prior to germination at both positions on the dune (Table 1.2 with post-hoc contrasts also at p<0.0001, Fig. 1.2).

Relation to vegetation:

There were significantly more plants on the lower transects of the dune than the upper transects (ANOVA, F=22.1, p<0.0001, n=200). There was no significant difference in plant density along any one transect at different surveys (ANOVA, p>0.05). Lower transects had a significantly

more diverse plant species composition, but evenness was not significantly different between the two positions (ANOVA at p<0.05).

There was no relationship between the density of adjacent vegetation in the field and the seed masses subsequently collected on the same transects (regression analyses, p>0.05). In the greenhouse, the seed mass planted from each core (either seed size analyzed separately or total seed mass) did not predict the total density of seedlings that germinated (regression analyses, p>0.05) while the cores from the lower position on the dune still had significantly more seeds germinate (ANOVA, F=236.9, p<0.0001, n=1000 with contrast analyses; ANCOVA with seed mass planted as covariate was also p<0.0001).

Discussion

Seed banks are generally considered clumped, although most studies test for vertical spatial structure or measure the spatial pattern of seeds at a large scale that may not be ecological relevant to the seed bank (Bigwood & Inouye, 1988; Baskin & Baskin, 1998). The small-scale spatial structure likely determines the distribution of the plants particularly in communities that strongly rely on the seed bank such as Mediterranean grasslands (Bartolome, 1979; Maranon & Bartolome, 1989; Maranon, 1998). We tested for spatiotemporal patterns in the seed bank that might influence the plant community. In the first three questions, we explicitly tested for small-scale spatial changes in the seed bank. Using geostatistics, we found that the seeds in the seed bank were consistently highly clumped. The maximum clump area of both large and small seeds was about 85 cm^2 (which means that there are patches of seed in the field about this size), but seeds were even more strongly clumped at smaller scales within this range. This is consistent with a limited number of studies that test for small-scale horizontal clumping of seeds within the seed bank (Kellman, 1978 (150cm2); Thompson, 1986 (49cm2); Elberling, 2000 (100cm2); Houle *et al.*, 2001 (<1m)). While these studies all detected significant clumping of seeds in the soil, only Kellman (1978) collected contiguous cores to specifically test for horizontal spatial pattern (and only 20 50x50mm cores were collected). Thompson (1986) cut 128 7x7cm segments of turf from an acidic grassland and mapped the fine scale pattern of germination within the samples in a laboratory; Elberling (2000) collected sets of 30 samples of soil at three distances from adult plants, in dessication cracks, or at random; and Houle et al. (2001) marked 10 or 18 contiguous 50x50cm quadrats on each transect and collected 4.4cm diameter soil cores from each quadrat. Therefore, for at least three reasons it is critical that more studies be conducted to document the small-scale spatial pattern of seed banks. Seeds are clumped at small-scales (Kellman, 1978; Thompson, 1986, our study; Elberling, 2000; Houle

et al., 2001); increased sample sizes are needed (at least $\underline{n}$ = 100) to better detect spatial pattern and seed density in the soil (Webster & Oliver, 1992; Aubry & Debouzie, 2001); and spatial associations can be particularly important in small aggregated neighbourhoods (Garrett & Dixon, 1998) or in reducing abiotic stress through facilitation (Callaway, 1995; Pugnaire & Haase, 1996; Callaway, 1998).

Notably, the spatial pattern of the seeds did not significantly vary between positions on the dune or between large and small seeds even though the total amount of seed changed in both instances. Either consistent features of micro-topography, such as depressions (Boeken & Shachak, 1994; Boeken *et al.*, 1995; Gutterman, 1997), or properties of the seeds themselves, such as a mucilaginous layer which adheres the seed to the soil near the mother plant (Gutterman *et al.*, 1990; Gutterman, 1993), are strong determinants of the spatial pattern of the seeds regardless of seed mass, seed size, or degree of stress in the habitat. The consistent spatial pattern of the seed bank could be an extremely important finding because it is possible that this small-scale clumping of the seed bank generates specific associations between different species of seeds which then translates into associations at the emergent plant level. If this is true, then the structure of the plant community that ecologists typically measure (at least at germination) may be a direct product of the spatial pattern of seeds. We are not able to directly test for this in our current study. Further work with plant communities that are seed bank-based would strongly benefit from a better understanding of the spatial pattern of seeds and a test for associations between species of seeds due to the spatial pattern of the seed bank.

While we are unable to test for compositional similarities, we tested for density effects and detected no relationship between the density of plants in the field and the mass of seed present or the density of plants that germinated from cores in the greenhouse. There are several possible explanations to account for this low predictability: seed dormancy was not entirely broken for all seeds in our greenhouse germination experiment, a proportion of the seeds were dead, or more likely species-specific differences in seed mass introduce variation into the analysis which we did not measure. This illustrates that there is a definite trade-off in experiments with seed bank studies. We were concerned primarily with spatial pattern and as such had large sample sizes repeated at different locations and over time. However, we were unable to test for species composition effects. Other studies that focus on composition generally have smaller and more detailed sample sizes, but do not measure spatial pattern. Both approaches should be applied to the study of seed banks concurrently. For instance,

collecting many cores contiguously (or in any design that can be used to test for spatial pattern) and recording the species composition of the seeds in a smaller sub-sample would allow detection of spatial pattern and test for interactions between pattern and composition.

We also detected a temporal structure to the seed bank in our study system. The mass of seeds and strength of clumping was lowest just prior to germination. A decrease in the mass of seed present within the system from primary dispersal in April to secondary dispersal in late October is not surprising as granivory and strong winds over the summer reduce the seed present on a dune. However, the apparent increase in seed mass from just prior to germination to post-germination is difficult to reconcile. The weaker degree of aggregation of seeds prior to germination is also likely due to granivory and winds which may spread the seeds out across the dune. This suggests that although the seed bank we studied has a consistent small-scale spatial pattern it still has the potential to change the amount of seed present and degree of aggregation over time though interactions with the environment.

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Table 1.1. Model parameters for the fitted semivariograms shown in Fig. 1.1 to visualize the spatial dependence of seeds in the seed bank. Survey 1 was immediately after primary seed dispersal, survey 2 was prior to subsequent germination the following growing season, and survey 3 was after germination. The model fit was selected based on minimizing the residual sum of squares. The nugget, sill, and range are explained in text.

Survey	seed size	position	model	nugget	sill	range	spatial dependence (%)
1	large	lower	spherical	0.3	0.5	11	40.0
1	large	upper	spherical	0.028	0.046	12	39.1
2	large	lower	spherical	0.016	0.023	12	30.4
2	large	upper	spherical	0.0048	0.006	12	20.0
3	large	lower	spherical	0.09	0.14	10	35.7
3	large	upper	spherical	0.045	0.065	12	30.8
1	small	lower	exponential	0.03	0.06	11	50.0
1	small	upper	exponential	0.003	0.011	12	72.7
2	small	lower	spherical	0.0075	0.014	18	46.4
2	small	upper	spherical	0.00037	0.00055	12	32.7
3	small	lower	exponential	0.015	0.03	12	50.0
3	small	upper	spherical	0.0045	0.0061	12	26.2

Table 1.2. A full-factorial repeated measures ANOVA for mass of seeds. There were three surveys (primary dispersal, prior to germination of subsequent season, and post-germination), two sets of transects (top and bottom of dune), and two seed sizes (large and small). Contrast analyses were used to test for differences between groups (p>0.05).

Effect	DF	SS	F Ratio	P-value
Model	11	309.57	375	<0.0001
Survey	2	91.32	608.41	<0.0001
Transect	1	127.13	1693.9	<0.0001
Seed size	1	5.46	72.7	<0.0001
Survey x Transect	2	59	393.25	<0.0001
Survey x Seed size	2	9.26	61.68	<0.0001
Transect x Seed size	1	6.13	81.7	<0.0001
Survey x Transect x Seed size	2	10.8	72.1	<0.0001
Error	2987	532.9		

Figure 1.1. Semivariograms of small and large seeds collected along two transects on a semi-stabilized sand dune. The seeds were collected at three times (1) primary dispersal of seed, (2) pre-germination of following season, and (3) post-germination of following growing season. Each semivariogram was calculated using 250 soil core samples each 3cm in diameter and 2cm deep (therefore a lag distance of 1 is equivalent to $7.1cm^2$). The semivariograms show the calculated variance for a number of pairs of samples at each possible lag distance apart (up to 50 apart). Each lag class had at least 250 pairs.

Figure 1.2. The mean seed mass per core (+ or − 1 SE) for seed collected at three different times and at two locations on the sand dune (n=250 per point). Large and small seeds are shown together here as they expressed the same trends.
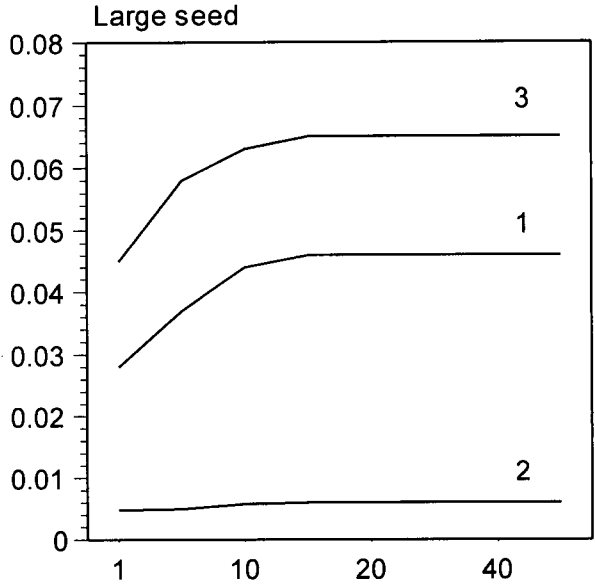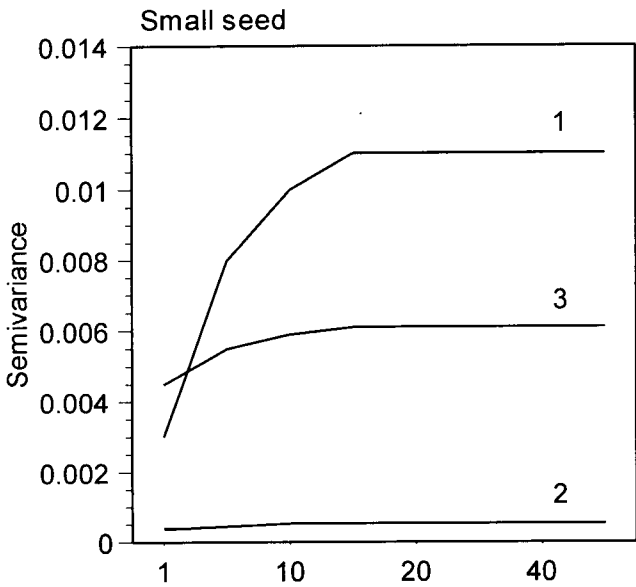
Upper transect
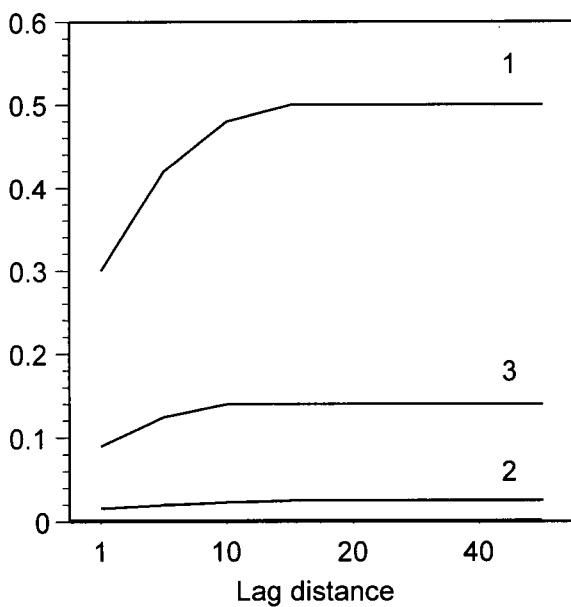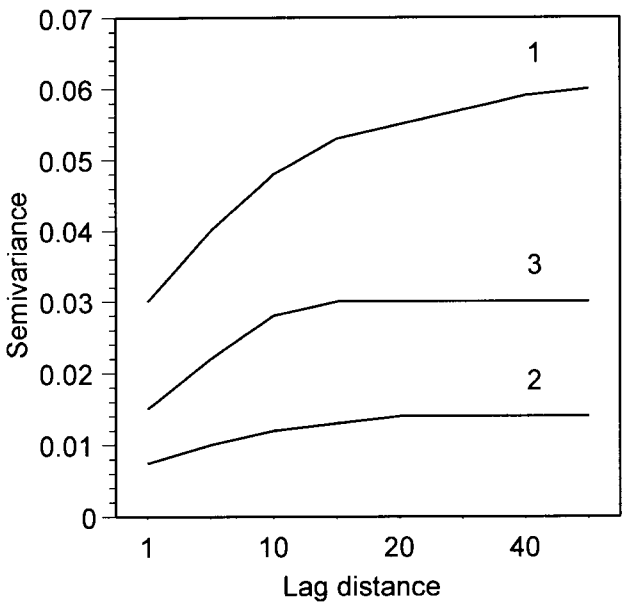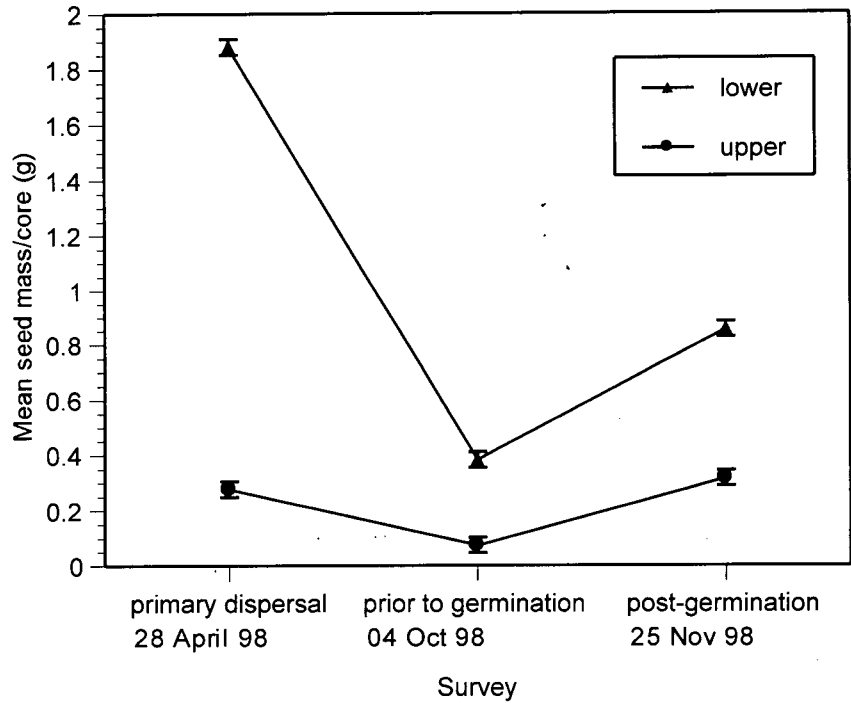
Chapter Two: Implications of local seed density and seed spatial pattern on community-level interactions.

Abstract

Field surveys and preliminary single-species experiments suggest that variation in local seed density (small patches of seed) and spatial pattern of the patches can influence subsequent plant community structure. However, there are few experiments that experimentally manipulate the fine scale density or spatial pattern of seeds. As such, we test the following three questions: (i) do changes in local seed density affect the performance of patches or entire plots (global level), (ii) do changes in the number of adjacent patches with seeds affect a target patch, and (iii) do these factors interact? In an outdoor experimental garden, we planted 0.5 x 0.5m plots partitioned into 10 x 10cm cells with well mixed seed bank collected from a desert semi-stabilized sand dune. The global seed density (plot level) was held constant and the local seed density was varied <u>within</u> plots (i.e., cells planted at 1x, 2.5x, & 5x). The spatial arrangement of the cells was also varied to include 0, 2, 3, & 4 adjacent cells planted with seed. Increasing local seed density generally had negative effects on plant performance at the patch and plot level. However, patches of seed with no adjacent patches planted had fewer plants at the 5x local seed density and lower survival at 1x and 5x seed densities. Hence, both local seed density and spatial arrangement of patches of seed influence the plant community, but their combined effects demonstrate that interference between seeds or plants within a patch can be mitigated by positive interactions between patches.

Keywords: annual plants, arid environments, competition, facilitation, fine scale, interactions, local seed density, patchiness, seed bank, spatial pattern.

Introduction

Seed banks in a wide range of habitats are generally clumped at least at the horizontal scale of a few meters (Kellman 1978, Schenkeveld and Verkaar 1984, Thompson 1986, Bigwood and Inouye 1988, Henderson et al. 1988, Crawford and Young 1998, Jutila 1998a, b, Maranon 1998, Mitchell et al. 1998, Perez et al. 1998, Arroyo et al. 1999). While global densities at this scale can be quite similar among adjacent patches, there will be patches of locally higher and locally lower seed densities which may be more relevant for interactions among plants. Furthermore, these changes in local seed density will also be accompanied by changes in spatial pattern of the patches of seeds. Hence, we define local density as the number of neighbours (seeds or plants) within a patch or neighbourhood that are likely to directly interact and global density as the total number of individuals within a group of adjacent patches. For instance, Miller et al. (1994) planted seeds of *Prunella vulgaris* in three different spatial arrangements creating a range of local seed densities (within 5cm), and this was repeated at three global densities, i.e., 40 – 400 seeds per 25 x 52cm flats. Increasing global density decreased mean plant aboveground biomass, while increasing local density increased the fitness of plants and selection for earlier emergence times. This demonstrates that local density and global density can have different effects on the germination of individuals within a population. Thus, the location of a seed within a local neighbourhood and the global density of the patches can both influence germination and perhaps growth and survival. However, studies that have tested seed density effects generally do so either at larger global scales such as at the square meter level (Goldberg et al. 2001) or for single species (Palmblad 1968, Linhart 1976, Waite and Hutchings 1978, 1979, Inouye 1980, Soetono and Puckridge 1982, Smith 1983, Adler et al. 1993, Miller et al. 1994, McMurray and Jenkins 1997, Murray 1998). The germination and establishment phases in plant communities are likely the most vulnerable life-stages (Harper 1977), and these experiments suggest that manipulation of local density at the community-level would be revealing. As such, we manipulate the local seed density of the entire seed bank for an annual plant community in this study.

To test for the effects of local seed density however, it is necessary to hold global seed density constant while varying local seed density. The problem is that to change local seed density (or number of local neighbours), one is also forced to change the spatial arrangement of seeds (Linhart 1976, Miller et al. 1994). However, the arrangement of the seeds or spatial pattern of the patches of seeds might also independently influence how the seeds germinate or the seedlings grow. For instance, clumps of seeds might have a higher tolerance for salinity (Waite and Hutchings 1978), or might allow roots of seedlings to, en masse, penetrate the soil more

effectively thereby having higher germination rates (Linhart 1976). Conversely, gaps or unoccupied spaces might increase the rate at which a species spreads in a community (Bergelson et al. 1993). Bergelson et al. (1993) demonstrate a purely spatial effect by investigating the effect of available space or refuges on colonization and found that large underdispersed gaps were best for seed dispersal. Recently, Houle (1998) and Houle et al. (2001), using non-experimental field surveys, demonstrated that the spatial pattern of the seeds in the seed bank have a significant influence on the density of the final plant community. There was a consistent correlation between the spatial pattern of emergent seedlings and mature individuals (Houle 1998, Houle et al. 2001). In the first experimental study of its kind, Stoll and Prati (2001) explicitly manipulated the fine scale species aggregation of four species of annuals at two densities. They found that the spatial arrangement of plants significantly affected competitive interactions with the weakest competitors benefiting from aggregated distributions and the competitively superior species having reduced performance with aggregation. Hence, local seed density and spatial pattern may both be important factors in shaping plant communities that are seed bank based but neither have been thoroughly tested. As Stoll and Prati (2001) also point out, further research needs to experimentally address these two factors under natural field conditions with communities which are more species rich and across a wider range of spatial scales. In this study, we have directly addressed these recommendations by testing the effects of local seed density and spatial arrangement of patches using the entire seed bank for an annual plant community, using an outdoor experimental garden similar and proximal to the field site, and by using a spatial scale that approximates fine scale patches of seed.

Based on preliminary experimentation and field surveys (Lortie and Turkington, unpublished), we explicitly define local density as the density of seeds or plants in 10 x 10cm patches, a scale that is likely relevant to immediate single plant-plant interactions, and global density is defined as the total density of a group of patches in a 0.5 x 0.5m plot. By holding global seed density (plot level, 0.5 x 0.5m) constant for the entire plant community and changing either the local density of small patches within the plots or the arrangement of the patches, we test the following three questions.

1. Do changes in local seed planting density affect the performance of individuals in patches (density of plants that emerge and aboveground biomass of patch) or the performance of the entire plot (plot density, survival, and aboveground biomass)?
2. Do changes in the number of adjacent planted patches affect the performance of plants in a target patch (density, survival, and aboveground biomass of plants in a patch)?

3. Do changes in <u>both</u> local seed density and spatial pattern of seed patches interact?

In general, we predict that both the fine scale interactions (between seeds and plants within a patch) and the interactions between patches will signficantly influence the structure of the plant community.

Methods

<u>Site description</u>

Seed was collected at Bir Asluj in the Holot Mashabim Nature Reserve (31°00.212'N, 34°44.474E). This is a semi-stabilized sand dune system in the central Negev desert, Israel, where the herbaceous vegetation is predominantly annual and entirely seed bank based. The seed bank is clumped (Lortie and Turkington unpublished), and easily collected and manipulated. There are typically about 20 species of annual plants in this community (area of sand dune about 1ha) (Dyer et al. 2001, Goldberg et al. 2001). Rainfall occurs primarily in winter months (December to April) and has a 30 year average of 110 mm per year (Goldberg et al. 2001).

<u>Seed collection</u>

Seed was collected November 5th, 1999. We used a modified shovel which removes the top 2 cm of sand (Goldberg et al. 2001) and effectively samples at least 99% of the total seed bank (Venable 1989). The seed was separated from the sand using a 0.5<u>mm</u> sieve and subsequently thoroughly mixed. This sieve size captures virtually all of the seed (Goldberg et al. 2001). Natural seed bank density was calculated for the field site (based on amount of seed collected per unit area) to be approximately 600 g/m². The mixed seed was then weighed into appropriate aliquots for each treatment according to the experimental design (see below).

<u>Experimental garden description</u>

Experiments were performed in an outdoor garden at the Institute for Desert Research, Sede Boker Campus (25 km SE of the field site). The experimental garden is composed of 1m x 1m and 1.2m deep corrugated plastic boxes sunk into the ground filled with seedless sand - for a detailed description see Goldberg et al. (2001). In order to avoid border effects, each box was comprised of a 0.5 x 0.5m plot surrounded by an unplanted 25cm wide buffer strip.

<u>Experimental design</u>

Seed was planted February 9th, 2000 in fifty 0.5 x 0.5m plots. The following factors were varied using a grid of 10 x 10cm cells (patches) within a plot: (i) local seed density (amount of seed

added to a cell), and (ii) spatial aggregation of the cells (adjacent cells planted or empty). To change local seed density, global seed density was held constant and seed was planted into different amounts within each plot. To test for spatial effects, local seed density was held constant (i.e., 1x or 5x) and the cells within the plots were planted in different spatial arrangements at each density (Fig. 2.1). Treatments were replicated 10 times, and a randomized block design was used to assign treatment levels to each plot. In addition to natural precipitation, plots were watered ten times at 1.5L per plot, which is equivalent to an overall annual precipitation of 100mm.

1. Local seed density treatment

The local density of seeds within a plot was changed by sowing seed at a constant global density corresponding to the natural seed bank density in the field (i.e. 1x = 150g in each 0.5 x 0.5m plot) but concentrating the seeds to smaller patches within each plot and creating three different local seed densities: 5 patches of 30g of seed per cell (5x), 10 patches of 15g per cell (2.5x), and 25 patches of 6g of seed per cell (1x) (Fig. 2.1a).

2. Spatial treatment

To test for spatial effects, local seed density was held constant at either 1x or 5x, and the number of planted cells adjacent to a target cell were varied within a plot. To test for the interaction between local seed density and spatial effects, we planted plots with 9 cells at 5x local density and 5 cells at 1x density to generate the full range of possible adjacent cells planted with seed (Fig. 2.1b). For instance, the 5x local seed density had 5 cells planted in a plot with no adjacent cells planted (Fig. 2.1a). The planting of 9 cells at 5x was arranged so that each target cell had 2, 3, and 4 adjacent planted cells (Fig. 2.1b). This design allows us to test whether the establishment of seedlings, with the same local seed density, is influenced by the number of occupied surrounding patches.

Dependent variables and statistical analyses

Both global (0.5 x 0.5m) and local (10 x 10cm cells) plant densities of each plot were recorded. For simplicity, we refer to global density as plot density and local density as cell density. Density was measured four times during the growing season by recording the total number of individuals of all species in each planted cell. Survival to subsequent census, total number of species present, and diversity measures (richness, reciprocal of Simpson's D, and E_{var} (Smith and Wilson 1996)) were also calculated. Differences between groups for all response variables were tested using Repeated Measures ANOVAs. The changes in cell plant density with the

increased seed density treatments (2.5x and 5x) were also tested against 1x plant densities as an expected null; i.e., does adding 2.5x more seed result in 2.5x more plants? At the end of the growing season, all aboveground vegetation was harvested by cell and weighed after drying for 48h at 60°C in a ventilated oven. Total aboveground biomass per plot, aboveground biomass per cell, and mean plant size (grams) were compared for the different local seed densities using one-way ANOVAs. The significance level for multiple tests was adjusted using Sequential Bonferroni corrections (Rice 1989).

To specifically test for spatial effects, we compared plant density, survival, aboveground biomass, and mean plant size (aboveground biomass / plant density) of the following target cells: cells with 0, 2, 3, and 4 planted adjacent cells. The same number of replicates (60 per group per seed density) were selected randomly from plots to maintain a balanced design for analysis (with cells nested within plots). Repeated measures ANOVAs were used for plant density and survival and ANOVAs for aboveground biomass and mean plant size. There was no significant effect of census, so only ANOVAs for the final census are reported for all response variables. All analyses were done using JMP 4.02 (SAS 2000).

Results

1. Local seed density effects

The local density of seeds has a significant effect on cell plant density with the 1x local seed density cells having significantly less plants per cell than the higher local seed density cells (Table 2.1, Fig. 2.2a). However, adding 2.5 times (or 5 times) the seed did not proportionally increase the density of emergent plants relative to the 1x cells (ANOVAs, $p > 0.05$, Fig. 2.2a). At the plot level, lower local seed densities had significantly higher plot plant densities (Table 2.1, Fig. 2.2b). Local seed density had no significant effect on plant aboveground biomass per cell, plant size (Table 2.1), or on any plot species diversity measures (one-way ANOVAs at the $p<0.05$ level); nevertheless, the proportion of individuals surviving and total plot aboveground biomass were significantly higher in the 1x local seed density plots (Fig. 2.2c, d). The differences in density were consistent throughout the growing season (Table 2.1) while survival signficantly decreased mid-season for the 5x local seed density plots (post-hoc contrast analyses, $p < 0.05$).

2. Spatial effects

As expected, the 5x local seed density cells always had significantly higher plant density per cell than the 1x local density cells (Table 2.2, Fig. 2.3a), but not a five-fold increase (ANOVA, p >

0.05). There was a reduction in density for the 5x local seed density cells with no adjacent cells planted relative to the other 5x density cells with occupied adjacent cells (contrast analysis, Fig. 2.3a). At 1x local seed density, there were no significant differences between target cell densities (Fig. 2.3a). Survival at both seed densities was lowest for cells with no adjacent cells planted (Table 2.2 and additional contrast analyses, Fig. 2.3b). There were no significant differences in plant aboveground biomass per cell (Fig. 2.3c) or mean plant size (Table 2.2).

Discussion

In the field, fine scale variation in seed density is highly likely, and while it is a relatively common notion in plant ecology that fine scale differences influence community structure (Harper 1977), there are relatively few direct manipulative tests of this notion (Waite and Hutchings 1979, Fowler 1984, Miller et al. 1994, Rees et al. 1996, Stoll and Prati 2001). Hence, the first question we tested addresses this issue by determining if local changes in seed density have effects at the fine scale and at larger scales. If fine scale changes in seed density were unimportant to the plant community, then we would predict that changes in local seed density within a plot do not change global plant density (as seed density was held constant at the plot level). Not surprisingly, changes in local seed density indeed affected plant density at the fine scale patch level. Higher local seed density patches had higher densities of plants but less than expected based on the amount of seed added (i.e., 5x seed density cells did not have 5 times more plants relative to 1x cells). At the plot level, local seed density significantly affected global plant density. This suggests that fine scale changes in the density of small patches of seeds can influence the emergent plant community. Similar to the few other studies that tested for interactions between seeds, we found that increasing seed density generally had negative effects on plant performance in terms of plant density, survival, and aboveground biomass (Inouye 1980, Shaw and Antonovics 1986, Miller et al. 1994, Murray 1998, Goldberg et al. 2001). Other studies have also reported that seeds may have the ability to detect and respond to neighbouring seed densities by changing emergence time (Miller et al. 1994, Murray 1998, Dyer et al. 2001). Here, the key implication is that these fine scale interactions between seeds can potentially structure the emergent plant community at larger scales and that the interactions between seeds may generally be negative.

It is important to differentiate between local density effects and spatial effects as both can vary concurrently. For instance, Linhart (1976) changed both spatial pattern and density by planting seeds separated from one another (5 – 7mm) and seeds contiguously - while changing the number of seeds planted in each group. Bergelson et al. (1993) also reported that colonization

by *Senecio vulgaris* was sensitive to both gap size and spatial distribution of the gaps (underdispersed versus overdispersed) in experimental arrays in the field. However, the community-level consequences of changing the arrangement of patches of seeds has not been tested (Murrell et al. 2001, Stoll and Prati 2001). In this study, there were significant spatial effects with neighbouring patches increasing the performance of target patches (survival) particularly at higher local seed densities (increased plant density per patch and survival). This difference was most strongly expressed with the decreased performance of patches with no neighbouring planted patches, while patches with 2, 3, or 4 nearby patches generally did not differ. This suggests that nearby cells buffer each other perhaps in the form of reduced evapotranspiration. Facilitation of this sort is typically demonstrated in shrub-understorey systems where a larger plant such as shrub provides a habitat for understorey plants that has higher soil moisture, nutrients, or lower evapotranspiration due to shade (Callaway 1995, Pugnaire and Haase 1996, Pugnaire 2001). However, to the best of our knowledge only one other study has detected a positive effect of the entire neighbourhood itself (i.e., a patch effect of a group of plants versus a single larger plant) where a canopy of neighbours stabilized environmental conditions in a subalpine plant community (Kikvidze 1996). In short, the spatial arrangement of patches in this study significantly influenced the plant community but was dependent on the local seed density. The effects were also consistent throughout the growing season which suggests that, similar to Houle et al. (2001), the underlying initial pattern of the seeds can contribute to the pattern of mature individuals in a plant community (entire annual plant community in our study and a rare annual, *Floerka proserpinacoides*, in Houle et al. (2001)).

The third question we posed was whether the effects of local seed density and spatial pattern of patches interacted. Similar to Stoll and Prati (2001), we found that spatial pattern was more important at higher local seed densities; the 5x patches with planted neighbouring patches resulting in increased survival and density of target patches. However, the effect of independently increasing local seed density was generally negative within the patches and at the plot level. Hence, the interaction between seeds within a patch differs from the interactions between the patches. This is an intriguing result as it suggests that competition (or interference) between seeds or plants may be mitigated by facilitation through spatial effects of the patches of seed or vegetation. The implication of positive spatial effects at the patch level and negative density effects among individuals is that the vast majority of competition studies, which focus on individual species interactions, have detected negative effects of increasing density (Goldberg and Barton 1992). However, this study and that of Stoll and Prati (2001) suggest that other

processes at the patch level also influence the performance of the community. Further research should thus encompass multiple scales of interactions and where possible tease apart the relative importance of different processes that influence community structure when moving up from the intimate interactions among individual plants to that of larger patches - particularly in arid systems where the vegetation is commonly found in patches (Fowler 1986).

Finally, mean plant size and diversity measures were relatively unaffected by either local seed density or spatial arrangement of patches. This suggests that (i) interactions between plants within this system were predominantly expressed through differences in establishment and survival rather than through individual differences in size, and (ii) that the representation of species within the community is independent of seed density or spatial pattern. As Goldberg et al. (2001) speculate, interactions within this arid sandy community function to determine who persists essentially in a discrete fashion given that the annual plants are very were small, i.e., between 15 – 40cm tall (Lessin et al. 2001). The lack of differences in diversity may be attributed to the thoroughness of the mixing of the seed bank in our experiment prior to planting. Under strict field conditions, it is likely that the seed bank is not mixed and spread evenly over the dune, and that fine scale spatial heterogeneity may even further exacerbate the effects of patchiness within the natural plant community that we detected here and potentially also influence diversity and species coexistence (Murrell et al. 2001, Stoll and Prati 2001).

Conclusions

Both local seed density and seed spatial pattern significantly influenced the subsequent annual plant community of a semi-stabilized sand dune in the Negev desert, Israel. The effect of increasing local seed density was generally negative while the spatial aggregation of patches of seed and vegetation was generally positive. Hence, the direction of interactions within a plant community are more complex than interpretation by a strictly individual-based approach suggests. Only multiple scales of responses, i.e., local and global plant density responses, that incorporate tests of potential spatial effects particularly at the patch level can lead to the integration of our understanding of community-level patterns and processes.

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Table 2.1. Repeated Measures ANOVAs and ANOVAs for response variables to changes in local seed density. The aboveground biomass measures refer to the final census. Significant p –values are in bold (after Sequential Bonferroni corrections for 2 tests of density and 3 for aboveground biomass).

Measure	Effect	DF	SS	F Ratio	P-Value
Cell density	Census	3	159.2	1.51	0.22
	Seed density	2	982.7	14	**0.0001**
	Census x Seed density	6	47.6	0.22	0.97
Plot density	Census	3	27318	2.12	0.10
	Seed density	2	248717	28.9	**0.0001**
	Census x Seed density	6	19924.9	0.77	0.59
Proportion	Census	2	7.6	13.9	**0.0001**
surviving	Seed density	2	2.6	4.7	**0.012**
	Census x Seed density	4	3.9	3.6	**0.01**
Plot aboveground biomass	Seed density	14	50.5	12.3	**0.0012**
Aboveground biomass per cell	Seed density	14	0.00001	0.76	0.49
Mean plant size	Seed density	14	0.0007	2.4	0.13

Table 2.2. Two-way ANOVAs for final response variables to changes in spatial arrangement. Significant p –values are in bold (after Sequential Bonferroni correction for two tests of aboveground biomass).

Measure	Effect	DF	SS	F Ratio	P-Value
Cell density	Local seed density	1	4993	117.7	**0.0001**
	No. of adjacent cells planted	3	415.5.	3.3	**0.022**
	Seed density x Adjacent cells	3	1246.5	9.8	**0.0001**
Proportion	Local seed density	1	0.13	5.0	**0.026**
surviving	No. of adjacent cells planted	3	0.26	3.3	**0.019**
	Seed density x Adjacent cells	3	0.12	1.7	**0.19**
Cell aboveground biomass	Local seed density	1	0.17	1.5	0.22
	No. of adjacent cells planted	3	0.77	2.3	0.08
	Seed density x Adjacent cells	3	0.45	1.3	0.27
Mean plant size	Local seed density	1	0.23	2.7	0.1
	No. of adjacent cells planted	3	0.08	3.2	0.026
	Seed density x Adjacent cells	3	0.01	0.6	0.62

Figure 2.1. The planting design used in an outdoor garden to test for the effects of different local seed densities and spatial arrangement of patches of seed on plant community structure. A plot is 0.5 x 0.5m in size with a 25cm buffer strip around each, and patches (or cells) are 10 x 10cm. On the left panel (a), the planting arrangement of three local seed densities (1x, 2.5x, 5x) are shown. On the right panel (b), the two additional spatial arrangements are shown. Using the two plots shown at either 5x (30g) or 1x (6g) local seed density, we are able to compare cells with 0, 2, 3, or 4 planted cells adjacent to target cells (numbered to show number of planted neighbour cells).

Figure 2.2. The effect of three different local seed densities on the local (cell) and global (plot) plant density. Cell plant density is the number of individuals per 10 x 10cm cell sown with seed, and plot plant density is the total number of individuals per plot (0.5 x 0.5m). Density and survival show the mean values from Repeated Measures ANOVA and aboveground biomass shows the mean values from an ANOVA at final census. Bars with different letters are significantly different ($p < 0.05$, ANOVA with Tukey multiple comparisons).

Figure 2.3. The effect of different spatial arrangements of patches (cells) of seed on changes in local seed density. A plot is 0.5 x 0.5m and a cell 10 x 10cm. The number of adjoining planted cells around a given cell within a plot allows us to test the effect of adjoining cells. The three response variables shown are for the final census at the end of the growing season. Open triangles are 1x local seed density, and closed circles are 5x.

a. Local density effect

b. Additional spatial treatments

5x local seed density
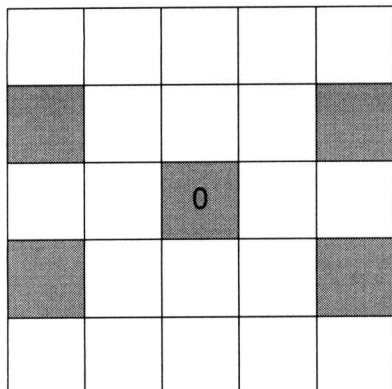
30.0g per cell

2.5x

1x

6.0g per cell
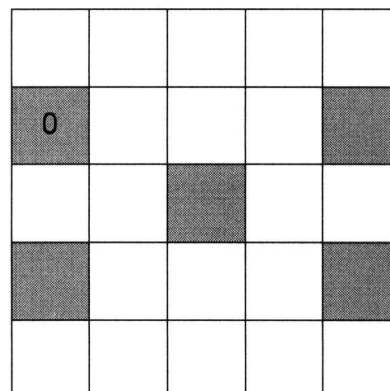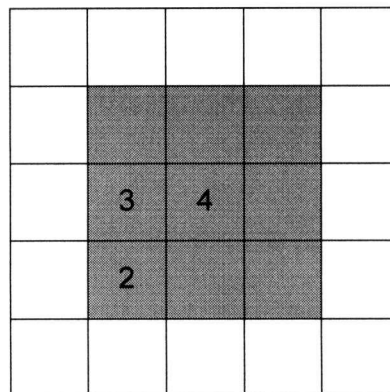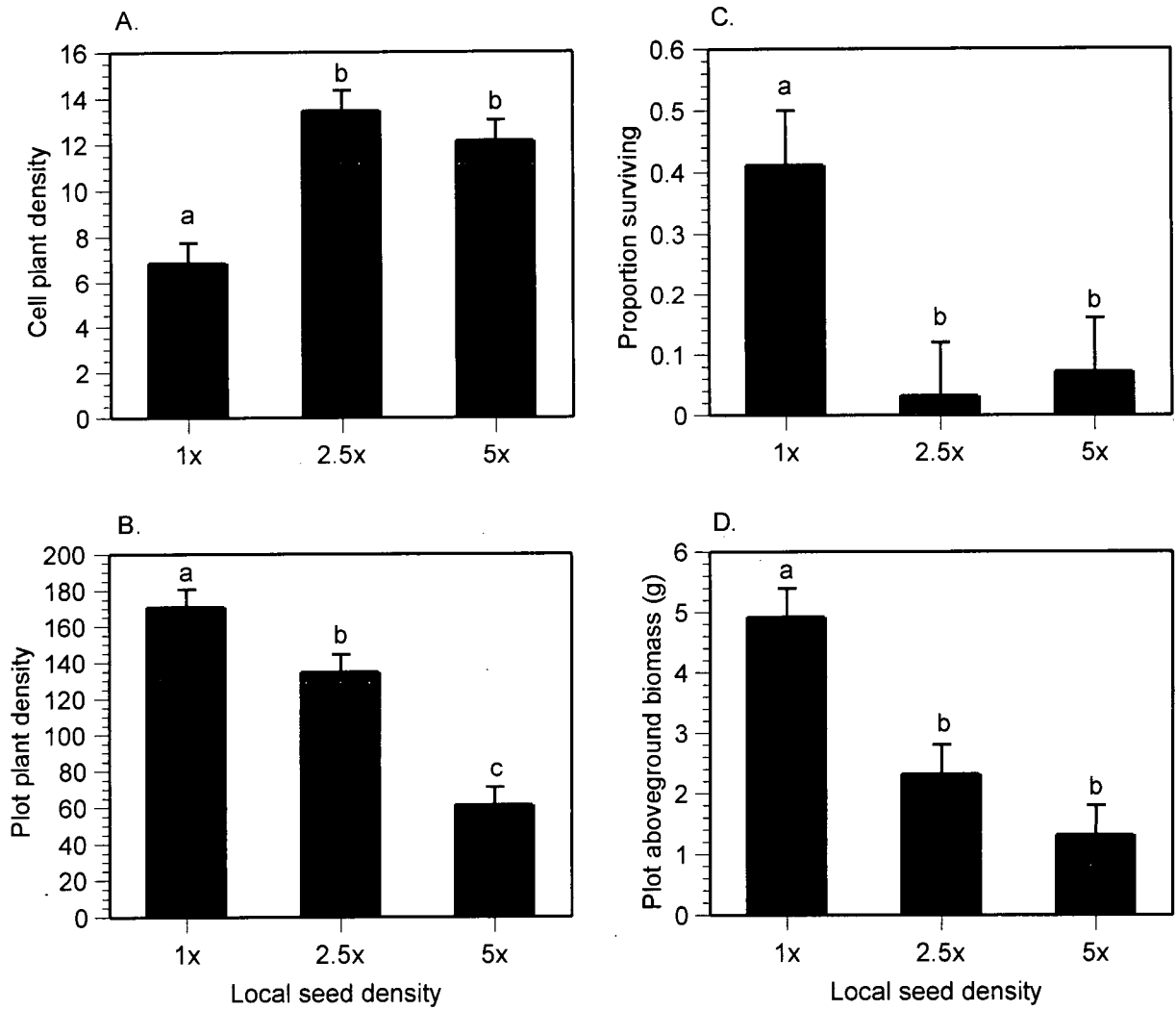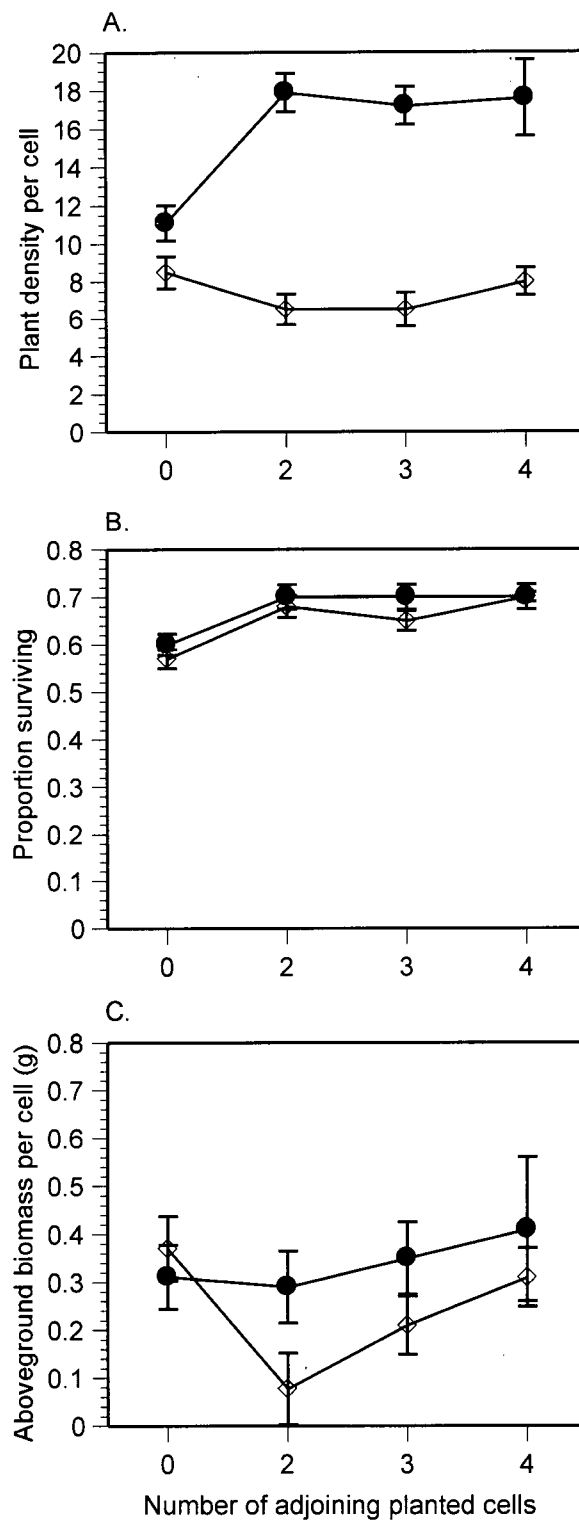
Section A: Conclusions for seed level effects

The seed bank was clumped at a fine spatial scale ($85cm^2$). However, contrary to the predictions tested, this fine scale spatial pattern did not vary significantly with seed size or position on a semi-stabilized sand dune. The consistency of this spatial pattern could have significant consequences for the emergent plant community in that this clumping may generate specific species associations that may either be positive or negative, i.e. species found growing together more often, or less often respectively, than chance. Further research should directly test whether the spatial pattern of seeds affects the species composition of the emergent plant community. There was significant variation in the temporal structure of the seed bank, i.e., amount of seed present at different times between and within growing seasons. This is likely due to either granivory, wind, or movement of sand over time. There was no effect of mass of seed on the density of plants that emerged in the field or the greenhouse. However, this experiment (chapter 1) only crudely tested for relationships between seeds and emergent plants (the study described in the following chapter specifically tested this). Hence, there was a fine scale spatial pattern of the seed bank and temporal variation in the amount of seed present on the dune.

The second experiment (chapter 2), which tested the effects of changes in local seed density and spatial arrangement of patches of seeds, closely approximated the natural variation we detected in the field. The patch size used, 10 x 10cm, is similar to the consistent natural patch size of seeds - $85cm^2$. The results demonstrated strong negative effects of increasing local seed density within these small patches on the performance of the emergent plant community (namely survival and aboveground biomass). The effects were negative at both the patch and plot level. The effect of the spatial aggregation of patches of seed was however positive and was more strongly expressed at higher local seed densities. Hence, the negative effects of increased seed density within a patch may be offset by the spatial arrangement of patches of seed. This may be particularly important since chapter 1 showed that patch size was consistent while the density of seed on the dune may change with time which suggests that there is variation in the local density of seeds within patches on the dune. However, further research should quantify the spatial pattern of the seed bank at larger spatial scales to determine if patches of seeds are aggregated on the dune, i.e., use a stratified random approach to sampling seed to measure the frequency of patches of seed and distance between these patches. These studies have demonstrated a consistent fine scale spatial pattern of a seed bank and detected significant effects of this variation on an emergent plant community.

Section B: Preface for density effects

There are very few direct experimental tests of density dependence. Furthermore, the importance of density in potentially regulating populations or influencing community structure is highly questionable and hotly debated in the literature. In chapter 3, a recently developed experimental approach of varying the density of the entire community was used to test (i) whether initial seed density influences the emergent and established plant community and (ii) whether there are density dependent processes operating within the emergent and established plant community. Hence, the ultimate, ideal, goal of this chapter would be to determine if regulation (via density dependence) or if limitation by the environment is more important in structuring this desert annual plant community.

The experimental design used in chapter 3, sowing the seed bank at different densities within 0.5 x 0.5m plots, and successive measurements of performance at the community level, also allowed the following hypotheses to be tested. Initial seed density is important at both life-stages (emergent and established plants); life-stage will affect the role seed density and density dependence play within the vegetation; level of stress will affect the role of density; and year will affect the role of density. This study is a significant extension to the single published experimental study of this sort as it tested for density dependence in the field, distinguished between the effects of seed density and density dependence within the established vegetation, and distinguished between community-level regulation and limitation by the environment.

Chapter Three. The effect of initial seed density on the structure of a desert annual plant community.

Abstract

Few experiments directly test the role of density dependence in natural plant communities. We tested (i) whether different initial seed densities of the entire seed bank of an annual plant community affected performance (emergence, mean plant aboveground biomass, and survival) and (ii) whether density dependent processes were operating within the communities. We also tested whether stress, both on a natural gradient and in an experimental manipulation, life-stage, and year influenced the effects of seed density. To address these questions, we divided the life-cycle of the plants into two distinct phases: seed to emergent seedlings, and emergent seedlings to established plants. The seed bank was collected, sieved, and replanted at the same study site, a semi-stabilized sand dune in the Negev Desert, Israel, at four different initial seed densities: 1/16x, 1/4x, 1x (natural seed density), and 2x. The experiment was repeated for three consecutive growing seasons. Emergence of seedlings was significantly influenced by initial seed density in all three years with higher initial seed densities having lower rates of emergence. Mean final plant size was negatively density dependent (by plant density) and consistently unaffected by the initial seed density sown. In general, there were no strong interactions of stress level with initial seed density or density dependent processes within the vegetation, and the experimental addition of water did not influence any of the performance measures tested. Density dependent processes vary from year-to-year, while stress effects do not. We conclude that seedling emergence and some processes in the established plant community are density dependent, but other processes, such as limitation by the environment, are also occurring in the established plant community.

Keywords: annual plants, abiotic stress, competition, density dependence, life-stage.

Introduction

Crawley (1997) argues that populations without density dependent processes will go extinct, yet White (2001) denies the very existence of density dependent regulation. Perhaps the debate was foreseen by Murray (1994) who suggested that while we make general predictions using the concept of density dependence, we do not address specific questions about how regulation might occur. In addition, Goldberg *et al.* (2001) specifically identify three major short-comings in the density dependence literature: (i) most studies only consider variations in density of a single focal species, (ii) most studies that address density dependence in the field only do so nonexperimentally, and (iii) typically, only changes in the effects of density of single species are considered in the field. However, different species of plants interact in the field, and as such, the whole community may be subject to density dependent regulation. Hence, processes related to the community may regulate parameters such as birth or death rates and thereby influence community structure (Crawley 1997); however, this general concept and the evidence itself are equivocal (Goldberg & Barton 1992; Murray 1994; Goldberg *et al.* 2001; White 2001). We are interested in determining if community-level manipulations of density, through experimental manipulation of the seed bank, directly influence interactions within the community.

The potential range of responses by a community to manipulation of total density include negative density dependence (Goldberg & Barton 1992; Goldberg *et al.* 2001), positive density dependence (Bertness & Callaway 1994; Callaway 1995; Callaway & Walker 1997; Goldberg *et al.* 2001), and density independence. Negative density dependence is typically interpreted as either competition through interference or exploitation, and positive density dependence is typically attributed to facilitative nontrophic interactions such as reduced stress or protection from herbivores (Bertness & Callaway 1994; Callaway 1995, 1998). The most direct methods used to test for these effects are either through removal of the entire neighbourhood surrounding a focal species or sowing different densities of the community. The first approach is common in both the competition and facilitation literature (Goldberg & Barton 1992; Callaway 1995) while the second approach is less common (Goldberg *et al.* 2001). Directly changing the initial sowing density of the community provides a test of density dependence while maintaining the proportion of species represented. This approach also provides a test for interactions between seeds and if these seed-seed interactions influence the early emergent and final established plant community. Competition studies have been criticized for recording only the final outcome of interactions by measuring net effects at the end of the growing season (Gibson *et al.* 1999; Connolly *et al.* 2001) because it is highly likely that the outcome of interactions

between plants is dependent on life-stage, primarily due to changes in plant size (Callaway & Walker 1997). Hence, the manipulation of initial community density (Goldberg *et al.* 1995; Goldberg *et al.* 2001) and monitoring changes in the subsequent plant community over time allows us to distinguish between interactions and final outcomes (i.e., density dependent processes and density dependent effects).

In addition to changes in density dependent effects with life-stage, the level of environmental stress is also predicted to influence the outcome of interactions between plants. Stress is inversely related to productivity where low productivity represents a high stress site. The predictions are highly debated (Goldberg & Barton 1992; Goldberg *et al.* 1999), but in general, competition is either predicted to decrease with increasing stress (Grime 1973, 1977; Bertness & Callaway 1994) or remain relatively constant (Newman 1973; Tilman 1988). It has also been argued that the type of stress gradient tested (natural vs. manipulative) may significantly influence whether the outcome is positive or negative (Goldberg & Barton 1992; Kadmon 1995). Furthermore, the level of stress may also vary from year to year depending on local weather conditions such as total amount or frequency of rainfall (Kadmon 1995; Tielborger & Kadmon 2000; Novoplansky & Goldberg 2001). Manipulating both stress and density concurrently tests whether density dependent processes are influenced by stress level.

Using the initial community density approach (developed by Goldberg *et al.* 1995) by sowing the seed bank at different densities, we ask the following questions: (i) does the initial seed density influence the emergent and the established plant community, and (ii) are density dependent processes important at these different life-stages. To specifically address these questions, we test the following four hypotheses.

1. Density effects. Initial seed density influences the plant community both at the emergent seedling stage and in its final form, and interactions within the established plant community will also be density dependent.

2. Life-stage. The effect of initial seed density and density dependent effects will vary with life-stage.

3. Stress. The effect of initial seed density and density dependent effects will vary with position on a natural stress gradient and manipulation of amount of water.

4. Temporal effect. The effect of initial seed density and density dependent effects will vary between years.

Methods

Study site and annual plant community

The study site is a semi-stabilized sand dune dominated by winter annuals and sparsely distributed shrubs at Bir Asluj in the Holot Mashabim Nature Reserve (31°00.212'N, 34°44.474E) in the central Negev desert, Israel. There are typically about 20 species of annual plants in this community (area of dune about 1ha) (Dyer *et al.* 2001; Goldberg *et al.* 2001). Rainfall occurs primarily in winter months (December to April) and has a 30 year average of 110 mm per year (Goldberg *et al.* 2001). The three years tested were very different in the total amount and frequency of rainfall they received during the growing season. In 1998, there were 27 rainfall events totalling 96.82mm; in 1999, there were 10 rainfall events totalling 39.6mm, and in 2000, 14 rainfall events totalling 35.7mm (data provided by the Blaustein Meteorological Unit). Hence, the years sampled included one relatively wet year (96.82mm) and two dry years (1/3 the MAP). The experiment was performed on the south face of the dune with prevailing winds from the north-west. The bottom of the dune is typically less stressed (lower temperatures and higher soil moisture) than the top of the dune (Lortie and Turkington, underlined data). The growth of natural vegetation and transplants confirm that the top of the dune is less suitable for plant growth (lower productivity) than the bottom.

Experimental design

To test for density dependence, initial seed density was varied by sowing different amounts of the entire seed bank (thereby maintaining a constant proportion of the different species of seeds). The experiment was repeated during the winters of 1998, 1999, and 2000 at two different positions on the dune (high and low stress). The same approach of changing initial seed density at two stress levels was repeated each year but with slight modifications in two of the seasons. In 1998, the species abundance was recorded throughout the growing season to determine if seed density affects diversity. In 1999, half of the plots at each position received additional water to further test if level of stress influenced density dependent processes.

Seed was collected early each November using a modified shovel that removes the top 2 cm of sand. This depth effectively samples at least 99% of the total seed bank (Venable 1989). Seed was separated from the sand using a 500um sieve. This sieve size captures virtually all of the seed (Goldberg *et al.* 2001). Natural seed bank density (1x) was then calculated for the field site (based on amount of seed collected per unit area) and was found to be 600.8 g/m² in 1998, 625.3 g/m² in 1999, and 597 g/m² in 2000 (mean = 607.7 g/m²). Each plot was 0.5 x 0.5m, and

all were sown at one of four seed densities: 1/16x, 1/4x, 1x, and 2x the natural seed density for the field (1998 value used for all three seasons).

On the same semi-stabilized sand dune, two transects (50m apart) were marked, one at the bottom (lower stress) and one higher (higher stress). On each transect, 10 blocks each with 5 plots (0.5 x 0.5m) were marked. The blocks were spaced at least 1m apart on the transects, and plots had a 20cm buffer between them. The sand from four of these plots was collected and sieved to remove seed from that plot. The seed collected and weighed to one of the four densities was thoroughly mixed with the seedless sand and replanted in each plot. Densities were randomly assigned to plots within each block. In 1998, 10 additional 0.5 x 0.5m plots were cleared, sieved, and the seedless sand replaced to test the effectiveness of sieving in the field and to determine if additional seeds were dispersed into plots prior to germination.

Each year, the emergent vegetation was surveyed three times: early season (the end of January after the maximum number of seedlings had emerged as determined by 2-3 initial censuses), mid-season (the end of February), and at the end of the growing season (the 2nd to 3rd week of April). Abundance of each species was recorded in 1998, and total plant density was recorded in 1999 and 2000 for each census. At the end of the growing season, all aboveground vegetation was harvested and sorted into either dicots or monocots. The plants were dried at 60°C for 48 hrs and weighed. In 1998, all sand (top 2cm) and remaining material were also collected from the plots (and sieved) to provide an estimate of seed production per plot.

In 1999, half of the blocks at the top and bottom of the dune were randomly assigned to an additional water treatment. Control blocks received only natural rainfall, and treatment blocks were hand watered with a very fine nozzle early in the morning receiving 1L of water per plot every 10 days (equivalent to 100mm of precipitation in addition to rainfall).

<u>Analyses</u>
The effect of seed density on the plant community was primarily tested by comparing the emergence index, proportion surviving, and mean final plant size. The emergence index is a measure of the probability of emerging and was calculated by dividing the total number of emergent seedlings by the number of seeds added. The mass of seed added to each plot was converted to seed number by dividing by the calculated mean number of seeds per gram (based on trial counts and average seed sizes). The proportion surviving (from the total number of

emergent seedlings) was calculated for the mid and end of season censuses, and mean plant size (aboveground biomass/density) was calculated at the end of the growing season. Differences between groups for each of the three response variables were tested by repeated measures ANOVAs (for proportion surviving throughout the season) or ANOVAs (emergence index and mean final plant size) using JMP 4 (SAS 2000). The main effects were initial seed density, stress level, census (for proportion surviving) and their interactions. All assumptions were tested. In 1998, species diversity, E_{var}, (Smith & Wilson 1996) was also tested as a response variable. The diversity data were also compared to a null model of increasing diversity with increased seed density due to a sampling effect (Goldberg & Estabrook 1998). In 1999, water addition was also tested as a main effect in the ANOVA models.

Density dependence within the plant community at different life-stages and stress levels was tested each year using regression analyses similar to Goldberg *et al.* (2001). The emergence index was regressed against initial seed density, and mean plant size and final proportion surviving were regressed against final plant density. It was predicted that the population level response (final proportion surviving) should increase linearly with the number of seeds planted if there is no density dependence in the community (null). Nonlinearity indicates density dependence (concave – competition, and convex – facilitation). For the individual performance measures, the emergence index and mean plant size, the null relationship is a slope of zero, i.e., probability of an individual seed emerging or plant size are independent of density. A significant positive slope suggests facilitation and a negative slope competition.

Differences between years in the effects of initial seed density on all performance measures were also compared using ANOVAs. The main effects were year, stress level, seed density, and interactions with year. Only comparable plots were included in these analyses, i.e., plots in 1999 that received additional water were not included.

Results

Control plots

The seedless sand controls tested in 1998 had a small number of seeds germinate (mean = 8 plants), but these numbers were significantly less than the emergent plant density of all other plots (ANOVA, F = 21.09, p = 0.0001, n = 20, with contrast analyses p < 0.001, mean for lowest emergent plant density from a treatment plot , 1/16x = 50.3 plants).

Density effects

1. Initial seed density effects

The analyses of initial seed density effects within each year and between years demonstrated a range of responses from no effect of low initial seed density to significant negative effects at higher seed densities. In all three years, the emergence index generally decreased with higher seed densities (Fig. 3.1a, Table 3.1). The proportion surviving throughout the season and mean plant size were in most cases unaffected by initial seed density in all three seasons (Fig. 3.1c,d, Table 3.1). There were some differences, but they were not consistent across years and the correction for multiple tests made most trends statistically non-significant (Table 3.1). Plant diversity was also unaffected by initial seed density (ANOVA's, $p > 0.25$). Although species diversity increased in higher seed densities (data not shown), observed diversity was frequently much lower than the null expected at a given density ($p > 0.05$, same approach as Goldberg and Estabrook 1998).

2. Density dependence within the plant community

Regression analyses detected both density dependent and independent processes. In 1998, only the emergence index demonstrated density dependence (Fig. 3.2a, Table 3.2); the relationship was negative (slope of line significantly different from 0 and negative) and shows competition at higher densities. There were no differences between the two positions on the natural stress gradient for any response variable in 1998 (ANOVAs, $p > 0.05$). In 1999, the emergence index and mean plant size were negatively density dependent for both the high and low stress sites (Fig. 3.3a-d, Table 3.2). The proportion of individuals surviving in 1999 was density independent (linear fit) for the low stress site and positively density dependent (non-linear fit and convex) for the high stress site suggesting facilitation (Fig. 3.3e,f, Table 3.2). However, this positive effect is not significant after a Sequential Bonferroni correction (Table 3.2). In 2000, the emergence index and mean plant size were negatively density dependent for the high stress site and density independent for the low stress site (Fig. 3.4a-d, Table 3.2) while proportion surviving was density independent at both positions (Fig. 3.4e,f). To summarize, the emergence index and mean plant size were generally negative while proportion surviving was density independent or possibly facilitative.

Life-stage

Earlier life stages were generally more affected by initial seed density (i.e., negative effects on seedling emergence, Fig. 3.1a) while later life-stage variables such as final mean plant size were unaffected by initial seed density in all three years (Fig. 3.1d). Density dependent

processes within the plant community were generally negative early in the growing season (i.e., the emergence indices). However, measures later in the season were negatively density dependent (mean plant size in most cases), density independent (proportion surviving in most cases), and in one case positively density dependent (proportion surviving for the high stress site in 1999, Fig. 3.3f). The proportion of individuals surviving within each season significantly decreased with time (Table 3.1 with contrast analyses for census times, $p < 0.01$).

Stress

The level of natural stress influenced the plant communities by significantly reducing growth in the high stress site at the top of the dune (Fig. 3.1e,f,g,h) but generally did not interact with initial seed density (Table 3.1). Similarly, density dependent processes within the community generally did not differ with natural stress, except in 2000 when the emergence index and mean plant size were negatively density dependent only at the high stress site (Table 3.2). There was also no effect of water addition in 1999 on any performance measure for initial seed densities or density dependence within the community (Table 3.1).

Temporal effect:

There were significant differences between the three years tested in terms of the emergence index, plant density, proportion surviving, and mean plant size (Table 3.3). The highest emergence and final plant density were in 1999 (Fig. 3.1a,b), and the largest plants and the highest proportion surviving (high stress site only) in 1998 (Fig. 3.1c,d). Density dependent processes within the community also changed between years (Figs 3.2-4). The two dry years (1999, 2000) were similar insofar as they expressed differences between the high and low stress sites, while the wettest year, 1998, did not.

Discussion

Density dependent processes are typically tested at the population level. Unfortunately, a shortcoming of this approach is that the target population is typically embedded in a matrix of neighbouring species, i.e., a community. This shortcoming has been addressed by Goldberg *et al.* (2001) by using the initial community density approach (Goldberg *et al.* 1995) which generates a range of population densities for all of the species typically represented in the community. A second difficulty is that common perceptions about density dependent processes are not explicitly formulated nor tested, e.g., what parameter is being regulated, are density dependent responses linear, and what range of population densities are affected (Murray 1994). Thirdly, interactions between species (i.e., effects of one species on another) and the outcome

of these interactions (e.g., which species dominates) are not clearly differentiated, and density dependent processes likely also vary throughout the life cycle (Connolly *et al.* 2001). We specifically addressed these difficulties by making specific testable predictions, by measuring interactions within the community and final outcomes, and by differentiating between early season and late season effects by measuring both initial effects of seed density and density dependent interactions throughout the growing season. This experimental approach thus determines if density dependent processes such as emergence or survival have an impact at the community level.

Two questions emerge when we consider a growing plant community. First, do initial starting conditions influence the subsequent structure of the early emergent and later established community? In essence, this question asks if initial treatments are as important as subsequent interactions on the final plant community? Second, what impact do interactions occurring within the developing plant community have on final plant community structure? To address these questions, we divided the life-cycle of the plants into two distinct phases: seed to emergent seedlings, and emergent seedlings to established plants. The early phase includes the process of emergence with the end product being density of seedlings, and the later phase includes survival and growth rate with the end products being density and dry weight of survivors. The experimental manipulation of seed density generates a range of seed and seedling densities. Increasing seed density decreased the probability of a seedling emerging while measures of growth or survival at later life-stages were unaffected by the initial seed density. Later in the season as the plants grow, interactions between these established plants are more important than the initial seed densities sown (Callaway & Walker 1997; Holmgren *et al.* 1997). However, the community in which these density dependent processes occur are established by early interactions between seeds and seedling. Analysis of the established plant community showed that there was significant divergence from the emergent plant community. Density dependent processes at later life-stages, chiefly growth rate, determined the final plant community, while initial seed densities did not.

The initial effects of seed density and subsequent interaction between plants may not always act in the same direction. For instance, increased seed density reduced the proportion of seeds that emerged in all three seasons and subsequent survival may, on occasion, increase at higher plant densities (high stress site in 1999 only). Depending on the strength of the interactions at different life-stages, we may see net positive or net negative outcomes for the final plant community. Admittedly, very few studies have tested for, and detected, interactions between

seeds or seedlings. Nonetheless, there are a growing number of studies that have identified both positive (Linhart 1976; Waite & Hutchings 1978; Smith 1983; Adler *et al.* 1993) and negative effects (Palmblad 1968; Murray 1998; Goldberg *et al.* 2001) of manipulating seed density on germination and emergence. It has been shown that seeds may 'sense' other seeds, e.g., by the release of leachates (Murray 1998), and change patterns in germination either through accelerated emergence (Dyer *et al.* 2000) or by reduced emergence (Murray 1998; Goldberg *et al.* 2001). Although we can only speculate, the negative effects of increased seed density may also be a product of chemical inhibition by some of the species of seeds within the community (Qadir & Abbasi 1971). Therefore, it is crucial to measure initial effects of seed density in terms of possible seed-seed interactions and interactions between seedlings to understand what processes influenced the emergent plant community.

The second question we address was the overall importance of density dependence within the establishing plant community. It appears that competition influences this plant community through its effects on differential growth rate rather than on differential survival. This would typically be interpreted as regulation. But is it regulation or limitation? White (2001) would argue that decreases in mean plant size could be attributed to limitation of a key resource such as water and that competition is a consequence of shortage in a resource - not a cause. This may be true, particularly since mean plant size was density independent in the wet year and negatively density dependent in the two dry years. However, the negative density dependence for emergence of seedlings is not likely related to a limitation in water for the following reasons. The experimental addition of water had no effect on seedling emergence, and emergence did not differ between years (one wet year and two dry years). Density dependent emergence is thus more reasonably attributed to regulation via interference through the production of leachates as discussed earlier. The density independence of survival throughout the growing season similarly suggests that resource limitation is not important in determining survival once the plants are established. Hence, regulation and limitation may both be important in structuring this plant community with regulation determining establishment, and limitation determining growth. White (2001) is correct in stating that we often assume that the number of individuals in a population or community are regulated below the capacity of the environment without testing if limitation is actually determining the numbers instead. Our results suggest that it is perhaps premature to conclude that there is no regulation at the community level, but that we do need to be cautious when interpreting single measures as either limitation or regulation.

This leads us to briefly discuss the hypothesis that the effects of initial seed density and density dependence vary with life-stage. The effects of initial seed density were important early in the growing season but had little effect thereafter. It has been proposed that facilitation is more important for earlier life-stages and that as plants increase in size they are more likely to compete (Callaway & Walker 1997). Our results do not support this prediction with negative density dependent emergence occurring in all three growing seasons and decreases in mean final plant size with increasing plant density detected in 1999 and 2000. Actually, the only example of facilitation occurred at later life-stages (proportion surviving at high stress site in 1999), and it is possible that this result is spurious due to multiple tests for a single effect. Hence, life-stage strongly influences the net interactions within a plant community, with earlier life-stages being negative while later life-stages were either negative or density independent. Perhaps, the generality of detecting competition versus facilitation in the plant ecology literature has been due in part to measurement at single life-stages. Successive measurements provide a viable solution. Further work that incorporates experimental changes in density at different life-stages for the same community would also be revealing. For instance, changes in initial seed density coupled with experimental changes in plant density at later life-stages would test for differences in the importance of density at each life-stage. The experimental manipulations could include not only reductions in density via removal of seedlings or adult plants but the addition of seeds or seedlings to emergent plant communities (Fowler 1986).

We predicted that stress, and year, would signficantly interact with density effects. Whilst the natural stress gradient affected the plant community (higher stress site reduced performance), stress did not interact with the effects of initial seed density. Furthermore, the lack of a clear difference between sites in the patterns of density dependence support the hypothesis that the net effect of changing stress (or productivity) is a relatively constant intensity of competition (Newman 1973; Tilman 1988). The regular addition of water to plots also had no appreciable effect on performance or influence the effects of density which suggests that either competitive intensity is relatively constant or that the pulsing of the resource (i.e., frequency of rainfall and duration between events) is more important than the actual magnitude (Novoplansky & Goldberg 2001). The natural stress gradient we used (i.e., position on dune) encompassed a greater range of abiotic stress than the experimental stress gradient (i.e., water addition) as there were reductions in survival and growth at the naturally higher stress site but no differences with water addition. The type of gradient tested thus has the potential to influence our ability to detect plant interactions (Goldberg & Barton 1992; Kadmon 1995). In this dune system, natural stress affects the plant community but does not directly influence the interactions within the

plant community (i.e., density dependent processes). Further work that tests the effects of timing and frequency of limiting resources will determine whether stress can affect interactions between plants in this capacity.

Conclusions

Initial seed density and density dependence within the emergent vegetation were important processes in shaping this annual plant community. However, initial seed density affected the early emergent plant community but not later life-stages, and competition or negative density dependence within establishing plants occurred both early and late season. Regulation and limitation both played a role in structuring this dune community with regulation determining emergence and limitation determining growth rates. Level of stress, life-stage, and year were also important; however, life-stage and year were more important than the stress gradients tested here, both natural and experimental. The year-to-year variation and lack of strong consistent effects of stress gradients suggest that interactions within the plant community may not necessarily change with productivity (at least aboveground). These results suggest that it is critical to use successive measurements spanning the entire life-stage of plants and that community-level net outcomes may differ from interactions between seeds, seedlings, or within the established plant community.

Acknowledgments

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Table 3.1. ANOVAs for final response variables to initial seed density manipulations in 1998, 1999, 2000. Sequential Bonferroni corrections were applied for each response variable and only significant values are in bold. Differences in proportion surviving were tested with a full-factorial Repeated Measures ANOVA since proportion surviving to each census was calculated. There were no significant interaction effects with census so only the main effect is reported here. Stress refers to position on the dune (high and low stress), and water to the addition of water to half the blocks (1999 only).

Measure	Effect	DF	1998			1999			2000		
			SS	F Ratio	P-value	SS	F Ratio	P-value	SS	F Ratio	P-value
Emergence index	Stress	1	0.0000	8.1	0.012	0.0007	1.5	0.23	0.0003	15.9	**0.0003**
	Seed	3	0.0004	54.6	**0.0001**	0.05	37.1	**0.0001**	0.0008	12.43	**0.0001**
	St. x Sd.	3	0.0000	4.3	0.021	0.0001	0.49	0.69	0.0005	7.6	**0.0004**
	Water	1				0.0001	0.14	0.71			
Proportion	Stress	1	0.001	0.03	0.86	0.0000	0.0001	0.99	2.24	38.9	**0.0001**
	Seed	3	0.15	1.6	0.21	1.3	6.23	**0.0005**	0.6	3.43	0.021
	St. x Sd.	3	0.09	0.96	0.42	1.5	7.2	**0.0001**	0.69	4.0	0.011
	Census	1	0.53	17.4	**0.0002**	15.1	109.0	**0.0001**	1.63	28.45	**0.0001**
	Water	1				0.06	0.83	0.36			
Mean plant size	Stress	1	0.32	6.43	0.022	0.22	0.93	0.34	0.008	3.5	0.07
	Seed	3	0.43	3.0	0.064	0.71	1.0	0.40	0.014	1.94	0.14
	St. x Sd.	3	0.16	1.1	0.39	0.7	0.99	0.41	0.003	0.47	0.7
	Water	1				0.24	1.02	0.32			

Table 3.2. Regression coefficients for interactions within the emergent vegetation. Stress refers to position on the sand dune. In 1998, the two sites were pooled as there were no significant differences between them for any of the response variables (ANOVAs, p > 0.05). The p-values reported here refer to whether the slope of the line is significantly different from zero. Significant p-values are in bold after Sequential Bonferroni corrections. The degrees of freedom reported are for the total model (model + error).

Year	Stress	Measure	DF	Intercept	Slope	R^2	P-Value
1998	Pooled	Emergence index	23	0.007	-4.3×10^{-8}	0.45	**0.0003**
		Plant size	23	0.38	0.001	0.005	0.73
		Prop. surviving	23	0.44	0.002	0.04	0.37
		Seed production	22	0.0005	-0.000005	0.002	0.85
1999	Low	Emergence index	39	0.05	-3.0×10^{-7}	0.35	**0.0001**
		Plant size	75	0.002	-0.000003	0.41	**0.0001**
		Prop. surviving	39	0.69	0.00012	0.015	0.45
1999	High	Emergence index	39	0.05	-2.5×10^{-7}	0.38	**0.0001**
		Plant size	79	0.016	-0.0001	0.44	**0.0001**
		Prop. surviving	39	0.56	-0.0007	0.17	0.03
2000	Low	Emergence index	23	0.0025	-9.2×10^{-9}	0.09	0.15
		Plant size	21	0.018	-0.00007	0.03	0.43
		Prop. surviving	23	0.58	0.00043	0.003	0.8
	High	Emergence index	23	0.012	-6.5×10^{-8}	0.26	**0.012**
		Plant size	23	0.14	-0.005	0.46	**0.0017**
		Prop. surviving	22	0.16	0.0009	0.08	0.18

Table 3.3. ANOVAs for final response variables to initial seed density manipulations across three seasons (1998 – 2000). Interactions between main effects such as stress x seed density are reported in the analyses for each year. Significant p-values are in bold (Sequential Bonferroni correction did not change table-wide p < 0.05 significance).

Measure	Effect	DF	SS	F Ratio	P-Value
Emergence index	Year	2	0.02	47.9	**0.0001**
	Stress	1	0.0001	0.41	0.52
	Seed density	3	0.24	34.1	**0.0001**
	Year x Stress	2	0.001	2.42	0.09
	Year x Seed density	6	0.023	16.6	**0.0001**
Final plant density	Year	2	2158248	180.2	**0.0001**
	Stress	1	317776	53.1	**0.0001**
	Seed density	3	320235	17.82	**0.0001**
	Year x Stress	2	318451	8.86	**0.0001**
	Year x Seed density	6	521787	43.6	**0.0001**
Proportion surviving	Year	2	0.59	8.38	**0.0004**
	Stress	1	1.4	39.73	**0.0001**
	Seed density	3	0.59	5.55	**0.0013**
	Year x Stress	2	0.23	1.1	0.38
	Year x Seed density	6	1.1	15	**0.0001**
Mean plant size	Year	2	5.64	195.4	**0.0001**
	Stress	1	0.16	11.11	**0.0011**
	Seed density	3	0.24	5.5	**0.0014**
	Year x Stress	2	0.68	7.8	**0.0001**
	Year x Seed density	6	0.48	16.8	**0.0001**

Figure 3.1. A comparison of initial seed density effects and position on a natural stress gradient on the performance of an annual plant community. Seed densities are the different masses of seed sown prior to the growing season (November) in each of 1998, 1999, and 2000. 1x is the natural seed density in the field. The low and high stress sites are positions on a natural stress gradient (bottom and top of dune respectively). The emergence index is the proportion of seeds that germinated based on total (maximum) emergence. The proportion surviving is the number of individuals at each census / total emergence density (from repeated measures ANOVAs with no significant interactions between census and seed density, $p > 0.05$). Mean plant size was calculated as total aboveground biomass per plot / total final plant density. The error bars are + 1 S.E. Graphs are arranged in pairs horizontally sharing the sharing response variable (i.e. A & E, B & F, etc.).

Figure 3.2. The effects of density on the performance of an annual plant community in the 1998 growing season. High and low stress sites are combined. Regression coefficients are reported in Table 3.2. Graphs are arranged in pairs horizontally sharing the sharing response variable (i.e. A & B, C & D, etc.).

Figure 3.3. The effects of density on the performance of an annual plant community in the 1999 growing season. Regression coefficients are reported in Table 3.2. Low and high stress refers to the position on a natural stress gradient.

Figure 3.4. The effects of density on the performance of an annual plant community in the 2000 growing season. Regression coefficients are reported in Table 3.2. Low and high stress refers to the position on a natural stress gradient.

A.

B.

C.

Low stress

High stress
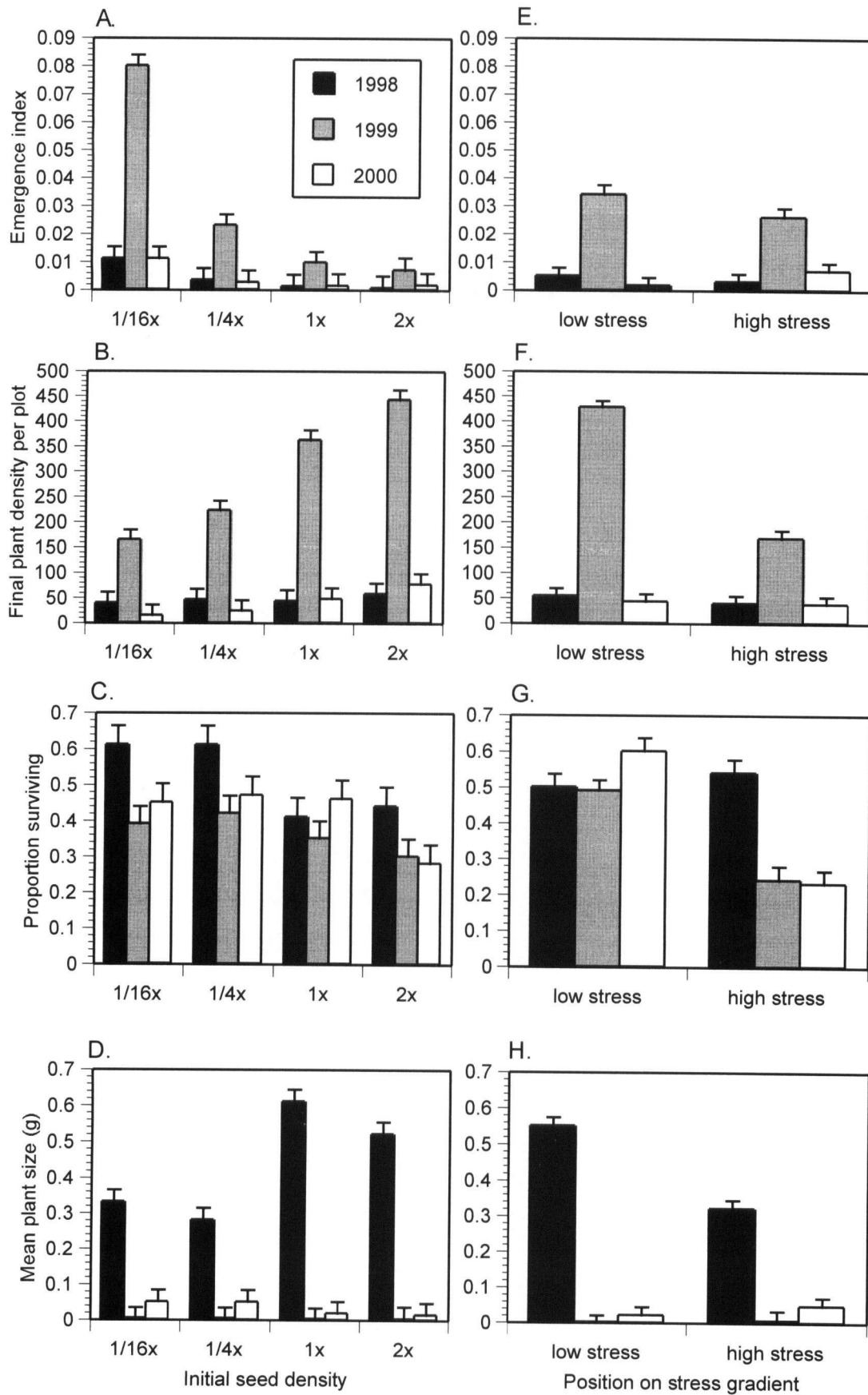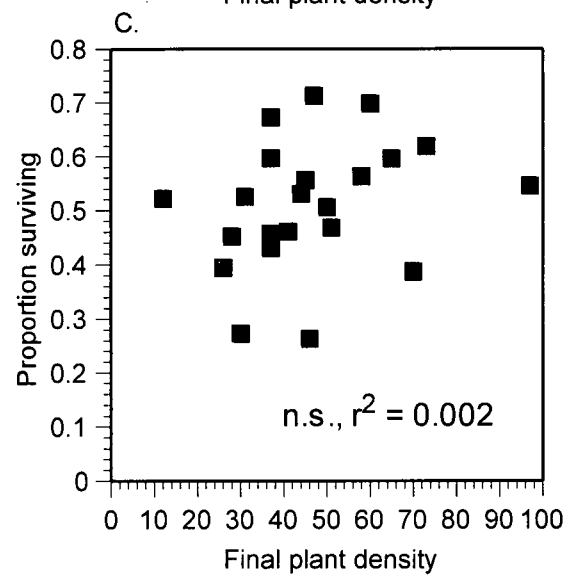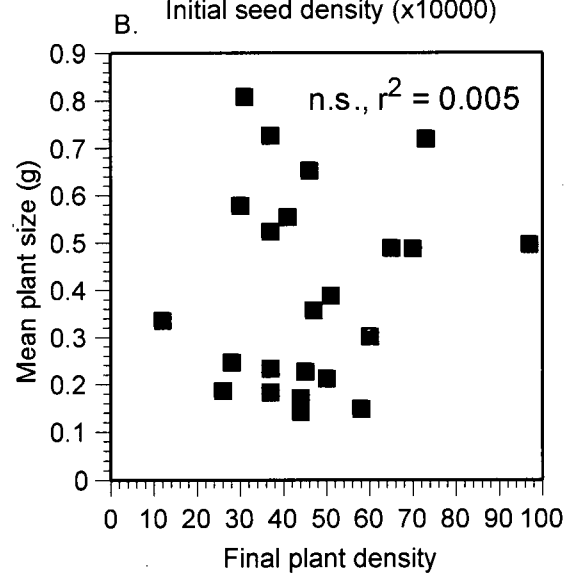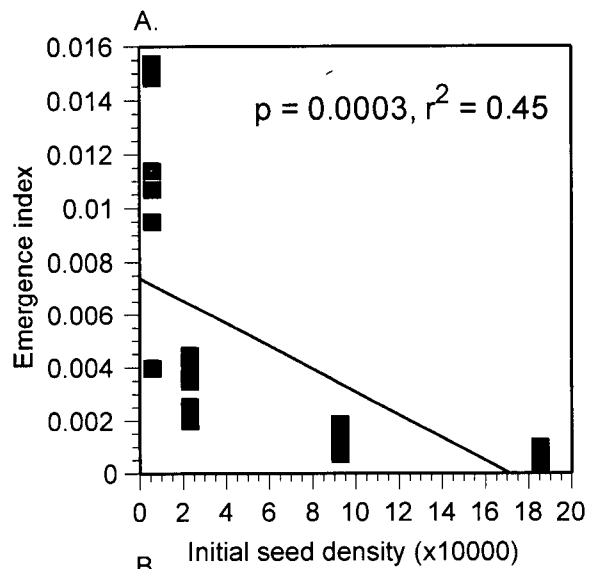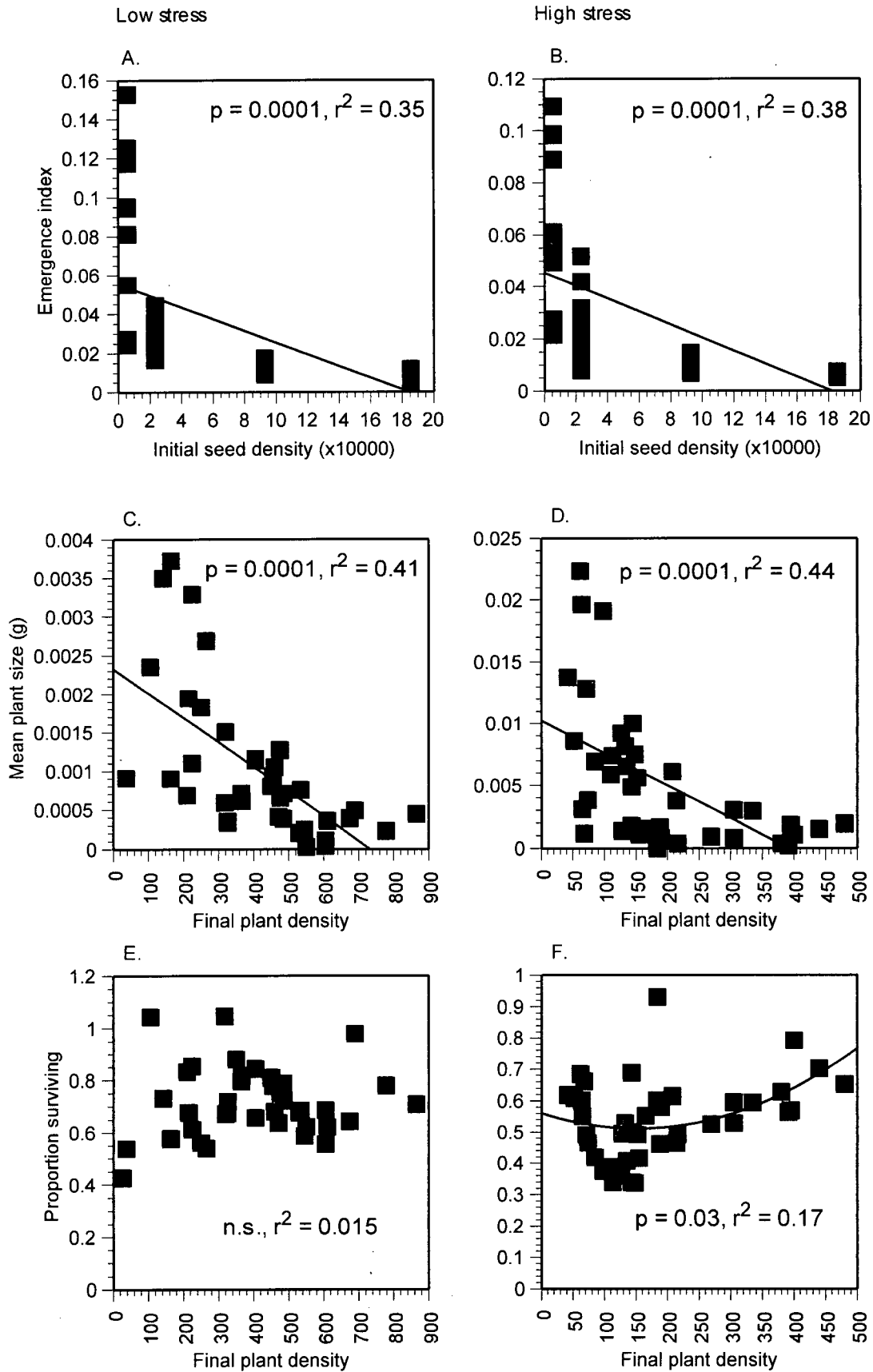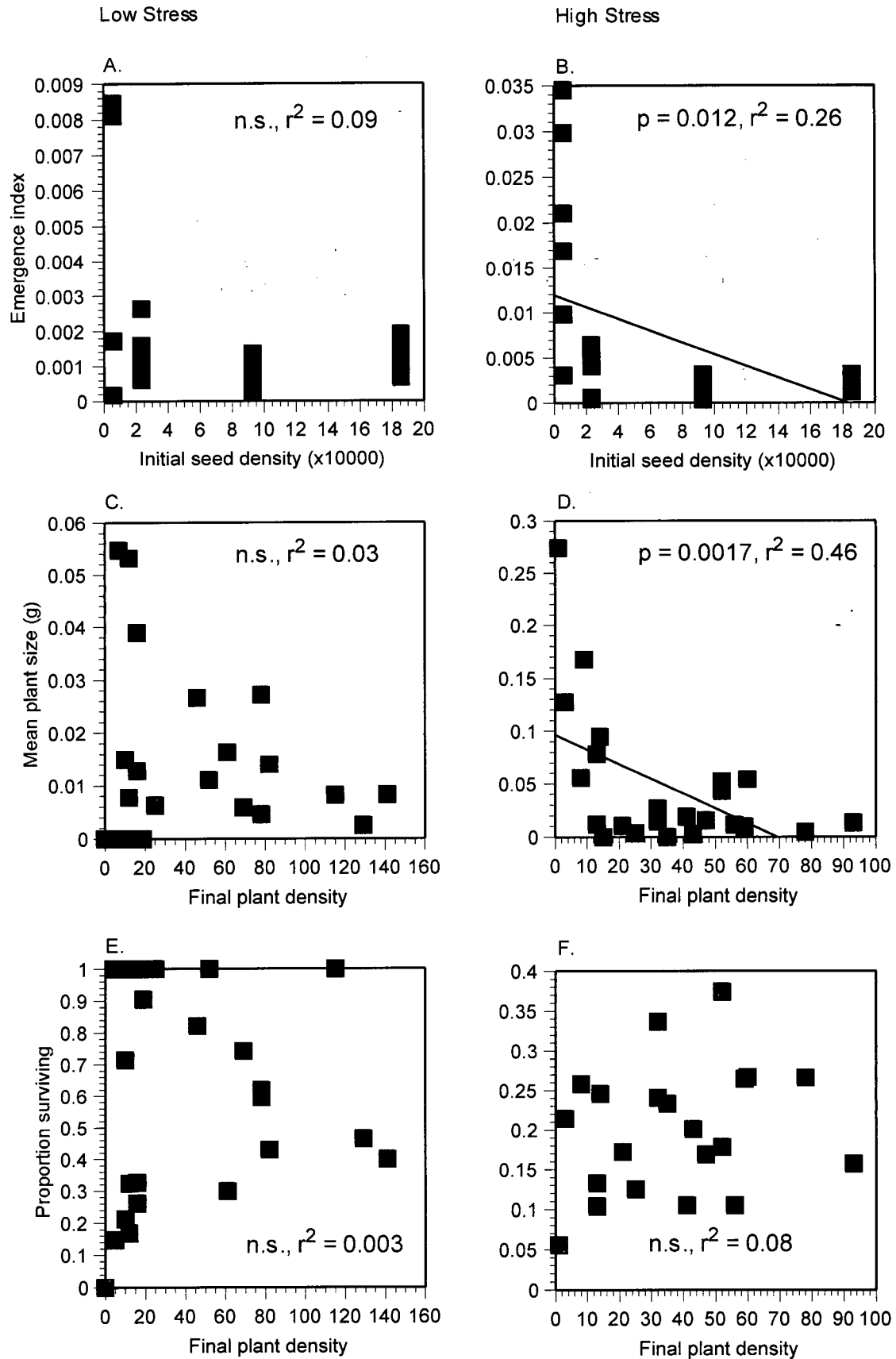
Section B: Conclusions for density effects

Initial seed density influenced the emergent plant community but not the established plant community. This is not surprising given that later in the season as plants grow, interactions between these established individuals becomes more important than the initial seed density sown. However, interactions between seeds or seedlings need not function in the same direction as interactions between larger established adult plants. In this study, increasing seed density had negative effects on emergence; while within the established vegetation, mean plant size was negatively density dependent and survival density independent. At a superficial level, chapter 3 suggests that both seed density and density dependent processes are important in structuring the emergent and established plant community.

However, are the effects of density we detected a product of regulation or limitation? Here, I conclude that both regulation and limitation may be functioning within this plant community. Negative density dependence at early life-stages is more reasonably attributed to interference between seeds and seedlings rather than a limitation imposed by the environment, and at later life-stages, limitation may be functioning to reduce mean plant size (i.e., shortage in limiting resource such as water). A second conclusion that density dependent processes vary from year-to-year, while the effects of stress do not. These novel findings further our understanding of density dependence and challenge typical notions about whether populations are regulated or limited. Chapter 3 suggests that both regulation and limitation are important processes, but that it is crucial to consider different phases of the life-cycle and to clearly distinguish between interactions and net outcomes for the plant community with changes in density.

Section C: Preface for specific species effects

This section, explicitly tests whether a particular species has negative or positive effects on the plant community. The primary prediction is that the largest and earliest germinating annual species, *Erodium laciniatum*, acts as a benefactor species to smaller subordinate annual plant species. The second main prediction is that life-stage is important in determining the role that this larger species may play in the plant community. This approach is innovative because the majority of studies that test for facilitation have done so through non-manipulative studies that use natural associations of species to test for a benefactor effect and have typically used shrub-understorey systems where the benefactor species is considerably larger than the beneficiary species and with significantly different life-histories.

In chapter 4, the effect of adding seed and seedlings of *Erodium* and another large annual (*Erucaria pinnata*) on subsequent germination and performance of the seed bank was tested, and chapter 5 investigated the effects of removal of *Erodium* on the neighbouring annuals. The main prediction is that addition of *Erodium* would increase the germination of neighbours and also increase final plant community measures such as density or aboveground biomass. We also predicted that removal of *Erodium* would have negative effects on density and survival of the neighbouring annuals. This chapter tested whether the effects of *Erodium* are specific to this species or a direct product of being a larger plant, and if the importance of *Erodium* changes with level of abiotic stress.

Chapter Four. The facilitative effects by seeds and seedlings on emergence from the seed bank of a desert annual plant community.

Abstract

Facilitation is important in structuring plant communities in harsh environments although most studies are descriptive and focus on adult plant interactions. Here, we test for facilitative effects by seeds and seedling of *Erodium laciniatum* and *Erucaria pinnnata* on the emergence and subsequent community structure from an annual plant seed bank in the Negev desert, Israel. We specifically test whether (i) the addition of seeds or seedlings of these species increases emergence from the seed bank, (ii) the addition of seeds or seedlings increases final plant community measures, and (iii) the effect of each species is specific. Both *Erodium* seeds and seedlings acted as a benefactor for the rest of the plant community by increasing density and aboveground biomass of the neighbourhood germinated from the seed bank. *Erodium* seedlings had a stronger positive effect than seeds early in the growing season. *Erucaria* (seeds and seedlings) did not influence the plant community. This suggests that species interactions are specific and that interactions between seeds and between plants and seeds can influence community structure.

Keywords: facilitation, nurse-plant, focal species, annuals, stress, *Erodium laciniatum*, *Erucaria pinnnata*.

Introduction

Most research on facilitation, outside the context of succession, has focused on interactions among adult plants (Callaway, 1995). This bias restricts the understanding of facilitation among species and does not provide insight into size-related effects, temporal effects, or interactions between other life-history stages such as seeds and seedlings. An additional bias occurs if measurements are recorded only at the end of an experiment because these fail to distinguish between the outcome of facilitation and the process by which this outcome was produced; this distinction has also been noted in competition studies (Gibson *et al.*, 1999). In this paper, we address both of these aspects and explicitly test for facilitative effects by seeds and seedlings on emergence from the seed bank of an annual plant community including subsequent effects measured throughout the growing-season. This allows us to test for more subtle interactions among congeneric (similar-sized) annual plant species at the seed and seedling level rather than at the shrub-understorey level that is common in the facilitation literature (Callaway, 1995).

There are two general types of studies that have tested small-scale seed-related effects. The effect of different seed densities has generally detected density-dependent emergence (Palmblad, 1968; Linhart, 1976; Waite & Hutchings, 1978, 1979; Adler *et al.*, 1993; Murray, 1998) and accelerated emergence times (Ross & Harper, 1972; Inouye, 1980; Miller, 1987; Bergelson & Perry, 1989; Dyer *et al.*, 2000). The second category of experiment has focussed on either the effect of previously planted seeds on the germination of other seeds (Black & Wilkinson, 1963; Bergelson & Perry, 1989) or on the effect of existing adult vegetation on the germination of planted seeds (Ross & Harper, 1972; Shaw & Antonovics, 1986; Fowler, 1988; Bergelson, 1990; Ryser, 1993). While this category of experiment is unique in that specific effects of particular seeds or plants are tested (seed-seed and plant-seed interactions), unfortunately, only two-species mixtures were used and only germination, and not subsequent emergence, was tested.

In this study, we investigate the following hypotheses to test for specific effects, both early and mid-season, of two of the largest annual species in our system (hereafter called focal species) on emergence from the seed bank of a desert annual community.

1. The addition of seeds or seedlings of the focal species will increase emergence of seeds from the seed bank.
2. The addition of seeds or seedlings of the focal species will increase some final plant community measures such as total adult plant density, final aboveground biomass, or final aboveground biomass/plant.

3. The effects of each focal species on the seeds and emergent vegetation are species specific.

Methods

<u>Seed collection:</u>

Seed was collected in November 1998 and 1999 at Bir Asluj in the Holot Mashabim Nature Reserve (31°00.212'N, 34°44.474E, MAP 110mm) in the central Negev desert, Israel. The herbaceous vegetation is entirely annual and seed bank based. There are typically about 20 species in this community (area of sand dune about 1ha) (Dyer *et al.*, 2001). We collected the top 2 cm of sand which effectively samples at least 99% of the total seed bank (Venable, 1989). Virtually all the seed was separated from the sand with 500um sieves, and the natural seed bank density (hereafter called1x) was calculated for the field site and was 600.8 g/m² (Goldberg *et al.*, 2001). The experiments were done in pots in an open air greenhouse at the Mitrani Department for Desert Ecology Research, 25km SE of the site where the seed was collected.

<u>Experiment 1 (1999). Addition of seeds of the focal species to the seed bank:</u>

We tested the effect of adding additional seeds of the two focal species, *Erodium laciniatum* and *Erucaria pinnata*, to the 1x density seed bank (with no other seeds of these focal species present). We sowed seed in the following ways: 1x seed bank spread evenly + a group of 5 seeds of *Erodium* <u>or</u> *Erucaria* placed in the middle of each pot; 1x seed bank + 5 seeds of *Erodium* <u>and</u> *Erucaria* combined, and seed bank only (no additional focal seed). We also sowed 5 seeds of each of the focal species by themselves without seed bank allowing us to test for a reciprocal effect of the seed bank on the focal species. We repeated all plantings 10 times at two irrigation levels using a randomized block design. The low water treatment was equivalent to the long term annual average of 110mm, and the high water was equivalent to 150mm per annum, levels similar to that applied by Goldberg et al. (2001) using seed from the same study site.

Seed was sown on January 15th, 1999 in 15cm diameter pots filled with seedless sand from the sand dune where the seeds were collected. The planting density of seed was equivalent to the natural seed density in the field, i.e., 6g of seed per 10x10cm. A buffer strip of 4.5cm was left in each pot to minimize edge effects. The pots were surveyed once a week for the first three weeks and every two weeks thereafter to the end of the natural growing season in mid-April.

To control for the effects of disturbance caused by emergence of the larger focal species, i.e., cotyledonous leaves disturbing the sand, an artificial mimic of the disturbance was also applied to the seed bank planted without any of the seeds of the focal species specifically added. In these pots after the seed bank was in place, a 15 x 3cm wooden tongue depressor was used to gently stir up the middle of the pot. The depressor was then "planted" and left in the middle of the pot for the remainder of the experiment.

Analyses for Experiment 1:

The effect of adding seeds of the focal species (and mimic) on emergence and subsequent plant density, aboveground biomass, and number of species was analyzed with repeated measures ANCOVA with census and water level as main effects (and interaction effects) and the mass of the additional 5 or 10 seeds as the covariate. To test for the effect of the seed bank on the emergence of the focal species, we compared the mean emergence of *Erodium* or *Erucaria* with seed bank, both sown together with seed bank, and each sown independently with ANOVAs at both water levels and interaction effects. Specific differences between groups were tested with post-hoc contrast analyses at $p<0.01$.

Experiment 2 (2000). Addition of seedlings of the focal species to the seed bank:

We tested the effect of adding seedlings of three focal species to ungerminated and germinated seed bank at two different times – beginning of the natural growing season and 6 weeks later in mid growing season. We planted the seed bank at natural seed density (1x) in 15cm pots and followed the same procedures as in the first experiment. Controls received only seed bank while treated pots received seed bank and a seedling of the focal species planted in the middle of the pot. These seedlings were germinated individually in pots two weeks in advance. Three species of seedlings were independently added on January 15th, 2000 to the pots with seed bank, *Erodium laciniatum*, *Erucaria pinnata*, and *Trifolium tomentosum*. The first two are large annuals in the field and the latter a smaller annual.

To add the seedlings of the focal species to the neighbourhoods (in both early and mid-season), we used a small metal spatula to place the seedling and a core of seedless sand with intact roots into the pots with seed bank. For the mid-season seedling additions, we placed the seedlings and core as close as possible to the middle of the pots without disturbing the vegetation growing in that region. In both cases, we recorded the density of the neighbourhood in the pot once a week for the first two weeks and every two weeks thereafter for the remainder of the growing season to mid-April.

Analyses for Experiment 2:

We compared the density of the neighbourhoods (for all surveys) between the different treatment groups within each addition experiment (early and mid) by repeated measure ANCOVAs with seedling mass as covariate. The main effect tested was treatment group. Total neighbourhood aboveground biomass and neighbourhood aboveground biomass/plant at the end of the experiments was compared by ANCOVAs also with seedling mass as covariate. Specific differences between groups were tested with post-hoc contrast analyses at $p<0.01$. All statistical analyses for both experiments in 1999 and 2000 were performed with JMP 4.02 (SAS, 2000).

Results

<u>Expt. 1. Seed addition and mimic.</u>

At high water, total plant density was significantly greater in the +*Erodium* pots throughout the growing season (Table 4.1, water x treatment with additional contrast analyses, $p<0.01$; Fig. 4.1). The combined addition of *Erodium* and *Erucaria* seeds significantly increased plant density at low but not high water (Fig. 4.1). There was no significant difference between the other treatment groups at each water level. The addition of seeds of the focal species only weakly affected the final plant community; total aboveground biomass per plot or mean aboveground biomass/plant was not significantly different between the treatment groups (ANCOVAs $p>0.05$) while number of species was only marginally increased by the addition of *Erodium* seeds (ANCOVA, F(treatment)=2.5, $p=0.04$).

The seed bank also had a significant effect on the focal species. *Erodium* sown with seed bank had higher mean germination (and density of *Erodium* throughout season) than *Erodium* sown alone (Repeated measure ANOVA with contrast analyses, F(treatment)=3.3, $p=0.039$, n=100, Fig. 4.2). There was no inhibition of *Erodium* germination by *Erucaria* seeds(Fig. 2). *Erucaria* germination was however strongly inhibited by *Erodium* seeds but unaffected by the general seed bank (Repeated measure ANOVA, F(treatment)=6.1, $p=0.0034$, n=100, contrast analyses, Fig. 4.2). There were no significant water x species interactions ($p<0.05$)

The density or aboveground biomass of neighbourhoods treated with the artificial mimic were not significantly different from the control seed bank in the greenhouse (Fig.4.1).

<u>Expt. 2. Seedling addition.</u>

The addition of *Erodium* seedlings significantly increased the emergence (and density throughout season) from the seed bank if the *Erodium* was planted early in the growing season (Table 4.2 with additional contrast analyses, $p < 0.01$; Fig 4.3a). There was no significant difference between the other additions and the controls (contrast analyses, $p > 0.05$; Fig. 4.3a). *Erodium* seedlings planted early in the season resulted in an increased total density (Fig. 4.3a), total neighbourhood aboveground biomass (Fig. 4.3b), and mean neighbourhood aboveground biomass/plant (Table 4.3).

Mid-season addition of the three focal species to the germinated seed bank did not have any effect on the density or aboveground biomass of the plant community (Fig 4.3, Repeated measure ANCOVA and ANCOVA respectively, all $p > 0.05$). *Trifolium* and *Erucaria* did not have significantly different effects (Fig. 4.3).

Discussion

Facilitation has been shown to strongly influence certain plant communities particularly shrub understorey systems in highly stressed environments (Callaway, 1995). Nonetheless, the central focus of plant ecology has been on testing for and understanding negative plant interactions (Goldberg, 1990). The majority of research on facilitation has been descriptive (Callaway, 1995) while competition studies have largely been experimental both in the greenhouse (Gibson *et al.*, 1999) and in the field (Goldberg *et al.*, 1999). If we are to develop our understanding of the importance of facilitation on structuring plant communities it is crucial to conduct well replicated field experiments and to test for specific species effects (Callaway, 1998). Seed-seed or plant-seed interactions could be extremely important positive effects within plant communities, particularly if positive interactions are more important at earlier life-stages.

In this study, we predicted that the two largest annual plant species, which coincidentally also germinate earlier, influence the entire plant community. The first hypothesis we tested, that the addition of seeds or seedlings of these focal species would increase emergence from the seed bank, was supported for the addition of both *Erodium* seed and seedlings. The additional seeds of this species increased total neighbourhood density throughout the growing season while aboveground biomass was not significantly affected. *Erodium* seeds thus had a positive effect on the community. Adding seeds can change the chemical properties of the soil through leachates from seeds or release of CO_2 which subsequently affects the germination of other seeds (Qadir & Abbasi, 1971; Inouye, 1980; Bergelson & Perry, 1989; Murray, 1998). Only two

other studies have tested for, and found, a positive effect of adding seeds on subsequent germination of the seed neighbourhood but both studies focussed on either intraspecific effects (Black & Wilkinson, 1963) or interactions between two species (Bergelson & Perry, 1989). Using pair-wise tests of species of seeds in petri dishes, Qadir and Abbasi (1971) also found many cases of one-way stimulation by a given species of seeds on the germination of another species. Our study is the first to test the effect of specific seeds on a complete community of seeds and measure seed-seed interactions as they influence the final plant community (albeit annuals).

The planting of *Erodium* seedlings had a stronger positive effect on emergence from the seed bank than did the sowing of *Erodium* seeds. This suggests that *Erodium* acts as a nurse-plant for other seedlings rather than through a direct mechanism associated with its emergence such as breaking the sand crust. Thus, we have a general picture of *Erodium* seeds promoting germination of seeds of other species, and as *Erodium* grows, it continues to have positive effects on the local neighbourhood by acting as a nurse-plant. It would also appear that these effects are specific to *Erodium* since the use of a mimic of the disturbance effect of a large annual germinating did not influence subsequent germination. The effect of *Erodium* seedlings is species specific and not simply a product of some physical perturbation. The shrub nurse-plant effect is well documented in the facilitation literature and possible effects include reduced water stress, reduced temperature by shading or generating a boundary layer, or increased soil moisture (Callaway, 1995; Pugnaire & Haase, 1996; Callaway, 1998; Holzapfel & Mahall, 1999). Very few studies have tested for nurse-plant effects between herbaceous dicots and grasses. Studies are few, so that no consistent pattern has emerged, but the literature reports examples of positive effects of herbs and grasses on emergence of a second species (Fowler, 1988; Ryser, 1993) and negative effects of established individuals on germination of seed (Ross & Harper, 1972; Shaw & Antonovics, 1986; Bergelson, 1990). While we detected a strong positive effect of *Erodium* seedlings on seed bank emergence, the other tested species (*Erucaria*) had no such effect. Hence, the choice of species is likely important in many systems when testing for interactions between plants. Positive interactions are probably more infrequent and species specific than competitive interactions, making them more difficult to detect (Callaway, 1998). The probability of two species coevolving traits that increase fitness and also benefit another species is significantly less than the probability of simply developing a trait that increases one's own fitness. In our study system, the small number of species and clear size hierarchy made it easy to select larger focal species; however, further research in more complex systems should also test for positive interactions between herbs and grasses on germination and emergence.

The second hypothesis, that the positive effect of focal species will persist until the end of the growing season, was (generally) supported. The addition of *Erodium* seeds (at high water) and a mixture of *Erodium* + *Erucaria* seeds (at low water) resulted in an increase in plant density until the end of the growing season while the addition of *Erodium* seedlings also increased density, aboveground biomass, and aboveground biomass/plant. Early positive effects of species on the community can thus strongly influence the final plant community structure in some situations. In a shrub-understorey experiment in the Mojave desert, Holzapfel and Mahall (1999) also reported that the annual plants benefited most from the presence of a shrub early in the growing season. In our study, there was no additional positive effect of adding seedlings to the plants once the seeds had germinated. This is surprising as we expected that there would be some effect, likely interference, of adding larger plants to a mixture of existing plants. The interactions among different species in our community are important early in the growing season because these initial interactions had an influence on the final structure of the neighbourhood. This is comparable to other studies which reported that early asymmetries in plant communities (plants that emerge first are the largest) can be exaggerated in the adult plants (Miller, 1987; Bergelson & Perry, 1989; Gibson *et al.*, 1999). We have demonstrated here that the effect of these asymmetries on the community can be positive. It would be interesting to investigate further what these communities would look like without facilitative effects by selecting high and low density plots and introducing focal species into both. We would predict that the focal species might function positively (facilitation) at lower densities but negatively (competition) at higher densities.

We tested the response of the community at high and low irrigation levels to determine if the effects of focal species were consistent. We expected that at higher water (lower stress) the effect of focal species would be less than at lower water (high stress) (Bertness & Callaway, 1994). The assumption here is that as stress decreases, the plants increase in size and are more likely to compete and interfere (Bertness & Callaway, 1994). Therefore, we argue that as the level of stress increases in a community, there should be a higher probability of detecting facilitation. This means that focal species which act as benefactors to the community reduce abiotic stress and that this effect is more important as stress increases. Our results show that the situation is more complex. We detected facilitation at both irrigation levels, but the effects were not always by the same species thereby supporting our third hypothesis that the effects of each focal species differ. *Erodium* was the most consistent benefactor species, but abiotic stress may affect the focal species differently from the rest of the plant community. In our system, it is possible that reduced water stress increases the size of focal species (*Erodium*)

which actually increases its ability to facilitate rather than shifting to competition as the general hypothesis would predict (Bertness & Callaway, 1994). We recommend careful use of this framework to predict where facilitation should be most important as focal benefactor species may uniquely respond to stress which in turn influences the rest of the plant community.

By sowing the seeds of *Erodium* and *Erucaria* independently without seed bank we tested whether there were effects of the community on the large annuals. The two species were not affected in the same way. *Erodium* had higher germination when a seed bank was present while *Erucaria* was unaffected. *Erodium*, while generally being a benefactor species to the seed bank, inhibited germination of *Erucaria*. This suggests that species interactions are specific and that for *Erodium* there is no cost to being a benefactor species to the greater plant community. In a field-based target-neighbourhood competition experiment (non-manipulative) at the site where we collected the seed, Lessin et al. (2001) also found that there was no significant relationship between density or aboveground biomass of the neighbourhood and target plant performance (6 target species tested including *Erodium laciniatum*). However, the maximum size of target plants was influenced by the neighbourhood, and when the analysis was restricted to the largest target plants, a competitive response was detected (Lessin *et al.*, 2001). This supports our finding that there may be no cost to being a benefactor species but suggests that the net-outcome of the interactions with the community may shift based on benefactor size (or species identity). At least in this simple plant community, experimental manipulation involving the addition of focal species (similar to competition experiments with targets but testing the effect on the neighbourhood instead) provided valuable insight into how facilitation might influence community structure.

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Table 4.I. A repeated measures ANCOVA for plant density throughout the growing season. The different treatments included sowing the 1x seed bank without additional seeds, with additional *Erodium* seeds, with additional *Erucaria* seeds, or with an artificial mimic. The covariate was seed mass added.

Effect	DF	SS	F Ratio	P-value
Model	29	51570.9	1778.3	<0.0001
Census	2	19685.3	12.9	0.069
Water	1	23626.2	31.8	0.03
Treatment	4	2432.5	2.7	0.11
Census x Water	2	1487.4	3.6	0.028
Census x Treatment	8	1790.7	1.1	0.37
Water x Treatment	4	2051.3	2.5	0.04
Census x Water x Treatment	8	746.95	0.45	0.89
Error	569	116784.17		

Table 4.2. A repeated measures ANCOVA for plant density throughout the growing season. The treatments included planting two-week old seedlings (of the focal species) to the seed bank at the start of the growing season. The covariate was mass of seedling planted. Three species of seedlings were added, *Erodium*, *Erucaria*, and *Trifolium*.

Effect	DF	SS	F Ratio	P-value
Model	7	4753.9	679.1	<0.0001
Census	1	990	10.9	0.04
Treatment	3	3492.4	12.9	0.03
Census x Treatment	3	271.4	2.1	0.1
Error	159	11295.8		

Table 4.3. Summary of ANCOVA for total neighbourhood aboveground biomass and aboveground biomass/plant for the emergent vegetation at the end of the growing season. The treatments were planting two-week old seedlings of the focal species to the seed bank at the start of the growing season. The covariate was mass of seedling planted for three species of seedlings *Erodium*, *Erucaria*, and *Trifolium*.

Effect	DF	SS	F Ratio	P-value
Total aboveground biomass				
Treatment	3	15.1	8.2	<0.0001
Aboveground biomass/plant				
Treatment	3	0.017	2.8	0.04

Figure 4.1. The effects of different seed additions, and mimic, on the final total plant neighbourhood density under low and high watering levels. Differences between groups were analyzed with repeated-measures ANCOVA (with contrast analyses at p<0.05 denoted by different letters). Erodium and Erucaria refer to the seeds added to the 1x seed bank.

Figure 4.2. The effect of different sowings of seeds of focal species on the mean germination of the focal species -- Erodium and Erucaria. Treatments refer to the identity of focal species added to the seed bank (5 seeds of each species sown). Different letters denote significant contrast analyses (p<0.05).

Figure 4.3a. The effect of seedling additions of focal species on the total neighbourhood plant density throughout the growing season. Seedlings were added at the beginning of the growing season and mid-season. Different letters denote significant contrast analyses (p<0.05).

Figure 4.3b. The effect of different seedling additions on the final total plant biomass of the neighbourhood. Seedlings were added at the beginning of the growing season and mid-season. Different letters denote significant contrast analyses (p<0.05).

a.

b.
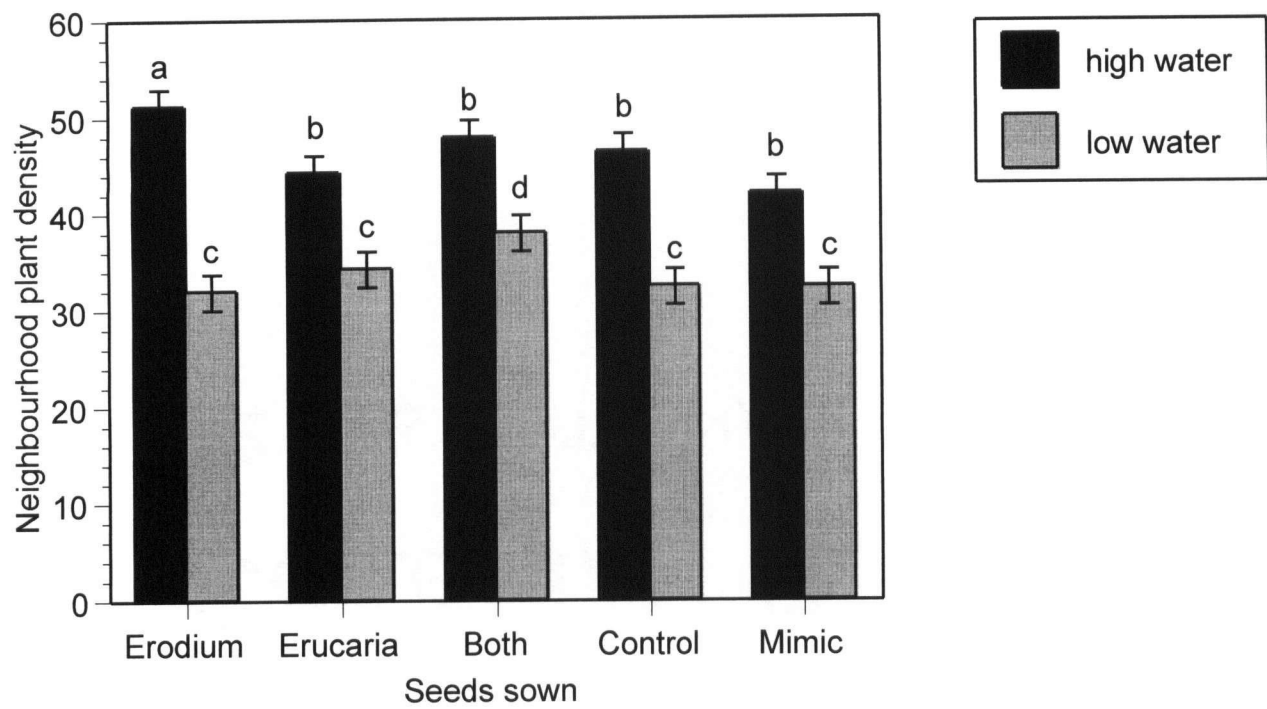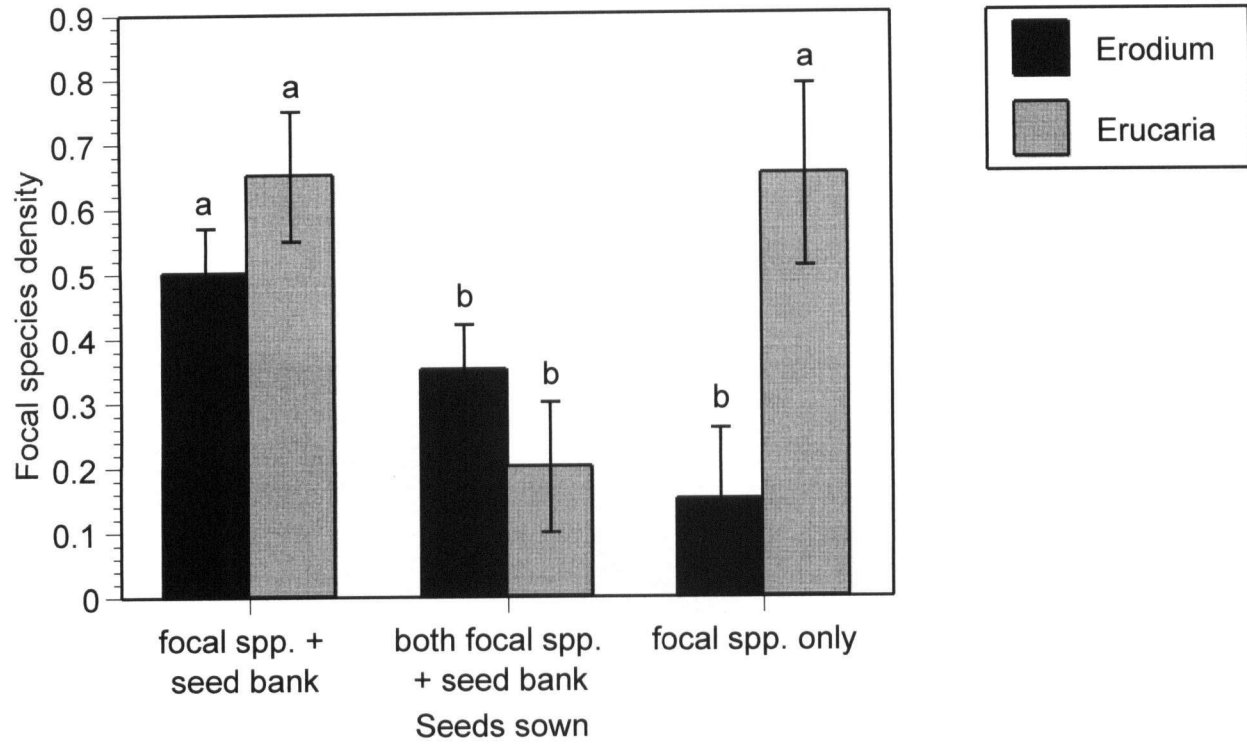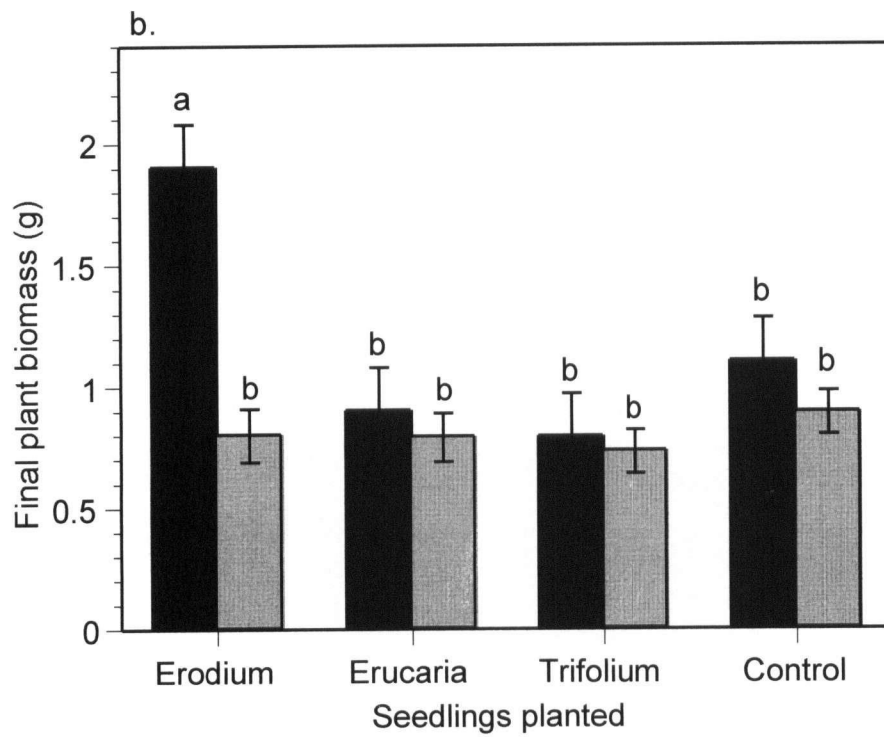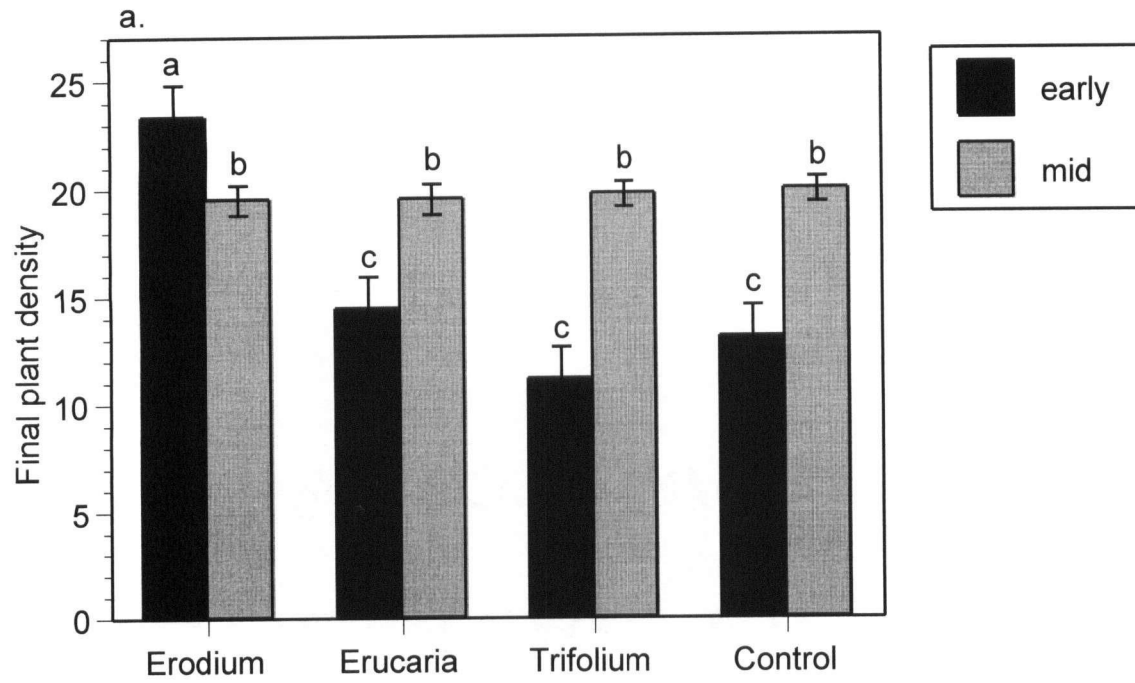
Chapter Five. A benefactor effect by *Erodium laciniatum* on subordinate annuals.

Abstract

Facilitation is an important process structuring plant communities, particularly in harsh environments. In the Negev Desert, Israel, we tested for facilitation within an annual plant community. *Erodium laciniatum* germinates earlier and is generally larger than most of the other annual species. We predicted that this dominant annual facilitates the germination and establishment of other annuals thereby acting as a nurse-plant much like shrubs. A pairwise design involved removal of *Erodium* from half the plots at two times, early and mid growing season. Density, survival, and final aboveground biomass of the neighbouring plants were recorded. The experiment was replicated at two extremes of a natural stress gradient. The presence of *Erodium* increased survival of neighbours and increased mean neighbour plant size. The positive effect was more strongly expressed early in the season, and it is not a direct result of *Erodium*'s size. Conversely, the neighbouring plants did not affect the performance of *Erodium*. This is interpreted as an example of commensalism similar to many shrub-understorey studies. In this study however, the nurse-plant was another congeneric annual species which demonstrates that the effects typically detected at a larger scale are also occurring at smaller scales.

Keywords: annual, abiotic stress, commensalism, facilitation, nurse-plant, shrub-understorey, temporal, removal, reciprocal.

Introduction

Recent research has demonstrated that facilitation, or positive interactions between plants, are important in structuring plant communities (Hunter and Aarssen 1988, Bertness and Callaway 1994, Callaway 1995, 1997, 1998). The vast majority of studies that test for facilitation have focused on shrub-understorey systems (Callaway 1995, 1998). These studies typically compare the performance of herbaceous plants underneath a canopy of shrubs to conspecifics in the open, and typically those living in the understorey have a benefit (Tielborger and Kadmon 1995, Pugnaire et al. 1996, Tielborger and Kadmon 1997, 2000b, Pugnaire and Luque 2001, Tewksbury and Lloyd 2001). Another experimental approach to measuring shrub-understorey effects has been to directly manipulate the influence of the shrubs by using aboveground mimics and removing neighbours. Such studies also detected a positive effect of shrubs on the understorey annuals (Holzapfel and Mahall 1999). The possible benefits of a shrub canopy include shade, reduced water stress, or increased nutrient levels through litter deposition (Callaway 1995, 1998, Holzapfel and Mahall 1999). Nonetheless, we need to more thoroughly test (i) if the effects occurring at the larger shrub-understorey scale are also occurring at a smaller scale such as between congeneric (more similar sized) plant species, and (ii) whether there are any reciprocal effects on the benefactor species. As such, we are interested in determining if a larger annual plant species could positively influence the subordinate species within a patch in much the same way as a shrub.

The most current shrub-understorey studies typically test for differences in the benefit of the shrub along natural stress gradients and through time (Tielborger and Kadmon 1997, Holzapfel and Mahall 1999, Pugnaire and Luque 1999, 2001, Tewksbury and Lloyd 2001). Generally these studies support the prediction of Bertness and Callaway (1994) that the importance of facilitation increases with increasing abiotic stress. Similarly, a strong temporal effect (year-to-year variation) has been detected in studies of facilitation by shrubs (Tielborger and Kadmon 1997, 2000b). Hence, we test the following hypotheses.

1. Benefactor effect. The presence of a larger annual species within a plot will increase the performance of its neighbours (density, survival, and mean plant size) within that plot.
2. Temporal effect. The effect of a dominant annual on the neighbourhood varies throughout the growing season.
3. Reciprocal effect. The neighbourhood of subordinate plants will influence the performance of the larger dominant annual (number of leaves and aboveground biomass).
4. Stress gradient effect. The intensity of facilitation by the benefactor will be stronger in the higher stress plots (Bertness and Callaway 1994).

Methods

Study site and species description:

The study site is a semi-stabilized sand dune dominated by winter annuals and sparsely distributed shrubs, the *Artemesia monosperma* and *Stipagrostis scoparia* associations (Orshan and Zohary 1963) at Bir Asluj in the Holot Mashabim Nature Reserve (31°00.212'N, 34°44.474E) in the central Negev desert, Israel. There are typically about 20 species of annual plants in this community (area of dune about 1ha) (Dyer et al. 2001, Goldberg et al. 2001). Rainfall occurs primarily in winter months (December to April) and has a 30 year average of 110 mm per year (Goldberg et al. 2001). The experiment was done on the south face of a dune with prevailing winds from the North-west. The bottom of the dune is typically less stressed (lower temperatures and higher soil moisture) than the top of the dune and about 50m lower (Lortie and Turkington, *unpublished data*). *Erodium laciniatum* is typically among the largest of the annual plants in size, is erect with a height to 40cm, (Lessin et al. 2001) and is also one of the first to germinate (*personal observation*).

Experimental design:

We used a simple removal design of *Erodium*. We included the following treatments: control/removal, removal time (early season or mid-season), and stress (one of two positions on dune). We recorded effects of removal on the neighbourhood and the effects of the neighbourhood on the *Erodium* plants (in control plots). Successive measurements were taken throughout the growing season.

On January 12th, 2000, after early rains when most of the seedlings had emerged, two transects were positioned on the dune -- one at the top (higher stress) and a second at the bottom (lower stress). On each, we selected 120 adjacent pairs of *Erodium* that were similar in size. The two individuals of a pair were always less than 1m apart. The number of leaves of *Erodium* were measured at this time, and a 15cm diameter ring was placed with *Erodium* in the center to record neighbourhood density. A neighbourhood size of 15cm conservatively samples the maximum sphere of influence by an annual plant of this size (Mack and Harper 1977).

After the initial measurements, 60 pairs of *Erodium* per transect were randomly assigned to one of two removal times - early season or mid-season. Within each pair, one plant was randomly assigned to a treatment group - control or removal. *Erodium* were removed on January 12th (early, immediately after germination) or March 3rd (mid-season removal). *Erodium* was removed by gently pulling it out of the sand which effectively removed most of the belowground

and all of the aboveground biomass. The sand on the dune is loose with no crust, and the removal treatment did not significantly disturb the sand any more than wind or other natural disturbance agents. In an independent test of small-scale disturbance in the field, we detected no effects of disturbance on germination or performance of nearby plants (Lortie and Turkington, *unpublished data*).

The density of the neighbourhood within 7.5cm of the target (i.e. inside the 15cm diameter ring) was recorded six times for the early season removals and three times for the mid-season removals (about 2 weeks between each census). The number of leaves of *Erodium* was also recorded at each census. At the final census (April 15th, 2000), all aboveground biomass was collected from the neighbourhood. Unfortunately, due to many species having very small seeds and asynchronous timing of production, it was not feasible to collect seeds. However, seed production is highly correlated with aboveground biomass in annual plants (Miller 1987). Therefore, we use final plant aboveground biomass as a measure of performance. Control *Erodium* plants were also harvested (aboveground). Plants were dried at 60°C for 48h and weighed.

To investigate reciprocal effects of the neighbourhood on *Erodium*, we selected 60 additional *Erodium* on each transect that were naturally growing without neighbours (within 7.5 cm radius). The number of leaves for these *Erodium* was measured and they were harvested, dried, and weighed. We also recorded the density of *Erucaria pinnata* and *Rumex pictus* in the control plots throughout the season. *Erucaria* is the second largest annual in the system (up to 40cm tall) and *Rumex* a smaller annual (up to 15cm tall) (Lessin et al. 2001). By recording the density of these two species, we can test whether there is a specific effect of the density of another large annual or a smaller annual on *Erodium*. If size is important, then *Erucaria* should have a larger effect than *Rumex*.

Analyses

The beneficial effect of *Erodium* on its neighbourhood was tested by comparing density, survival, and mean plant size (aboveground biomass) of the plants within 7.5cm. From the second census to the end of the growing season, density was converted into a proportionate change in density calculated as (I-F)/I where I is the initial density (at time of removal) and F is the density at that subsequent census. Survival (proportion of individuals surviving) was calculated for each census, and mean plant size (density/aboveground biomass) was calculated for the final census. Differences between groups for each of the three response variables were

tested by ANOVAs or repeated measures ANOVAs using JMP 4 (SAS 2000). All assumptions were satisfied. The main effects were treatment, stress level, and census (for proportionate density and survival). Multiple tests were adjusted with a sequential Bonferroni correction (Rice 1989). For each pair of plots, the negative log response ratio was calculated (for mean neighbour aboveground biomass) to measure both the intensity and the direction of interactions (Hedges et al. 1999). The ratio is

$$- \ln(Xr/Xc)$$

where Xr and Xc correspond to the mean neighbour aboveground biomass for removal and control plots respectively. We compared the sample mean with the expected mean of 0 (no interactions with *Erodium*) using a two-tailed t-test (Hedges et al. 1999). We used the negative of this competition intensity index so that positive values indicate facilitative responses to removal of *Erodium* (i.e., control plots are being facilitated relative to removal plots) and negative values indicate competitive responses (Choler et al. 2001). Regression analyses were used to test *Erodium* size in control plots as a predictor of neighbourhood density, survival, or mean plant neighbour size. The analyses were repeated for both the early and the mid-season plant removals.

The reciprocal effect of neighbourhood plants on *Erodium* size (number of leaves and aboveground biomass for the final census) was tested using a regression analyses of neighbourhood density, total neighbourhood aboveground biomass, or mean neighbour aboveground biomass as independent variables. Following the analysis of Lessin et al. (2001) who also used *Erodium laciniatum*, we tested our data using a boundary constraint approach so as to facilitate comparison. We set boundaries in the aboveground biomass of the neighbourhood and regressed these values against the proportion of maximum *Erodium* aboveground biomass within each width class (of neighbours) and tested a range of different width classes (Lessin et al. 2001). The density of the two subordinate species, *Erucaria pinnata* and *Rumex pictus*, was also regressed against *Erodium* size. The size of *Erodium* plants naturally found without neighbours and those in the control plots was compared using ANOVA. All statistics were done using JMP 4 (SAS 2000).

Results

Initial census (set-up):

When *Erodium* were removed for both the early and mid-season treatments, there were no significant differences in the density of plots, the size of the *Erodium* removed, nor position on the dune (ANOVA, p > 0.05).

Beneficial effects:

In general, control plots with *Erodium* present had higher neighbourhood performance measures relative to plots with *Erodium* removed (Table 5.1; Fig. 5.1) but only with early season removals. Plants in the early season control plots had significantly lower proportionate decreases in density, higher survival, and higher mean neighbour plant size (Table 5.1; Fig. 5.1). The negative log response ratio was also significantly positive (and different from 0) for the early season removals (-LRR = 0.67, t = 3.82, p = 0.0004). Plants in the mid-season control plots did not differ in survival (Table 5.1). Furthermore, the negative log response ratio for the mid-season removals was not significantly different from 0 (mean – LRR for both positions = -0.01, t = -0.09, p = 0.93). *Erodium* size (number of leaves or aboveground biomass) in control plots did not significantly influence the three performance measures tested for either the early or mid-season removals at any census (Regression analyses, p > 0.05).

Temporal effects:

A positive effect by *Erodium* was detected in response to early season removals but not the mid-season (Fig. 5.1 and – LRR values reported above). There was also no interaction between treatment (*Erodium* removal) and census in either experiment for any response variable (Repeated Measures ANOVA, p > 0.05). This means that the differences detected between control and treatment plots in the early season removals emerged by the first census (within two weeks) after removal of *Erodium* and persisted throughout the growing season. Therefore, only final measures are reported here.

Reciprocal effect:

In the control plots, neighbourhood density only weakly decreased *Erodium* mass (Fig. 5.2A, B). However, there was no significant difference between *Erodium* grown with and without neighbours at the end of the season (ANOVA, F = 2.8, p = 0.067, n = 60; Fig. 5.2C), and no effect of neighbourhood aboveground biomass or mean neighbour aboveground biomass on *Erodium* performance (Regression analyses, all p > 0.39). Using the boundary constraint approach, the regressions for different categories of neighbourhood aboveground biomass also did not significantly predict *Erodium* mass (Regression analyses, in all cases p > 0.19). Neither mean neighbour plant size, *Erucaria* density, nor *Rumex* density significantly affected *Erodium* (number of leaves or aboveground biomass, Regression analyses, p > 0.05).

Stress gradient effect:

In both early and mid-season there was a significant effect of stress on the survival of the neighbourhood plants but no significant interaction with the removal treatment (Table 5.1). Survival was significantly greater at the high stress site (contrast analyses, $p < 0.01$).

Discussion

Interactions between species are commonly an interplay between competition and facilitation (Callaway and Walker 1997). Recent studies on facilitation in particular have considerably furthered our understanding of how this interplay might shift through time (Bertness and Callaway 1994, Casper 1996, Callaway and Walker 1997, Brooker and Callaghan 1998, Tielborger and Kadmon 2000b) or along stress gradients (Bertness and Yeh 1994, Greenlee and Callaway 1996, Callaway and Walker 1997, Brooker and Callaghan 1998, Tielborger and Kadmon 2000a, Pugnaire and Luque 2001, Tewksbury and Lloyd 2001). However, the detection of this interplay in the facilitation literature has been largely restricted to shrub-understorey plant communities. As Holzapfel and Mahall (1999) state, this is an obvious system to test for facilitation through reduced abiotic stress. Nonetheless, to significantly further our understanding of plant interactions (both positive and negative) we need to test plant communities that are composed of more similar-sized species particularly if we are to detect reciprocal interactions. In this study, we detected a strong positive effect of a larger annual on the plant community. This supports the hypothesis (#1) that the presence of a larger annual might act as a benefactor species to nearby subordinate annuals. This small-scale approach to testing for facilitation is extremely rare in the facilitation literature. To the best of our knowledge, only two other studies have tested for (and detected) facilitation among congeneric species in this way (Carlsson and Callaghan 1991, Aksenova and Onipchenko 1998). Carlsson and Callaghan (1991) tested the positive effect of two evergreen dwarf shrubs on *Carex bigelowii* by erecting plywood shelters and found that this mimic of sheltering by shrubs increased *Carex* performance. The latter study removed five dominant species from alpine tundra and found both positive and negative responses by the vegetation depending on the species (Aksenova and Onipchenko 1998). In combination, these initial results suggests that at least in abiotically stressed environments such as deserts (our study) or alpine communities (Carlsson and Callaghan 1991, Aksenova and Onipchenko 1998) the presence of a relatively larger species can significantly influence the outcome of the interaction between species within the community at a small scale.

We demonstrated that the positive effect of a larger annual plant was more important early in the growing season thereby supporting our second hypothesis that the effect of a dominant species varies throughout the growing season. Furthermore, the relative benefit of *Erodium* was expressed within 2 weeks of removal and persisted throughout the growing season. This suggests that these initial positive effects persist to ultimately influence the outcome of the interactions in the final plant community and that the lack of a response to mid-season *Erodium* removal is not due to a short experimental period. A growing body of literature supports the prediction that facilitation is more important early in the life-stage of plants (Fowler 1988, Callaway et al. 1996, Greenlee and Callaway 1996, Callaway and Walker 1997, Aksenova and Onipchenko 1998, Foster 1999, Holzapfel and Mahall 1999). Seedlings are thought to be more sensitive to abiotic stress (Foster 1999), and as plants increase in size, it is more likely that they will interact negatively via interference (Callaway and Walker 1997). Our study explicitly tested when the positive effect occurred by successive measurements and by removing the larger annual plant species at two different times. Hence, we can differentiate early season and mid-season interactions (when the subordinate species are larger) from the final outcome on the plant community. This essentially gives us two snap-shots of the effects of *Erodium*; it is a benefactor early in the growing season, and does not significantly interact with the subordinate species later in the season. That *Erodium* size did not directly influence the performance of the neighbourhood also demonstrates that the initial effects are crucial. Although we can not assert that seedlings are more sensitive to abiotic stress, it is clear that as plants grow there is no shift to interference. Based on the role *Erodium* plays in this system, early season interactions with seedlings are more important in structuring this plant community.

In a recent review of methods to study interspecific competition, Connolly et al. (2001) argue that questions regarding the outcome of competition are not sufficiently differentiated from questions regarding the effect neighbouring species have on each other. The third hypothesis, that the neighbourhood of subordinate species will influence the benefactor species, in this case *Erodium*, allows us to make this distinction. The neighbouring plants weakly interacted with the *Erodium* (negatively) but this did not significantly reduce *Erodium* size to a level different from *Erodium* without neighbours. Lessin et al. (2001) also reported no significant relationship between target species performance and density or aboveground biomass of the neighbourhood for six species of annuals including *Erodium*. However, they detected negative effects of the aboveground biomass of the neighbourhood when restricted into boundaries (size classes) on the maximum size of target plants in each size class (Lessin et al. 2001). We did not detect an influence of a second large annual (*Erucaria*) on *Erodium* or when we applied the

boundary constraint approach to detecting effects on *Erodium*. The results from our study suggest that the final outcome of interactions with *Erodium* is essentially commensal (+, 0), similar to a nurse-plant effect (Aksenova and Onipchenko 1998, Holzapfel and Mahall 1999) which effectively rejects our third hypothesis. Further tests for facilitation (and target-neighbourhood competition studies such as Lessin et al. (2001)) should address possible reciprocal effects and differentiate between species interactions and final outcomes. The consistency of detecting competition and facilitation may also strongly depend on different patterns of seasonal resource pulses such as rainfall in desert systems (Lessin et al. 2001).

It is predicted that the intensity of facilitation is directly related to the level of abiotic stress (Bertness and Callaway 1994, Callaway and Walker 1997, Brooker and Callaghan 1998), and there is evidence to support this prediction (Bertness and Yeh 1994, Greenlee and Callaway 1996, Tielborger and Kadmon 2000b, Pugnaire and Luque 2001, Tewksbury and Lloyd 2001). However, our study and that of Tielborger and Kadmon (2000a) did not detect a difference in the interactions between neighbourhoods with or without the benefactor species at different locations on a stress gradient. Surprisingly in our study, survival was greatest at the high stress site. The positive effect of *Erodium* on the community did not change with changes in abiotic stress within the habitat, and it is possible that competition between subordinate individuals may be even more intense at the lower stress site. Alternatively, the initial benefit of *Erodium* may function to reduce stress for seedlings and subsequent abiotic differences on the dune do not influence desert annual plants as they increase in size. Nonetheless, facilitation is important in this plant community but apparently is not directly related to abiotic stress which rejects our fourth hypothesis that the intensity of facilitation will be directly related to stress.

Conclusions

Two of our four initial facilitation hypotheses were supported by a dominant species removal experiment. *Erodium* acted as a benefactor species early in the season to the subordinate annuals in the community similar to a shrub-understorey (nurse-plant) effect. However, the scale tested was much smaller and among more similar-sized congeneric species of plants than typical nurse-plant studies. The positive effect of *Erodium* did not change with abiotic stress. There was a weak reciprocal interaction by the neighbourhood on the *Erodium*, but this interaction did not change the final performance of *Erodium*. This suggests that it is crucial to distinguish between interactions and final effects. The small scale manipulative approach used here (and in two other studies) should be tested for different plant communities to determine if facilitation is prevalent among congeneric species.

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Table 5.1. ANOVAs for final response variables of neighbourhood to early and mid-season *Erodium* removal. Values in bold indicate significance after Sequential Bonferroni correction.

measure	Effect	DF	Early removal			Mid-season removal		
			SS	F Ratio	P-Value	SS	F Ratio	P-Value
Change in density	Treatment	1	0.68	5.9	**0.02**	0.02	0.05	0.83
	Stress	1	0.03	0.28	0.6	2.4	5.7	0.027
	Tr. x Stress	1	0.01	0.10	0.75	0.3	0.68	0.41
Mean neighbour size	Treatment	1	0.05	4.01	**0.04**	0.001	0.01	0.92
	Stress	1	0.01	0.74	0.39	0.01	0.69	0.41
	Tr. x Stress	1	0.01	0.81	0.38	0.006	0.67	0.42
Survival	Treatment	1	11	20.2	**0.0001**	2.75	5.5	0.02
	Stress	1	12.6	23.1	**0.0001**	7.72	15.4	**0.0001**
	Tr. x Stress	1	0.003	0.007	0.94	0.13	0.25	0.62

Figure 5.1. The effect of *Erodium* removal on the final performance of a neighbourhood of subordinate annuals (within 15cm ring). Control plots had *Erodium* left in place, and treatment plots had *Erodium* removed either in early season or mid-season. A. Proportionate decrease in density was calculated by (I-F)/1, where I is initial emergent plant density and F is final density (or density at subsequent census). B. Survival ($\pm$ 1 S.E.) was calculated from the initial census. C. Mean plant size ($\pm$ 1 S.E.) was calculated by dividing total aboveground biomass of neighbourhood by total final density. Different lower case letters denote significance at the $p <$ 0.05 level for the early season experiment and $p < 0.016$ for the mid-season experiment (sequential Bonferroni corrections).

Figure 5.2. The effect of the neighbourhood on the final aboveground biomass of *Erodium*. Figures A. & B. show linear regressions for neighbourhood density on the mass of *Erodium* in the control plots. Figure 5.2C. shows the aboveground biomass ($\pm$ 1 S.E.) of *Erodium* naturally grown under different neighbourhood conditions (no neighbours within 15cm diameter ring and neighbouring plants within ring).

A. early control plots

r² = 0.12
p = 0.01
n = 60

B. mid-control plots

r² = 0.13
p = 0.03
n = 60

C.
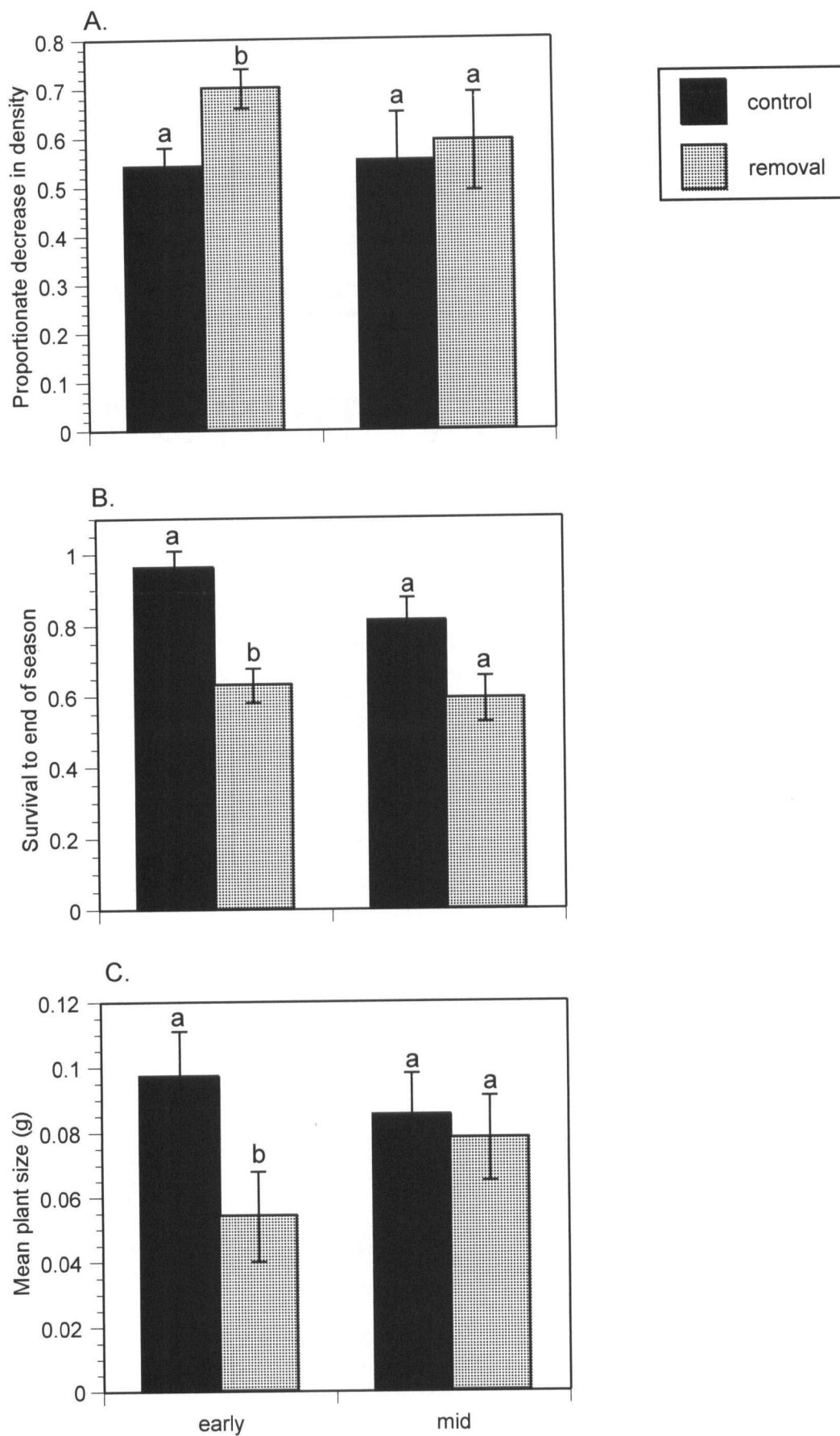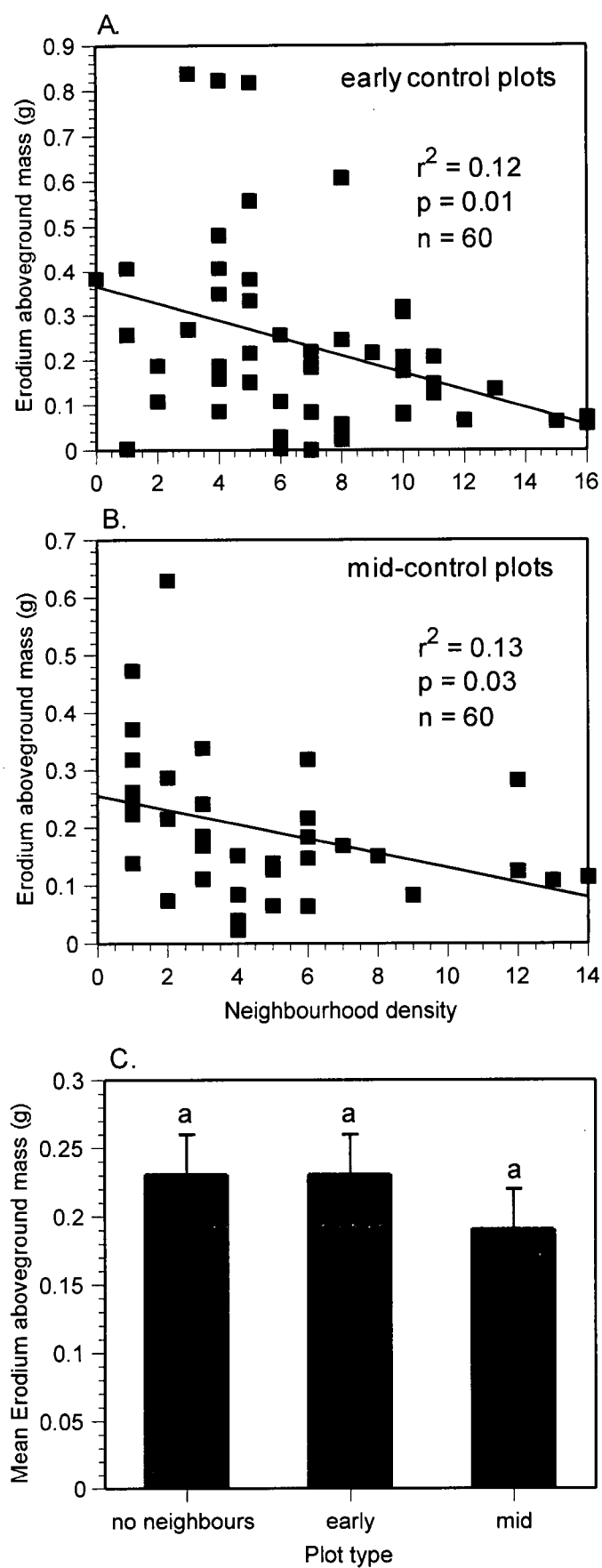
Section C: Conclusions for specific species effects

Erodium acted as a benefactor species in this plant community in both the greenhouse and the field. Seedlings of *Erodium* were more effective as benefactors than seeds; whereas, removal of *Erodium* was more important early in the growing season relative to mid-season. Hence, both initial predictions were supported with a larger annual species facilitating neighbouring annual species, and life-stage was important in determining the strength of the facilitation. The consistent positive effect of *Erodium* in both experiments (chapters 4 & 5), coupled with a lack of effect by *Erucaria* (chapter 4), suggest that *Erodium* had a significant influence in structuring this plant community.

This species may thus function in a manner similar to a nurse-plant typically demonstrated in shrub-understorey systems. However, the effects detected are at a much smaller scale and amongst plant species with similar life-histories. Interestingly, its importance was generally independent of benefactor plant size and stress. These novel findings further our understanding of the prevalence of facilitation in plant communities and challenge the general notion in the facilitation literature that the intensity of facilitation is directly related to the level of abiotic stress.

General Discussion

Context

Although is has been speculated that there may be very little competition in arid environments (Grime 1977, 1979, Fowler 1986, Bertness and Callaway 1994), plant interactions, both positive and negative likely play a role in structuring these plant communities. In general, the importance of competition in determining community structure has been extensively studied (Goldberg and Barton 1992) while the influence of facilitation has only recently been addressed outside the context of plant succession (Callaway 1995). The primary objective of this thesis was to test whether interactions (positive or negative) between seeds and plants significantly influence an annual plant community in the Negev Desert, Israel. The most effective approach to addressing this question was to incorporate the different themes reviewed in the general introduction: test for an interaction between competition and facilitation, incorporate different life-stages into experiments, and test different densities and stress levels all at the community-level. A review of the facilitation literature also reveals that there is a lack of tests for interactions between similar-sized species with comparable life-histories. As such, the research reported in this thesis focused exclusively within the annual plant community and did not incorporate experimental designs that involved shrubs.

This overall approach, encompassing multiple spatial scales, life-stages, and stress levels to test for community-level effects, lends itself well to addressing a more fundamental question: are patterns in community structure better explained by individuals (Gleason 1926, Gleason and Cronquist 1964) or the community as a whole (Clements 1916)? An individualistic based approach to understanding (and therefore testing) community structure is currently the dominant paradigm in plant community ecology and argues that the independent tolerances of species on environmental continua (i.e., gradients) determine community structure at a particular point in space (Callaway 1997). Hence, competition studies which test for the individual competitive effects or responses of species to explain community composition prevail. Communities are thus viewed as assemblages of individual species interacting. However, the community-based approach views these assemblages as interdependent collections of species with emergent (holistic) properties (Clements 1916). The question then becomes, do assemblages of species exhibit interdependence, and if so, how much (Callaway 1997)? Here, I will address this general question, and in brief, evaluate the success of the approaches used to understand plant community structure.

In general, I will address the following questions:

1. Are interactions between seeds, and between plants important in this desert annual plant community?

2. Does facilitation function among congeneric species?

3. Does life-stage, density and level of abiotic stress influence plant interactions?

4. Does the community structure suggest independence of species or interdependence?

Importance of interactions

Chapters 2, 3, and 4 all demonstrated that competition and facilitation influence the subsequent plant community. However, the response of the community was generally limited to changes in density and performance of individuals (i.e., aboveground biomass and survival) within the community and not to differences in diversity. Intuitively, one would predict that facilitation increases diversity by reducing stress or promoting germination for certain species while competition decreases diversity by eliminating weaker competitors. Neither increasing seed density (chapters 2 and 3), changing the spatial pattern of patches of seed (chapter 2), nor addition and removal of a larger annual species, *Erodium laciniatum* (chapters 4 and 5), changed the representation of species within the plant community. Coupled with the general lack of significant effects by life-stage or level of abiotic stress, it seems that the structure of this annual plant community is relatively constant. Furthermore, the experiments described here were done in three very different growing seasons (average MAP to $1/3^{rd}$ MAP), and the study described in chapter 3 was repeated in each season without differences in diversity for the global seed densities tested. The spatial pattern of the seed bank was also relatively constant. Hence, the interactions between seeds and plants within this community determine structure but only insofar as structure pertains to overall aboveground productivity. It is possible that these effects could structure the plant community over a longer term, and it would be interesting to test whether these processes, which influence productivity, in turn influence diversity or ecosystem function. The results reported here suggest that the use of species diversity as a predictor of ecosystem function may not be appropriate for arid or lower productivity systems (Aarssen 1997, Austin 1999, Hector et al. 1999, Tilman 1999, Bednekoff 2001, Hector 2001). Admittedly, the use of a seed bank-based system may, to a certain extent, also buffer the community from significant changes in diversity over time, at least in the short-term. This in itself would be interesting to test from a diversity-ecosystem function perspective. To conclude, interactions between seeds and between plants are important in this plant community, thereby rejecting the hypothesis that competition is unimportant in arid environments (Fowler 1986).

Facilitation among congeneric species

Chapters 2, 4, and 5 are among the first studies to detect facilitation among congeneric species (but see Carlsson and Callaghan 1991, Kikvidze 1996, Aksenova and Onipchenko 1998, Kikvidze and Nakhutsrishvili 1998). The positive effects were manifested in one of two ways: (i) a relatively larger annual acting as a nurse-plant, and (ii) patches of seeds or plants increasing the performance of neighbouring patches. These results suggests that the shrub-understory systems which generally serve as the model for understanding facilitation between plants can also be applied to smaller-scales. Fine scale manipulation of congeneric species (additions and removals) have been used in the competition literature (Goldberg and Barton 1992, Goldberg 1996, Goldberg et al. 1999, Connolly et al. 2001) but not in the facilitation literature (Callaway 1995, 1997, 1998). It would be worthwhile to test whether species-specific effects change with overall density or with the distribution of neighbouring patches. For instance, we might predict that increasing plant density increases interference within the plant community. However, selective removal of dominants may either increase or decrease the intensity of competition depending on whether the species has positive or negative effects on nearby plants. The effects of local density and spatial arrangement of patches of seed might also be influenced by the presence of a particular species. It is also possible that some species may actually determine spatial pattern or local density depending on its effect on neighbour plants, i.e., acting as a nurse-plant or wind-trap for blowing seed. The experimental manipulation of the patterns detected in the field, i.e. clumping of seeds or presence of a larger, earlier germinating annual, demonstrated that facilitation among congeneric species does occur. The approach that most directly lead to detecting facilitation was viewing the community as a collection of neighbourhoods or patches and testing for interactions between them or testing the species that seemed to most commonly occur within patches.

Life-stage, density, and level of abiotic stress

Life-stage significantly influenced interactions between individuals (seeds and plants) in this plant community. In general, both positive and negative interactions were more intense between seeds and seedlings than between adult plants. Following emergence, interactions between adults plants generally did not influence the performance of individuals. These findings support the general notion in plant ecology that the earlier life-stages are more vulnerable to abiotic stress and competition through interference (Harper 1977). However, the hypothesis that facilitation is more important at earlier life-stages and that as plant increase in size they are more likely to compete (Callaway and Walker 1997) was not supported. As Connolly et al. (2001) propose, competition studies that focus on a single snap-shot approach at the end of the

growing season may miss significant interactions between species, and as speculated in chapter 5, the bias in detecting negative interactions between plants may be due to this snap-shot effect. Based on the studies in this thesis, I strongly recommend that successive measurements be used so that we can measure both interactions between species and final outcomes, and differentiate between them. In addition to successive measurements, the experimental manipulation of the presence/absence of certain species at different life-stages or manipulation of density at different life-stages would be interesting to pursue further. In this thesis, life-stage was generally categorized into two distinct phases – seeds to emergent seedlings, and established plants to the end of the growing season. While it was not possible to identify species as they germinated, interactions between seeds under more controlled conditions should be tested (Qadir and Abbasi 1971, Murray 1998) to determine how seeds interact.

Manipulation of both local (chapter 2) and global (chapter 3) seed density generally demonstrated negative effects of increasing density. The correspondence between the two levels of response by the plant community in two separate experiments (experimental garden and the field) and in several growing seasons suggest that negative density dependence is an important process in shaping this plant community. This is similar to other studies in this system which also detected negative effects of seed and plant density (Goldberg et al. 2001, Lessin et al. 2001). However, the field based approach used suggests that interactions between patches of seed, which are likely to occur in the field, may serve to reduce the intensity of competition, and that limitation by the environment may also be important in determining the performance of individuals within the plant community. Density effects are thus important but other processes should also be further considered. For instance, how do changes in both density and spatial arrangement interact, i.e., high density patches near lower density patches, and can we more effectively distinguish limitation from regulation (White 2001)?

Stress is generally defined as any external factor that limits the rates of resource acquisition, growth, or reproduction of an organism (Grime 1989, Parker et al. 1999). In this study system, there were higher levels of stress at the top of the sand dune relative to the bottom with plants at the top of the dune having lower plant densities and decreased survival (chapter 3). However, the effects of the level of abiotic stress (natural or experimental) did not interact with any of the main treatments, i.e., spatial pattern, seed density, or removal of *Erodium*. Hence, the hypothesis that the importance of facilitation will increase with increasing stress (Bertness and Callaway 1994, Callaway 1995) was not supported. It is also possible that the pulsing of

the resource (i.e., frequency of rainfall and duration between events) is more important than the actual magnitude (Novoplansky and Goldberg 2001). Alternatively, other resources such as nitrogen may have been limiting in this system although it was not within the scope of this research programme to adequately test for differences in nutrient levels and limitations thereof. Nonetheless, future facilitation studies in this system would strongly benefit from detailed measurements of nutrient levels within the habitat along the natural moisture stress gradient and from tests for differences between species or treatment levels which may be expressed through different belowground biomass allocation strategies.

Implications for community structure

The simplest way to differentiate between the individual-based approach and the community-based approach is in terms of dependence between species within the community. If the majority of the interactions detected suggest that species function independently, then it might be more productive to continue with experiments that test for differences between species to understand plant communities. If the majority of interactions were interdependent, i.e., interactions between species depended more on neighbouring species, then perhaps more effort should be devoted to understanding communities as a whole (although much more complex experimentally). The experimental approaches used here allow us to only indirectly infer the degree of dependence between species.

In chapters 2 and 3, the effects of changing seed density for the entire plant community were tested. If species functioned independently, we would predict that increases in the density of individuals would increase overall competition intensity within the community, whereas, if the species were interdependent, then we would predict that there might be effects of groups of species. The conclusions from these two chapters suggest that independence and interdependence of species were both important. There were negative effects of increasing density but positive effects between patches of species. One explanation is that patches of species interact positively by reducing abiotic stress for nearby patches. However, this is not compelling evidence for interdependence between species but does suggest that there are emergent properties for groups of aggregated species.

In chapters 4 and 5, the effects of *Erodium* on neighbouring species were tested. In this situation, if species were interdependent we might predict that a single species affects a group of species. The presence of *Erodium* increased the performance of all species within the neighbourhood thereby affecting a group of species which suggests interdependence. There

was also no significant difference in the representation of species in plots with *Erodium* relative to plots without *Erodium* (chapters 4 and 5). Additionally, the overall lack of differences in species diversity in any experiment suggest that species may function interdependently as groups within this plant community even when a larger benefactor species is not present. It is possible that the association of species is so strong that the effects tested (or position on the natural stress gradient) do not disrupt species' associations. Preliminary experimental work (Lortie and Turkington, unpublished) also suggests that there are strong consistent positive associations between species in the field. Based on sampling effects, use of multiple tests, and replicated experiments alone, we would expect that species diversity might sometimes be different for a treatment we applied. Nonetheless, community composition remained relatively consistent supporting the idea that communities can function as whole units.

In summary, individual species effects are important and species compete and likely have direct independent effects. Community-level effects that result from the interdependence of species may however be more important in structuring (or maintaining structure) for this plant community. This may be a product of the fact that in arid systems the vegetation is typically clumped (Fowler 1986) as is also the seed bank (chapter 1). Further research that includes patch-level effects for communities that are not seed bank-based and communities that have higher species richness is necessary to determine whether community-level effects are widespread or restricted to communities with strong spatial structure such as those in arid environments.

Conclusions

The four general questions I posed here in the general discussion can simply be distilled to three simple hypotheses which are essentially the themes that run throughout the thesis.

1. The scale used to test for interactions strongly determines the net outcome (i.e. individuals to neighbourhoods).
2. Interactions between species will change with life-stage (i.e. seeds versus established plants).
3. The intensity of competition and facilitation change with level of abiotic stress.

As I have discussed, the first two hypotheses are clearly accepted while the third is rejected. Scale and life-stage strongly influence the net outcome of interactions between species but level of abiotic stress does not. The experiments described here are also among the first to demonstrate facilitation similar to shrub-understorey systems but at a much finer and more subtle spatial scale.

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Appendix A. Mean (± s.e.) of some traits of the annual plant species commonly found at Bir Asluj in the Holot Mashabim Nature Reserve in the central Negev desert, Israel. Data adapted from Dyer et al. (2001).

Species	Family	N	Seed mass	Emergence time	No. of leaves	Leaf area	Root mass	Shoot mass	Height
Ammochloa palaestina	Poaceae	6	0.021	9.0 (0.00)	1.8 (0.17)	0.011 (0.003)	0.302 (0.072)	0.333 (0.061)	31.6 (3.03)
Bromus fasciculatus	Poaceae	16	0.11	6.6 (0.51)	2.0 (0.00)	0.063 (0.019)	1.431 (0.381)	0.881 (0.079)	63.4 (3.92)
Crucianella membranacea	Rubiaceae	6	0.058	6.7 (0.64)	6.7 (0.67)	0.133 (0.008)	0.233 (0.034)	0.533 (0.076)	19.0 (3.26)
Ctenopsis pectinella	Poaceae	4	0.022	7.5 (1.04)	1.8 (0.25)	0.006 (0.003)	0.18 (0.100)	0.275 (0.111)	38.9 (3.53)
Cutandia dichotoma	Poaceae	8	0.075	6.4 (0.53)	1.0 (0.00)	0.005 (0.001)	0.838 (0.134)	0.675 (0.049)	76.1 (2.62)
Diplotaxis harra	Brassicaceae	7	0.028	4.4 (0.78)	3.6 (0.20)	0.105 (0.015)	0.271 (0.047)	1.171 (0.143)	27.3 (3.07)
Erodium laciniatum	Geranaceae	4	0.174	5.4 (1.32)	5.0 (0.41)	0.982 (0.265)	0.925 (0.364)	3.85 (0.997)	25.4 (5.57)
Erucaria pinnata	Brassicaceae	5	0.121	7.0 (1.43)	4.8 (0.37)	0.496 (0.072)	0.66 (0.194)	3.02 (0.439)	20.4 (1.39)
Filago desertorum	Asteraceae	3	0.006	6.3 (1.43)	5.0 (0.58)	0.04 (0.003)	0.07 (0.063)	0.233 (0.088)	9.4 (1.30)
Hippocrepis multisiliquosa	Fabaceae	6	0.154	5.4 (1.29)	5.2 (0.17)	0.107 (0.013)	0.75 (0.297)	1.167 (0.143)	26.0 (1.58)
Mathiola livida	Brassicaceae	3	0.012	3.5 (0.50)	4.3 (0.33)	0.048 (0.005)	0.01 (0.00)	0.20 (0.058)	10.0 (1.30)
Picris asplenoides	Asteraceae	5	0.018	5.3 (0.48)	3.4 (0.40)	0.109 (0.40)	0.028 (0.018)	0.34 (0.075)	8.5 (0.85)
Plantago cylindica	Plantaginaceae	6	0.136	8.0 (1.73)	3.8 (0.31)	0.228 (0.047)	0.333 (0.084)	1.733 (0.296)	21.9 (2.43)
Plantago ovata	Plantaginaceae	2	0.013	4.5 (0.29)	4.0 (0.00)	0.039 (0.006)	0.01 (0.00)	0.30 (0.00)	7.9 (0.90)
Reboudia pinnata	Brassicaceae	7	0.022	4.6 (0.46)	3.4 (0.20)	0.095 (0.015)	0.189 (0.090)	0.571 (0.078)	17.9 (1.41)
Rumex pictus	Polygonaceae	8	0.054	5.3 (0.94)	3.4 (0.32)	0.13 (0.029)	0.214 (0.034)	0.588 (0.119)	13.1 (2.84)
Trifolium tomentosum	Fabaceae	16	0.044	4.1 (0.59)	6.0 (0.27)	0.16 (0.015)	0.519 (0.073)	0.925 (0.099)	20.5 (1.79)
Trigonella arabica	Fabaceae	12	0.161	4.3 (0.74)	4.8 (0.60)	0.286 (0.033)	0.609 (0.088)	1.625 (0.180)	34.1 (3.49)
Vulpia brevis	Poaceae	7	0.084	7.9 (1.13)	2.0 (0.00)	0.018 (0.009)	0.473 (0.107)	0.571 (0.097)	55.2 (3.52)
Vulpia myuros	Poaceae	8	0.062	7.3 (0.94)	1.9 (0.13)	0.01 (0.004)	0.400 (0.071)	0.40 (0.063)	47.4 (2.83)

Appendix B. Two of the common species, (A) *Erodium laciniatum* and (B) *Rumex pictus*, and the study site at Bir Asluj in the Holot Mashabim Nature Reserve in the central Negev desert, Israel. The dune with lower and upper 50m transects along with inset topographical representation are shown in Figure C.
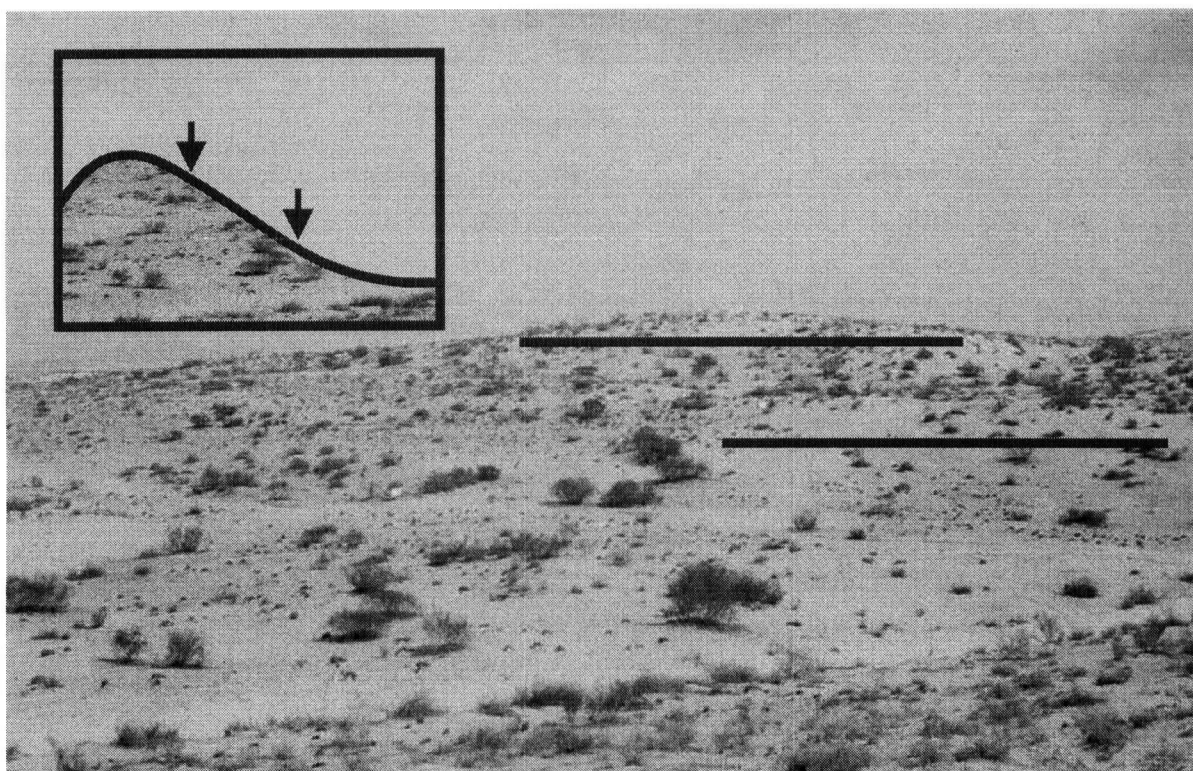
A. *Erodium laciniatum*.

5cm

B. *Rumex pictus.*
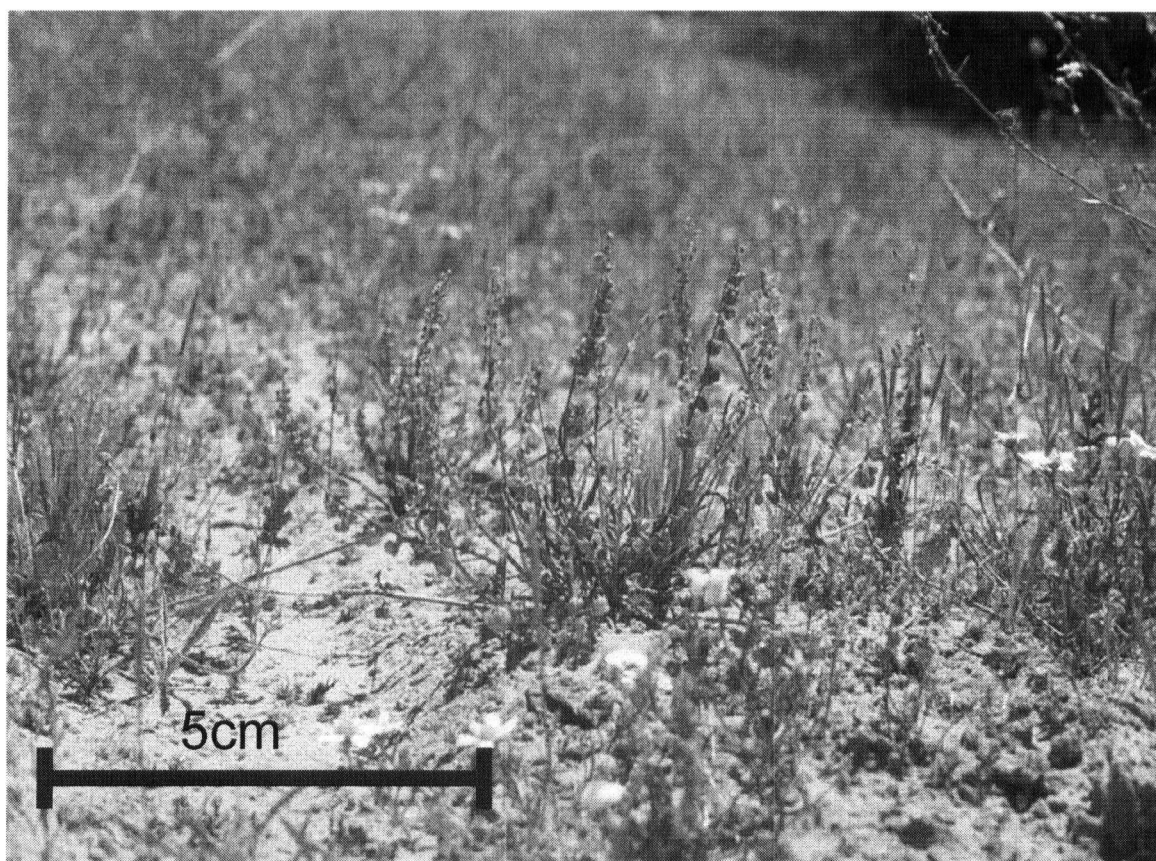
C. The dune at Bir Asluj.

Appendix C. Rainfall data at weather station 25km SE of Bir Asluj in the Holot Mashabim Nature Reserve in the central Negev desert, Israel. Data from the Blaustein Meteorological Unit.

Month	Season 1997-1998		Season 1998-1999		Season 1999-2000	
	Monthly sum (mm)	Rainy days per month	Monthly sum (mm)	Rainy days per month	Monthly sum (mm)	Rainy days per month
October	8.20	2	0.00		0.25	1
November	0.60	2	0.00		0.00	
December	12.00	5	1.50	1	0.38	2
January	45.20	5	8.15	4	20.80	4
February	32.32	5	17.75	3	1.40	2
March	7.40	6	3.50	1	12.85	5
April	2.20	1	8.20	1	0.00	
May	0.90	1	0.00		0.00	
Annual Total (mm)	**96.82**		**39.6**		**35.68**	

Appendix D. Soil nutrient data for the semi-stabilized sand dune where experiments were done at Bir Asluj in the Holot Mashabim Nature Reserve in the central Negev desert, Israel (1998 only). The mean reported in each case is from five replicates collected over the length of the north face of the dune. NH_4 is presented as mmol g^{-1}, all other nutrients are mg g^{-1}. Data from Goldberg, Turkington, and Dyer (unpublished).

Nutrient	Mean	Standard deviation
NH_4	1.3	0.46
NO_3	0.82	0.20
P	18.02	4.27
K	2.4	1.00
Mg	11.16	0.79
Ca	65	10.07
Cl	75.6	15.29