Investigating the role of biotic interactions in determining the degree of local adaptation and thus the potential response of plants to climate change

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„It is not the strongest of the species that survives, nor the most intelligent, but the one most responsive to change"

– Charles Darwin

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Abstract

Efficiently predicting the response of plant species to climate is one priority issue of present research, especially for Eastern Mediterranean systems, which according to predictions will be subject to increasing aridity. Current predictions on the future response of species to the expected change, though, are based on abiotic factors and virtually neglect the role of biotic interactions.

The present thesis investigated the combined effect of climate, soil abiotic properties and biotic interactions on plant populations and community performance. Firstly, the performance of plant species was investigated in the field performing a reciprocal seed sowing and soil transplant experiment combined with neighbour treatments between two sites along an aridity gradient. Four species were chosen in order to investigate species-specific effects. Secondly, a similar experiment was performed under controlled conditions with an additional soil treatment to tease apart the effects of soil abiotic properties and soil biota on plants. Thirdly, the change in species composition and richness of the communities emerging from transplanted soils under different climatic conditions was monitored in order to investigate potential community shifts under changed climate.

The study was based on an integrated approach in which two types of biotic interactions that have been so far addressed separately were combined and analysed jointly. By focusing on different spatial scales we were able to single out some important factors driving population and community performance. Furthermore, by integrating field and greenhouse experiments it was possible to analyse how different levels of stress can act as a lever on the relative importance of abiotic factors *vs.* biotic interactions.

Our overall findings show the prominence of biotic interactions over climatic factors and suggest that in the short term plant species might be able to successfully buffer the expected climate change. Nonetheless, climate acted as a filter on the outcome of biotic interactions suggesting a complex interplay between biotic and abiotic factors. Namely, under more stressful conditions abiotic factors might override the effect of biotic interactions; whereas under low stress, neighbours and soil biota

effect might have a higher influence on plant fitness. Similarly, at the community level, soil was the main determinant of species composition and the effect of climate was ancillary. Although a shift in species richness and community composition was observed, it is suggested that species might be able to withstand the predicted change as long as wetter years are periodically occurring. In synthesis, this thesis presents a novel approach to the study of species adaptive potential and how this could be integrated in future predictions for population and community.

Declaration of my own working contribution to the present thesis

The present manuscript is based on the work I carried out in the field in Israel and in the greenhouse in Germany during my PhD.

For the second and third chapter I carried out the statistical analyses myself, for the fourth chapter I received great help from Mark Bilton in performing statistical analyses.
In general I have discussed statistical approaches and alternatives with my colleagues Merav Seifan, Mark Bilton and Michal Gruntman.

I wrote the first draft of the entire thesis myself, but I have received and incorporated comments and suggestions from my supervisors Katja Tielbörger and Wim Van der Putten. In the case of chapter four comments were provided by Katja Tielbörger and Mark Bilton.

Throughout the course of this research I was advised by my supervisors Katja Tielbörger and Wim Van der Putten.

Chapter 1

General introduction

Over the past decades the often negative effects of climate warming have taken their toll on several species and habitats (Grabherr *et al.* 1994; Penuelas & Filella 2001; Parmesan & Yohe 2003), urging researchers to conduct studies aimed at estimating species potential to adapt and survive. Thus, studies have been carried out to predict the consequences of climate change on plant species survival and community composition. Research has highlighted that the response of plant species to climate change can have different outcomes: plants can migrate compatibly with dispersal ability and resource availability (Walther *et al.* 2002); they can go extinct when their habitat have shrunk to a critical threshold (Parmesan 2006), or they can adapt to the new habitat conditions (Penuelas & Filella 2001; Walther *et al.* 2002; Parmesan 2006; Aitken *et al.* 2008) through e.g. phenological shifts or rapid evolution. Several approaches, spanning from field experiments and climate manipulations to large scale distribution models, have been used to tackle the question of how species will respond to a climatic shift. Statistical models have been built on the assumption that at large spatial scales species distribution can be well described by climate (Turesson 1930), and thus plant species will be able to track their climatic niche and to adjust their distribution range accordingly (Thuiller *et al.* 2008; Bellard *et al.* 2012). This assumption though is inaccurate, as it overlooks that a species realized niche is determined by the joined action of climate and other non-climatic factors (Morin *et al.* 2007; Lavergne *et al.* 2010) such as life strategies, local adaptation and biotic interactions (Davis *et al.* 2005; Lavergne *et al.* 2010). Thus, by ignoring these parameters, the complex interplay between ecological responses and evolutionary processes is virtually unexplored (Thuiller *et al.* 2008).

The issues associated with predictions that rely solely on climate emerge particularly when focusing on regional scales, where the influence of non-climatic factors on species response becomes especially relevant (Joshi *et al.* 2001). In such cases reciprocal transplants experiments and climate manipulations have provided

insight on the complexity of mechanisms influencing plants response to climate change. For example it has been shown that plants life strategies (Salguero-Gomez *et al.* 2012) or the degree of local adaptation and genetic diversity (Etterson 2004; Sambatti & Rice 2006; Jump *et al.* 2009) can have a strong impact on species persistence under a changing climate. Particularly, the study of populations distributed across ecological gradients has shown that genetic diversity, being the 'raw material of evolution', is fundamental to ensure adaptation to a wide range of abiotic conditions (Jump *et al.* 2009), and that conversely, narrow local adaptation can have deleterious effects on overall fitness and adaptive potential (Sambatti & Rice 2006). As such, the study of local adaptation and biotic interactions allows to integrate our understanding of evolutionary processes and ecological response (Parmesan 2006) and can thus shed light on the complexity of species responses to climate at regional scales. Regrettably, local adaptation has rarely been integrated in climate change studies despite the growing awareness of its importance both in the context of plant evolution and climate change.

Local adaptation

Local adaptation is defined as an adaptive differentiation in response to local environmental conditions (Galloway & Fenster 2000). Species adaptive responses can manifest either through genetic variation, determined by mutations or selection of genotypes (Salamin *et al.* 2010), or through plasticity which occurs as short term response within an individual's lifetime (Charmantier *et al.* 2008). In absence of other constraints, the interaction between genotype and environment determines selection of traits so that confer higher fitness to a population in its home environment than to populations from other habitats (Kawecki & Ebert 2004). However, species are not isolated entities, and their performance and distribution are affected by their interaction with the surrounding plant community (Brooker & Callaghan 1998) and with soil biota (Van der Putten *et al.* 2010). The outcome of such interactions and the consequent fitness of a species throughout its distribution range are affected by the trade-off between abiotic stress and biotic interactions (Keddy 1989; Brown *et al.* 1996; Guisan & Zimmermann 2000).

An optimal approach to study local adaptation is through reciprocal transplants, which aim to evaluate the performance of different ecotypes by

reciprocally sowing them in home and away sites along an ecological gradient. Hence, by indirectly manipulating climatic conditions it is possible to investigate whether or not a population may persist under predicted scenarios of climate change. Reciprocal transplant in the field are ideal to uncover the often unpredictable array of interactions occurring in natural systems, especially given the inherent difficulties of recreating them artificially. Concurrently, greenhouse experiments offer the possibility to monitor the fitness of different ecotypes under controlled conditions excluding potentially confounding effects that cannot be accounted for in the field (Gibson *et al.* 1999). Therein, a general overlook on the multiple processes affecting plant performance can be complemented by honing in on specific factors of interest that couldn't be otherwise disentangled under natural conditions.

The general prediction in local adaptation studies is that if local adaptation exists, a genotype will perform better in its home environment compared to any other genotype originating from a different habitat (Kawecki & Ebert 2004). A home-site advantage can be assessed in two different ways: either by comparing the performance of the 'local' genotype with a 'non-local' one, or by comparing the performance of a genotype in its 'home' environment as opposed to its 'away-from-home' environment (Kawecki & Ebert 2004). Even though the first method is often claimed to be the most appropriate in traditional local adaptation studies, the aim of the present research is to assess how local adaptation can affect plant fitness in novel environmental conditions, and thus we adopted the second criteria.

Traditionally, local adaptation experiments have mainly focused on abiotic variables disregarding that, when in a new habitat, an ecotype experiences not only a new climate, but also a new community of neighbours (Callaway 1992) and new abiotic (Olsson & Agren 2002), as well as biotic soil conditions (Kulmatiski *et al.* 2008). Despite some studies have combined reciprocal transplants with neighbour manipulations (e.g. Ariza & Tielbörger 2011) or with soil transplants among sites (e.g. Link *et al.* 2003), very few have adopted an integrated approach (Biere & Verhoeven 2008) addressing more than one type of biotic interactions simultaneously.

Biotic interactions and gradients of stress

Despite the evident interdependence between plants and soil, plant ecology and soil ecology have been regarded as separate disciplines for a long time (Wardle *et al.* 2004). Consequently, knowledge about soil processes has been scarcely available to plant ecologists and *vice-versa*. Obviously, such a separation has hampered the necessary integration of the two fields, which is fundamental to grasp the complex interaction between above-ground and below-ground processes. Only over the past few decades the study of soil community has been closely integrated into plant ecology research (Bardgett *et al.* 2005) beyond the simple measurement of soil properties and their correlation to plant performance. A further step toward the integration between the two fields is offered by the study of local adaptation that relatively recently has focused on biotic interactions alongside abiotic factors.

Research on plant-plant interactions along ecological gradients delivered the Stress Gradient Hypothesis (SGH) which predicts that the outcome of plant-plant interactions spans from competition, under favourable conditions, to facilitation, under stressful conditions (Bertness & Callaway 1994). Because of the different methodologies employed, and the diverse habitats and species addressed, the outcome of such studies has not been unambiguous. Some studies validated the predictions of SGH (e.g. Jurjavcic *et al.* 2002; Schiffers & Tielbörger 2006; Stanton-Geddes *et al.* 2012), whereas others found competition to be the prevalent interaction at both ends of the gradient (Maestre & Cortina 2004). The explanation that has been provided for such discrepancies is that competition and facilitation are two forces coexisting in nature and the observed outcome is the net result of their interaction, but their increment along ecological gradients may not be linear (Malkinson & Tielborger 2010). Facilitation occurs when a plant, defined as 'nursing', provides nutrient or alleviates the level of stress for another plant defined as 'nursed' (Bertness & Callaway 1994). Such kind of interaction is generally stronger when nursing and nursed plants belong to different life forms or life stages such that they are likely to exploit a different set of resources (Callaway 1995; Callaway & Walker 1997; Brooker *et al.* 2008; Leger & Espeland 2010). Additionally, several studies have

shown that beyond a certain threshold, the level of stress becomes too intense for the nursing plant to facilitate other plants, so that, even under stressful conditions the dominant interaction observed is competition (Michalet *et al.* 2006; Forey *et al.* 2010). Therefore, depending on the species, the life stage targeted, the level of stress experienced by the system and the considered measure of fitness, studies can address the same question but produce dissimilar results (Maestre *et al.* 2009).

It is even less straightforward to predict how plant-soil interactions change along ecological gradients, as their interactions are much more varied. Soil communities are characterized by an enormous variety of organisms with different functions and degree of species-specificity, so that it is difficult to disentangle the effect of each organism on different species in a community (Ettema & Wardle 2002). Parasite, pathogens and root herbivores remove nutrients from the soil with a consequent negative effect on plant growth (Bever *et al.* 1997), in contrast mycorrhizal fungi can provide access to rare nutrients (Bolan 1991), while the effect of soil microbes spans from mutualistic to pathogenic (Reynolds *et al.* 2003). Additionally, the abundance and the effect of pathogens and mycorrhizal fungi depend on the identity of species within a community (Grime *et al.* 1987; Bezemer *et al.* 2003; Klironomos 2003). Thus soil community composition is tightly related to plant community structure (Grime *et al.* 1987; van der Heijden *et al.* 1998). Despite the difficulties that such gamut of organisms and processes pose to studying the interaction between soil biota and plants performance, some trends have been outlined. Productive soils harbouring a rich plant community are associated with a likely dominance of negative plant-soil feedback (Kardol *et al.* 2006; Kulmatiski *et al.* 2012), conversely unproductive soils with a poor plant community are more likely to be characterized by positive plant-soil feedback (Reynolds *et al.* 2003). Therefore, when considering simultaneously the effects of plant-plant interactions, plant-soil interactions and abiotic filters on plant performance, it is possible to disentangle different components that until now have never been regarded separately in the framework of local adaptation.

Plant functional groups

Studies addressing communities are highly needed in ecology, where the majority of the research is conducted at small scale (Kareiva & Anderson 1988). In particular, when trying to predict how a community will respond to climate change, one of the main challenges is represented by the lack of knowledge on how single species will respond, thus a way to tackle the problem is to aggregate species into functional groups (Smith *et al.* 1993), that encompass the large diversity of species into a limited number of traits (Woodward & Cramer 1996). A functional group can be defined as a non-phylogenetic classification in which organisms that show similar responses to certain environmental factors are assembled (Smith *et al.* 1993). Over the past decades, several classifications have been proposed; Root (1967) defined as 'guilds' groups exploiting the same kind of resources or sharing similar niche requirements; Stebbins (1974) referred to common phylogenetic origin, Friedel *et al.* (1988) based its classification on the modality of response to a perturbation and Grime (1977) relied on strategies. The objective of such efforts was to reach a common ground for integrating the study of natural processes at different scales. Particularly, at the community scale, the use of a trait based approach is a powerful strategy that allows to visualize patterns and to predict the direction of responses. As such, assessing the degree of resilience of each functional group to a potential climatic shift, would allow casting predictions on the response of the entire community to environmental change.

Thesis objective and outline

The main scope of this thesis was to investigate the effect of abiotic and biotic factors in determining plant fitness and local adaptation in the Eastern Mediterranean region. Such scope was achieved through an integrated approach that investigated the combined effect of two different types of biotic interactions (plant-plant and plant-soil) and two abiotic filters (climate and soil properties) on plant fitness. The study was carried out during two subsequent years, first in the field and then in a greenhouse. The results of this research are particularly relevant because of the relatively novel approach and the originality of the results. We show how in our system the effect

biotic interactions on plants fitness was prominent compared to climate alone; furthermore we stress the importance of a multispecies approach and provide suggestions and guidelines for future studies on plants response to climate change. Our experimental set up, consisting of reciprocal seed sowing and soil transplants combined with neighbour removal treatments, allowed to investigate the effect of multiple factors on both single species and communities without the confounding effects often arising in traditional reciprocal transplant studies.

The second and third chapters focus on the response of four plant species to abiotic and biotic factors, and examine species-specific strategies and behaviour. The fourth chapter delves into the effect of climate and soil on entire plant communities and resorts to the use of functional groups to draw predictions on community responses to climate change.

The final chapter summarizes the overall results, discussing the main implications of the entire project and outlining future possible lines of inquiry. In order to allow independent reading of single chapters, each of them was written individually, thus causing in some segments repetitions of some background information.

Chapter 2 – Separating abiotic and biotic components of local adaptation – an experimental approach exemplified with drylands annual plants

The second chapter examines the strength and direction of plant-plant interactions and plant-soil interactions along an ecological gradient under different climates. A reciprocal seed sowing and soil transplant of two ecotypes was performed along a steep climatic gradient, and to further evaluate the role of plant community, neighbour removal treatments were added. The effect of climate on plant reproductive output was negligible compared to the role of biotic interactions and soil. In general the response of plants to abiotic factors and biotic interactions suggested no local adaptation, but rather an adaptation to the highly unpredictable environment.

Chapter 3 - An integrated approach to the study of biotic interaction for plants performance

The third chapter examines the effect of neighbours, soil abiotic and biotic properties, on plants performance under optimal conditions. A greenhouse experiment was set up to replicate the field experiment (Chapter 2), with the addiction of soil

treatments in order to separate the effect of soil biota from soil abiotic properties. Without the main limiting factor (i.e. water availability), the effect of soil biota on plant performance was larger than soil abiotic properties. Additionally, highly species-specific responses of species to soil biota were highlighted.

Chapter 4 - Effect of soil and climate on community composition along a steep climatic gradient in the Eastern Mediterranean region

The fourth chapter looked at the composition, richness and density of plant communities emerging from two different soil origins, either Mediterranean or semi-arid, transplanted in home and away climate conditions. By adopting functional groups based on species rainfall niche the role of soil and climate in community composition changes was further clarified and some general predictions about short term responses to the predicted climatic shift were drawn. Despite the abundance of functional groups changed according to climate conditions, the results suggest that the community may be able to buffer a climatic shift in the short period, provided that periodical rainy years occur in between drier ones.

Chapter 2

Separating abiotic and biotic components of local adaptation - an experimental approach exemplified with dryland annual plants

Abstract

At regional scales, the response of plant species to changing environment is influenced by both abiotic factors and biotic interactions. Therefore, understanding how such factors interact along ecological gradients is of essence. Many studies have focused on plant-plant interactions or plant-soil interactions alone, but rarely adopted an integrated approach. We performed a reciprocal seed sowing and soil transplant experiment combined with neighbour manipulations along a steep climatic gradient in Israel. Four winter annual species representing different taxonomic groups were selected for this study. The results show that biotic interactions were prominent compared to climate. In particular, plant-soil interactions were important to plant performance and their effect was likely determined by both abiotic properties and soil biota; furthermore neighbour interactions were always negative. Overall, there was no indication of local adaptation to climate, neighbours or soil. In general, besides illustrating the importance of including biotic interactions in local adaptation studies, the present study suggests that in the short term plants might be able to successfully withstand the impact of climate change. Such conclusions though need to be considered with caution because our results show also that, despite not having a direct influence on plants performance; climate affects the outcome of biotic interactions and eventually plant fitness.

Introduction

Plant species distribution at large scales can be well predicted by climate (Turesson 1930). Therefore, intuitive and simple predictions of species distribution under climate change have focused on matching future climate maps with the currently observed climatic niche (Thuiller *et al.* 2008). However, the realized distribution is not only a function of climate but of many other abiotic variables and, more importantly, biotic interactions with other organisms of the same or different trophic level (Lavergne *et al.* 2010). Also, studies relying only on the distribution range of a species may erroneously associate a wide distribution with large tolerance to climate change, disregarding local adaptation and its influence on the potential of a species to adapt to a changing environment (Etterson & Shaw 2001). For example, it has been shown that narrow local adaptation may impede a species' performance under new habitat conditions (Sambatti & Rice 2006), indicating that the study of local adaptation is of crucial importance for determining a species' response to climate change. So far though, climate niche modelling has rarely distinguished the role of climate from biotic interactions. Nonetheless several studies pointed out that interactions with neighbour plants (Brooker & Callaghan 1998), and with aboveground and belowground biota (Van der Putten *et al.* 2010) may greatly affect the range of conditions to which species are adapted and must therefore be considered when determining the adaptive potential of plant species (Etterson & Shaw 2001; Lavergne *et al.* 2010).

Though most studies on local adaptation have focused on abiotic conditions (Antonovics & Bradshaw 1970; Nagy & Rice 1997), an increasing body of research has addressed the contribution of biotic interactions such as plant-plant interactions (Briones *et al.* 1998; Ariza & Tielbörger 2011) or plant-soil interactions (Macel *et al.* 2007; Schweitzer *et al.* 2008). However, despite recognition of the reciprocal and highly complex influence between plant-plant interactions and plant-soil interactions (Wardle *et al.* 2004; Bardgett *et al.* 2005; Reinhart & Callaway 2006), classical experiments have rarely attempted to disentangle the interaction types (Biere & Verhoeven 2008).

A standard way to investigate local adaptation in plants is to conduct reciprocal transplants with ecotypes originating in habitats of different conditions, where their performance is compared between 'home' and 'away' conditions (Galen *et al.* 1991; Linhart & Grant 1996). Such studies are ideal for revealing whether or not a home site advantage exists that would point to locally adapted ecotypes (Linhart & Grant 1996; Kawecki & Ebert 2004). Furthermore, reciprocal transplants allow to indirectly manipulate climatic conditions and to investigate whether or not a locally adapted population may persist under predicted scenarios of climate change. However, when an ecotype is transplanted into a new habitat it not only experiences a new climate, but it is also subject to a new community of neighbours (Callaway 1992) and new abiotic (Olsson & Agren 2002) as well as biotic soil conditions (Kulmatiski *et al.* 2008). Therefore, a handful of studies has combined reciprocal transplants with neighbour removal treatments (Ariza & Tielbörger 2011) or soil transplants among sites (e.g. Link *et al.* 2003). However, no study to date has attempted to look at the combined effect of neighbours and soil on plants fitness and separate it from climate effects. Yet, such studies are crucial for making realistic estimates of plant response to climate change, because even when fully tracking its climatic niche, a plant will be inevitably confronted with novel biotic interactions, which may determine whether or not the plant may persist. Such a scenario is a very likely possibility as it is known that climatic conditions and intensity and direction of biotic interactions are closely related (Keddy 1989).

Community ecological theory may help predicting how climate change will influence biotic interactions and consequently plant performance. For example, it has been shown that intensity and direction of plant-plant interactions change along stress gradients (Callaway & Walker 1997). The degree to which such changes are predictable is still debated (e.g. Maestre *et al.* 2009), but a commonly used assumption is that competition prevails at the favourable end of an ecological gradient and facilitation dominates at the stressful end (Bertness & Callaway 1994; Brooker & Callaghan 1998). Therefore, depending on whether or not climate change aggravates abiotic stress, there would be either an increased dependence on positive interactions (e.g. in dry and hot environments with scenarios of decreasing precipitation), or an increased probability of competitive exclusion (e.g. in cold and wet environments with increasing temperatures).

Similarly, previous studies on plant-soil feedback showed that such interactions span from negative to positive according to soil productivity and successional stage (Reynolds *et al.* 2003; Kardol *et al.* 2006). Namely, in unproductive soils with a sparse plant cover, the soil community has been shown to be less abundant and diverse and positive plant-soil feedback to be more common (Reynolds *et al.* 2003); conversely in fertile soils, characterized by a denser plant community, the soil community has been shown to be extremely rich and plant-soil feedback more likely to have negative outcomes (Kardol *et al.* 2006; Kulmatiski *et al.* 2012). Thus, ecotypes from favourable environments are more likely to experience an away-from-home-soil advantage when transplanted, because of release from natural enemies, whereas ecotypes from harsh environments are likely to experience a home-soil advantage. In addition, such responses may be highly species-specific because some plant groups depend much less on positive soil effects such as mycorrhizal interactions (e.g. Brassicaceae, Newman & Reddell 1987). Tests for local adaptation to climate that ignore biotic interactions and their specificity could therefore be entirely misleading; either indicating increased or decreased performance under simulated climate change. Because climate change can have different impacts on different types of biotic interactions and possibly decouple them (Gonzalez-Megias & Menendez 2012), integrated studies targeting the role of both types of biotic interactions in determining local adaptation are urgently needed.

Here, we used a system that combines a number of desirable properties for conducting such a complex study. The experiment was performed in Eastern Mediterranean annual plant communities along a steep climatic gradient with high species richness and conservation concern (Sala *et al.* 2000), an attested high probability for decreasing precipitation (Smiatek *et al.* 2011), a large dependence of plants on rainfall and thus high responsiveness to change (Sala *et al.* 2000). We selected plant species with a wide distribution range and considerable ecotypic differentiation with respect to climate (Feinbrun-Dothan 1986); furthermore, the annual life cycle greatly facilitated the manipulation of the entire plant community. In order to separate the various abiotic and biotic factors for local adaptation of four different plant species, a field reciprocal transplant experiment combining climate (semi-arid *vs*. Mediterranean), neighbours (present or absent), soil and seed origin (semi-arid *vs*. Mediterranean) was set. The following hypotheses were tested: 1) ecotypes from low stress environments perform better in away-from-home soil, as

opposed to ecotypes from stressful environments that perform better in home soil; 2) plants experience stronger competition when climatic conditions are favourable (wet) than when they are stressful (dry); 3) ecotypes from favourable environments will have a higher competitive ability than ecotypes from dry environments; 4) plants perform better under home climate conditions, irrespective of biotic interactions

Methods

Study Area

The study was conducted along a steep North-South climatic gradient in Israel (Appendix 1) at two sites with Mediterranean and semi-arid climate, in areas of approx.100 m x 250 m each. The sites share the same calcareous bedrock, southern aspect, elevation and mean annual temperatures, and differ only in average and variation of annual rainfall, and in vegetation composition (Holzapfel *et al.* 2006). The Mediterranean site (M), is located south west of Jerusalem (N 31° 42' E 35° 3') at the elevation of 620 m a.s.l. on Terra Rossa soil and receives an average of 540 mm annual rainfall. The semi-arid site (SA) is situated about 30 km north east of Be'er Sheva (N 31° 23' E 34° 54') in the Northern Negev, at 590 m a.s.l., on Light Brown Rendzina soil. Mean annual rainfall is 300 mm.

At both sites, rainfall varies greatly among years, with a coefficient of variation of 31% at the M site and 38% at the SA site, respectively (Lampei & Tielborger 2010). The soil texture is characterized by an increased proportion of clay at the M site (Zwikel *et al.* 2007), resulting in a higher water holding capacity (Baver 1956; Zwikel *et al.* 2007) and a higher wilting point (Saxton *et al.* 1986).

The vegetation at both sites is characterized by shrublands dominated by the dwarf shrub *Sarcopoterium spinosum* (L.) and a community of winter annuals with partially overlapping species composition between the two sites (Tielbörger et al, unpublished data). Annual plant cover is 60% at the M site and 10% at the SA site (Schiffers & Tielbörger 2006; J. Kigel, unpublished observations).

Species

The four native winter annuals chosen as target are widely distributed across Israel (Feinbrun-Dothan 1986), reach high abundance at both study sites and belong to different taxonomic groups with potentially different response to biotic interactions: *Biscutella didyma* (L.) is a Brassicacea flowering between February and April, and belongs to a non-mycorrhizal family (Gerdemann 1968); *Urospermum picroides* (L.) F.W. Schmidt is a Composita flowering between March and May, whereas *Brachypodium distachyon* (L.) P. Beauv. and *Stipa capensis* Thunb. are both Poaceae flowering between March and May. *B. didyma*, *B. distachyon* and *U. picroides* are characterized by Mediterranean distribution, whereas *S. capensis* has a desert distribution.

Experimental set up

To test for local adaptation to neighbours, soil and climate, we set up a reciprocal seed sowing and soil transplant experiment between M and SA sites, while removing neighbours manually where planned. At each site, the experiment was set up with ten replicates in a full factorial manner with seed origin (M, SA), soil origin (M, SA) and neighbours (presence, removal) as factors, resulting in eight treatment combinations at each site (Fig 1).

In spring 2010, seed sampling of roughly 80 randomly chosen individuals for each species was carried out at both sites. At least 10 seeds per individual were sampled and each seed family was bagged individually. In July 2010, we excavated soil from eighty plots per site with a surface area of 400 cm^2, a depth of 3 cm each and a minimum distance of 20 cm. Heavily disturbed areas, large rocks and patches under shrubs were avoided to minimize confounding effects. The species pool, dominated by winter annuals that are stored as seeds in the soil seed bank during the dry season, could be transferred together with their soil without any damage to plants, seeds and soil organisms, as both seeds and soil organisms are inert in the dry season. Because small-scale heterogeneity in plant abundance is substantial (Siewert & Tielborger 2010) and may mask subtle treatment effects, the removed soil from each origin was homogenized. Soil and seeds were stored in a net house in Rehovot (Hebrew University of Jerusalem, Israel) to over summer. After the summer, the soil

was taken back to each field site, where half of the plots were selected randomly to be filled with M soils and the other half with SA soils.

Each plot was divided into four equally sized sections; at the centre of each we sowed ten seeds of each species randomizing the position of the species within the plots. Based on an established procedure, seeds of individual seed families were sowed within a 5 cm diameter plastic ring to avoid secondary seed dispersal during the first major rainstorm (see Ariza & Tielbörger 2011). Additionally, to prevent contamination from the surrounding environment, the surface of the plots was covered with a layer of organza, a see-through synthetic fabric, which was removed shortly after the first major rain, when soil was settled. Previous experiments indicated that the organza cover neither inhibited germination nor altered the amount of water and light reaching the soil surface (Petrů & Tielbörger 2008).
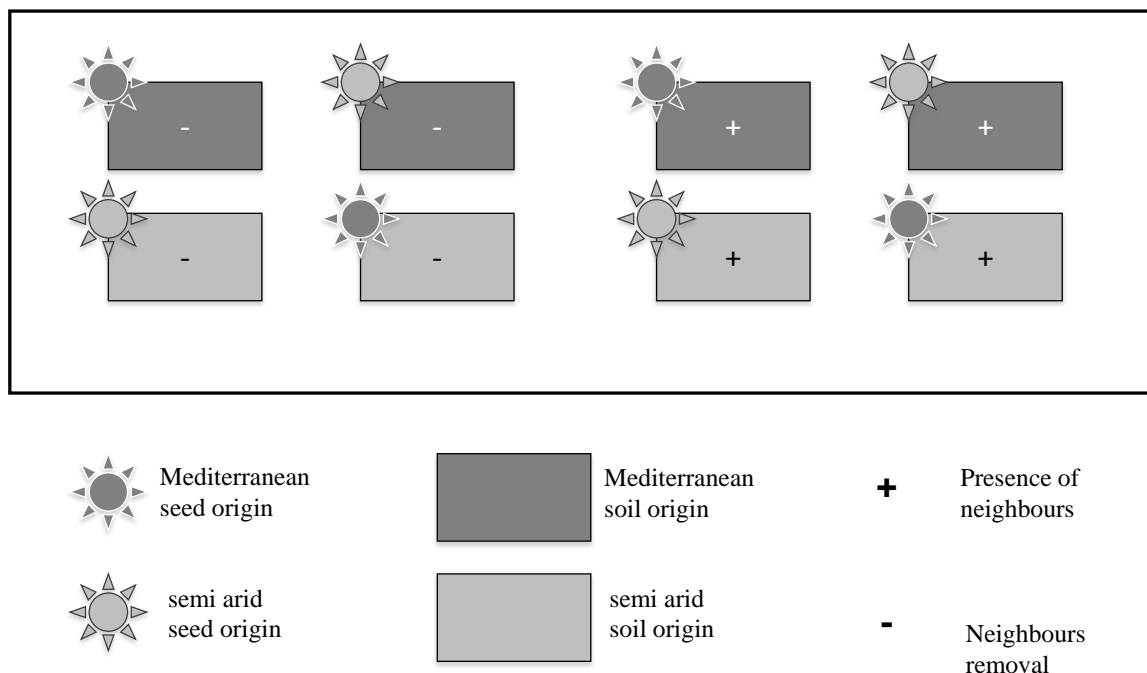

Figure 1: Graphic representation of a replicate/block in which each experimental unit is present. Each replicate was repeated ten times at each site. Each treatment was divided in different sectors where species were sown separately.

Germination of the first main cohort was monitored. After three weeks, when no more germination occurred, the plastic rings were carefully removed and all but one randomly chosen seedling, which served as the target for further observations, were removed as well. In the neighbour removal treatments, all individuals that emerged around the target from the soil seed bank were removed from the plots by

cutting the base of the stem with a scalpel so as to avoid disturbance to the soil or the plant roots. As all neighbours were annuals, they died immediately after being cut, such that there were also no below-ground plant-plant interactions. At the end of the reproductive season, the seeds produced by each target plant were collected. Seed production was chosen as response variable because it best corresponds to fitness (and thus best indicates adaptation), and it is unlikely to be influenced by maternal effects (Weiner *et al.* 1997).

The field study was carried out during the growth season 2010/2011, a particularly dry year, with the M and SA sites receiving only 66% (356 mm) and 60% (177 mm) of their long-term average rainfall, respectively.

Statistics

The hypotheses were tested separately for each species with seed production as response variable. Generalized Linear Models (GLM) with Poisson distribution and log link function were applied and over-dispersion was corrected for by scaling the data.

Climate, seed origins, soil origin and neighbour treatments were treated as independent variables. Full factorial models were employed in order to test the predictions and also to look for other potential relevant interactions. Then, to compare significant variables and their interactions post-hoc tests with multiple comparisons were performed using the least significant differences as a comparison method. All analyses were carried out using the statistical software IBM SPSS Statistics 19.

Source	B. didyma		B. distachyon		S. capensis	
	df	P	df	P	df	P
Main effects						
site	1	**.000**	1	.449	1	.090
neighbours	1	**.033**	1	**.000**	1	**.005**
seed origin	1	.311	1	.763	1	.418
soil origin	1	**.002**	1	**.000**	1	**.023**
Interactions						
site x neighbours	1	.658		.532	1	.557
site x seed origin	1	.717	1	.969	1	.794
site x soil origin	1	.378	1	.407	1	.077
neighbours x seed origin	1	.259	1	.385	1	.618
neighbours x soil origin	1	.226	1	**.037**	1	**.038**
seed origin x soil origin	1	.528	1	.687	1	.753
site x neighbours x seed origin	1	.571	1	.495	1	.754
site x neighbours x soil origin	1	.439	1	.814	1	.493
site x seed origin x soil origin	1	.362	1	.461	1	.506
neighbours x seed origin x soil origin	1	.711	1	.861	1	.384
site x neighbours x seed origin x soil origin	1	.464	1	.598	1	.228

Table 1: statistical table of results for GLM determining the effects of site, soil origin, seed origin and neighbour treatments on number of seeds produced by *B. didyma*, *B. distachyon* and *S. capensis*.

Results

Overall, the effect of climate was negligible for local adaptation when compared to the effects of soil and biotic interactions. Because of extremely low survival in the field, data of *U. picroides* were excluded from the analyses.

All three species performed significantly better in SA soil (Table 1, Figure 2), regardless of climate and seed origin. The interaction seed origin x soil origin though was not significant for any of the species.
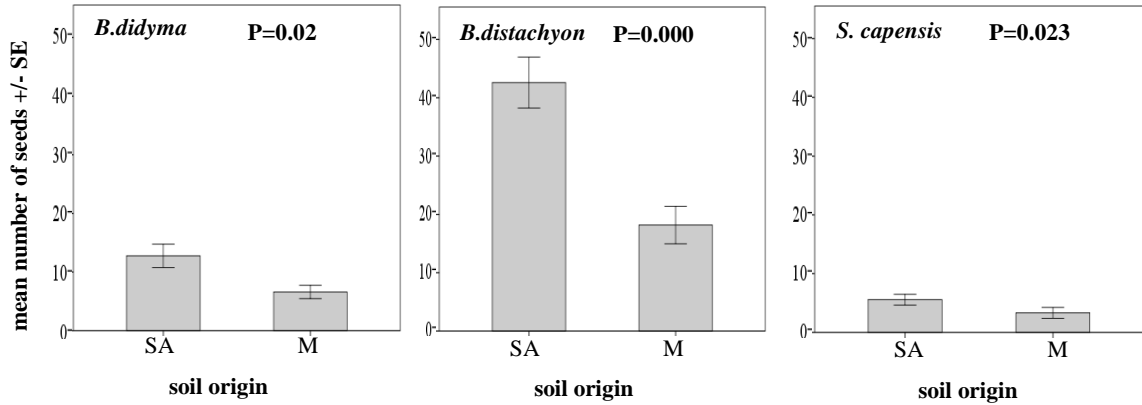
Figure 2: Average (+/- SE) number of seeds per plant in response to soil origins for the three target species. SA indicates semi-arid soil origins and M Mediterranean soil origins.
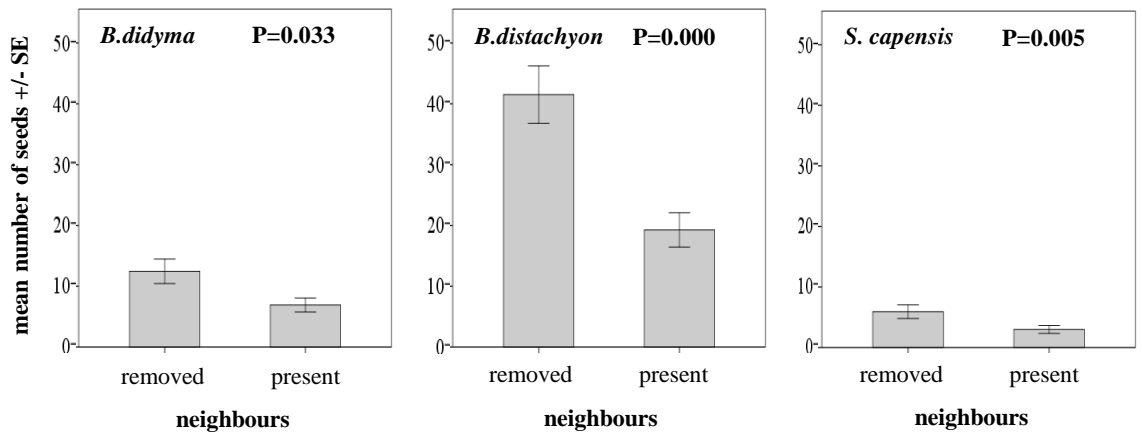

Figure 3: Average (+/- SE) number of seeds per plant in response to neighbour treatments for the three target species.

The presence of neighbours had a very consistent and significantly negative effect on seed production of all species (Table 1, Fig 3). This was unaffected by ecotype since there was no difference among seed origins in competitive ability (i.e. no significant seed origin x neighbour interaction).

Seed production did not differ among sites either (Table 1, Figure 4), but tended to be greater under M climatic conditions for all species (Table 1, Figure 5) even though this trend was significant only for *B. didyma*.
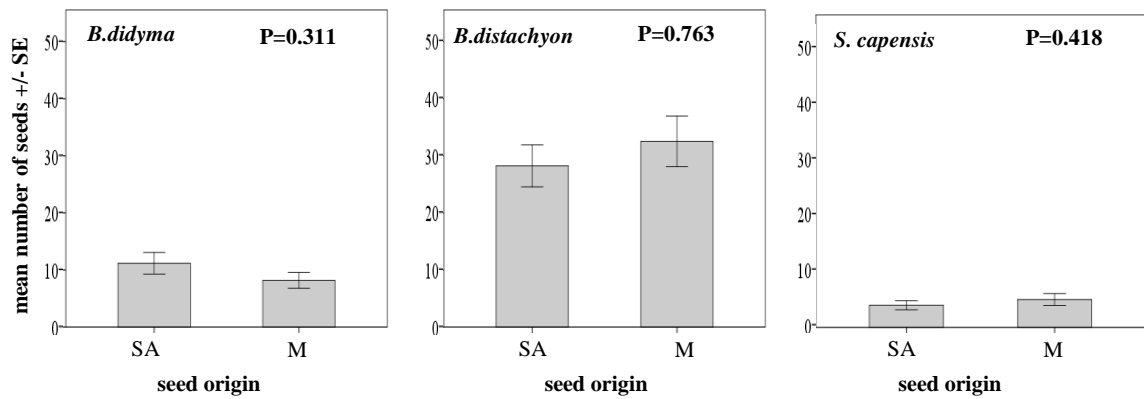
Figure 4: Average (+/- SE) number of seeds per plant in response to seed origins for the three target species. SA indicates semi-arid seed origins and M Mediterranean seed origins.
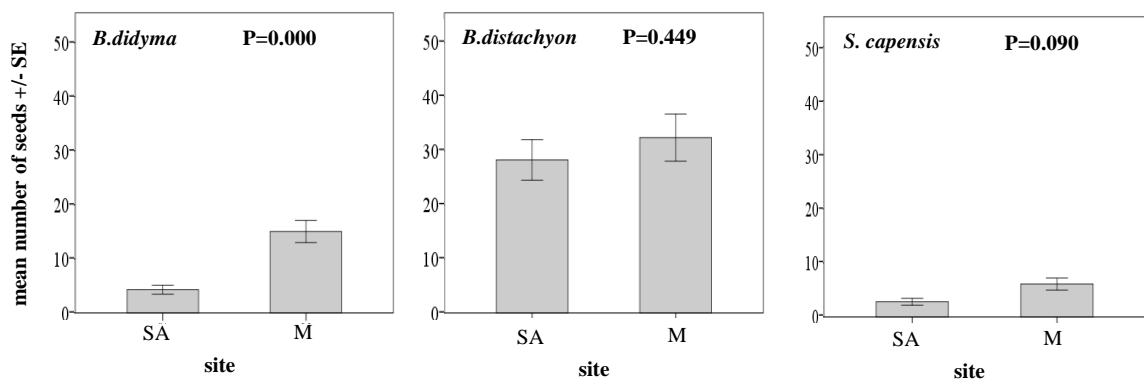

Figure 5: Average (+/- SE) number of seeds per plant in response to climate for the three target species. SA indicates semi-arid sites and M Mediterranean sites.

Additionally, there was no indication for local adaptation to climate, indicated by a lack of significant seed origin x site interaction (Table 1).

Finally, a significant soil origin x neighbour interaction for *B. distachyon* and *S. capensis* (Table 1, Figure 6), suggested that the negative effect of neighbour presence on target individuals was significantly larger in M soil origins. Conversely, *B. didyma* showed a different pattern, even though not significant, with a higher effect of competitive release in SA soil origins.
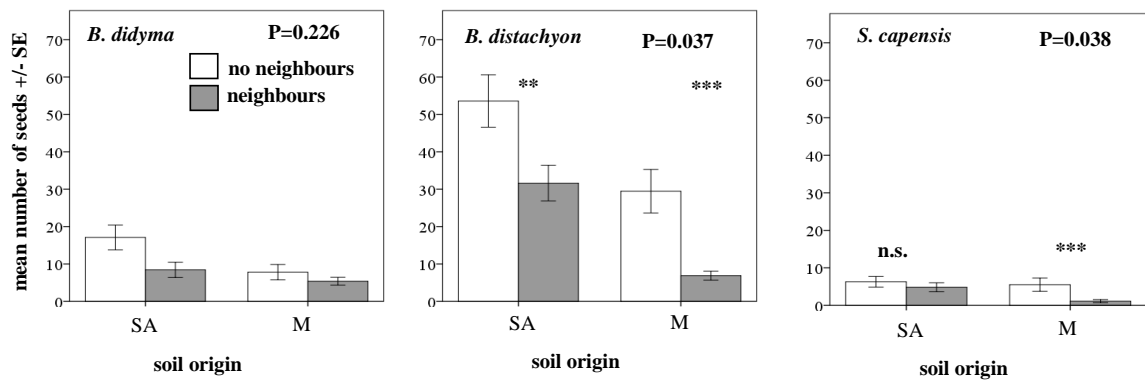
Figure 6: Average (+/- SE) number of seeds per plant in response to soil origins and according to neighbour treatment for the three target species. SA indicates semi-arid soil origins and M Mediterranean soil origins. Asterisks indicate significant differences according to post-hoc tests.

Discussion

In general the results of the present study show that climate was unimportant compared to biotic and soil effects. No local adaptation was found; on the contrary plants' response to climate suggested a relatively plastic behaviour that could have derived by an adaptation to the extreme climatic variability of the region. This indicates that in the short term species might be able to buffer the predicted climate change more successfully than originally thought. In the following, results will be discussed according to relevance, starting with climate, seed origins and moving onto biotic interactions with neighbours and soil.

Based on ecological theory and previous findings along a similar gradient (Liancourt & Tielborger 2011), we had expected that ecotypes from more favourable sites would have been better adapted to competition. However, the results obtained in the field contradict this hypothesis and might point at a more plastic response to abiotic and biotic factors than expected. The response to climate leads to a similar conclusion, suggesting a rather plastic response to climate more than an adaptation to local conditions. Only in the case of *B. didyma* there was a significant difference in performance between climates and in this case our results pointed to a benefit induced by the higher water availability at the M site. A possible explanation to such outcome resides in the reduced resistance to drought observed in non-mycorrhizal families (Auge 2001) such as Cruciferae.
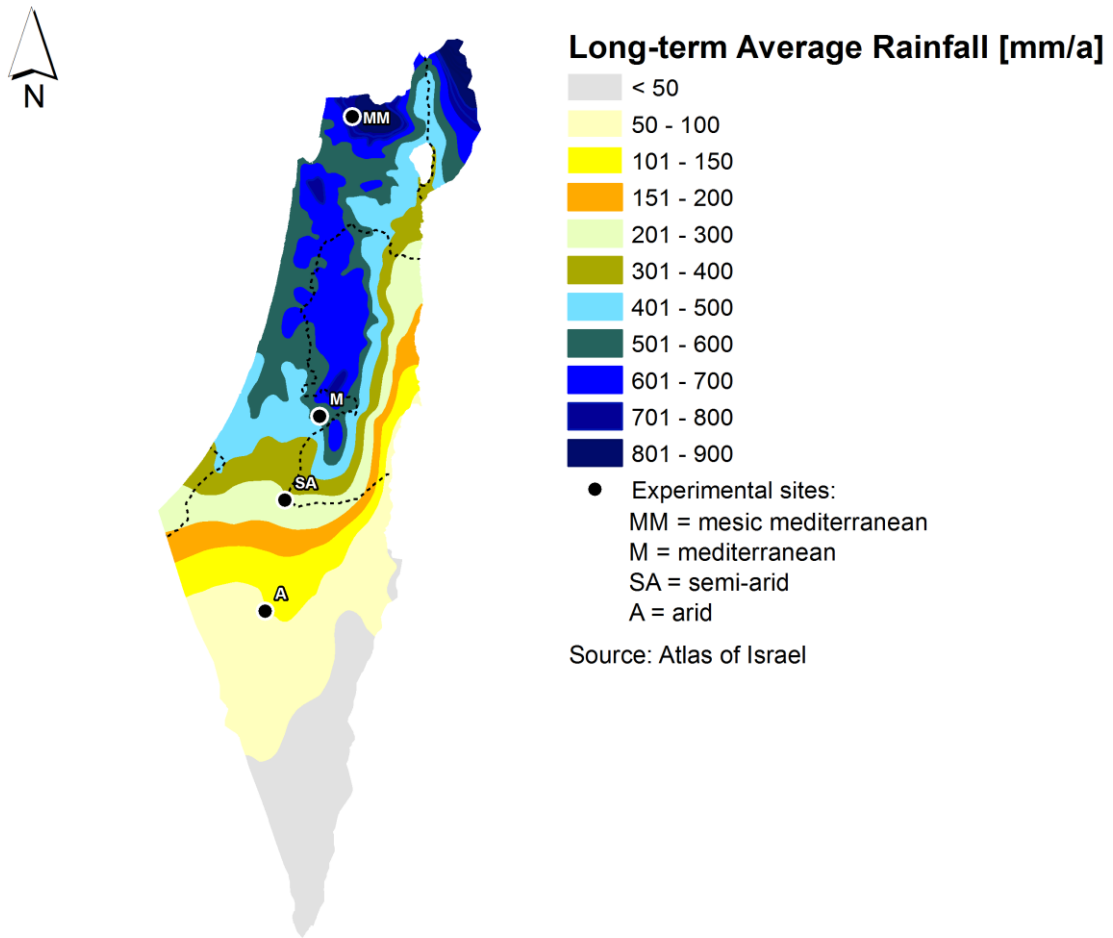
The presence of neighbour plants had a strong negative effect on seed production, clearly showing the importance of competitive interactions in plant performance. This result was unexpected because we would have anticipated that less intense competition or even facilitation would occur under more stressful conditions i.e. at the semi-arid site (Bertness & Callaway 1994) as demonstrated by several studies (Callaway *et al.* 2002; Jurjavcic *et al.* 2002; Stanton-Geddes *et al.* 2012). The prevailing effect of competition at both ends of the stress gradient, although counterintuitive, is not novel and has been previously explained. Under stressful conditions, the benefactor species itself may suffer from stress, which may in turn reduce its ability to act as facilitator (Maestre & Cortina 2004). Consequently, the net balance between facilitative and competitive effects may be in favour of the latter ones, so that only competition is observed (Michalet *et al.* 2006). Although previous studies conducted along our same gradient showed a shift from positive to negative net neighbour effects from the semi-arid to the Mediterranean site (Holzapfel *et al.* 2006; Schiffers & Tielbörger 2006), the particularly dry conditions of the year during which this field experiment took place might have exacerbated the effect of competitive interactions at both sites.

The unexpected interaction between soil origin and neighbours, showing higher competitive release in Mediterranean soils, could be an artefact caused by differences in seed density between soils, had we not found an opposite, yet not significant, trend in *B. didyma* that shows a higher competitive release in semi-arid soils. For that species, negative effects of the soil biota could be the main factor explaining why it performed so poorly in Mediterranean soils. This would indicate that negative plant- soil feedbacks were more important for determining the performance of *B. didyma* than competitive effects of neighbouring plants, pointing again to the importance of studying both types of interactions simultaneously.

The higher performance of species in semi-arid soils could be explained by the fact that plants in high stress environments are more likely to be facilitated by positive soil feedback and by a sparse soil community (Reynolds *et al.* 2003), whereas in richer plant communities their performance is hampered by higher interspecific competition, a richer soil community (van de Voorde *et al.* 2011; Kulmatiski *et al.* 2012) and generally stronger negative plant-soil feedback (Kardol *et al.* 2007). If so, Mediterranean seed origins would benefit from enemy release in semi-arid soil, and semi-arid seed origins would experience a home soil advantage. Additionally, the

higher clay content of Mediterranean soil is disadvantageous when water is limiting, because evaporation from fine-textured soils is much higher than from coarse soils (Salter & Williams 1965; Noy-Meir 1973; Petrů & Tielbörger 2008) and it increases water holding capacity, thereby reducing the amount of available water for plants (Baver 1956; Zwikel *et al.* 2007). Therefore, under field (i.e. dry) conditions, species performance may have been limited by drought in Mediterranean soils. Nevertheless our field experiment did not allow to clearly teasing apart abiotic soil properties and plant-soil biota interactions. Consequently, it is still unclear whether plant's enhanced performance in semi-arid soil origins was determined by the effect of climate on soil physical properties or by biotic interactions or by the combined action of both.

An extremely interesting finding of our study is the prominence of biotic interactions over climate. In general, we suggest that the apparent resilience to climate observed in all target species results from a pre-adaptation to the high climatic variability that characterizes both climates, i.e. plants have adapted to persist in a relatively unpredictable environment, which allows them to buffer for a deviation from the average conditions, at least in the short term. For example, in wet years climatic conditions observed at the semi-arid site may resemble the average rainfall amount for the wetter site or *vice-versa*. Thus, it is likely that plants have adapted to persist over a very large range of climatic conditions, and species might be able to endure better than expected a climatic shift over a short time scale. Nonetheless, experiments monitoring the performance of plants over a longer time span might be a good complement to our observations. In general, thanks to our integrated approach we highlighted the complex response of plant performance to different types of biotic interactions and stressed their importance to local adaptation. We therefore conclude that a number of interacting factors (such as soil properties, plant-plant and plant-soil biota interactions) will affect the potential of species to adapt. As suggested by previous research, predictions on future response to climate that rely on abiotic factors alone neglecting biotic interactions and species-specific requirements will most likely be inefficient (Davis & Shaw 2001; Jump & Penuelas 2005).

Long-term Average Rainfall [mm/a]

- < 50
- 50 - 100
- 101 - 150
- 151 - 200
- 201 - 300
- 301 - 400
- 401 - 500
- 501 - 600
- 601 - 700
- 701 - 800
- 801 - 900

● Experimental sites:
MM = mesic mediterranean
M = mediterranean
SA = semi-arid
A = arid

Source: Atlas of Israel

Appendix 1: Map of Israel showing the rainfall distribution and the climatic gradient. The map shows the clear climatic gradient with increasing rainfall from south to north. The two sites considered in the present study are marked by the letters 'M' (i.e. Mediterranean climate) and 'SA' (i.e. semi-arid climate)

Chapter 3

An integrated approach to the study of biotic interactions for plant performance

Abstract

Biotic interactions strongly affect local adaptation of plants and consequently their adaptive potential to novel conditions. However, studies on local adaptation that consider both abiotic and biotic factors often focus on only one kind of biotic interaction, e.g. plant-plant interactions or plant-soil biota interactions. Rarely so far, the study of biotic interactions has adopted an integrated approach. We investigated the effect of plant-plant interactions, plant-soil biota interactions and soil abiotic properties on the reproductive success of two ecotypes in four annual species originating from different climates. We tested the following hypotheses: **1)** plant fitness is increased in home soil when positive interactions are prevalent compared to negative ones; **2)** plant and soil community have an effect on plants performance, in particular under optimal conditions: a) the effect of soil biota on plant performance varies according to species-specific characteristics, e.g. mycorrhizal dependence, b) plants perform better in the absence of neighbours (competitive release); **3)** ecotypes differ in competitive ability, in particular ecotypes from favourable environments are better competitors than ecotypes from stressful environments.

Our results showed no differences in competitive ability between ecotypes. Additionally, under optimal abiotic conditions, the influence of biotic interactions on plant performance was prominent compared to abiotic factors and the outcome varied according to species. As such, future studies should focus on disentangling the combined effect of abiotic factors and biotic interactions on plant fitness.

Introduction

Biotic interactions are an important determinant of species realized niche and can have a fundamental role in population and community dynamics at small scales (Joshi *et al.* 2001; Lavergne *et al.* 2010). Previous studies conducted at regional and local scales have shown that biotic interactions such as plant-plant or plant-soil interactions affect plant fitness and their adaptive potential to future conditions (Petrů & Tielbörger 2008; Yang *et al.* 2011). The effect of the interactions between plants and their neighbours has been connected to availability of resources (Goldberg 1996; Goldberg *et al.* 1999), density of the community (Novoplansky 2009), life stage (Schiffers & Tielbörger 2006) and life form (Holzapfel *et al.* 2006) of the interacting plants, and plant competitive ability (Novoplansky 2009). Similarly, studies have shown that the effect of plant-soil interactions is constrained by soil properties (Snaydon & Bradshaw 1962; Antonovics & Bradshaw 1970; Bradshaw 1984), density and richness of the plant community (Kulmatiski *et al.* 2012) and species composition of soil biota (Wardle *et al.* 2004; Macel *et al.* 2007). Such diverse factors can affect the strength and direction of biotic interactions with a consequent spill over on plant fitness, nonetheless ecological research has largely underexploited the relevance of biotic interactions in ecological and evolutionary processes (Lavergne *et al.* 2010; Thorpe *et al.* 2011).

The notion that abiotic conditions, strength and direction of biotic interactions are deeply related (Keddy 1989; Callaway & Walker 1997) is not novel, although the degree of predictability of such relation is still debated (e.g. Maestre *et al.* 2009). Theory suggests that at the favourable end of a productivity gradient competition prevails, whereas at the stressful extreme facilitation is expected to dominate (Bertness & Callaway 1994; Brooker & Callaghan 1998). Such trade-off in the outcome of biotic interactions is often mirrored by different life strategies among plants (Grime 1977), so that in favourable environments a larger number of competitors is expected to occur, whereas under stressful conditions plants are more likely to exhibit high tolerance to stress.

Despite the plethora of studies conducted on soil microcosms, it is less straightforward to infer the outcome of plant-soil interactions compared to plant-plant interactions because of the vast number of microorganisms involved and the different degree of specialization characterizing each species of soil organism (van der Heijden *et al.* 1998; Bezemer *et al.* 2003; Wardle *et al.* 2004). Soil ecology has shown how productivity and successional stage of a plant community are linked to the prevailing outcome of plant-soil feedback (Reynolds *et al.* 2003; Kardol *et al.* 2006). In unproductive soils, often associated with a sparse plant cover, the soil community has been suggested to be less abundant and diverse, and positive plant-soil feedback to be more frequent (Reynolds *et al.* 2003). Productive soils instead are characterized by a denser plant community which harbours a relatively rich soil community and is characterized by prevailing negative plant-soil feedback (Kardol *et al.* 2006; Kulmatiski *et al.* 2012). According to theory, it would be logical to assume that ecotypes experiencing strong negative plant-soil interactions would benefit from enemy release and experience an away-from-home-soil advantage when transplanted into a new soil. Clear predictions though might be further complicated by the fact that such responses can be influenced by species-specific characteristics such as obligate dependence on mycorrhizal interactions (e.g. Brassicaceae, Newman & Reddell 1987).

As such, the overall performance of a plant is affected by the synergy between plant- plant interactions, plant- soil biota interactions and soil properties (Wardle *et al.* 2004). Therefore, knowing the effect of biotic interactions on species might help to fine tune predictions about their performance under changed conditions. A widely used approach to investigate the interplay between abiotic factors and biotic interactions is to conduct reciprocal transplant experiments in the field or under controlled conditions. Such experiments allow to compare the performance of ecotypes with and without neighbours (Brooker & Callaghan 1998; Ariza & Tielbörger 2011) or in different soils (Link *et al.* 2003). Both the field and the greenhouse approach present a series of advantages and shortcomings: in the field it is possible to monitor plant performance in their natural environment avoiding biases caused by imposed artificial conditions. However, greenhouse experiments grant a higher degree of control over abiotic and biotic conditions, thus managing a number of potentially confounding effects found in the field (Gibson *et al.* 1999). In particular, greenhouse experiments not only allow to single out the effect of the investigated

interactions, but also enable to focus on more subtle and complex interactions that would otherwise be overlooked or not properly disentangled.

Here, we used a system that combines a number of desirable properties for conducting such a complex study. We selected a steep climatic gradient in the Eastern Mediterranean region characterized by annual plant communities with high species richness and conservation concern (Sala *et al.* 2000) because of the attested high probability for decreasing precipitation (Smiatek *et al.* 2011). We selected four plant species with a wide distribution range and considerable ecotypic differentiation with respect to climate (Feinbrun-Dothan 1986); furthermore, the annual life cycle greatly facilitated the manipulation of the entire plant community. In order to assess the combined effect of different types of biotic interactions we set up a greenhouse transplant experiment combining seed origins (semi-arid *vs*. Mediterranean), soil origin (semi-arid *vs*. Mediterranean), soil- and plant- community treatments namely i) presence of neighbours + natural soil, ii) absence of neighbours + natural soil, iii) absence of neighbours + sterilized soil.

We hypothesized that under homogeneous conditions, **1)** seed origins perform better in home soil (home soil adaptation) when positive interactions are prevalent compared to negative ones, **2)** soil biota and plant community have an effect on plant performance, in particular under optimal conditions: a) the response of plants to soil biota varies according to species-specific requirements e.g. mycorrhizal dependence b) plants perform better in the absence of neighbours (competitive release), **3)** seed origins differ in competitive ability, in particular seed origins from favourable environments are better competitors than seed origins from stressful environments.

Methods

Seed origins

The seeds were collected in two sites along a steep North-South climatic gradient in Israel with Mediterranean (M) and semi-arid climate (SA), respectively. The sites, already used for a previous field study (see Chapter 2), have the same calcareous bedrock, are situated on slopes with southern aspect and share similar elevation and mean annual temperatures, whereas they differ in average annual

rainfall, vegetation composition and soil properties (Holzapfel *et al.* 2006). The Mediterranean site (M) is characterized by Terra Rossa soil, a mean annual temperature of 17°C and 540 mm average annual rainfall. The semi-arid site (SA) is located on Light Brown Rendzina soil and is characterized by a mean annual temperature of 18.4°C and 300 mm average annual rainfall. The two soil origins present texture characteristics classified as clay (M site) and clay-loam (SA site) (Zwikel *et al.* 2007), that under field conditions are associated to higher field capacity and higher wilting point for M soil (Saxton *et al.* 1986).

Species

We tested four native winter annuals that are widely distributed across Israel (Feinbrun-Dothan 1986) and abundant at both study sites. *Biscutella didyma* (L.) is a Brassicacea flowering between February and April and is not mycorrhizal (Gerdemann 1968; Newman & Reddell 1987); *Urospermum picroides* (L.) F.W. Schmidt is a Composita flowering between March and May. *Brachypodium distachyum* (L.) P. Beauv. and *Stipa capensis* Thunb. are both Poaceae flowering between March and May. *B. didyma*, *B. distachyon* and *U. picroides* are characterized by Mediterranean distribution, whereas *S. capensis* has a saharo-arabian distribution.

Experimental set up

We set up a greenhouse experiment focused on disentangling the role of biotic interactions for local adaptation. We combined seed origins (M, SA), soil origins (M, SA), and three community treatments comprising i) presence of neighbours + live soil, ii) neighbour removal + live soil, iii) neighbour removal + sterilized soil. Factors were mixed in a full factorial manner with the exception of sterilization and neighbor presence; such coupling was not possible because we used the natural seed bank present in each soil origin to recreate the community, which during the process of sterilization was destroyed. The full factorial combination resulted in twelve treatments that were replicated twelve times for each species.
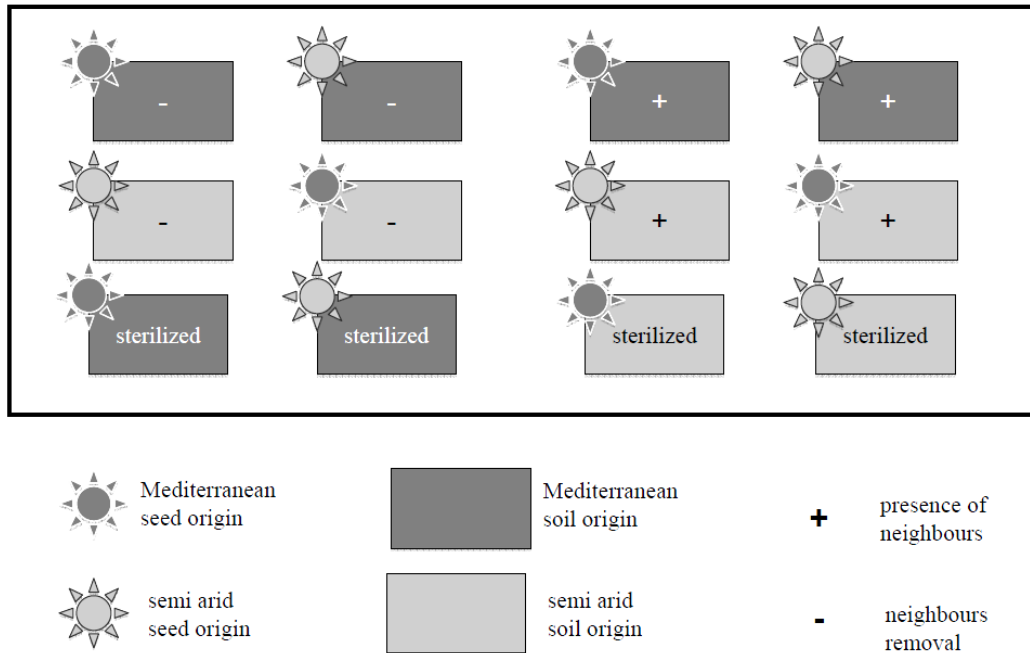
Figure 1: scheme of a block. The entire combination of treatments was applied to each species, so that a block would be composed of four sub-blocks. Each treatment was replicated 12 times for each species, with the result of 12 blocks.

During October and November 2011, the experiment was set up in a greenhouse at the University of Tübingen, Germany. Pots of 10x10x10 cm were filled with soil previously excavated at each site and over-summered in Israel. We excavated approximately 500 kg of soil up to a depth of 10 cm and kept separate the top layer (0- 3cm) which contained the seed bank, and the remnant soil from greater depth which was used for filling the bottom of the pots. Soil and seeds were stored in a net house in Rehovot (Hebrew University of Jerusalem, Israel) to over summer, and after such time they were shipped to Germany. To test the effect of the soil community we sterilized one third of the soil by autoclaving it at 121°C two times for a total of 48 hours, with a pause of 24 hours in between. In each pot, 5 seeds of a single species were sown within a plastic ring. The experimental set up was divided in twelve blocks, each corresponding to a replicate of all treatments combinations (Figure 1) repeated for the four species. The pots were placed 10 cm apart in order to avoid any interaction between plants of different treatments. The position of blocks and treatments within blocks was periodically randomized during the entire experiment. Once the set up was completed, all pots were watered to saturation and germination was monitored. After two weeks, when no more germination occurred,

the plastic rings were carefully removed and all but one randomly chosen seedling, which served as the target for further observations, were removed as well. In the neighbour removal treatments, all individuals emerging from the seed bank around the target were removed from the plots by cutting the base of the stem with a scalpel so as to avoid disturbance to the soil or the plant roots. As all neighbours were annuals, they died immediately after being cut, such that there were also no below-ground plant-plant interactions. Throughout the entire experiment temperature was kept at values consistent with the averages expected in the field.

In order to collect all diaspores and prevent accidental loss of seeds, organza bags were used to wrap all target individuals at seed set, except for *U. picroides* where manual collection of ripe achenes prior to dispersal was feasible.

Statistics

We first applied Generalized Linear Models (GLM) with Poisson distribution and log link function, using seed production as response variable against soil origin, seed origin and community (i.e. neighbours and soil biota manipulations) as independent variables. The data were scaled for over dispersion. To better investigate the influence of the community treatments, we split the data and analysed separately the effect of neighbour treatments in live soil and the effect of soil community in live versus sterilized soil. To compare significant variables and their interactions we then performed post-hoc tests with multiple comparisons using the least significant differences as a comparison method. All statistical tests were conducted using the software IBM SPSS Statistics 19.

Source	B. didyma		B. distachyon		S. capensis		U. picroides	
	df	P	df	P	df	P	df	P
Main effects								
Soil origin	1	**.000**	1	.444	1	.236	1	**.064**
Seed origin	1	.610	1	.745	1	.734	1	.716
Neighbours	1	**.000**	1	**.000**	1	**.000**	1	**.000**
Interactions								
Seed origin x soil origin	1	.488	1	.752	1	.962	1	.347
Seed origin x neighbours	1	.935	1	.784	1	.552	1	.710
Soil origin x neighbours	1	.465	1	**.000**	1	**.015**	1	.584
Seed origin x Soil origin x neighbours	1	.842	1	.777	1	.577	1	.154

Table 1: statistical table of results for GLM determining the effect of soil origin, seed origin and neighbour treatment on the number of seeds produced by *B. didyma*, *B. distachyon*, *S. capensis* and *U. picroides*.

Source	B. didyma		B. distachyon		S. capensis		U. picroides	
	df	P	df	P	df	P	df	P
Main effects								
Soil origin	1	**.001**	1	**.015**	1	.122	1	**.000**
Seed origin	1	.509	1	.470	1	.828	1	.181
Sterilization	1	**.017**	1	**.000**	1	.131	1	/
Interactions								
Seed origin x soil origin	1	.581	1	.872	1	.386	1	**.000**
Seed origin x sterilization	1	.159	1	.504	1	.812	1	/
Soil origin x sterilization	1	.103	1	.196	1	.485	1	/
Seed origin x Soil origin x sterilization	1	.312	1	.844	1	.965	1	

Table 2: statistical table of results for GLM determining the effect of soil origin, seed origins and sterilization treatment on number of seeds produced by *B. didyma*, *B. distachyon*, *S. capensis* and *U. picroides*.

Results

The effect of soil origin on plant performance varied according to species (Fig 2); *B. didyma* (Tables1, 2; Appendix 1) showed a higher performance in SA soil, whereas *U. picroides* (Tables A1, 1, 2,) and partly *B. distachyon* (Table 2) produced more seeds in M soil origins. Even though not statistically significant, also *S. capensis* showed a trend of higher seed production in M soil (Table 1, 2). A significant seed origin x soil origin interaction was found only for *U. picroides* which was consistent with the preference for M soil shown in the main effect (Table 2).
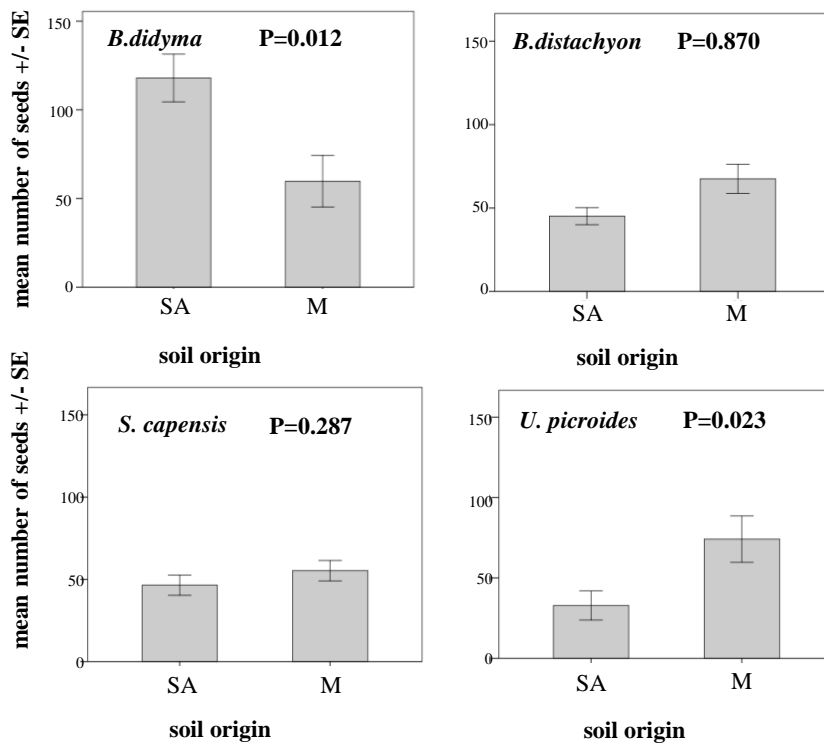
Figure 2: Average (+/- SE) number of seeds per plant in response to soil origins for the four target species. SA indicates semi-arid soil origins and M Mediterranean soil origins

Soil sterilization significantly affected seed production, but the strength and direction of the effect varied considerably between species (Table 4, Fig 2). *B. didyma* produced more seeds in sterilized soil, as opposed to *B. distachyon*, that produced more seeds in live soil, and *U. picroides* of which no individual survived to seed production in sterilized soil. *S. capensis* instead did not show any significant difference between treatments. The effect of sterilization did not vary according to soil or seed origin (i.e. no significant interactions between soil origin and sterilization treatment or between seed origin and sterilization treatment).
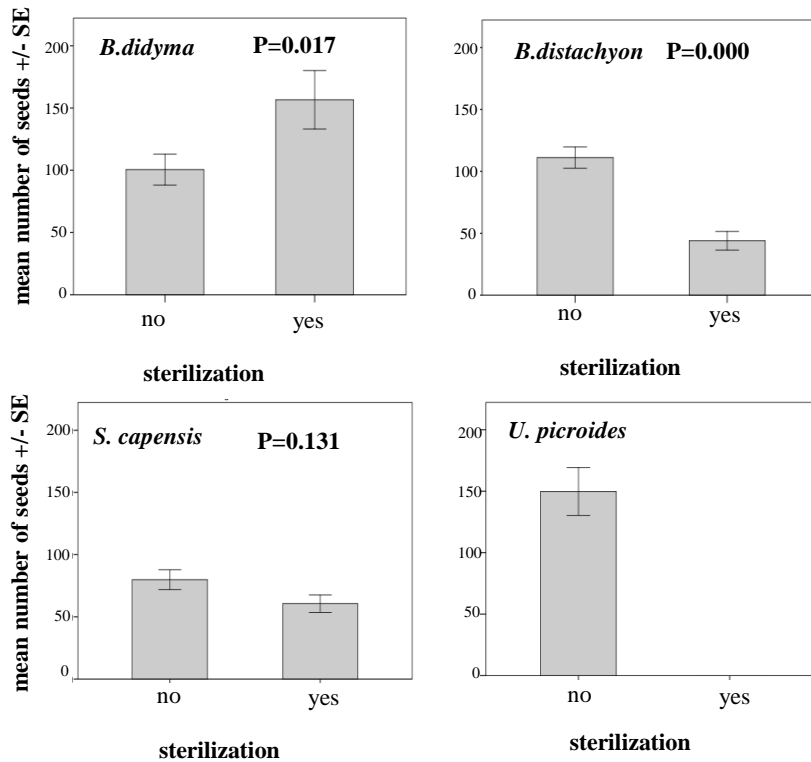
Figure 3 Average (+/- SE) number of seeds per plant in response to soil biota manipulation for the four target species. 'no' indicates natural soil, 'yes' indicates sterilized soil

The presence of neighbours had a significantly negative effect on seed production (Table A1,1, 2; Fig 4). In all species seed production was consistently higher in the absence of neighbours, regardless of seed origin. Seed production did not differ significantly across seed origins in any of the target species (Table A1, 1, 2, Fig 5), and the non-significant interaction between seed origin x neighbour indicated no differences in competitive ability between seed origins (Table A1, 1, 2).
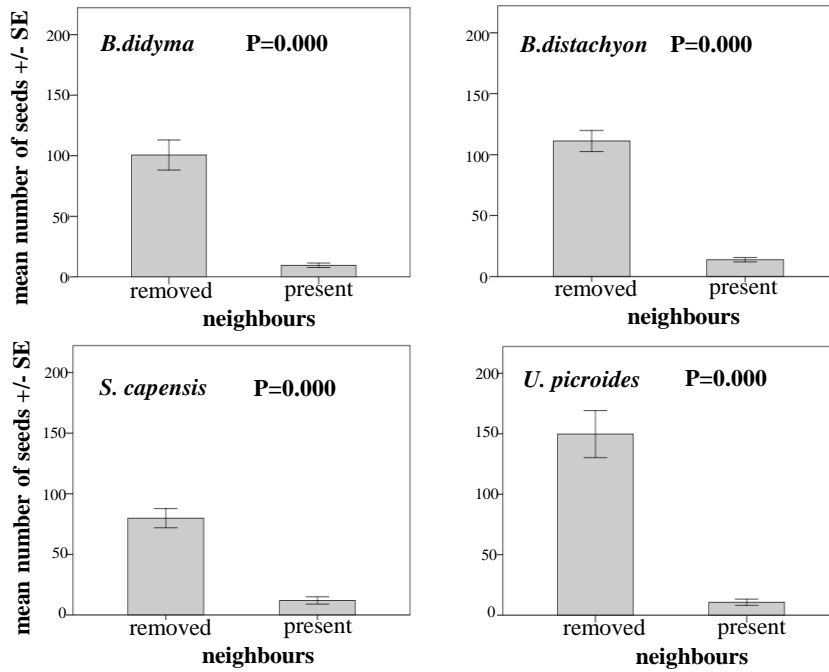
Figure 4 Average (+/- SE) number of seeds per plant in response to neighbour treatments for the four target species. 'Removed' indicated the absence of neighbour plants, 'present' indicates the presence of neighbour plants
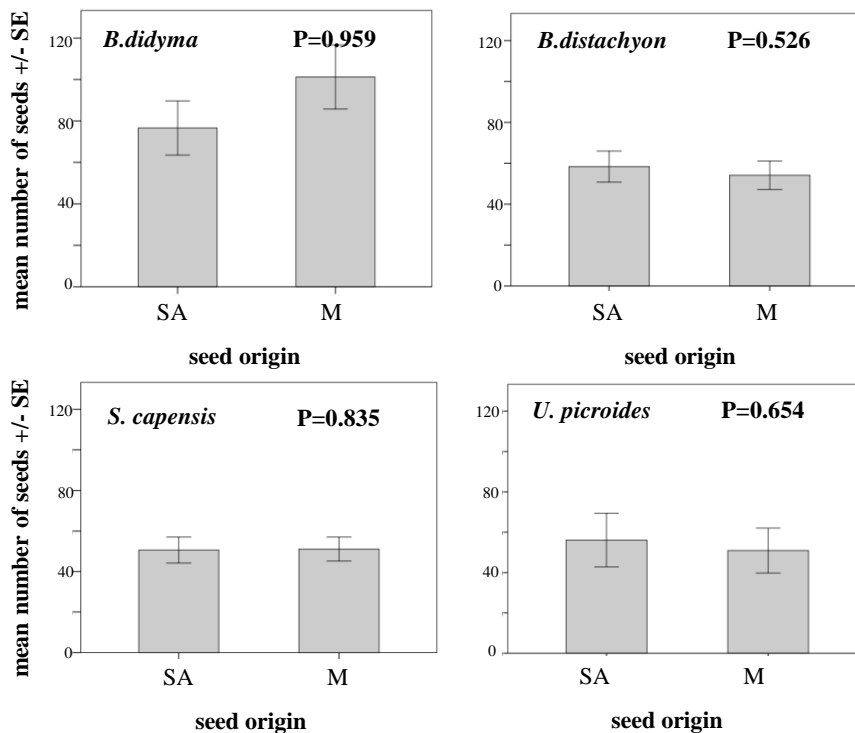

Figure 5 Average (+/- SE) number of seeds per plant in response to seed origins for the four target species. SA indicates semi-arid seed origins and M Mediterranean seed origins

A significant soil origin x neighbour interaction was observed for *B. distachyon* and *S. capensis* (Table 2, Figure 6), indicating a larger negative effect of

neighbours in M soil origins. Similarly, *U. picroides* showed a similar trend even though not significant, as opposed to *B. didyma* that showed a higher competitive release in SA soil, also in this case not strong enough to be significant.
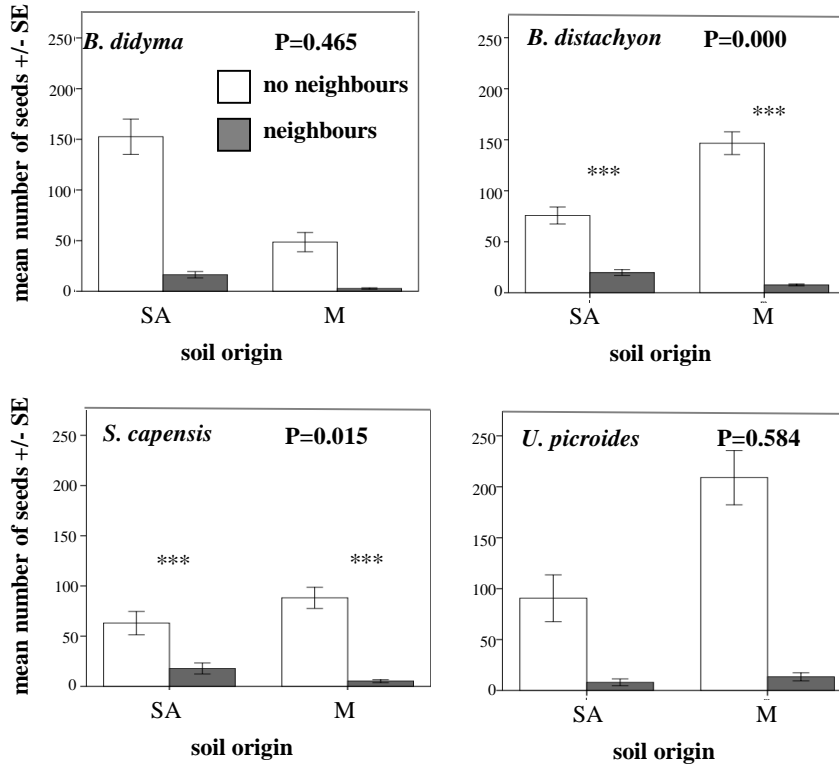

Figure 6 Average (+/- SE) number of seeds per plant in response to soil origins and according to neighbour treatment for the four target species. SA indicates semi-arid soil origins and M Mediterranean soil origins. Asterisks indicate significant differences according to post-hoc tests.

Discussion

Our experiment showed that, under no limiting abiotic factors, biotic interactions take on a significant role in determining plant performance. Particularly, we observed a highly species-specific response to soil and soil biota which was likely determined by different degrees of mycorrhizal dependence among species. We now proceed to discuss the results according to relevance, starting with seed origins and moving on to interactions with neighbours, soil and soil biota.

Based on the results obtained by previous studies conducted in the same region from which our target species originate (Liancourt & Tielborger 2011), we

hypothesized that Mediterranean ecotypes would be adapted to a more favourable environment and thus would be better competitors than semi-arid ecotypes. However, our results contradicted this hypothesis showing that neither reproductive output nor competitive ability changed between ecotypes for any of the target species, thus pointing to a rather plastic response of both ecotypes to the new abiotic and biotic conditions. A possible explanation is that under optimal conditions both ecotypes were able to maximize their performance and consequently potential differences were evened out. Similarly, under such beneficial conditions the competition between the targets and the plant community might have been exacerbated for both ecotypes.

The detrimental effect of neighbours was consistent with our predictions and showed in all species a persistently negative performance associated with the presence of neighbours. Furthermore, the significant soil origin x neighbour interaction found in *B. distachyon* and *S. capensis* suggests a higher effect of competitive release in Mediterranean soils. This outcome is likely determined by the higher density of neighbours typical of Mediterranean soils. This interpretation is also corroborated by the similar trend found in *U. picroides*, although not significant. The opposite pattern observed in *B. didyma* instead is possibly due to the fact that, in this case, soil origin had a stronger effect than neighbours (see further in the discussion).

Our results did not point to either local adaptation to soil as shown by other previous studies (Sherrard & Maherali 2012; Smith *et al.* 2012) or to away-from-home soil advantage (e.g. Crémieux *et al.* 2008). However, there was a distinct difference in response to soil among species; *B. didyma* performed better in semi-arid soil, whereas *B. distachyon* and *U. picroides* showed a significantly better performance in Mediterranean soil and *S. capensis* showed a slightly higher, although not significant, seed production in Mediterranean soil. The improved performance of *B. didyma* in semi-arid soil can be explained by the higher occurrence of positive plant-soil feedback usually observed in unproductive soils (Reynolds *et al.* 2003) which are characterized by a scarce plant community and a relatively poor soil community. Belonging to a non-mycorrhizal family (Gerdemann 1968; Newman & Reddell 1987), it is likely that *B. didyma* generally experiences mainly negative plant-soil feedback, which in Mediterranean soil origins are possibly augmented. It has been shown that soil origins associated to high productivity and elevated plant density, are characterized by intense plant-plant competition (Schiffers & Tielbörger 2006) and high occurrence of negative plant-soil interactions (Kardol *et al.* 2006; van de

Voorde *et al.* 2011; Kulmatiski *et al.* 2012). Hence, in the case of *B. didyma*, Mediterranean ecotypes would benefit from enemy release in semi-arid soil, and semi-arid ecotypes would experience a home soil advantage. While previous literature supports the results observed in *B. didyma*, it does not explain what observed in the other species. Such discrepancies though are clarified when looking at the response of plants to soil biota manipulations. Intriguingly, sterilization treatments elicited a different response in *B. didyma* compared to all other species. *B. didyma* performed better in sterilized soil, where the absence of natural enemies provided a favourable environment. Differently, *B. distachyon*, *U. picroides* and *S. capensis* are mycorrhizal species and their performance was either hampered or fatally compromised in sterilized soil, probably because of the absence of necessary symbionts. Therefore, the improved performance observed in live soil and in Mediterranean soil origins suggests that in these cases the presence of necessary mycorrhizal fungi might have overridden the presence of relatively stronger negative plant-soil feedback compared to semi-arid soil origins. These results suggest that few necessary positive plant-soil biota interactions may be prominent compared to a higher proportion of negative plant-soil biota interactions. Hence, not only intensity but also importance of biotic interactions should be addressed in future studies.

The importance of biotic interactions to plant fitness is further highlighted when field (Chapter 2) and greenhouse results are compared. In the field, where all target species experienced high water stress, larger reproductive output was consistently observed in semi-arid soil origins. These results could be explained as either an effect of soil biota or as a consequence of Mediterranean soil origins poor performance under dry conditions (Salter & Williams 1965; Noy-Meir 1973). However, when comparing observations from the field and the greenhouse, it appears that under limiting water availability abiotic stress might prevail, whereas under optimal conditions biotic interactions might become prominent. As such, these results allow inferring that under different climatic scenarios, i.e. increased or decreased rainfall, the relative importance of biotic interactions might change and thus determine different outcomes on species fitness.

Overall, our experiment showed that under homogenous non-limiting abiotic conditions, the performance of plants was deeply affected by biotic interactions with neighbour plants and soil biota. By separating abiotic soil properties from soil biota we were able to parse the species-specific outcome of plant-soil interactions. Without

teasing apart such components, the interpretation of our results might have been biased or worse completely incorrect. As such biotic interactions should be kept into account when planning further experiments that aim to predict the performance of species under novel conditions.

Appendix 1: statistical table of results for GLM determining the effect of soil orogin, seed origin and community treatment on number of seeds produced by *B. didyma*, *B. distachyon*, *S. capensis* and *U. picroides*.

Source	*B. didyma*		*B. distachyon*		*S. capensis*		*U. picroides*	
	df	*P*	*df*	*P*	*df*	*P*	*df*	*P*
Main effects								
Soil origin	1	**.012**	1	.870	1	.287	1	**.023**
Seed origin	1	.959	1	.526	1	.835	1	.654
Community	2	**.000**	2	**.000**	2	**.000**	2	**.000**
Interactions								
Soil origin x seed origin	1	.728	1	.928	1	.882	1	.247
Soil origin x community	2	.102	2	**.005**	2	**.066**	2	.500
Seed origin x community	2	.224	2	.735	2	.821	2	.647
Soil origin x seed origin x community	2	.479	2	.944	2	.820	2	.079

Chapter 4

Effect of soil and climate on plant community composition along a steep climatic gradient in the Eastern Mediterranean region

Abstract

Studies about the effects of climate change on arid plant communities are rare and generally limited to few species. In a novel study, we reciprocally transplanted soil origins comprising their seed banks between two climates. By clustering species according to climatically relevant pseudo-traits we aimed to explain the properties of the emergent communities under different climatic conditions. We hypothesized that 1) species pool and local conditions mainly determine community composition, 2) climate is a filter for the background species pool within each community. We found that species composition was highly related to both soil origin, due to the influence of home conditions on the species pool, and to climate, suggestive of adaptive responses of the communities. Higher species richness and density of individuals were found in the wetter climate regardless of soil origin. Communities from drier soils exhibited a larger change in composition between climates, thus suggesting a more plastic response to rainfall. Therefore, in the face of increasing aridity, species groups associated with dry origins may increase dramatically. However, the periodical occurrence of some wet years might grant persistence of species groups from wet origins, thus providing a potential mechanism for increased community resistance in dryland habitats.

Introduction

Accurately predicting the future response of plants to climate change is essential for understanding the future structure and functioning of ecosystems. This could be especially important for ecosystems that are limited by extremes in temperature or precipitation (e.g. alpine, arctic or desert systems). In such regions, even small changes in climatic conditions are expected to have a dramatic impact on plant species composition and biodiversity (Sala & Lauenroth 1982; Rustad *et al.* 2001; Miranda *et al.* 2011; Wu *et al.* 2011; Salguero-Gomez *et al.* 2012) with consequential rearrangement of ecosystems, including the creation of novel communities (Williams *et al.* 2007; Pereira *et al.* 2010). While in systems limited by low temperatures climate change may release stress factors (IPCC 2007b), in subtropical deserts the predicted increase in temperatures accompanied by decreasing precipitation (Cubasch *et al.* 1996; IPCC 2007a; Smiatek *et al.* 2011) could drive the collapse of the community due to excessive stress (Forey *et al.* 2010). Unfortunately, not only are studies about the effects of climate change on arid plant communities rare in general (Bellard *et al.* 2012), but, as is the case for other climatic regions, they are mostly limited to only a few species.

A commonly applied correlative approach to studying climate change impacts on plant communities is the "bioclimatic envelope model" (BEM). This approach though neglects the role of local adaptation in plants response to climate change and has often failed to generate comprehensive predictions (Hampe 2004; Gilman *et al.* 2010). An interesting option to improve BEMs, other models, and our predictions of community response in general, is represented by field experiments such as field climate manipulations or reciprocal transplants, which provide clear insights into the roles of local adaptation and biotic interactions under an altered climate. Field climate manipulations allow monitoring the whole community response to artificially created conditions. Such experiments have given a valuable viewpoint into the vulnerability of different ecosystems (e.g.Liancourt *et al.* 2012; Lurgi *et al.* 2012); however, because they should be carried out over large areas and over long time scales, they are extremely expensive and labour intensive, thus often leading to a lack of replication. Reciprocal transplant experiments are a valid but underexploited way to explore local adaptation in plants under changed climatic conditions (Van Tienderen 1992; Nagy &

Rice 1997; Link *et al.* 2003; Stanton-Geddes *et al.* 2012). They often consist of the sowing of different ecotypes in home and foreign locations, allowing for comparison of performance under changed, but natural, climates. Besides saving the inconvenience of artificially altering climate with costly structures, reciprocal transplants can reduce the risk of unwanted secondary effects determined by the manipulations themselves. Ultimately, they provide an insight into the mechanisms of adaptation expressed by the plants, and into their potential persistence in an altered climate within a novel community of neighbour plants.

However, even classical reciprocal transplants are not immune to confounding effects (Macel *et al.* 2007). Plants are usually transferred into new regions without separating the climatic differences among sites from the local abiotic or biotic conditions (Petrů & Tielbörger 2008; Shaw & Etterson 2012). Hence the direct effect of climate might be confounded with the effect of the new soil properties, the new soil community and new plant-plant interactions (Petrů & Tielbörger 2008). Furthermore, transplant studies have focused on individual species, whereas much less is known about whether such adaptation translates into community effects (Weißhuhn *et al.* 2012) and how species interactions might change. Ideally, for accurately predicting plant community response solely to climate change, one should fully separate climate from the other afore mentioned factors. This can be achieved by transplanting entire communities with their soil and exposing them to different climates. In this scenario, both the soil properties and the soil community would be preserved, and the community performance could be assessed without drastically altering the original plant species composition. The observed local species composition could thus be examined as the result of the interaction between the regional species pool (determining the species that are 'available' for establishment), climate (determining whether the species will be within its climatic niche), soil as a resource (determining other abiotic aspects of the plant's niche such as water holding capacity and nutrient status), plant-soil and plant-plant interactions (affecting species fitness under different conditions). With enough overlap of species pools between sites of transfer, changes in dominance through alterations in community components would be more likely observed. Furthermore, such approach would allow extending the concept of local adaptation from the single individual to entire communities. Hence the community performance and its changes in composition, richness and abundance could be used as a test for investigating the potential of the community to respond and/or buffer a

change in environmental conditions. Nevertheless, transplanting entire microcosms that include soils and entire plant communities will inevitably suffer from the danger of damaging plants during transport and cause a great deal of disturbance. Thus for many plant communities, this kind of experiment is virtually impossible.

Here we present, for the first time, an experimental approach where we reciprocally transplanted entire plant communities, including their soil, between two very different climates. Our study system, which is located along a steep aridity gradient in Israel, combined a set of ideal conditions for our study aims. First, the species pool can be transferred without any damage to the plants because plant communities are dominated by winter annuals that for most of the year are stored as seeds in the soil seed bank and thus are easily transferable together with their soil. Second, being soils transferred during the dry season, when seeds and soil organisms are dormant, no damage to roots or components of the soil community can be done. Third, the plant communities are extremely species-rich with partial overlap of the species pool among climatically different sites, consequently the potential to observe compositional shifts is large; finally the presented scenario is compatible with the climate shift projections relative to this area (Giorgi 2006; Smiatek *et al.* 2011). Such an experimental set up allows not only observing the entire community response to a different climate without confounding effects, but also supports the investigation of local adaptation at a higher level than the single species, so that the community is regarded as an organic unit responding to environmental shift.

In general, the potential for a species to persist under changing conditions is a function of both the present climate and its specific adaptations to climate (Shaw & Etterson 2012). Therefore, to fully explore a community transplant approach, it would be useful to identify species traits or strategies which are directly related to climate change and observe if these components are selected for within the emerging communities. Unfortunately, to identify species traits related to climate adaptation is often difficult and laborious, hence any comprehensive study of adaptive potential may require a very extensive comparative approach (Diaz & Cabido 1997; Grime 1997). However, previous studies carried out for over nine years along the afore-mentioned aridity gradient have identified different traits and strategies associated to species from sites with different climatic conditions. In general, at the more favourable end of the gradient (characterized by abundant rainfalls) plants displayed traits typical of high competitive ability, whereas at unfavourable sites (characterized

by scarce and unpredictable rainfall) plants exhibited high adaptation to variability and stress (Petrů & Tielbörger 2008; Liancourt & Tielbörger 2011). Furthermore, long term rain manipulation experiments showed a higher abundance of species associated with drier habitats under diminished rainfall conditions, as opposed to a higher frequency of species associated with wetter habitats under increased rainfall regimes (Bilton *et al*, unpublished data). In a recent study, plant species were grouped according to their rainfall niche, which has proved a successful pseudo-trait in identifying shifts to climate and a potentially powerful predictor of future persistence of plants (Bilton *et al*, unpublished data). The resulting classification - based on Observed Rainfall Niche (ORN) organizes together potentially diverse sets of traits onto one axis of predictive response to changing climate.

Using the combined strengths of the whole community transplant experiment and a known 'trait' for accurately predicting response, we aimed to be able to determine the properties of communities emerging under different climatic conditions, and whether their response differs among regions of origin, without confounding effects such as soil or new neighbours.

We hypothesized that 1) the species pool, represented by the seed bank within each soil, is influenced by the historical local conditions and would be the main determinant of species composition; 2) climate would act as a filter for the background species pool determining which particular species emerge and survive within each plant community. Therefore the species which are naturally more abundant in the drier or wetter climates will be favoured under the corresponding drier or wetter climatic conditions of the experiment.

Methods

Study area

This study was conducted in Israel along a steep North-South climatic gradient. Two sites with respectively Mediterranean (M) and semi-arid (SA) climate were selected (Appendix 1 in Chapter 2). The sites share the same calcareous bedrock, southern aspect, elevation and mean annual temperatures, whereas they differ in average and variance of annual rainfall and in vegetation composition.

The M site is located 18 km south west of Jerusalem, near Matta (N 31° 42' E 35° 3') at 620 m a.s.l., on Terra Rossa soil. The climate is characterized by 540 mm average annual rainfall and 17°C mean annual temperature. The SA site is located near Lahav (N 31° 23' E 34° 54') between the southern Judean Mountains and the Northern Negev, at 590 m a.s.l., on Light Brown Rendzina. Average annual rainfall is 300 mm with mean annual temperature of 18.4°C. (Holzapfel *et al.* 2006)

The plant communities at both sites are semi-natural shrubland, dominated by the shrub *Sarcopoterium spinosum* (L.) Spach, and by a community of winter annuals which contribute approximately to 85% of all species. The annual plant cover is 60% at the M site and 10% at the SA site (Schiffers & Tielbörger 2006; J. Kigel, unpublished observations). Despite a large overlap of species among sites the relative quantities and richness differ between communities.

Experimental set up

In order to test the response of whole communities to different climates, we set up a reciprocal soil transplant experiment between the two sites (M and SA) which exposed the natural soil seed banks to the different climates. This resulted in four treatments characterized and named firstly by two levels each for soil/seed bank origin and then by the climate of the planting site (see Table 1).

soil	Site- climate	ID
Mediterranean	Mediterranean	M-M
Mediterranean	semi-arid	M-SA
semi-arid	Mediterranean	SA-M
semi-arid	semi-arid	SA-SA

Table 1: Abbreviation system for treatments composed by the combination of soil origin first and site (i.e. climate) second.

In July 2010, we excavated soil out of forty plots (surface area: 400 cm^2, depth: 3cm) from each site. The plots were randomly distributed around the whole site (area approx. 100 m x 250 m) with a minimum distance of 20 cm, avoiding heavily disturbed areas, rocky spots and patches in proximity to shrubs, to prevent confounding effects. After removal, soil from each origin was homogenized with a mixing machine at the Rehovot Agricultural Campus of the Hebrew University of Jerusalem (Israel). Half of each soil origin was taken back to each field site and 20 randomly selected plots each refilled by either local soil or soil from the other site,

respectively. To prevent contamination from the surrounding environment, a layer of thin tissue paper was placed at the base of each plot in order to separate the transplanted and natural soil. In addition, we covered the plot's surface with a layer of organza which was removed after the first rainfall that triggered germination. Previous trials indicated that the cover does not inhibit germination (Petrů & Tielbörger 2008). The resulting set up was characterized at each planting site (i.e. climate) by 2x20 replicates of soil/seed bank from each origin. At peak development, in spring 2011, we recorded the emerging species identity and density (number of individuals) in each plot. In order to prevent edge effects, we avoided sampling the vegetation growing in the outer 1 cm margin of each plot.

In addition, we wanted to test the response of different climatically relevant functional groups within the experimental set-up. For this purpose we obtained independent biological records data for the occurrence of each species recorded across Israel from the BioGIS – Israel Biodiversity Website (http://www.biogis.huji.ac.il/). Overlaying a GIS layer for rainfall (function in BioGIS) we could assign average rainfall values to each species defining their observed rainfall niche – a trait which has been shown to have strong predictive power for species response to rainfall (Bilton *et al.* unpublished data). Following the protocol of Bilton *et al.* (unpublished data), species were divided into four functional groups, called ORN (Observed Rainfall Niche), from ORN1, containing species whose distributions are associated with low rainfall patterns; to ORN4, containing species distributed in areas with high rainfall patterns.

Statistical analyses

To analyse density of individuals and species richness in the communities, we applied generalized linear models (GLM) with negative binomial distribution using the MASS package within the R software version 2.14. We modelled each parameter testing for the effect of soil (i.e. seed bank origin), site (i.e. climate) and their interaction. The significance of the model was assessed with a Type 3 ANOVA.

For testing species composition responses determined by soil origin and climate we used Redundancy Analysis (RDA) in the R package 'vegan' (Legendre *et al.* 2011). Their interaction term was included in a full model and confirmed using a step-wise approach. The data was Hellinger transformed (Legendre & Gallagher 2001)

and scaled within plots. Significance of the models was tested using 999 permutations. To test if the patterns of species composition could be explained by particular traits we assessed the RDA outputted 'species mean values' against the 'average rainfall niche' of each species, both for individual species and for the ORN groupings. Furthermore, we performed a further RDA on the community weighted means (CWM) of the 'average rainfall niche' trait value (Garnier *et al.* 2007). Finally, to test for responses of the different ORN functional groups to the experiment, we applied generalized linear models (GLM) with negative binomial distribution. The full model described ORN group density according to soil origin, climate, ORN functional groups, and their 2- and 3-way interactions. To best illustrate the interactions we used log ratios of the mean values so that the relative difference in density between treatment types resulted in positive values for greater densities in SA soil and climate, and negative values for higher densities in M soil and climate.
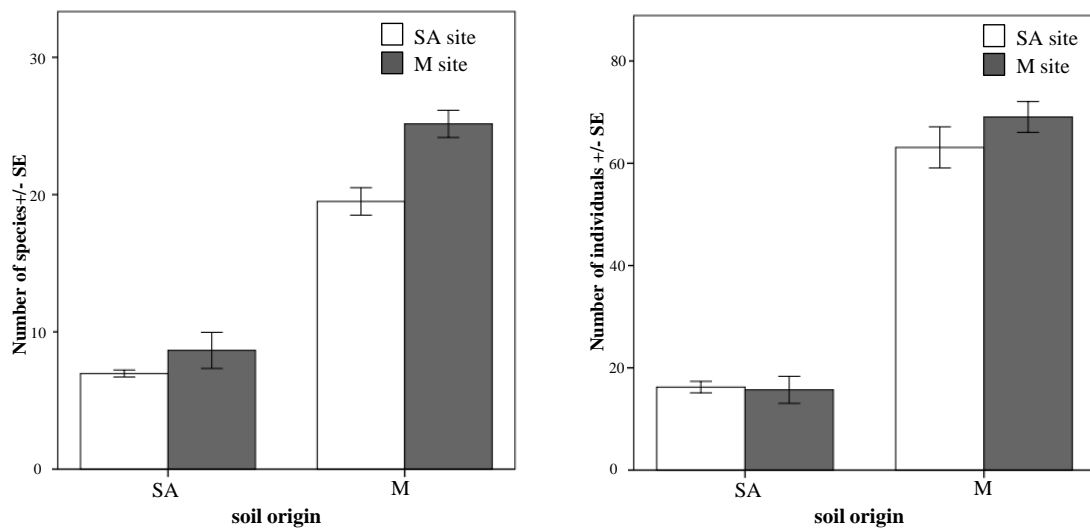

Figure 1: Mean species richness (measured as number of species) and mean density of plants (measured as number of individuals) in communities established from soils of two origins ('M' Mediterranean; 'SA' Semi-Arid) grown in two climates/sites ('M' Mediterranean; 'SA' Semi-Arid)

Results

Overall, 109 species were recorded (Appendix 1), among which 15% were Poaceae, 21% Fabaceae and 64% belonged to other groups. Sixteen species were recorded emerging from both soil seed banks under M and SA climate. The GLMs showed that the density of individuals was significantly higher in communities emerging from M soil seed banks (Figure 1, Table 2). Species richness of the emergent communities (Figure 1, Table 3) was significantly higher in soils of M origin and marginally significant in soils grown in M climate.

Effect	*df*	*P*
Soil	1	0.0000
Site	1	0.1254
ORN	3	0.0000
soil x site	1	0.0787
soil x ORN	3	0.0000
ORN x site	3	0.0000
soil x ORN x site	3	0.0000

Table 2: Statistical table of results for GLM determining the effect of soil origin, climate and ORN group on plant densities.

Effect	*df*	*P*
Soil	1	0.0000
Site	1	0.2913
soil x site	1	0.9320

Table 3: Statistical table of results for GLM determining the effect of soil origin and climate on plant species richness.

For species composition, the multi-variate analyses showed four distinct communities emerging from the respective treatments. Species composition was more varied among the plots from SA soil origin than those from M origin. The RDA model showed a significant effect of soil and climate on species assembly, as well as a significant interaction in these terms (Figure 2 a,b). We obtained three main RDA axes describing the species composition. Using simple correlations we assessed which plots/species were changing and having most impact on each axis. For plot mean values: RDA1 (9.2% explained; Figure 2 a, b) was most highly related to general overall differences to both soil and climate, with larger differences shown between

soil origins; RDA2 (3.3%; explained Figure 2a) was highly related to the effect of climate on M soil; and RDA3 (1.7%; explained Figure 2b) was highly related to the effect of climate on SA soil communities. The constrained RDA2 and RDA3 axes were likely distinguishing the climate x soil interaction term. For species mean values, RDA1 was highly positively correlated to average rainfall niche values, and RDA2 and RDA3 were also positively but less strongly correlated. This is highlighted in figure 2c, where the species belonging to the different ORN groups have been identified.
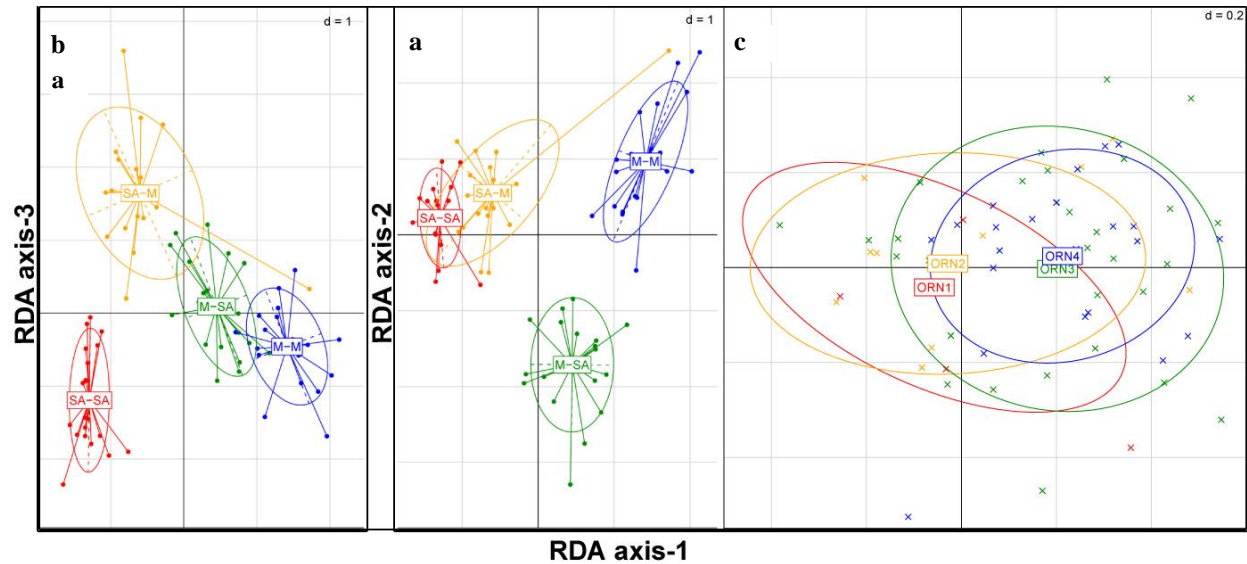
Figure 2: RDA (Redundancy Analysis) of species composition change according to the communities emerging from each treatment. Indicated on each graph are the plots/species (crosses) centroids of each treatment/species group (e.g. M-SA, ORN1) and the 95% confidence intervals for the grouping (ellipses). Plot mean values: a) RDA axes 1 (x) and 2 (y). RDA1 is representative of overall difference between plots. RDA2 is representative of the differences among plots due to climate on M soil; b) RDA axes 1 (x) and 3 (y). RDA3 represents the distance among plots due to climate on SA soil. Species mean values: c) shift in ORN group abundances across RDA axes 1 (x) and 2 (y) indicate association between dry rainfall niche groups (ORN1 and ORN2) in semi-arid conditions, and wet rainfall niche groups (ORN3 and ORN4) in Mediterranean conditions.

On both RDA axis 1 and 2, ORN groups increase in ranked order from driest species group (ORN1) to wettest species group (ORN4) associated respectively with the driest community types (SA-SA) to the wettest communities (M-M). To further validate these results, the RDA performed on the community weighted mean trait (CWMT) using average rainfall niche as trait, showed significant soil and climate effects. In combination, these results suggest that rainfall niche was partially responsible for some of the significant effects seen in species composition.

When analysing individual densities in relation to ORN groups, the GLMs (Table 2) showed a significant overall soil effect (Figure 3 'Raw Density'), but no overall climate nor soil x climate effect, consistently with the results on total densities.

There was an overall ORN effect ($p < 0.001$) which merely reflected different densities of the ORN groups emerging from the seed banks, with ORN 3 contributing with the highest density to overall communities. Two 2-way interactions, ORN x site and ORN x soil, were highly significant ($p < 0.001$, table 2). Firstly, soil x ORN, which is expressed by log-ratios in Figure 3, shows that all ORN groups emerged in proportionally greater numbers from the M soil seed banks than SA soils. However, plants belonging to ORN3 and ORN4 groups emerged in relatively greater numbers from M soils than ORN1 or ORN2 species. Secondly, climate x ORN (Figure 3) showed that individuals from ORN1 and ORN2 consistently emerged in greater numbers in SA climate, regardless of soil origin, whereas ORN3 and ORN4 consistently emerged in greater numbers in M climate. Finally, there was also a significant 3-way interaction between soil, climate, and ORN (Table 2, Figure 3). The log-ratios highlight that in both soil origins results were consistent with what observed in the climate x ORN interaction (Figure 3), with ORN 1 and ORN2 more abundant in SA climate, as opposed to ORN 3 and ORN 4 more abundant in M climate. In addition, the 3-way interaction shows that there was a relatively larger proportional change across all ORN groups for communities emerging from SA origin soils than from M soils.
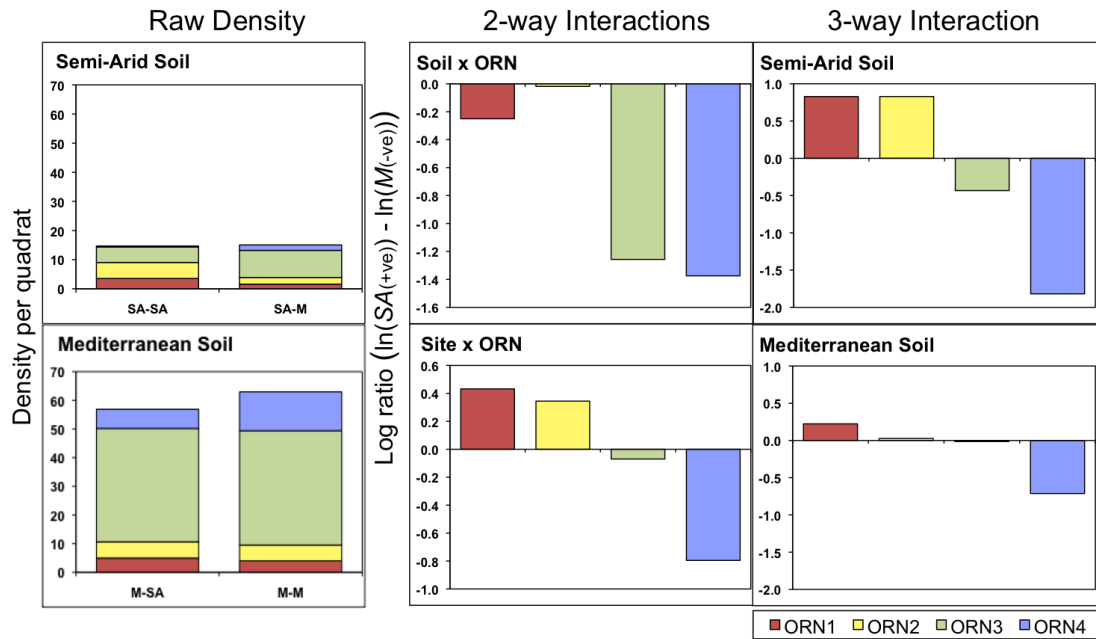
Figure 3: Graphical representation of the impact of soil origin, climate and ORN group on plant community densities establishing from seed banks. 'Raw density' shows the total mean density and relative abundance of ORN groups within each treatment. To best illustrate any interaction between factors in the communities (see table 2 for statistical results) we used log ratios of the mean values to give the relative difference in density between treatment types (+ve for greater values in SA; -ve for greater values in M). '2-ways interactions' shows how the composition of the community according to ORN groups changes respectively between soil origins and sites when M (soil or site) is compared to SA (soil or site). '3-way interactions' shows how the composition of the community according to ORN groups changes between M site and SA site respectively in SA soil origin and M soil origins.

Discussion

Our novel experimental approach, by transferring entire communities within their soil into a new climate, successfully separated the direct effect of climate from other key factors. Overall, our results revealed that the response of the community was related to soil origin (i.e. seed bank composition), climate and their reciprocal interactions with plant traits. We showed that the species pool is highly influenced by historical conditions, and that climate does influence which species will establish. Most notably, the shift in species composition in response to climate was most varied for the communities originating from the drier semi-arid site, suggesting inherent adaptive strategies to a more unpredictable environment, which may be important for future climate change predictions.

Soil origin was the strongest factor influencing species establishment within our communities, regardless of the parameter we looked at (density, richness, composition and ORN groups). This is consistent with previous studies (Fridley *et al.* 2011; Agra & Ne'eman 2012) and with our own prediction that the species pool is one of the main determinants of species composition. This result is not surprising because home environment was the main determinant of the seed bank contained in the transplanted soils and, only successively, did other factors operate further selection on the species pool. The differences in species richness, density and array observed among communities are likely the result of the different rainfall availabilities under which Mediterranean and semi-arid soils have evolved (Harel *et al.* 2011). Thus, as a consequence of the higher water availability and more favourable conditions, Mediterranean soils are characterized by more species and more abundant seed banks. Consistently, the multi-variate analysis showed communities as clear clusters separated by soil origin, furthermore by incorporating the ORN groups into the analysis, we gained further information about the characteristics of the species present in the species pool. We showed that in the Mediterranean soil there was a predominance of species belonging to ORN 3 and 4 groups (i.e. species from wetter climates) as opposed to a more solid presence of species from ORN 1 and 2 (i.e. species from drier climates) in semi-arid soil.

The significant effect of climate on all the response variables, except species density, proved that climate (i.e. rainfall) is one of the main limiting factors in dryland environments and can affect species richness (Adler & Levine 2007), both at the community and functional levels (Yang *et al.* 2011) by impacting upon the establishment of species (Bazzaz 1996).

Overall species richness was lower in the drier climate at the semi-arid site, consistently with the humpback model (Grime 1973), which predicts that under stressful conditions and low productivity, communities should express lower richness. Differently, density is the result of a multifold process, including seed bank composition, plant strategies and biotic interactions, in which climate is only one of the many acting factors and this might explain why no significant correlation was found between climate and plant density. The effect of climate on species composition is well represented by the shift in relative abundance of different ORN groups. Species from the wetter climates (ORN3 and 4) performed much better in the Mediterranean climate than in the semi-arid, whereas species from the drier climates (ORN1 and 2) performed better in semi-arid climate than in Mediterranean one. This result is consistent with what observed by Bilton *et al.* (unpublished data) from a 9-year-long experiment conducted in the same sites and might suggest co-evolution of plants historic traits with their climates. As such, this information might be used as a predictive tool for the future response of species according to their rainfall niche and the climate they are usually found in. For example it is likely that under changed climatic conditions, species with pre-adaptation to the new climatic conditions will have a better chance to occur in the new community composition. Unexpectedly though, our results showed that species belonging to dry climates (ORN1 and 2) did not increase in density under wetter and more favourable conditions. This suggests that the dry ORN groups might have evolved specific adaptations (e.g. drought tolerance strategies) which enable them to achieve greater fitness than the wet ORN groups under dry climatic conditions, but that such stress tolerant strategy is counterbalanced by a low competitive ability, as predicted by the CSR model (Grime 1977).

Despite our overall results suggest that the communities are adapted to their home conditions, the significant interaction between soil origin and climate in the compositional analysis indicates that some species within each community may actually be selected for and achieve higher fitness in a given climate. In that, ORN groups might be a valuable instrument to discern species ability to successfully persist under future climate. Intriguingly, the results of the three way interaction (soil x climate x ORN) suggest that not only do the dry species groups perform better in dry climates and that wet species groups perform better in wetter climates, but that the response to climate is stronger for plants from semi-arid origin. Indeed it is commonly hypothesized that under drier and more variable conditions (e.g. at semi-arid site) plants develop a higher ability to cope with unpredictability in the form of increased phenotypic plasticity (Bradshaw 1965; Sultan 2003; Garzon *et al.* 2011). Consistently with this theory, it appears that semi-arid communities showed greater plasticity with climate in

terms of overall abundance response. Thus, because of the predicted increase in aridity and variability of climate for our study region (Smiatek *et al.* 2011), these inherent adaptations to variability could be highly valuable in the event of future climate change.

With our study we showed for the first time the effect of climate on an entire community free from other confounding effects, such as soil. Not only did this provide unique insights into the relative importance of regional (species pool) and local (climate) effects on species assembly, but enabled us to derive specific predictions about plant community response to prospective climate change. Namely, the species pool from the soil seed bank determined species composition at large, and also defined the limits within which the local community responded to changes in rainfall. Dry species groups were definitely more abundant when the communities were grown in the drier climate, which is an effect comparable to what might happen during dry years. The increasing occurrence of dry years predicted for the Eastern Mediterranean region (Smiatek *et al.* 2011) is likely to gradually select for the drier species within the community. Although the more plastic response of dry species groups may at first suggest that such species may adapt more rapidly to a climatic shift, it is important to note that even species adapted to wetter climate have likely adapted to the high climatic variability of the region. Therefore occasional wet years might allow the persistence, even though reduced, of wetter species even under a drier climate. Eventually, such pre-adaptation to variability may determine a generally high resistance of the communities to climate change and a lower than expected vulnerability (Grime *et al.* 2008; Tielborger *et al*. unpublished data; Bilton *et al*. unpublished data).

Our results showed the importance of species traits that can group the community in meaningful categories with predictive power for future responses to climate. On one side, climate is an important filter for diversity and structure of natural communities, but on the other side, species local adaptation, phenotypic plasticity and biotic interactions within a community hold the key to reliably predict the future of a community.

Appendix1: List of recorded species with their average rainfall and ORN group

Species	Average rainfall (mm/year)	ORN group
Adonis dentata	227	1
Aegilops peregrina	478,9	3
Allium sp		
Althaea hirsuta	602,23	4
Anagallis arvensis	429,5	3
Asphodelus sp		
Asteracea sp		
Astragalus asterias	278,2	2
Astragalus tribuloides	115	1
Atractylis cancellata	361,1	2
Avena sterilis	460,9	3
Biscutella dydima	449,4	3
Brachypodium distachyon	413,1	3
Bromus fasciculatus	302,9	2
Bromus japanicus	503,8	4
Calendula sp	349,1	2
Carrhictera annua	203,2	1
Cephalaria joppensis	574,02	4
Cichorium sp	471,9	3
Clipeola jonthalspi	391,2	3
Convolvolus pentapetaloides	498,3	4
Convolvolus siculus	428,4	3
Coronilla scorpioides	473,6	3
Crepis sancta	466,1	3
Crithopsis delileana	302,1	2
Crupina crupinastrum	535,6	4
Cruscianella macrostachya	494,3	4
Cuscuta campestris	488,1	1
Erodium crassifolium	108,15	1
Erodium malachioides	472,2	3
Erodium sp		
Erucaria rostrata	168,45	1
Filago contracta	342,9	2
Filago desertorum	122,5	1
Filago palestina	344,4	2
Gagea reticultata	143,74	1
Galium judaicum	485,7	3
Galium sp		
Geranium rotundifolia	544,9	4
Geropogon hybridus	520,8	4
Gynandriris sisyrinchium	399,35	3
Hedipnois rhagadioloides	405	3
Hedysarum spinosissimum	347,64	2
Helianthemum aegytiacum	360,2	2
Helianthemum salicifolium	347,9	2
Helianthemum stipulatum	373,68	3
Hordeum bulbosum	540,26	4
Hymenocarpus circinnatus	443,6	3
Hyppocrepis unisiliquosa	341,3	2

Lasiopogon muscoides	83,65	1
Linum corimbosum	569,2	4
Linum pubescens	557,9	4
Linum strictum	464	3
Lolium multiflorum	382,88	3
Lolium rigidum	481,4	3
Lotus peregrinus	508,5	4
Malva sp		
Medicago coronata	458,7	3
Medicago rotata	506,4	4
Medicago sp		
Medicago truncatula	484,1	3
Medicago turbecolata	484,6	3
Mentha longifolia	547,39	4
Mentha sp		
Mercurialis annua	504,1	4
Mesembryanthemum nodiflorum	127,15	1
Nigella ciliaris	547,5	4
Onobrychis caput-galli	479,5	3
Onobrychis crista-galli	279,3	2
Onobrychis-squarrosa	509,7	4
Pallenis spinosa	572,5	4
Phalaris brachystachys	496,86	4
Picris damassena	165,7	1
Plantago afra	403,7	3
Plantago coronopus	185,5	1
Plantago cretica	468,2	3
Plantago lagopus	474,6	3
Poa annua	527,96	4
Pteranthus dichotomus	120,29	1
Pterocephalus plumosus	408,22	3
Ranunculus asiaticus	437,9	3
Reichardia tingitana	179,3	1
Rhagadiolus stellatum	446,7	3
Rostraria cristata	469,5	3
Sarcopoterium spinosum	511,27	4
Scabiosa palestina	515,2	4
Scandix hiberica	604,9	4
Schysmus arabicus	146,9	1
Scorpiurus muricatus	447,8	3
Sedum pallidum	438,8	3
Senecio vernalis	526,9	4
Stachys neurocalycina	576,9	4
Stachys sp		
Stipa capensis	221,7	1
Theligonum cynocrambe	515,6	4
Torilis tenella	474	3
Trifolium campestre	502,2	4
Trifolium pilulare	575,2	4
Trifolium purpureum	526,7	4
Trifolium scabrum	545,2	4
Trifolium sp		
Trifolium stellatum	533	4
Trigonella monspeliaca	438,5	3

Tripodium tetraphyllum	536,7	4
Trisetaria macrochaeta	244,25	2
Urospermum picroides	457,6	3
Valantia hispida	448	3
Vicia palestina	561,12	4
Vulpia ciliata	445,2	3

Chapter 5

General conclusions

Plant communities of the Eastern Mediterranean region face a steep increase in aridity according to climate predictions (Sala *et al.* 2000) with consequent high risk of extinction and conservation priority (Giorgi 2006). The major objective of this thesis was to assess how soil properties and biotic interactions can affect local adaptation and potentially implement future predictions of plant responses to climate change both at the population and community level. In the following, considerations on our results at the level of population and community are set forward.

Population (chapters 2 & 3)

The most salient finding regarding the population level is that climate resulted unimportant compared to biotic interactions and soil properties in determining plant performance and local adaptation. Nevertheless, we were able to show that climate affects the direction and intensity of biotic interactions and thus may have strong impacts on species performance. In the following, we discuss the results for seed origins, climate, and biotic interactions with neighbours and soil biota separately.

Based on ecological theory and previous findings (Galen *et al.* 1991), we expected that ecotypes from more favourable sites would be better adapted to competition and less well adapted to drought stress and vice-versa. However, ecotypes performance did neither differ among climates nor was there a differential response to neighbour removal. This indicates a more plastic response to abiotic and biotic factors than expected.

The relatively plastic response to climate observed in the target species could indicate adaptation to the naturally large climatic variation in the region, rather than to mean climatic conditions. The higher performance observed in *B. didyma* under Mediterranean climatic conditions is probably a direct effect of the higher water availability and not an indication of

local adaptation. The larger response of this species compared to the other targets may lie in the reduced resistance to drought observed in non-mycorrhizal families (Auge 2001).

The presence of neighbour plants had a consistently strong negative effect on seed production, indicating the large importance of competitive interactions for plant performance. Although this finding was not surprising in the greenhouse (Chapter 3) where we kept all treatments under homogenous and optimal conditions, we had expected that in the field (Chapter 2) competition would be less intense and that facilitation would occur under more stressful conditions (Bertness & Callaway 1994; Jurjavcic *et al.* 2002; Stanton-Geddes *et al.* 2012). While competition indeed appeared to be less intense in the field than in the greenhouse (Table 1, Figure 1), the prevalence of competition at both ends of the stress gradient in the field was in contradiction with previous studies conducted along our same gradient (Holzapfel *et al.* 2006; Schiffers & Tielbörger 2006). Although counterintuitive, our finding is not novel and has been previously explained. Under stressful conditions, the benefactor species itself may suffer from stress, which may in turn reduce its ability to act as facilitator (Maestre & Cortina 2004). Consequently, the net balance between positive and negative neighbour effects may be tipped in favour of competition at both ends of a stress gradient (Michalet *et al.* 2006).
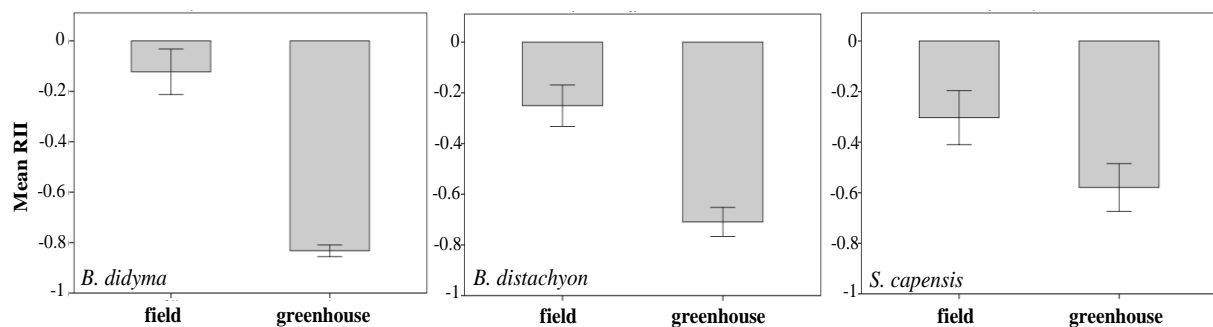

Figure 1: RII: relative interaction index was calculated according to (Armas *et al.* 2004) using the number of seed produced. RII= (number of seeds without neighbours - number of seeds with neighbours) /(number of seeds without neighbours + number of seeds with neighbours). RII has values spanning between -1 and +1, with negative values indicating competition and positive values indicating facilitation.

Source	df	F	Sig
Experiment	1	39.493	**0.000**
Species	3	0.540	0.655
Experiment * Species	2	2.655	0.072

Table 1: Statistical table of results for the ANOVA conducted on the RII values for each species in order to test whether there is a significant difference in interaction intensity between the field and the greenhouse experiment.

Positive, rather than negative plant-soil feedbacks were probably the reason for the higher performance of species in the field in semi-arid soil. As shown by previous studies, high stress environments are more likely to exhibit positive soil feedback (Reynolds *et al.* 2003) as opposed to low stress ones, characterized by high productivity, elevated plant density and high occurrence of negative interactions between plants and soil organisms (Kardol *et al.* 2006; Kulmatiski *et al.* 2012). Furthermore, plants from highly diverse communities are usually vulnerable to the attack of specialized pathogens and less susceptible to generalist soil organisms (Reynolds *et al.* 2003). Though these explanations appear straightforward and support our results from the field experiment, when comparing them to the greenhouse experiment a particularly intriguing and more complex pattern emerges. Only *B. didyma* showed a preference for semi-arid soil in the greenhouse and all other species performed better in Mediterranean soil. This discrepancy between field and greenhouse results may be explained when separating the abiotic and biotic components of soil. In the field, the higher clay content of Mediterranean soil is disadvantageous when water is limiting, because evaporation from fine-textured soils is much higher than from coarse soils (Salter & Williams 1965) and it increases the water holding capacity, thereby reducing the amount of available water (Baver 1956; Zwikel *et al.* 2007). Therefore, under field conditions, species performance in Mediterranean soils may have been limited by drought. Conversely, when water is not limiting, fine grained soils provide better conditions for plant growth because of the larger amount of available water. Thus, in the greenhouse, without the filter of abiotic stress (i.e. limiting water availability); the main factor influencing plant performance was the soil community.

Intriguingly, the effect of sterilization treatments not only allowed separating the role of soil biota from soil abiotic properties, but shed additional light on the species-specificity of responses to the soil community. The only species that benefitted from soil sterilization (*B. didyma*) belongs to a non-mycorrhizal family (Newman & Reddell 1987), and was the only species that in live soil likely experienced mostly negative soil feedbacks. Therefore, sterilized soil, being free of natural enemies, provided a more favourable environment for this species. This conclusion is also supported in live soils where *B. didyma* performed better in semi-arid soil which should be characterized by a scarce soil community and more abundant positive plant-soil interactions (Reynolds *et al.* 2003; Kulmatiski *et al.* 2012). In contrast to the Brassicacea, *B. distachyon*, *U. picroides* and *S. capensis* are mycorrhizal species and their performance was either hampered or fatally compromised in sterilized soil, indicating the

absence of necessary symbionts. Additionally for these species, Mediterranean soil origins provided a consistently more favourable environment, most likely because mycorrhizal fungi were able to offset the stronger negative plant-soil feedback (Borowicz 2001).

This consistent and prominent difference in performance observed between mycorrhizal and non-mycorrhizal species could only be detected by combining the field with the greenhouse study. Furthermore, a comparison between the field and the greenhouse enabled us to separate the biotic from the abiotic component of local soil conditions and evaluate their relative importance for plant performance and local adaptation. While in the field, water stress due to abiotic soil properties overrode the importance of soil biota, in the greenhouse, under non-limiting water conditions, soil biota was more important than soil abiotic properties in determining plant success. This suggests that not only the outcome, but also the importance of biotic interactions change along ecological gradients (Seifan *et al.* 2010), with biotic interactions being comparatively more important under low abiotic stress.

In summary, the most interesting finding of our study at the population level is the species-specific response to biotic interactions and the general dominance of biotic interactions in affecting plant fitness over responses to climatic conditions.

Community (Chapter 4)

One of the main findings of this study was that the species pool was highly affected by historic conditions (i.e. seed bank composition) and that climate acted as a filter in determining species richness. The use of ORN groups as a species pseudo-trait well represented the shift in community composition caused by climate. While ORN 1 and 2, associated with drier climate, emerged in relatively larger proportions from semi-arid soil origins and under semi-arid climatic conditions, species belonging to ORN 3 and 4 were more abundant in Mediterranean soil origins and under Mediterranean climatic conditions. In particular, we found a larger compositional shift in response to climate in semi-arid communities, suggesting that such communities are better adapted to the extreme climatic variability and thus capable of more plastic response (Aronson *et al.* 1992). Thus, in response to the predicted increase in aridity and climate unpredictability, species belonging to dry groups (ORN 1 and 2) might be selected for. However, the occurrence of some wet years

might grant that even though in reduced number, species from wet groups (ORN 3 and 4) might still persist. Our results are in contrast with previous long term studies (Grime *et al.* 2000, Tielbörger et al. unpublished data) , where a bigger variance in the response of plants to climate was observed in more fertile soils. Such discrepancy might be explained by the different temporal scale of the studies, and stresses the importance of studies targeting the response of the entire community at both short and large temporal scales. While our study illustrates the potential response of species to the predicted climatic shift in the short run, it is important to bear in mind that in the long term a community will withstand only a change consistent with the range of climatic variation to which it is adapted (Anderson 2008, Tielbörger *et al*. unpublished data).

Future predictions

Both at the population and community level, climate acted more as a filter on other factors than as a direct driver of species response. This may indicate that species, as they are not narrowly adapted to a certain climate, could be relatively resistant to climate change. Namely, regional models suggest a decrease in average annual precipitation of approximately 25% over the next 50 to 80 years (Smiatek *et al.* 2011), which is well within the range of rainfall differences applied in this study and the variation naturally experienced by the species on a year-to-year basis. However, caution must be taken when formulating such optimistic scenarios precisely because our findings suggest a detectable and complex effect of climate on biotic interactions, soil and soil seed bank, which in turn affect species performance and community composition.

Each of our experiments stretched for only one year and longer term observations might produce more exhaustive results; nonetheless we were able to clearly show that climate had a marginal role in determining plants performance. Had we performed our experiment without manipulating both abiotic and biotic factors such as climate, soil properties, soil biota and neighbours, our conclusions about the response of plants to climate change would have been entirely different. Our overall findings therefore support previous suggestions in that predictions about response to climate change should be implemented with information on local adaptation and adaptive potential (Davis & Shaw 2001; Jump & Penuelas 2005; Lavergne *et al.* 2010; Bellard *et al.* 2012).

Bibliography

Adler P.B. & Levine J.M. (2007). Contrasting relationships between precipitation and species richness in space and time. *Oikos*, 116, 221-232.

Agra H. & Ne'eman G. (2012). Composition and diversity of herbaceous patches in woody vegetation: The effects of grazing, soil seed bank, patch spatial properties and scale. *Flora*, 207, 310-317.

Aitken S.N., Yeaman S., Holliday J.A., Wang T.L. & Curtis-McLane S. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol. Appl.*, 1, 95-111.

Anderson T.M. (2008). Plant compositional change over time increases with rainfall in Serengeti grasslands. *Oikos*, 117, 675-682.

Antonovics J. & Bradshaw A.D. (1970). Evolution in closely adjacent plant populations part 8 clinal patterns at a mine boundary *Heredity*, 25, 349-362.

Ariza C. & Tielbörger K. (2011). An evolutionary approach to studying the relative importance of plant-plant interactions along environmental gradients. *Funct. Ecol.*, 25, 932-942.

Armas C., Ordiales R. & Pugnaire F.I. (2004). Measuring plant interactions: A new comparative index. *Ecology*, 85, 2682-2686.

Aronson J., Kigel J., Shmida A. & Klein J. (1992). Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water-stress. *Oecologia*, 89, 17-26.

Auge R.M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11, 3-42.

Bardgett R.D., Bowman W.D., Kaufmann R. & Schmidt S.K. (2005). A temporal approach to linking aboveground and belowground ecology. *Trends Ecol. Evol.*, 20, 634-641.

Baver L.D. (1956). *Soil physics*. John Wiley and Sons, Inc., New York.

Bazzaz F.A. (1996). *Plants in changing environments: Linking physiological, population, and community ecology*. Cambridge University Press, The Pitt Building, Trumpington Street, Cambridge CB2 1RP, England; Cambridge University Press, 40 W. 20th Street, New York, New York 10011-4211, USA.

Bellard C., Bertelsmeier C., Leadley P., Thuiller W. & Courchamp F. (2012). Impacts of climate change on the future of biodiversity. *Ecol. Lett.*, 15, 365-377.

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

Bever J.D., Westover K.M. & Antonovics J. (1997). Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *J. Ecol.*, 85, 561-573.

Bezemer T.M., Wagenaar R., Van Dam N.M. & Wackers F.L. (2003). Interactions between above- and belowground insect herbivores as mediated by the plant defense system. *Oikos*, 101, 555-562.

Biere A. & Verhoeven K.J.F. (2008). Local adaptation and the consequences of being dislocated from coevolved enemies. *New Phytol.*, 180, 265-268.

Bolan N.S. (1991). A critical review on the role of mycorrhizal fungi in the uptake of phosphorous by plants *Plant Soil*, 134, 189-207.

Borowicz V.A. (2001). Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology*, 82, 3057-3068.

Bradshaw A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advance Genet*, 13, 115-155.

Bradshaw A.D. (1984). *Ecological significance of genetic variation between populations. In: Dirzo R, Sarukhan J (eds) Perspective on plant population ecology. Sinauer, Sunderland, MA, pp213-228.*

Briones O., Montana C. & Ezcurra E. (1998). Competition intensity as a function of resource availability in a semiarid ecosystem. *Oecologia*, 116, 365-372.

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

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

Brown J.H., Stevens G.C. & Kaufman D.M. (1996). The geographic range: Size, shape, boundaries, and internal structure. *Annu. Rev. Ecol. Syst.*, 27, 597-623.

Callaway R.M. (1992). Effect of shrubs on recruitment of Quercus douglasii and Quercus lobata in California. *Ecology*, 73, 2118-2128.

Callaway R.M. (1995). Positive interactions among plants. *Bot. Rev.*, 61, 306-349.

Callaway R.M., Brooker R.W., Choler P., Kikvidze Z., Lortie C.J., Michalet R., Paolini L., Pugnaire F.I., Newingham B., Aschehoug E.T., Armas C., Kikodze D. & Cook B.J. (2002). Positive interactions among alpine plants increase with stress. *Nature*, 417, 844-848.

Callaway R.M. & Walker L.R. (1997). Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology*, 78, 1958-1965.

Charmantier A., McCleery R.H., Cole L.R., Perrins C., Kruuk L.E. & Sheldon B.C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, 320, 800-803.

Crémieux L., Bischoff A., Šmilauerová M., Lawson C.S., Mortimer S.R., Doležal J., Lanta V., Edwards A.R., Brook A.J. & Tscheulin T. (2008). Potential contribution of natural enemies to patterns of local adaptation in plants. *New Phytol.*, 180, 524-533.

Cubasch U., vonStorch H., Waszkewitz J. & Zorita E. (1996). Estimates of climate change in Southern Europe derived from dynamical climate model output. *Climate Research*, 7, 129-149.

Davis M.B. & Shaw R.G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, 292, 673-679.

Davis M.B., Shaw R.G. & Etterson J.R. (2005). Evolutionary responses to changing climate. *Ecology*, 86, 1704-1714.

Diaz S. & Cabido M. (1997). Plant functional types and ecosystem function in relation to global change. *J. Veg. Sci.*, 8, 463-474.

Ettema C.H. & Wardle D.A. (2002). Spatial soil ecology. *Trends Ecol. Evol.*, 17, 177-183.

Etterson J.R. (2004). Evolutionary potential of Chamaecrista fasciculata in relation to climate change. 1. Clinal patterns of selection along an environmental gradient in the great plains. *Evolution*, 58, 1446-1458.

Etterson J.R. & Shaw R.G. (2001). Constraint to adaptive evolution in response to global warming. *Science*, 294, 151-154.

Feinbrun-Dothan N. (1986). *Flora Palaestina*. Israel Academy od Sciences and Humanities, Jerusalem.

Forey E., Touzard B. & Michalet R. (2010). Does disturbance drive the collapse of biotic interactions at the severe end of a diversity-biomass gradient? *Plant Ecol.*, 206, 287-295.

Fridley J.D., Grime J.P., Askew A.P., Moser B. & Stevens C.J. (2011). Soil heterogeneity buffers community response to climate change in species-rich grassland. *Glob. Change Biol.*, 17, 2002-2011.

Friedel M., Bastin G. & Griffin G. (1988). Range assessment and monitoring in arid lands: the derivation of functional group to simplify vegetation data. *Journal of Environmental Management*, 27, 85-97.

Galen C., Shore J.S. & Deyoe H. (1991). Ecotypic divergence in alpine Polemonium viscosum genetic structure, quantitative variation and local adaptation *Evolution*, 45, 1218-1228.

Galloway L.F. & Fenster C.B. (2000). Population differentiation in an annual legume: Local adaptation. *Evolution*, 54, 1173-1181.

Garnier E., Lavorel S., Ansquer P., Castro H., Cruz P., Dolezal J., Eriksson O., Fortunel C., Freitas H., Golodets C., Grigulis K., Jouany C., Kazakou E., Kigel J., Kleyer M., Lehsten V., Leps J., Meier T., Pakeman R., Papadimitriou M., Papanastasis V.P., Quested H., Quetier F., Robson M., Roumet C., Rusch G., Skarpe C., Sternberg M., Theau J.P., Thebault A., Vile D. & Zarovali M.P. (2007). Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: A standardized methodology and lessons from an application to 11 European sites. *Ann. Bot.*, 99, 967-985.

Garzon M.B., Alia R., Robson T.M. & Zavala M.A. (2011). Intra-specific variability and plasticity influence potential tree species distributions under climate change. *Glob. Ecol. Biogeogr.*, 20, 766-778.

Gerdemann J. (1968). Vesicular-arbuscular mycorrhiza and plant growth. *Annual review of phytopathology*, 6, 397-418.

Gibson D.J., Connolly J., Hartnett D.C. & Weidenhamer J.D. (1999). Designs for greenhouse studies of interactions between plants. *J. Ecol.*, 87, 1-16.

Gilman S.E., Urban M.C., Tewksbury J., Gilchrist G.W. & Holt R.D. (2010). A framework for community interactions under climate change. *Trends Ecol. Evol.*, 25, 325-331.

Giorgi F. (2006). Climate change hot-spots. *Geophysical Research Letters*, 33.

Goldberg D.E. (1996). Simplifying the study of competition at the individual plant level: Consequences of distinguishing between competitive effect and response for forest vegetation management. *New Zealand Journal of Forestry Science*, 26, 19-38.

Goldberg D.E., Rajaniemi T., Gurevitch J. & Stewart-Oaten A. (1999). Empirical approaches to quantifying interaction intensity: Competition and facilitation along productivity gradients. *Ecology*, 80, 1118-1131.

Gonzalez-Megias A. & Menendez R. (2012). Climate change effects on above- and below-ground interactions in a dryland ecosystem. *Philos. Trans. R. Soc. B-Biol. Sci.*, 367, 3115-3124.

Grabherr G., Gottfried M. & Pauli H. (1994). Climate effects on mountain plants. *Nature*, 369, 448-448.

Grime J.P. (1973). Competitive exclusion in herbaceous vegetation *Nature (London)*, 242, 344-347.

Grime J.P. (1977). Evidence for the existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111, 1169-1194.

Grime J.P. (1997). Ecology - Biodiversity and ecosystem function: The debate deepens. *Science*, 277, 1260-1261.

Grime J.P., Brown V.K., Thompson K., Masters G.J., Hillier S.H., Clarke I.P., Askew A.P., Corker D. & Kielty J.P. (2000). The response of two contrasting limestone grasslands to simulated climate change. *Science*, 289, 762-765.

Grime J.P., Fridley J.D., Askew A.P., Thompson K., Hodgson J.G. & Bennett C.R. (2008). Long-term resistance to simulated climate change in an infertile grassland. *Proc. Natl. Acad. Sci. U. S. A.*, 105, 10028-10032.

Grime J.P., Mackey J.M.L., Hillier S.H. & Read D.J. (1987). Floristic diversity in a model system using experimental microcosms. *Nature*, 328, 420-422.

Guisan A. & Zimmermann N.E. (2000). Predictive habitat distribution models in ecology. *Ecol. Model.*, 135, 147-186.

Hampe A. (2004). Bioclimate envelope models: what they detect and what they hide. *Glob. Ecol. Biogeogr.*, 13, 469-471.

Harel D., Holzapfel C. & Sternberg M. (2011). Seed mass and dormancy of annual plant populations and communities decreases with aridity and rainfall predictability. *Basic Appl. Ecol.*, 12, 674-684.

Holzapfel C., Tielborger K., Parag H.A., Kigel J. & Sternberg M. (2006). Annual plant-shrub interactions along an aridity gradient. *Basic Appl. Ecol.*, 7, 268-279.

IPCC (2007a). *Climate change 2007: the physical science basis.*, Cambridge.

IPCC (2007b). *Climate change 2007: impacts, adaptation, and vulnerability.*, Cambridge.

Joshi J., Schmid B., Caldeira M.C., Dimitrakopoulos P.G., Good J., Harris R., Hector A., Huss-Danell K., Jumpponen A., Minns A., Mulder C.P.H., Pereira J.S., Prinz A., Scherer-Lorenzen M., Siamantziouras A.S.D., Terry A.C., Troumbis A.Y. & Lawton J.H. (2001). Local adaptation enhances performance of common plant species. *Ecol. Lett.*, 4, 536-544.

Jump A.S., Marchant R. & Penuelas J. (2009). Environmental change and the option value of genetic diversity. *Trends Plant Sci.*, 14, 51-58.

Jump A.S. & Penuelas J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol. Lett.*, 8, 1010-1020.

Jurjavcic N.L., Harrison S. & Wolf A.T. (2002). Abiotic stress, competition, and the distribution of the native annual grass Vulpia microstachys in a mosaic environment. *Oecologia*, 130, 555-562.

Kardol P., Bezemer T.M. & van der Putten W.H. (2006). Temporal variation in plant-soil feedback controls succession. *Ecol. Lett.*, 9, 1080-1088.

Kardol P., Cornips N.J., van Kempen M.M.L., Bakx-Schotman J.M.T. & van der Putten W.H. (2007). Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecol. Monogr.*, 77, 147-162.

Kareiva P. & Anderson T.M. (1988). Spatial aspects of species interactions: the wedding of models and experiments. In: *Community ecology*. Hastings, A. Berlin, Springer-Verlag, pp. 35-50.

Kawecki T.J. & Ebert D. (2004). Conceptual issues in local adaptation. *Ecol. Lett.*, 7, 1225-1241.

Keddy P.A. (1989). Effects of competition from shrubs on herbaceous wetland plants - A 4-year field experiment *Can. J. Bot.-Rev. Can. Bot.*, 67, 708-716.

Klironomos J.N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, 84, 2292-2301.

Kulmatiski A., Beard K.H. & Heavilin J. (2012). Plant-soil feedbacks provide an additional explanation for diversity-productivity relationships. *Proc. R. Soc. B-Biol. Sci.*, 279, 3020-3026.

Kulmatiski A., Beard K.H., Stevens J.R. & Cobbold S.M. (2008). Plant-soil feedbacks: a meta-analytical review. *Ecol. Lett.*, 11, 980-992.

Lampei C. & Tielborger K. (2010). Evolvability of between-year seed dormancy in populations along an aridity gradient. *Biol. J. Linnean Soc.*, 100, 924-934.

Lavergne S., Mouquet N., Thuiller W. & Ronce O. (2010). Biodiversity and Climate Change: Integrating Evolutionary and Ecological Responses of Species and Communities. In: *Annual Review of Ecology, Evolution, and Systematics, Vol 41* (eds. Futuyma DJ, Shafer HB & Simberloff D). Annual Reviews Palo Alto, pp. 321-350.

Legendre P. & Gallagher E.D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129, 271-280.

Legendre P., Oksanen J. & ter Braak C.J.F. (2011). Testing the significance of canonical axes in redundancy analysis. *Methods Ecol. Evol.*, 2, 269-277.

Leger E.A. & Espeland E.K. (2010). The shifting balance of facilitation and competition affects the outcome of intra- and interspecific interactions over the life history of California grassland annuals. *Plant Ecol.*, 208, 333-345.

Liancourt P., Spence L.A., Boldgiv B., Lkhagva A., Helliker B.R., Casper B.B. & Petraitis P.S. (2012). Vulnerability of the northern Mongolian steppe to climate change: insights from flower production and phenology. *Ecology*, 93, 815-824.

Liancourt P. & Tielborger K. (2011). Ecotypic differentiation determines the outcome of positive interactions in a dryland annual plant species. *Perspect. Plant Ecol. Evol. Syst.*, 13, 259-264.

Liancourt P. & Tielbörger K. (2011). Ecotypic differentiation determines the outcome of positive interactions in a dryland annual plant species. *Perspect. Plant Ecol. Evol. Syst.*, 13, 259-264.

Linhart Y.B. & Grant M.C. (1996). Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.*, 27, 237-277.

Link S.O., Smith J.L., Halvorson J.J. & Bolton H. (2003). A reciprocal transplant experiment within a climatic gradient in a semiarid shrub-steppe ecosystem: effects on bunchgrass growth and reproduction, soil carbon, and soil nitrogen. *Glob. Change Biol.*, 9, 1097-1105.

Lurgi M., Lopez B.C. & Montoya J.M. (2012). Novel communities from climate change. *Philos. Trans. R. Soc. B-Biol. Sci.*, 367, 2913-2922.

Macel M., Lawson C.S., Mortimer S.R., Smilauerova M., Bischoff A., Cremieux L., Dolezal J., Edwards A.R., Lanta V., Bezemer T.M., van der Putten W.H., Igual J.M., Rodriguez-Barrueco C., Muller-Scharer H. & Steinger T. (2007). Climate vs. soil factors in local adaptation of two common plant species. *Ecology*, 88, 424-433.

Maestre F.T., Callaway R.M., Valladares F. & Lortie C.J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J. Ecol.*, 97, 199-205.

Maestre F.T. & Cortina J. (2004). Do positive interactions increase with abiotic stress? - A test from a semi-arid steppe. *Proc. R. Soc. B-Biol. Sci.*, 271, S331-S333.

Malkinson D. & Tielborger K. (2010). What does the stress-gradient hypothesis predict? Resolving the discrepancies. *Oikos*, 119, 1546-1552.

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

Miranda J.D., Armas C., Padilla F.M. & Pugnaire F.I. (2011). Climatic change and rainfall patterns: Effects on semi-arid plant communities of the Iberian Southeast. *J. Arid. Environ.*, 75, 1302-1309.

Morin X., Augspurger C. & Chuine I. (2007). Process-based modeling of species' distributions: what limits temperate tree species' range boundaries? . *Ecology*, 88, 2280-2291.

Nagy E.S. & Rice K.J. (1997). Local adaptation in two subspecies of an annual plant: Implications for migration and gene flow. *Evolution*, 51, 1079-1089.

Newman E.I. & Reddell P. (1987). The distribution of mycorrhizas among families of vascular plants *New Phytol.*, 106, 745-751.

Novoplansky A. (2009). Picking battles wisely: plant behaviour under competition. *Plant Cell Environ.*, 32, 726-741.

Noy-Meir I. (1973). *Desert ecosystems environment and producers*.

Olsson K. & Agren J. (2002). Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb Lythrum salicaria. *J. Evol. Biol.*, 15, 983-996.

Parmesan C. (2006). Ecological and evolutionary responses to recent climate change. In: *Annual Review of Ecology Evolution and Systematics*. Annual Reviews Palo Alto, pp. 637-669.

Parmesan C. & Yohe G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37-42.

Penuelas J. & Filella I. (2001). Phenology - Responses to a warming world. *Science*, 294, 793-+.

Pereira H.M., Leadley P.W., Proenca V., Alkemade R., Scharlemann J.P.W., Fernandez-Manjarres J.F., Araujo M.B., Balvanera P., Biggs R., Cheung W.W.L., Chini L., Cooper H.D., Gilman E.L., Guenette S., Hurtt G.C., Huntington H.P., Mace G.M., Oberdorff T., Revenga C., Rodrigues P., Scholes R.J., Sumaila U.R. & Walpole M. (2010). Scenarios for Global Biodiversity in the 21st Century. *Science*, 330, 1496-1501.

Petrů M. & Tielbörger K. (2008). Germination behaviour of annual plants under changing climatic conditions: separating local and regional environmental effects. *Oecologia*, 155, 717-728.

Reinhart K.O. & Callaway R.M. (2006). Soil biota and invasive plants. *New Phytol.*, 170, 445-457.

Reynolds H.L., Packer A., Bever J.D. & Clay K. (2003). Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology*, 84, 2281-2291.

Root R.B. (1967). The Niche Exploitation Pattern of the Blue-Gray Gnatcatcher. *Ecol. Monogr.*, 37, 317-350.

Rustad L.E., Campbell J.L., Marion G.M., Norby R.J., Mitchell M.J., Hartley A.E., Cornelissen J.H.C., Gurevitch J. & Gcte N. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 126, 543-562.

Sala O.E., Chapin F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. & Wall D.H. (2000). Biodiversity - Global biodiversity scenarios for the year 2100. *Science*, 287, 1770-1774.

Sala O.E. & Lauenroth W.K. (1982). Small rainfall events an ecological role in semi arid regions *Oecologia (Berlin)*, 53, 301-304.

Salamin N., Wuest R.O., Lavergne S., Thuiller W. & Pearman P.B. (2010). Assessing rapid evolution in a changing environment. *Trends Ecol. Evol.*, 25, 692-698.

Salguero-Gomez R., Siewert W., Casper B.B. & Tielborger K. (2012). A demographic approach to study effects of climate change in desert plants. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 367, 3100-14.

Salter P.J. & Williams J.B. (1965). The influence of texture on the moisture characteristics of soils. II. Available-water capacity and moisture release characteristics. *J Soil Sci*, 16, 310-317.

Sambatti J.B.M. & Rice K.J. (2006). Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (Helianthus exilis). *Evolution*, 60, 696-710.

Saxton K.E., Rawls W.J., Romberger J.S. & Papendick R.I. (1986). Estimating generalized soil - water characteristics from texture. *Soil Sci. Soc. Am. J.*, 50, 1031-1036.

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

Schweitzer J.A., Bailey J.K., Fischer D.G., Leroy C.J., Lonsdorf E.V., Whitham T.G. & Hart S.C. (2008). Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. *Ecology*, 89, 773-781.

Seifan M., Seifan T., Ariza C. & Tielborger K. (2010). Facilitating an importance index. *J. Ecol.*, 98, 356-361.

Shaw R.G. & Etterson J.R. (2012). Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. *New Phytol.*, 195, 752-765.

Sherrard M.E. & Maherali H. (2012). Local adaptation across a fertility gradient is influenced by soil biota in the invasive grass, Bromus inermis. *Evol. Ecol.*, 26, 529-544.

Siewert W. & Tielborger K. (2010). Dispersal-Dormancy Relationships in Annual Plants: Putting Model Predictions to the Test. *American Naturalist*, 176, 490-500.

Smiatek G., Kunstmann H. & Heckl A. (2011). High-resolution climate change simulations for the Jordan River area. *Journal of Geophysical Research-Atmospheres*, 116.

Smith D.S., Schweitzer J.A., Turk P., Bailey J.K., Hart S.C., Shuster S.M. & Whitham T.G. (2012). Soil-mediated local adaptation alters seedling survival and performance. *Plant Soil*, 352, 243-251.

Smith T.M., Shugart H.H., Woodward F.I. & Burton P.J. (1993). *Plant functional types*. Chapman and Hall, Inc., 29 West 35th Street, New York, New York, USA; Chapman and Hall Ltd., 2-6 Boundary Row, London SE1 8HN, England.

Snaydon R.W. & Bradshaw A.D. (1962). Differences between natural populations of Trifolium repens in response to mineral nutrients. L Phosphate. *Jour Exptl Bot*, 13, 422-434.

Stanton-Geddes J., Tiffin P. & Shaw R.G. (2012). Role of climate and competitors in limiting fitness across range edges of an annual plant. *Ecology*, 93, 1604-1613.

Stebbins G.L. (1974). Flowering plants: evolution above the species level. *London: Arnold xviii, 399p.. Illustrations. General (KR, 197500089).*

Sultan S.E. (2003). Phenotypic plasticity in plants: a case study in ecological development. *Evol. Dev.*, 5, 25-33.

Thorpe A.S., Aschehoug E.T., Atwater D.Z. & Callaway R.M. (2011). Interactions among plants and evolution. *J. Ecol.*, 99, 729-740.

Thuiller W., Albert C., Araujo M.B., Berry P.M., Cabeza M., Guisan A., Hickler T., Midgely G.F., Paterson J., Schurr F.M., Sykes M.T. & Zimmermann N.E. (2008). Predicting global change impacts on plant species' distributions: Future challenges. *Perspect. Plant Ecol. Evol. Syst.*, 9, 137-152.

Turesson G. (1930). The selective effect of climate upon the plant species *Hereditas*, 14, 99-152.

van de Voorde T.F.J., van der Putten W.H. & Bezemer T.M. (2011). Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. *J. Ecol.*, 99, 945-953.

van der Heijden M.G.A., Klironomos J.N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., Wiemken A. & Sanders I.R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69-72.

Van der Putten W.H., Macel M. & Visser M.E. (2010). Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philos. Trans. R. Soc. B-Biol. Sci.*, 365, 2025-2034.

Van Tienderen P.H. (1992). Variation in a population of Plantago lanceolata along a topographic gradient *Oikos*, 64, 560-572.

Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., Fromentin J.M., Hoegh-Guldberg O. & Bairlein F. (2002). Ecological responses to recent climate change. *Nature*, 416, 389-395.

Wardle D.A., Bardgett R.D., Klironomos J.N., Setala H., van der Putten W.H. & Wall D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629-1633.

Weiner J., Martinez S., Muller-Scharer H., Stoll P. & Schmid B. (1997). How Important are Environmental Maternal Effects in Plants? A Study with Centaurea Maculosa. *J. Ecol.*, 85, 133-142.

Weißhuhn K., Prati D., Fischer M. & Auge H. (2012). Regional adaptation improves the performance of grassland plant communities. *Basic Appl. Ecol.*, 13, 551-559.

Williams J.W., Jackson S.T. & Kutzbach J.E. (2007). Novel and disappearing climates by 2100AD are concentrated in tropical regions: Implications for conservation biology. *Ecological Society of America Annual Meeting Abstracts*.

Woodward F.I. & Cramer W. (1996). Plant functional types and climatic change: introduction. *J. Veg. Sci.*, 7, 306-308.

Wu Z.T., Dijkstra P., Koch G.W., Penuelas J. & Hungate B.A. (2011). Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Glob. Change Biol.*, 17, 927-942.

Yang H.J., Wu M.Y., Liu W.X., Zhang Z., Zhang N.L. & Wan S.Q. (2011). Community structure and composition in response to climate change in a temperate steppe. *Glob. Change Biol.*, 17, 452-465.

Zwikel S., Lavee H. & Sarah P. (2007). Temporal evolution of salts in Mediterranean soils transect under different climatic conditions. *Catena*, 70, 282-295.

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