

Biotic and abiotic effects on species interactions in a  
water stressed ecosystem:  
the effect of precipitation and grazing on the  
survival and establishment of the Mediterranean  
dwarf shrub *Sarcopoterium spinosum*

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# Abstract

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The question of how biotic and abiotic factors interact in determining the structure and function of plant communities has been a central focus in ecological research. Global change such as climate change or land-use changes may shift the balance of importance between abiotic and biotic factors, and thus alter the natural productivity and species composition of plant communities. This may particularly apply to regions already experiencing extreme conditions, e.g. drylands. Drylands, such as the Eastern Mediterranean have been predicted to be severely affected by climate change. In particular, climate scenarios suggest that water availability will decrease substantially due to decreasing precipitation combined with increasing temperatures, and that extreme weather events will become more frequent. However, abiotic factors not only regulate plants directly through their effect on demographic processes, but also indirectly through alterations in the biotic environment of the entire community. This indicates that under climate change, not only the abiotic but also the biotic environment will change, requiring adaptation, plastic response or escape from altered conditions. Unfortunately, the majority of studies predicting the response of single species or whole plant communities have largely overlooked biotic interactions, and they are mostly based on abiotic factors.

In this study, I utilized a Mediterranean plant community which naturally occupies a large climatic range to test the combined effect of abiotic alterations and biotic interactions on shrub seedling dynamics. Since the region has been under grazing for millennia, I further tested how adding a second potential disturbance (here grazing simulated by clipping) alters the response of shrub seedlings to climate change and annual neighbours. I addressed these questions by conducting large field experiments along a natural climate gradient, complemented by Botanical Garden experiments. First, I evaluated if natural (spatial) stress gradients can serve as a proxy for predicting plant dynamics under future climate change. Secondly, I tested the interactive effect of changing abiotic and biotic factors on shrub seedling dynamics and in determining the intensity and direction of plant-plant interactions. Thirdly, I studied what will happen if grazing is added to such a system. Focal species of my experiment was a widely distributed dwarf shrub that occupies large areas in the Mediterranean and that is assumed to encroach if managed incorrectly (e.g. by overgrazing).

Overall I was able to show that abiotic and biotic factors are inevitably linked and in order to give reliable predictions about the future response of species they both need to be considered simultaneously. Particularly because of the complex interplay of abiotic and biotic factors, environmental gradients along an abiotic axis are a poor proxy for predicting the response of plant species to climate change. I furthermore showed that local direct interactions between shrub seedlings and annual neighbours were always more relevant to shrub seedling establishment than climatic conditions. Particularly, simulated climate change

never had any direct effect on shrub seedling establishment. However, climate change affected shrub seedling establishment indirectly via its effect on the growing conditions of the neighbouring community. Plant-plant interactions were always important and always negative. Grazing simulated by clipping significantly decreased biomass production by the annual plant community, resulting in a slight release from competitive interactions.

In synthesis, my research emphasizes the overriding importance of biotic interactions for determining plant population and community dynamics and therefore, they must be addressed in ecological climate impact studies.

# Declaration

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## **Declaration of my own working contribution to the present thesis**

The present manuscript is based on two years of field and Botanical Garden experiments I conducted in Israel. For all three studies I designed the experimental set-up in cooperation with my supervisors Merav Seifan, Marcelo Sternberg and Katja Tielbörger. I collected field data myself and performed statistical tests for all parts receiving great help from Merav Seifan. The main part of this thesis was written by me using relevant literature, but I have received and incorporated various comments and suggestions from my supervisors Merav Seifan, Marcelo Sternberg and Katja Tielbörger. Throughout the course of this research I was advised by my supervisors Merav Seifan, Marcelo Sternberg and Katja Tielbörger.



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# Literature overview

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Understanding the relative role of biotic and abiotic factors in regulating plant population dynamics is one of the oldest challenges in ecology (Walther *et al.* 2002). Because biotic and abiotic factors can modify the structure and function of plant communities by influencing its composition, growth, and productivity, changes in climate or management may have strong and even surprising effects on the ecosystem (Adler & HilleRisLambers 2008).

In this work I will focus on the effect of abiotic and biotic factors in determining shrub seedling dynamics of a common Mediterranean dwarf shrub. In particular, we studied the influence of both land-use (i.e. grazing) and climate change (i.e. change in precipitation) on the interactions between annual and perennial vegetation. Further, in the face of the universal increase in unpalatable woody vegetation in pasture areas due to overgrazing (i.e. shrub encroachment; Brown & Archer 1999; van Auken 2000), we studied the potential role of climate and grazing in tipping the current balance between coexisting plant species within the communities, which may lead to an increased abundance of the perennial vegetation.

## Climate change

Climate change and its effects on natural ecosystems have long intrigued scientists and it still remains one of the most studied topics in ecology. The evidence of climate change compromising natural ecosystems increases at an accelerating rate, further promoting research in this field of expertise (van Auken 2000; Walther *et al.* 2002; Thuiller *et al.* 2005; Thuiller *et al.* 2008). So far substantial evidence has been delivered that climate change causes alterations in the phenology, distribution range and composition of plant communities (Walther *et al.* 2002; Thomas *et al.* 2004; Thuiller *et al.* 2005; Parmesan 2006; IPCC 2007; Thuiller *et al.* 2008; Liancourt *et al.* 2012). And under the worst circumstances climate change can lead to the local or total extinction of a plant species (Thomas *et al.* 2004; Jump & Peñuelas 2005; Parmesan 2006; Peñuelas *et al.* 2013). Empirical studies seek to determine environments vulnerable to climate change and to predict the response of species to climate change in order to offer potential conservation strategies and to prevent the further degradation of natural ecosystems or single species.

Climate change describes alterations in temperatures, rainfall quantities as well as the frequency of rainfall, which may vary at a regional scale (IPCC 2007). Drylands, such as the eastern Mediterranean region, have been predicted to be severely affected by climate change (IPCC 2007; Suttle *et al.* 2007; Smiatek *et al.* 2011). More precisely, these ecosystems have been predicted to experience a decrease in water availability, as well as an increase in temperatures and between- and within-year variation of extreme weather events (Smiatek *et al.* 2011). However, especially these drylands are sensitive to a further reduction in water

availability as water is considered the main limiting factor for a successful plant production (Castro *et al.* 2004; Lloret *et al.* 2004; Castro *et al.* 2005; Holzapfel *et al.* 2006; Lloret *et al.* 2009; Matías *et al.* 2012).

There are several well established procedures for understanding and predicting the dynamics of ecological systems under climate change, e.g. natural gradients such as temperature gradients (Lloret *et al.* 2009) or rainfall gradients (Fukami & Wardle 2005; Sternberg *et al.* 2011). This approach predicts species distribution changes based on natural gradients which serve as a proxy describing temporal shift in abiotic factor.

A rather common theoretical modelling approach based on the same rationale are bioclimatic envelopes models (BEMs; e.g. Bakkenes *et al.* 2002; Thomas *et al.* 2004). These models are derived from the assumption that spatial gradients may serve as a proxy for temporal change allowing to predict novel distribution ranges of species based on their response to climatic variables (Huntley *et al.* 2010). Yet, these models have been heavily criticized as they generate rather unrealistic assumptions. Reasons for the models failure are that they ignore biotic interactions as well as the role of plasticity and adaptation (e.g. Davis *et al.* 1998; Pearson & Dawson 2003; Hampe 2004; Jump & Peñuelas 2005; Gilman *et al.* 2010). Aware of these shortcomings Fukami and Wardle (2005) proposed the combination of both, natural climatic gradients and local long-term climate manipulations. Specifically, the manipulations of single abiotic factors for multiple years allow us to overcome the earlier proposed shortcomings and provides a more mechanistic understanding (Grime *et al.* 2000; Suttle *et al.* 2007; Miranda *et al.* 2009). This approach provides a clear insight into the dynamics of a species under consideration of changing biotic interactions and local adaptation. However, there have been only a few attempts combining both approaches (e.g. Dunne *et al.* 2004; Liancourt *et al.* 2012). Hence further research combining both gradients has to be conducted in order to provide more reliable predictions about the response and future dynamics of species.

### **Interactions among plants and the stress gradient hypothesis (SGH)**

Besides the abiotic environment, biotic interactions play an important role in regulating and determining the dynamics, structure and composition of plant communities (Brooker *et al.* 2008). Biotic interactions are not static but are highly variable and their direction (facilitative vs. competitive) and intensity depend directly on the favourability of the abiotic environment (Bertness & Callaway 1994; Brooker 2006; Brooker *et al.* 2008). A common theoretical approach combining changes in the abiotic environment with biotic interaction is the stress gradient hypothesis (Bertness & Callaway 1994). The conceptual idea of the stress gradient hypothesis is that plant-plant interactions change depending on the favourability of the abiotic environment, i.e. competitive interactions dominate in benign conditions whereas facilitation dominates in harsh climatic conditions (Bertness & Callaway 1994; Brooker *et al.* 2008).

This conceptual approach offers the unique opportunity for investigating plant species responses to climate change, as natural geographical gradients imitate the gradual change towards harsher climatic conditions, as expected under climate change. Consequently, climate change will not only result in alterations of the abiotic environment but will also modify the biotic environment. This model and its generality has been heavily debated (Lortie & Callaway 2006; Maestre *et al.* 2006) and tested in the framework of several field experiments which either approved (e.g. Brooker & Callaghan 1998; Pugnaire & Luque 2001; Callaway *et al.* 2002) or contradicted the original hypothesis (Tielbörger & Kadmon 2000; Maestre & Cortina 2004; Maestre *et al.* 2005; Maestre *et al.* 2009).

Ever since this debate started several suggestions have been made to improve the predictive power of the stress gradient hypothesis. Maestre *et al.* (2009) suggested to distinguish between resource (e.g. water availability) and non-resource related (e.g. temperature) abiotic stress. Additionally, they predicted differences in interactions depending on the life history of species involved, i.e. if they are either stress tolerant or rather competitive. Smit *et al.* (2009) and Van der Putten *et al.* (2010) stressed out the importance of biotic interactions (here consumer pressure) as they further increase the complexity of plant-plant interactions. Namely, in productive environments, the presence of herbivory, and especially cattle which favour annual vegetation, is expected to reduce the competitive effect. Yet again, in unproductive environments grazing may counteract potential facilitative effects by the annual neighbouring community. The same shifts in interactions are expected for environments under climate change.

### **The role of biotic interactions and grazing**

Domestic grazing is known to have a strong influence on natural ecosystems, affecting the dynamics and composition of plant communities as well as the persistence of single species (Olf & Ritchie 1998). However, the magnitude of disturbance varies across ecosystems and in some regions it is also considered an important management tool for maintaining biodiversity (Noy-Meir 1995; Harrison *et al.* 2003; Callaway *et al.* 2005; Papanikolaou *et al.* 2011). Grazing can both, directly or indirectly influence natural plant communities, as it has the potential to influence the establishment success and productivity of single species and simultaneously alter the abiotic environment (Olf & Ritchie 1998). The Mediterranean region has been grazed for millennia, thus anthropogenic interferences have largely contributed to shaping the current landscapes (Noy-Meir 1995; Perevolotsky & Seligman 1998).

Grazing represents one of the most important factors altering natural ecosystems either directly or indirectly (Olf & Ritchie 1998). Direct effects comprise e.g. the removal of plant tissue (e.g. Noy-Meir 1993), nutrients heterogeneity due to manure and urine (e.g. Bokdam & Gleichman 2000) and landscape heterogeneity (e.g. Adler *et al.* 2001; Seifan & Kadmon 2006; Henkin *et al.* 2007); indirect effects include the alteration of the abiotic environment (Olf & Ritchie 1998). Yet, the direction and intensity of alterations

depends strongly on the intensity a certain ecosystem is grazed. Additionally grazing plays an important role in the establishment success of perennial species. Disturbances by trampling and the alterations of the annual neighbouring community create windows of opportunities which allows for the establishment of unpalatable, woody species (Perevolotsky *et al.* 2001; Callaway *et al.* 2005; Osem *et al.* 2007).

The effects of facilitation and competition between shrubs and annuals in dry regions have been the subject of many studies (e.g. Moro *et al.* 1997; Tielbörger & Kadmon 2000; Holzapfel *et al.* 2006), focusing on the effects of shrubs on annual plant species. Much less attention has been given to how the annual plants affect the performance of shrubs (but see Seifan *et al.* 2010), though these effects may be crucial in determining shrub encroachment at the expense of herbaceous vegetation. Generally, and in accordance with the stress gradient hypothesis, competition between annuals and shrub seedlings increases with increasing water availability (Maestre *et al.* 2003). Therefore, the presence of herbivores, and especially cattle which favour annual vegetation, is expected to reduce the competition between annuals and shrubs for the benefit of shrubs in wetter years. Interestingly, in a preliminary study in a similar ecosystem, Seifan *et al.* (2010) indicated positive drought effects mediated by interactions between annuals and shrub seedlings, i.e. annual vegetation promoted shrub seedling establishment in stressful years. Therefore, increasing aridity is expected to increase shrub establishment at the expense of annual plants and thus decrease the fodder value of the land (Maestre *et al.* 2003). However, due to the cattle preference of annual vegetation, grazing may counteract the positive effect of the annual on shrub establishment (Seifan & Kadmon 2006).

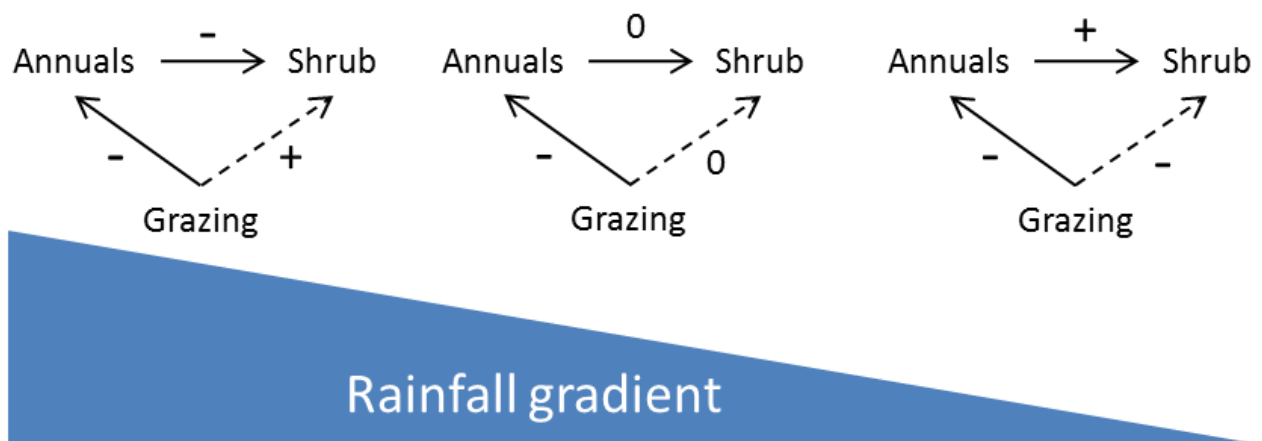


Figure 1. Conceptual model of the effect of rainfall on shrub seedling establishment and shrub seedling-annual interactions in a Mediterranean environment (rainfall gradient may be artificial or natural). Domestic grazing is expected to have an indirect effect on shrub seedling establishment by altering the interactions between annual neighbours and shrub seedlings. I.e. domestic grazing is expected to decrease competitive effects of the annual neighbouring community at the benign end of the gradient. However, at the harsh end of the gradient, domestic grazing might counteract potential habitat amelioration (i.e. facilitative effects) by the neighbouring community. The direction of the proposed relationship between various factors is indicated by plus, zero or minus indicating positive, neutral and negative relationships. Solid lines indicate the direct effect of one factor on the other. Dotted lines indicate indirect effects of a factor acting via a third factor. See text for further explanations. Adapted from (Seifan & Kadmon 2006).

According to these previous observations, it is clear that predictions concerning the combined effect of climate and land-use are complex, especially without particular knowledge of biotic interactions within the system. In Figure 1 I summarize the main pathways in annual-shrub dynamics that may be prone to changes in these factors.

### **Research significance**

Abiotic and biotic factors simultaneously affecting natural ecosystems are highly complex and predicting alterations in such systems are not straight-forward. Therefore, combining abiotic and biotic factors, namely climate change, grazing and the interactions among plants may significantly increase the precision and reliability of future predictions. In particular, there are three reasons for the understanding of how abiotic factors interact with biotic factors to affect plant community dynamics:

1) It has been repeatedly shown that the direction and intensity of biotic interactions, e.g. facilitation and competition among plants, depends directly on the favourability of the abiotic environment (Bertness & Callaway 1994; Brooker *et al.* 2008). According to Bertness and Callaway (1994) interactions between plant species shift along environmental gradients in a predictable manner such that competition prevails in less stressful environments and facilitation dominates in stressful environments. This indicates that under climate change, not only the abiotic but also the biotic environment will change and thus require adaptation, plastic response or escape from these conditions. However, because the experimental evidence for the stress gradient hypothesis is contradictory, we do not know to what extent it can be generalized.

2) Biotic interactions may often be imposed by artificial shifts in land-use, such as domestic grazing (Adler *et al.* 2001). If, as we assume, the response of plants to biotic interactions depends on abiotic conditions, knowing such complex interrelationships may provide to us means of managing ecosystems under climate change (e.g. via grazing management). Yet, our knowledge on the combined effect of climate change and management is rather limited. This is quite surprising, because of the high probability that areas prone to be affected by climate change will suffer the most from intense land use.

3) Finally, we are still lacking connection between the ecological mechanisms behind plant dynamics and the predictive tools in climate change studies. The dominant models used in current literature are mainly based on bioclimatic envelop models (Bakkenes *et al.* 2002; Thomas *et al.* 2004) that predict novel distribution ranges of species based on their response to climatic variables alone. These models have been heavily criticized not only because they ignore biotic interactions but also because they are purely correlative and not mechanistic (Davis *et al.* 1998; Pearson & Dawson 2003). Generally because climate change affecting whole plant communities may increase the complexity of plant interactions notably, predicting the response of single species is more complicated than expected by the models.

To address these questions we conducted a field and garden experiment in a semi-arid region. The combination of both natural climatic gradient and local climate manipulations are perfect conditions to investigate differential effects of the interacting abiotic and biotic factors on the system (Fukami & Wardle 2005).

### **Study system and focal species**

Israel with its highly diverse climate distributed over a small geographical area offers a unique possibility to investigate effects of climate change on the structure and functioning of ecosystems. Israel has a prominent spatial precipitation distribution ranging from 900 mm in the north to 30 mm annual rainfall in the south (Zangvil & Druian 1990). The focal species of this research is the unpalatable, thorny, dwarf shrub *Sacopoterium spinosum* (L.) Spach (Prickly burnet, Family: Rosaceae), which dominates large areas in the eastern Mediterranean region. The eastern Mediterranean scrub formation and *S. spinosum* were chosen for the following reasons:

1) The eastern Mediterranean is consistently predicted to be severely affected by climate change (IPCC 2007). Regional climate scenarios indicate that the climate will be more arid with increasing temperatures, decreasing average rainfall and an increase in (between and within) annual rainfall variability (Nahal 1981; Smiatek *et al.* 2011). Since water is the main limiting factor for plant performance in this region (Castro *et al.* 2004; Castro *et al.* 2005; Lloret *et al.* 2009), this will certainly have a dramatic effect on the plant communities.

2) The region has been under grazing for millennia, i.e. grazing is part of the dynamics of the ecosystems in the region (Perevolotsky & Seligman 1998). At the same time, increasing grazing pressure, but also abandonment from grazing threaten to affect the current status of the ecosystems and are likely to affect the balance between the annual and perennial vegetation (Perevolotsky *et al.* 2001).

3) The Mediterranean is a global biodiversity hotspot and thus the need to protect the unique flora and fauna under climate change is large (Médail & Quézel 1999).

4) The semi-arid and arid areas where grazing is not managed properly are commonly experiencing an increase of unpalatable species at the expense of the herbaceous vegetation (Brown & Archer 1999), thus decreasing the fodder value of the land (Maestre *et al.* 2003) and indicating the importance of studying shrub demography.

5) Our focal species, *S. spinosum* has been studied a lot and thus basic data on its life history is available (e.g. Litav *et al.* 1963; Litav & Orshan 1971; Reisman-Berman *et al.* 2006; Seifan & Kadmon 2006; Henkin & Seligman 2007; Reisman-Bermana & Henkin 2007; Seifan *et al.* 2010). Yet, although this particular shrub dynamics is important for the future of pasture land in the area, our knowledge about its potential

response to climate change, grazing management and the interactive role of these effects on the balance between shrubs and the annual vegetation is very limited.

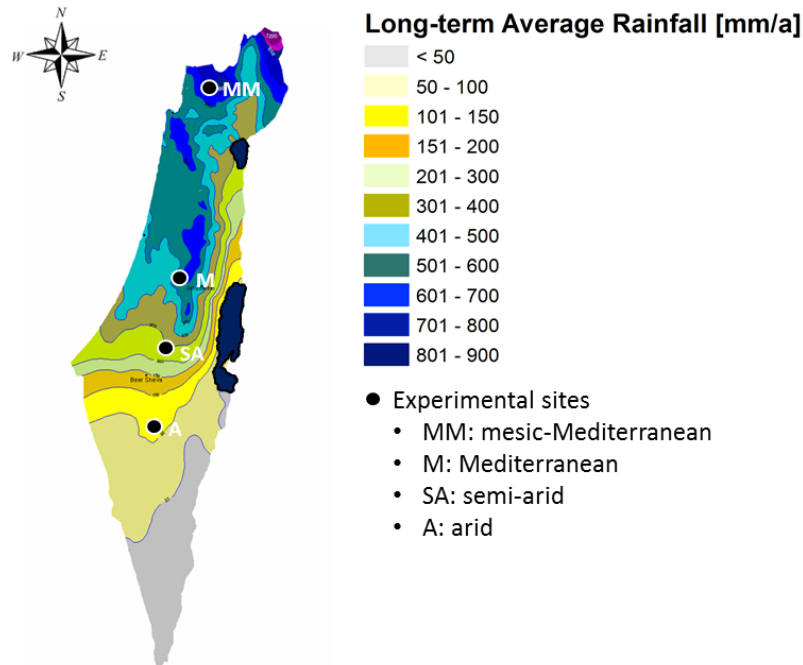


Figure 2. Distribution pattern of the average annual precipitation within Israel and the geographic location of the four research sites (Sternberg et al.).

The practical work of this study was conducted in Israel at the Department of Plant Science, Tel Aviv University. Field experiments and observations took advantage of the long term field stations constructed by the GLOWA Jordan River<sup>1</sup> project (directed by K. Tielbörger, Tübingen). The field stations are located in four points along the natural climatic gradient and represent four different climatic conditions (arid, semi-arid, Mediterranean and mesic-Mediterranean). The stations differ by approx. 200 mm average annual precipitation but have similar calcareous bedrock (Figure 2). At semi-arid and Mediterranean stations a further long-term manipulation of rainfall by means of additional irrigation and rain shelter exist, which enabled me to test expected differences in response to change along the geographical gradient.

### Thesis objectives and structure

The central aim of this thesis was to investigate the effect of various abiotic and biotic factors on the dynamics of a common Mediterranean dwarf shrub, enabling us to give predictions about the potential impact of climate change on the community structure of the region. This thesis is structured in three

<sup>1</sup> GLOWA Jordan River – Global Change and the Hydrological Cycle, a research program launched and financed by the German Federal Ministry of Education and Research (BMBF) (see [www.glowa-jordan-river.de](http://www.glowa-jordan-river.de)).



individually written chapters. In general, each chapter autonomously presents its own background and methods with the relevant focus on the specific question. In specific the thesis objectives were:

1. To determine whether a natural aridity gradient can serve as a proxy for determining shrub population dynamics under climate change (**Chapter 1**);
2. To study the simultaneous effect of climate change and consequential alterations in plant-plant interactions in order to give reliable predictions about changes in shrub seedling dynamics (**Chapter 2**);
3. Studying the effect of climate change and grazing as well as their interaction on shrub seedling dynamics and how they alter interactions with herbaceous neighbours (**Chapter 3**).

## **Key results**

**Chapter 1** – Shrub seedling dynamics under climate change - comparing the response along natural and experimental rainfall gradients.

Seedling survival of a common Mediterranean dwarf shrub was monitored in a mesic-Mediterranean, Mediterranean and semi-arid environment during two consecutive years. At the Mediterranean site further long-term climate manipulations were established, representing dryer and wetter climatic conditions. In order to cover the major source of small-scale heterogeneity we took seedlings growing in the open space between shrubs and under the shrub canopy of *S. spinosum* into account. Shrub seedling survival along the natural and the experimental rainfall gradient responded positive to increased soil moisture levels. However, shrub seedling survival responded differently to an increasing drought stress. Whereas shrub seedling survival further decreased along the experimental gradient, it exhibited a surprising increase towards the arid end of the natural gradient. Overall, we were able to show that natural gradients are a poor proxy for predicting the response of plant species to temporal shifts in climatic conditions, mainly as they ignore the complex interplay of abiotic and biotic factors.

**Chapter 2** – Neighbour effects on shrub seedling establishment override climate change impacts.

We conducted a field experiment along the same rainfall gradient with an additional garden experiment in order to study the effects of changing abiotic and biotic factors on shrub seedling establishment, as well as the direction and intensity of plant-plant interactions determining shrub seedling dynamics. Our results showed that the direct effect of climate on shrub seedling dynamics was negligible. Nevertheless, decreasing water availability altered the annual community, thus abiotic environment, which again affected shrub seedling dynamics. Overall, biotic interactions were always important and always negative, with slightly decreased negative interactions at the two extreme ends of the gradient. In general, our results suggest that interactions are based on decreasing water and increasing light availability along the aridity gradient.

### **Chapter 3** – Who cares about climate change impact if you have neighbours to deal with?

We conducted an experiment along the same natural gradient complemented by a Botanical Garden experiment to test the effect of water availability on the direction and intensity of shrub-annual interactions and to understand how grazing will affect such a system. Our results showed that direct effects of water were negligible. Interactions were always competitive. However, the additional factor grazing, simulated by clipping, counteracted this negative effect by increasing seedling establishment significantly, probably due to an increased light availability.

# Chapter 1

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Shrub seedling dynamics under climate change - comparing the response along natural and experimental rainfall gradients

## **Abstract**

Understanding and predicting the responses of plant communities to environmental changes is a key challenge in ecology. Here, we examined climate-related mechanisms influencing and regulating the seedling dynamics of a common Mediterranean dwarf shrub as yet our knowledge about mechanisms causing shifts in community composition and shrub dynamics are very limited. The objective of our study was to analyse the effect of water availability on seedling establishment and survival and to determine whether changes in plant community dynamics along a geographical gradient can serve as proxy for predicting local climate change effects. We conducted a field experiment along a natural rainfall gradient with additional long-term climate manipulations at one field site (a Mediterranean site) which provided a unique opportunity to investigate the relative importance and direction of biotic and abiotic factors. Survival showed the same increasing trend with increasing water availability both along the natural and the experimental rainfall gradient. However, with increasing drought stress, seedling survival varied greatly between the two gradients. Survival at the experimental Mediterranean site was clearly positively correlated with water availability. However, this pattern was not confirmed along the spatial climate gradient because seedling survival exhibited a surprising increase towards the arid end of the gradient. We attribute this pattern to biotic interactions, which appeared less negative at the dry end. Our results show that ignoring biotic interactions can result in highly misleading predictions about shifts in the distribution of species under climate change. In summary, the complex interplay of abiotic and biotic factors was responsible for our conclusion that spatial environmental gradients are a poor proxy for predicting the response of plant species to temporal shifts in climatic conditions.

## Introduction

Understanding and predicting the responses of plant communities to environmental change and disturbance represents a major challenge for ecology (Krebs 2009). Namely, increasing anthropogenic interference has made it crucial to predict responses of plant communities to man-made changes such as climate and land use change (Adler & HilleRisLambers 2008). A widely used approach for understanding the dynamics of ecological systems under climate change is the use of natural gradients varying in their climatic conditions, e.g. temperature gradients (Lloret *et al.* 2009), or rainfall gradients (Fukami & Wardle 2005; Sternberg *et al.* 2011). The underlying assumption of such an approach is that geographical gradients may serve as proxy for temporal shifts in climate, i.e. wetter or cooler sites represent conditions under a wetter or cooler future climate and *vice-versa*. A widely-used theoretical approach based on the same rationale, i.e. a space-for-time approach, are bioclimatic envelope models (e.g. Bakkenes *et al.* 2002; Thomas *et al.* 2004). These models predict future distribution ranges of species based on their current distribution range and correlation with climatic variables. However, these models are purely correlative and not mechanistic and they ignore the role of biotic interactions for determining species ranges (e.g. Davis *et al.* 1998; Pearson & Dawson 2003). Fukami and Wardle (2005) proposed that to overcome these shortcomings, both natural climatic gradients and local long-term climate manipulations should be combined to investigate the relative importance of biotic and abiotic factors. To the best of our knowledge there are only few studies that took this approach (e.g. Dunne *et al.* 2004; Liancourt *et al.* 2012). With this study, we attempted to fill that gap by studying shrub seedling responses to climate change in a water-limited system using both a steep rainfall gradient and rainfall manipulations.

Early plant life stages have been proposed to be most vulnerable to environmental alterations (Howard & Goldberg 2001; Fay & Schultz 2009). At the same time, they determine plant population dynamics, persistence and expansion (Harper 1977; Kitajima & Fenner 2002) and in many ecosystems, especially those characterized by extreme climatic conditions, early life history stages are considered a bottleneck for plant establishment (Leck *et al.* 2008). Especially for long-lived, woody plant species seedling establishment is the most vulnerable stage, because small seedlings do not yet exhibit the robustness of adult plants that may protect them from drought, high temperatures, or competition by neighbours (Castro *et al.* 2005; Mendoza *et al.* 2009; Matías, Zamora, *et al.* 2011). Seedling survival depends on a narrow spatial and temporal window ("safe site") with specific environmental conditions for a successful establishment (sensus Harper *et al.* 1961). At this early stage, seedlings appear to be particularly sensitive to a changing climate (Gómez-Aparicio, Gómez & Zamora 2005). Several studies conducted in drylands indicate that water availability is the main limiting factor for seedling establishment (Castro *et al.* 2004; Castro *et al.* 2005; Lloret *et al.* 2009). However, once shrub seedlings have been established, they appear to be less affected by abiotic and biotic factors (Davis *et al.* 1999; van Auken 2000).

Climate not only regulates plant performance directly through its effect on demographic processes, but also indirectly through alterations in the density and biomass of the entire community (Sternberg *et al.* 1999; Holzapfel *et al.* 2006; Seifan *et al.* 2010). If species-specific, such changes will be associated with modifications of biotic interactions, whose direction (positive vs. negative) and intensity depend directly on the favourability of the abiotic environment (Bertness & Callaway 1994; Brooker *et al.* 2008). For example, interactions between plant species often shift along environmental gradients in a predictable manner such that competition prevails in less stressful environments and facilitation dominates in stressful environments (Callaway & Walker 1997). This indicates that under climate change, not only the abiotic but also the biotic environment will change, requiring adaptation, plastic response or escape from altered conditions.

Shrubs are known to play an important role in water-limited systems, as they have the potential to influence (shrub) seedling establishment success through alterations of microclimatic conditions (see Callaway 1995). Ameliorations of stress include the improvement of soil moisture, soil nutrients, temperature and a reduction of high light intensities (Pugnaire *et al.* 1996; Pugnaire *et al.* 2004; Gómez-Aparicio, Gómez & Zamora 2005; Gómez-Aparicio, Gómez, Zamora, *et al.* 2005). However, the presence of adult shrubs can also negatively influence seedling establishment, e.g. by competition for light (Aguiar *et al.* 1992; Seifan *et al.* 2010). To date, it is virtually unknown how the direction and intensity of such biotic interactions will change under a new climate. If increased abiotic stress (e.g. by decreasing rainfall) is associated with decreasing biotic stress (e.g. competitive release), studying climate change and plant-plant interactions simultaneously is very important. Therefore, the objective of our study was to determine whether changes in plant community dynamics along a natural rainfall gradient can serve as proxy for predicting in-situ climate change effects. For this purpose, we studied establishment probabilities of a dominant shrub species in a water-limited system under different abiotic and biotic conditions. Specifically, we artificially manipulated rainfall availability in accordance with regional climate change scenarios (Smiatek *et al.* 2011) and monitored the establishment success of a common Mediterranean dwarf shrub in relation to herbaceous neighbours. These observations were compared to the shrub establishment success along a geographical rainfall gradient which served as spatial control for climate change scenarios (Sternberg *et al.* 2011). We predicted that with increasing site aridity and with experimental drought, shrub seedling survival will decrease. If shrub seedling survival along a natural and artificial rainfall gradient is indeed similar, this would be proof-of-principle for the use of geographical gradients and proxy for temporal change in climate. Moreover, we assumed that with decreasing rainfall, facilitation will become more important, i.e. shrub establishment will be higher in the presence of adult shrubs or herbaceous neighbours than without neighbours.

## Material and Methods

We conducted our study within the scrub formation of the Mediterranean region in Israel. Eastern Mediterranean scrub formations are characterized by highly heterogeneous plant communities and diverse environmental conditions spread over a small geographical area (Danin 1992). In Israel, this vegetation formation is found along a prominent spatial precipitation gradient across a small spatial distance ranging from 900 mm mean annual precipitation in the north to 30 mm in the south (Zangvil & Druian 1990), which is accompanied by an increasing temporal variability with decreasing rainfall (Nahal 1981). This combination offered a unique opportunity for investigating effects of climate change on the structure and functioning of ecosystems. The study was conducted at three sites along the natural climatic aridity gradient (183 km) (see: "Geographical gradient"), with additional rainfall manipulations at the Mediterranean site for testing potential climate change scenarios (see: "Climate change manipulations").

### *Climate change manipulations*

The Mediterranean site, located in the Judean Mountains (N 31°42', E 35°03') was further subjected to rainfall manipulations. Initially, the manipulations were done to cover the uncertainty of global circulation models and thus we both artificially increased and decreased rainfall. However, high-resolution models became available during the course of the study that predict a decrease in annual rainfall by 10-30% in the next decades (Smiatek *et al.* 2011). Therefore, while both treatments provide mechanistic information about the response of shrub seedlings to changes in rainfall, the drought treatment better mimics the most recent scenarios. Artificial irrigation increased rainfall by 30%, and maintained natural timing, frequency and intervals of rainfall at these sites (Sternberg *et al.* 2011). Rainout shelters (Yahdjian & Sala 2002) were constructed to exclude 30% of the natural precipitation. Five plots of 10 m x 25 m size were setup for each treatment and randomly located at each site. From here on we will refer to the treatments as irrigation, control and drought. For a more detailed description see Sternberg *et al.* (2011) and Talmon *et al.* (2011).

### *Geographical gradient*

North and south of the Mediterranean site, two additional research sites were established; a mesic-Mediterranean site (Galilee Mountains, N 33°0', E 35°14') and a semi-arid site (northern Negev desert, N 31°23', E 34°54'), respectively (Table 1, Harel *et al.* 2011). Average annual rainfall is 780 mm in the North, 540 mm in the central site, and 300 mm in the South. All sites are located on southern slopes of calcareous bedrock at similar elevations. They share the same Mediterranean climate characterized by mild, rainy winters and hot, dry summers and have similar annual mean temperatures (17.0-19.1 °C). The main growing season (November-May) is closely linked with the temporal distribution of rainfall. For further information about the sites see Holzapfel *et al.* (2006) and Sternberg *et al.* (2011).

The study was conducted over a period of two years. Rainfall in the two consecutive years (season 2009/10 and 2010/11) was 251 and 178 mm at the semi-arid, 357 and 533 mm at the Mediterranean and 832 and 711 mm at the mesic-Mediterranean site, respectively.

Table 1. Geographical extent and climatic ranges of the three sites along the geographical gradient of Israel (Harel *et al.* 2011).

Ecosystem type	Average Rainfall (mm $\pm$ CV)	Temperature (Mean in °C)	Elevation (a.s.l.)	Soil type
Semi-arid (N31°23' E34°54')	300 ( $\pm$ 37)	18.4	590m	Light Brown Rendzina
Mediterranean (N31°42' E35°3')	540 ( $\pm$ 30)	17.7	620m	Terra Rossa
Mesic Mediterranean (N33°0' E35°14')	780 ( $\pm$ 22)	18.1	500m	Montmorillonitic Terra Rossa

Long-term averages of seasonal rainfall ( $\pm$ CV) are presented.

### Study species

The thorny dwarf shrub *Sarcopoterium spinosum* (L.) Spach (Rosaceae), which dominates large areas in the eastern Mediterranean region (Litav & Orshan 1971), was the focal species of this research. *S. spinosum*, 30 – 60 cm in height, grows mainly on infertile, shallow soils in association with secondary succession and grazing systems (Litav *et al.* 1963; Litav & Orshan 1971). Seedlings develop taproots, reaching up to 40 cm, which remain dominant (approx. 2 – 4 years) until adventitious roots are formed (Litav & Orshan 1971). During this stage, the seedlings are very sensitive to drought and resource competition with neighbour plants (Litav *et al.* 1963).

### Environmental measurements

Soil moisture and light availability are among the main factors influencing shrub seedling establishment success (Seifan *et al.* 2010; Matías, Zamora, *et al.* 2011). In order to obtain a more complete understanding of their relative importance in controlling shrub seedling establishment dynamics we measured soil moisture and light availability, as well as soil temperature and biomass of the annual neighbouring community.

Because seedlings are highly affected by their immediate environment, we took all environmental measurements in the vicinity of seedlings while trying to minimize the disturbance effects of the measurements. Soil moisture was measured gravimetrically twice a year; at the onset and the end of the growing season, respectively. For each treatment and microhabitat, i.e. open space between shrubs and under the shrub canopy, respectively, three samples (0-5 cm depth; n = 15 per treatment) were collected with a 1.5 cm core. Soil temperature and light intensity were as well recorded for each quadrat and microhabitat. Soil temperature at a depth of 5 cm was measured at noon, using a digital thermometer. Simultaneous measurements of light intensity 5 cm above ground were carried out by using a lux meter. For



measuring light intensity under the shrub canopy the lux meter was placed 5 cm within the shrubs canopy. For measuring aboveground biomass of the surrounding annual community, we harvested three quadrats (20 x 20 cm) in each plot and treatment at the peak of biomass production. Overall, there were 75 quadrats of biomass samples, which were oven dried to constant weight at 75°C, and weighed.

#### *Seedling survival monitoring*

Seedling survival was monitored at each site along the geographical gradient and within the rainfall manipulations. Approximately 2-3 months after the main germination event, seedlings were marked with unique identifiers. In order to cover the major source of small-scale heterogeneity, seedlings growing in the open space between shrubs and under the shrub canopy of *S. spinosum* were marked (Holzapfel *et al.* 2006; Seifan *et al.* 2010). To fully characterize the range of variability in seedling survival processes across space and time, 200 seedlings in each treatment combinations and habitats were marked, making a total of 2000 target seedlings for the experiment.

At the time of the census, *S. spinosum* seedlings had cotyledons and one to three leaves. The presence of cotyledons certified that these seedlings germinated in the current season. Seedlings were examined again after the first initial rains of the following season, and characterized as either dead or alive.

#### *Data analysis*

In order to analyse differences in seedling survival we compared the effect of both water availability and habitat (open space vs. shrub understory) on seedling survival in two different models: one representing the experimental climate change conditions within the Mediterranean site (drought, control and irrigation) and the other the geographical gradient (semi-arid, Mediterranean and mesic-Mediterranean). The potential effect of these factors on seedling survival was analysed using generalized linear models (GLM) with binominal response variable and logit-link function. The effect of water availability and habitat on the local abiotic and biotic conditions such as soil moisture, light availability, soil temperature and biomass of the annual neighbouring community were tested using a GLM with a normal distribution and identity link. In all cases, normality was assessed with a Kolmogorov–Smirnov test.

To identify the most parsimonious model, different combinations of variables and their interactions were used and compared with the Akaike information criterion (Anderson 2008). Post-hoc comparisons with the least significant differences multiple comparison method were done when significant main effects or interactions were detected. All analyses were performed using SPSS 19.

## Results

### Environmental measurements

No significant differences in soil moisture were found between the climate change manipulations ( $\chi^2_2 = 1.27$ ,  $p = 0.53$ ). However, soil moisture was significantly higher in the shrub understory for all three treatments ( $\chi^2_1 = 25.1$ ,  $p < 0.001$ ; Figure 1a). Light intensity in the open space decreased with increasing water input, with a significant difference between the driest and the two wetter treatments ( $\chi^2_2 = 7.93$ ,  $p = 0.019$ ) (Figure 1b). Light intensity in the shrub understory was always significantly lower than in the open space ( $\chi^2_1 = 61.02$ ,  $p < 0.001$ ), irrespective of treatment. Soil temperature dropped with increasing water input, but only differences between habitats were significant ( $\chi^2_1 = 21$ ,  $p < 0.001$ ; Figure 1c). Aboveground biomass of the annual community increased with increasing water input ( $\chi^2_1 = 9.93$ ,  $p = 0.007$ ) (Figure 1d).

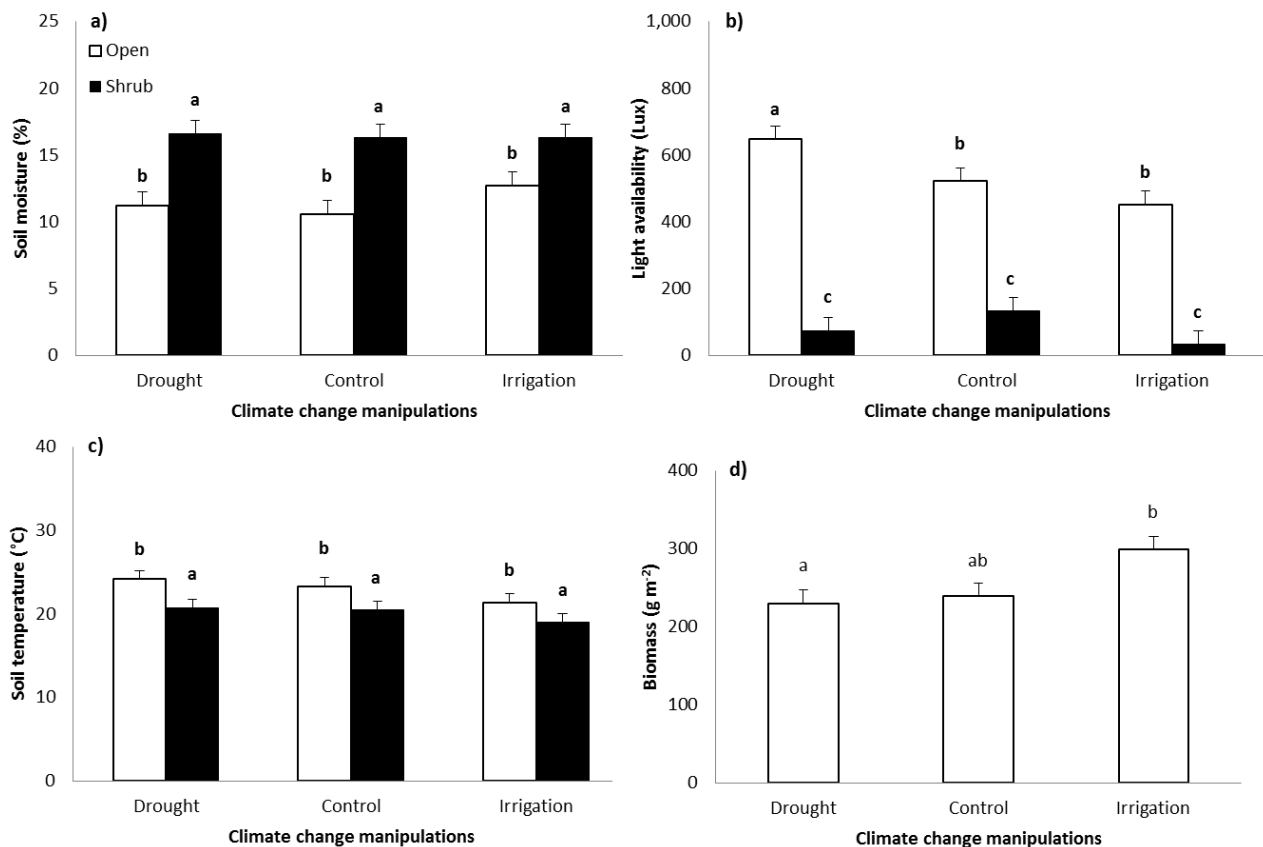


Figure 1. Abiotic and biotic conditions in the three different climate change manipulations (drought, control and irrigation) and two microhabitats (shrub vs. open areas) at the Mediterranean site ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ). a) soil moisture (%); b) light intensity (Lux); c) soil temperature (°C); and d) biomass ( $\text{g m}^{-2}$ ).

Soil moisture along the geographical gradient increased significantly with increasing rainfall ( $\chi^2_2 = 94.02$ ,  $p < 0.001$ ). In all three sites, soil moisture was always higher in the shrub understory than in the open space ( $\chi^2_2 = 14.48$ ,  $p < 0.001$ ; Figure 2a). Light intensity in the open space was significantly higher at the semi-

arid, compared to the Mediterranean and mesic-Mediterranean site ( $\chi^2_2 = 18.63$ ,  $p < 0.001$ ). As expected, light availability in the shrub understory was significantly lower, but did not differ between the three sites (Figure 2b). Soil temperature significantly decreased from the semi-arid to the mesic-Mediterranean site ( $\chi^2_2 = 12.12$ ,  $p = 0.002$ ) and was always lower in the shrub understory ( $\chi^2_2 = 39.09$ ,  $p < 0.001$ ; Figure 2c). The increasing water availability was associated with a significant increase in aboveground biomass production ( $\chi^2_2 = 43.19$ ,  $p < 0.001$ ; Figure 2d).

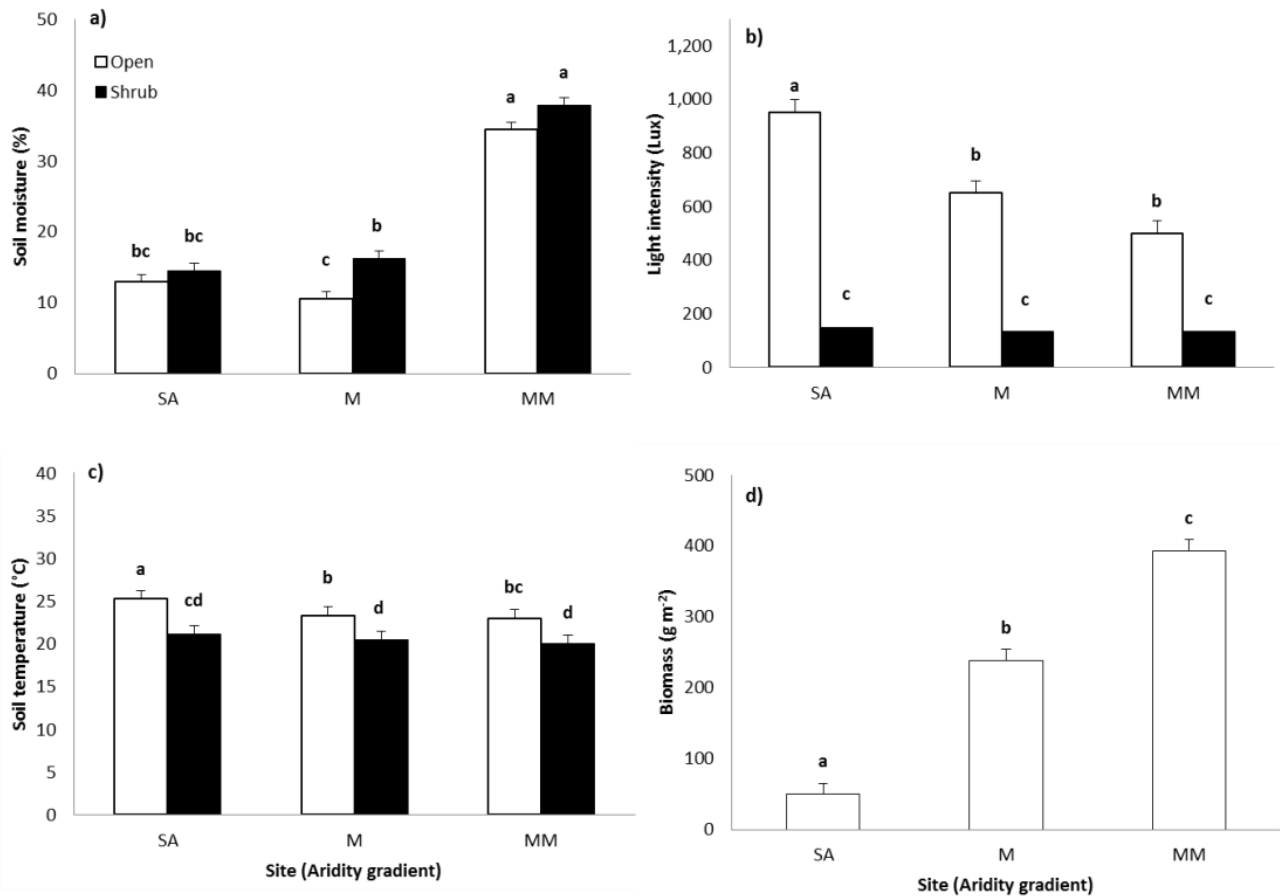


Figure 2. Abiotic and biotic conditions in the three sites indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) conditions, and two microhabitats (shrub vs. open areas) along the geographical gradient ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ). a) soil moisture (%); b) light intensity (Lux); c) soil temperature (°C); and d) biomass ( $g\ m^{-2}$ ).

### Survival

Extremely low annual rainfall and a prolonged drought in 2010 resulted in only 0.2% survival. We therefore analysed only data from the second year, i.e. data of 1117 seedlings.

In the experimental Mediterranean site, seedling survival increased significantly with increasing water input ( $\chi^2_2 = 14.97$ ,  $p < 0.001$ ; Figure 3). Shrubs had a positive effect on survival in all treatments but this effect was only significant in the irrigation treatment. No significant treatment and habitat interaction was observed ( $\chi^2_2 = 1.02$ ,  $p = 0.6$ ).

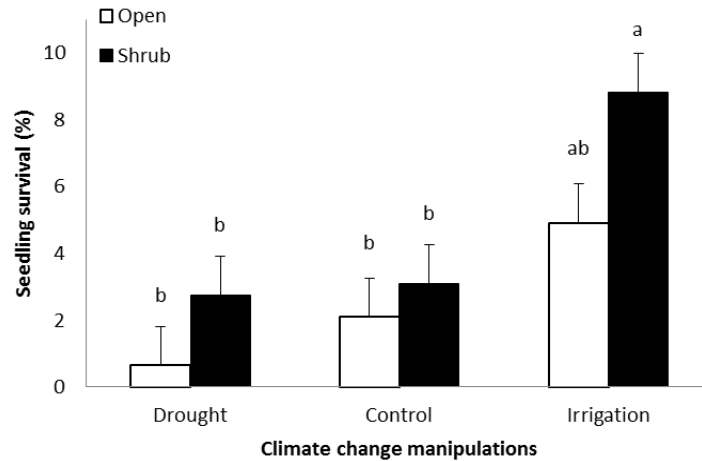


Figure 1. Seedling survival in the three different climate change manipulations (drought, control and irrigation) and two microhabitats (shrub vs. open areas) at the Mediterranean site ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).

In 2011, overall mean seedling survival ( $n = 114$ ) varied significantly between the three sites ( $\chi^2_2 = 52.86$ ,  $p < 0.001$ ). Seedling survival decreased from the semi-arid to the Mediterranean site and increased again towards the mesic-Mediterranean site (Figure 4). Seedling survival was significantly higher in the shrub understory in the semi-arid site, but there were no significant differences between survival in the shrub understory and open space in the two wetter sites. The interaction between site and habitat was marginally significant ( $\chi^2_2 = 5.98$ ,  $p = 0.05$ ). This interaction was created by two opposite trends: seedling survival showed an increasing trend in the shrub understory towards the dry end of the geographical gradient, while survival had an increasing trend in the open space with decreasing site aridity.

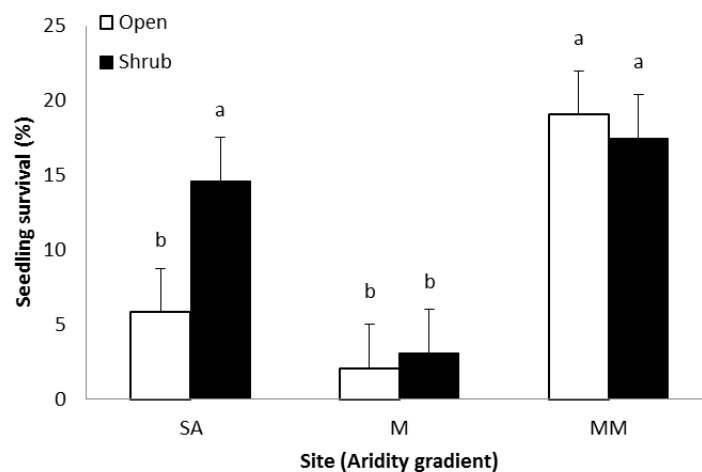


Figure 2. Seedling survival in the three sites indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) conditions, and two microhabitats (shrub vs. open areas) along the geographical gradient ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).

## Discussion

Our overall results show that climate gradients are a poor proxy for predicting the response of plant species to climate change, because of the complex interplay of abiotic and biotic factors. In particular, interactions with neighbouring plants play a crucial role in determining the distribution and establishment success of plants. Our findings of overall low survival indicate that seedling establishment is a bottleneck in shrub dynamics. Therefore, if future climate change alters shrub seedlings establishment, this may potentially compromise the long-term persistence of shrub populations (Garrido *et al.* 2007; Lloret *et al.* 2009). However, shrub seedling dynamics responded differently to an increase and decrease in water availability. Therefore, we discuss our results separately, starting with the response of shrub seedlings to increasing water availability, and followed by the response to increasing drought.

The fact that water availability was the main factor regulating shrub seedling establishment success was not surprising. The response of shrub seedlings growing along a geographical gradient and within local climate change manipulations followed a similar positive correlation with increasing water availability. This corroborates previous studies that suggested that stress alleviations via adding the most limited resource (here, water) boosts survival and leads to a significantly higher establishment success (Davis *et al.* 1999; Sternberg *et al.* 1999; Mendoza *et al.* 2009). Overall, these results emphasize the importance of water in determining seedling establishment, particularly in water stressed ecosystems (García-Fayos *et al.* 2000; Lloret *et al.* 2005).

More interesting than the direct effects of increasing water availability on survival were indirect effects that operated via the productivity of the annual community which in turn interacted with the shrub seedlings. Namely, the herbaceous neighbouring community benefitted from the higher water availability along the geographical gradient and climate change manipulations, as expressed by an increased biomass. A similar pattern was observed earlier by Holzapfel *et al.* (2006). As plant-plant competition is usually size-dependent (Weiner 1990), an increase in biomass is assumed to lead to an intensification of competitive interactions (Bertness & Callaway 1994; Callaway & Walker 1997; Holzapfel *et al.* 2006). We therefore hypothesized increasingly negative neighbour effects with increasing rainfall. Interestingly, our results showed an opposite trend contradicting not only the predictions by Bertness and Callaway (1994), but also several other studies that have been conducted in the same Mediterranean system (Holzapfel *et al.* 2006; Schiffers & Tielbörger 2006; Seifan *et al.* 2010; but see Ariza & Tielbörger 2011). Here, we found facilitative, rather than competitive interactions at the productive end of the natural as well as artificial environmental stress gradients. Although our work lacks a direct measurement of the interactions between the plants, similarly counterintuitive results from other studies support our observations. For example, Davis *et al.* (1999) detected only a small effect of herbaceous plants on seedling survival under wet conditions but a large negative effect under dry conditions with herbaceous neighbours present. They explained their findings

by assuming that the increased levels of the most limiting resource caused the resource level to exceed the requirements of the target plant and the surrounding herbaceous biomass at the same time, resulting in a reduced competition.

Under low general water availability, shrub seedling dynamics showed a more complex pattern which differed between the natural and artificial environmental stress gradient. For the geographical gradient, we observed an unexpected increased seedling survival towards the dry end. There are two reasonable explanations for our observations. First, shrub seedlings at the semi-arid site may be locally adapted to drier conditions (Jump & Peñuelas 2005). For example, physiological and morphological mechanisms to reduce the negative impacts of drought include rapid root elongation and/or increasing root size for expanding root absorption surface (Padilla & Pugnaire 2007). A second explanation could be a reduction in competition by neighbours, either by smaller negative effects of herbaceous neighbours, or by an increased function of adult shrubs as benefactors. The potential decrease in competitive effects by herbaceous neighbours could be due to the strong decrease in resource availability towards the dry end of the aridity gradient and subsequent biomass reduction. Indeed, productivity of the herbaceous community was significantly lower at the semi-arid site suggesting that competition was less intense. These findings are consistent with those of Seifan *et al.* (2010) who found that in dry years, shrub seedling establishment was facilitated due to release from competition by annual neighbours. Additionally, the potentially increased facilitative effect of adult shrubs was mainly observed through amelioration of the environmental conditions, such as reduced soil water loss through evaporation, lower soil temperatures and a reduced light intensity (see also Callaway 1995; Maestre *et al.* 2003; Castro *et al.* 2004; Castro *et al.* 2005; Holzapfel *et al.* 2006; Mendoza *et al.* 2009). These observations were furthermore verified by an increased seedling survival in the shrub understory at the dry end of the aridity gradient. This supports our above conclusion that water limitations associated with higher radiation and soil temperatures as in the open space seem to be the strongest causes for seedling mortality (Gómez-Aparicio, Gómez & Zamora 2005; Gómez-Aparicio, Gómez, Zamora, *et al.* 2005; Quero *et al.* 2006).

Intriguingly, shrub seedling dynamics in the experimental Mediterranean site responded differently to increased drought compared to the response along the geographical gradient. The drought treatment had a clear negative effect on shrub seedling establishment and in addition imposed a clear negative effect on the productivity of the entire plant community, consistent with our expectation (Sternberg *et al.* 1999; Fay & Schultz 2009). Our measurements of abiotic variables showed more stressful abiotic conditions in the dry treatment with higher light intensity and increased soil temperatures. In addition, the decreased seedling survival in the drought treatment could be explained by lower drought resistance of the local plants, as opposed to plants from the drier sites. Alternatively, the reduced survival of perennial species might also be a result of the ability of annual plants to use available water resources more rapidly (Krebs 2008), therefore compromising the water resources for perennial species.

In summary, this complex interaction between climatic effects and biotic effects demonstrates the potential dangers in using geographical gradients as proxies and highlights the importance of combining both experimental and correlative approaches for determining plant response to predicted climate change. Therefore, our study supports the suggestion made by Fukami and Wardle (2005) of combining natural and artificial environmental stress gradients in studies about environmental change. Our integrated approach enabled us to disentangle changes in climate only and local environmental conditions which are likely to be confounded (Dunne *et al.* 2004; Sternberg *et al.* 2011). Our study indicates that biotic interactions may actually reverse the direct effect of climatic change, i.e. change in water availability, on plant performance. Namely, a decrease in water availability is not necessarily detrimental for establishment if potential competitors are more sensitive to drought and thus competitive interactions diminished. In such a case, correlative models are not satisfactory for predicting species changes under climate change and may either over- or underestimate changes in species abundance. Another case where gradient studies fail to predict species response to climate change is when species are locally adapted to the current conditions, and when these conditions are unpredictable. Namely, the observed unimodal response to overall water availability along the gradient indicated some degree of adaptation to the dry and variable site condition. Therefore, in plant communities exposed to high intra- and inter-annual variations in rainfall, such as our systems, shrub seedlings may be preadapted to a large range of climatic conditions and thus exhibit a higher resistance to local drought.

## Chapter 2

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Neighbour effects on shrub seedling establishment override climate change impacts



## **Abstract**

Climate change effects on plant species composition, growth and productivity of ecosystems have long intrigued scientists. However, biotic factors and their interactions under climate change have been largely overlooked despite their importance in plant population dynamics. We conducted a field experiment along a prominent rainfall gradient in Israel in order to study the effects of changing abiotic and biotic factors on shrub seedling establishment, as well as the direction and intensity of plant-plant interactions determining shrub seedling dynamics. Our results show that biotic interactions override the effect of water availability, which commonly is considered the main limiting factor in shrub seedling establishment. In particular, decreasing soil moisture along the aridity gradient had no direct effect on shrub seedling establishment. However, decreasing water availability affected shrub seedling establishment indirectly by altering the biomass production of the neighbouring annual community, therefore changing the abiotic environment. Overall, biotic interactions were always negative, with highly competitive interactions at the intermediate site (Mediterranean) along the natural aridity gradient. We explain these patterns based on decreasing water and increasing light availability along the aridity gradient. Hence, at the wet end high levels of soil moisture compensated for insufficient light levels and the opposite effect was observed at the dry end of the gradient. At the intermediate site both factors are scarce, resulting in decreased survival and increased competitive interactions. Our study emphasizes the importance of incorporating biotic interactions in studies predicting changes in plant dynamics under future climate change.

## Introduction

Ever since climate change has been acknowledged as one of the main drivers influencing the species composition, growth and productivity of ecosystems, a large number of studies have attempted to understand and predict its influence on plant population dynamics (Walther *et al.* 2002; Thuiller *et al.* 2005). Changes in plant populations due to climate change are supposed to occur rather slowly and the responses between species vary significantly, comprising either adaptation or migration to climatic suitable areas, respectively, or extinction (Peñuelas *et al.* 2013). However, as abiotic factors are closely linked to biotic interactions, studying both factors simultaneously has been proven inevitable in order to give reliable predictions about alterations in plant population dynamics.

A common approach for studying species response to climate change involves natural geographical gradients, imitating the gradual change towards harsher climatic conditions, as expected under climate change. Thus, gradients of natural stress offer a unique experimental set up as they incorporate abiotic as well as biotic factors. Several studies investigating plant-plant interactions along gradients of natural stress have repeatedly shown that the direction and intensity of biotic interactions, e.g. facilitation and competition among plants, depend directly on the favourability of the abiotic environment (Bertness & Callaway 1994; Brooker *et al.* 2008). According to Bertness and Callaway (1994) interactions between plant species shift along environmental gradients in a predictable manner such that competition prevails in less stressful and facilitation dominates in stressful environments. Since then, a large number of field experiments tested these models (e.g. Brooker & Callaghan 1998; Pugnaire & Luque 2001; Callaway *et al.* 2002). Though there is also contradicting evidence (Tielbörger & Kadmon 2000; Maestre & Cortina 2004; Maestre *et al.* 2005; Maestre *et al.* 2009), the model by Bertness and Callaway (1994), called the stress gradient hypothesis (SGH), provides a useful framework for evaluating the relative importance of plant-plant interactions in a changing climate. Namely, if the climate will change to more harsh conditions (e.g. warming and reduced precipitation in water-limited systems), then increased facilitative interactions can be expected, while the opposite, i.e. intensified competition, would occur in habitats where climate change reduces abiotic stress (e.g. warming in habitats limited by cold temperatures).

To date, the majority of studies investigating plant interactions have focused on large, primarily woody benefactors (e.g. shrubs) ameliorating conditions for small herbaceous plants (Lloret *et al.* 2004; Holzapfel *et al.* 2006; Lloret *et al.* 2009; but see Tielbörger and Kadmon 2000). Yet again, studies researching the opposite, e.g. direct effects of herbaceous plants on shrub seedlings are very scarce (but see Holzapfel & Mahall 1999; Seifan *et al.* 2010). However, taking into account the important role of long-lived woody plants in shaping their habitats it is necessary to understand the intensity and direction of interactions between perennial species and herbaceous neighbours to explain and predict changes of natural plant communities prone to be affected by climate change.

Within the life cycle of woody species, early seedling establishment provides the best opportunity for understanding the potential effects of climate change and associated alterations in biotic factors. Long-lived woody plant species depend on a small spatial and temporal window (“safe site”) for a successful establishment (sensus Harper *et al.* 1961), in which they exhibit a high vulnerability to alteration in abiotic and biotic factors (Gómez-Aparicio, Gómez & Zamora 2005). The main abiotic factors influencing shrub seedling establishment are water and light availability (Davis *et al.* 1999; Seifan *et al.* 2010). This high dependency on available water resources (i.e. soil moisture) is particularly perceivable in water stressed ecosystems, which will be intensified under predicted climate change. However, in accordance with the stress gradient hypothesis we expect facilitative plant-plant interactions to increase, as limited water resources, the major abiotic constraint for perennial species in our study system, will be intensified in the future (Gómez-Aparicio *et al.* 2008).

Our study was designed to evaluate the relative importance of direct effects of climate change vs. indirect effects mediated by biotic interactions on shrub seedling establishment in a dryland ecosystem. To that end, we transplanted seedlings of a common shrub into three field sites along a natural rainfall gradient in Israel and combined these transplants with neighbour manipulation experiments. The three sites served as proxy for changes in abiotic and biotic factors according to expected climate change (Sternberg *et al.* 2011). Assuming different levels of adaptation to abiotic and biotic factors (Kawecki & Ebert 2004) and to give more realistic predictions about future shrub seedling dynamics we used seeds from the two extreme ends of the rainfall gradient. Shrub seedling survival was followed throughout two vegetative seasons (2011 and 2012). A complementary garden experiment, in which we manipulated water availability and neighbours, enabled us to better control environmental variations and to directly test the direct and indirect effect of the predicted major climate change effect on shrub seedling establishment. We tested the following specific hypotheses: a) increased drought, as predicted by climate models, reduces shrub seedling establishment; b) competition by annual neighbours reduces shrub seedling survival; c) negative effects by annual neighbours will be less pronounced under water limited conditions, i.e. climate change effects can be reverted due to less intensive negative interactions; and d) seeds originated from harsh environments are better adapted to abiotic stress whereas seeds from benign environments show resilience to high competitive levels.

## **Material and Methods**

### *Study sites*

The study was conducted at three sites along the natural rainfall gradient in Israel, covering mesic-Mediterranean in the north to semi-arid climatic conditions in the south. The mesic-Mediterranean site (Galilee Mountains, N 33°0', E 35°14') receives an average 780 mm of rainfall and is the wettest site. The Mediterranean site (Jerusalem Mountains, N 31°42', E 35°03') receives on average around 540 mm

precipitation, and the semi-arid site (northern Negev desert, N 31°23', E 34°54') receives only 300 mm average annual rainfall (details see Holzapfel *et al.* 2006; Sternberg *et al.* 2011). Annual fluctuations along the natural rainfall gradient are characterized by a coefficient of variation of  $\pm 22\%$  in the north and  $\pm 37\%$  in the south, respectively. These large fluctuations emphasize the importance of multiannual experiments, in order to capture this natural variation. During the first year of our experiment, annual rainfall in the Mediterranean was only 357 mm (66% of the long-term annual mean) and in the semi-arid site 178 mm (only 59% of the long-term annual mean). In the second year of the experiment, rainfall was slightly above average, with 575 mm and 318 mm precipitation, at the Mediterranean and semi-arid site, respectively. Rainfall in the mesic-Mediterranean site (832 mm in 2011 and 808 mm in 2012) was in both years above the long term average.

The vegetation at the three study sites is dominated by *S. spinosum*, associated with herbaceous annual vegetation (for details see Holzapfel *et al.* 2006; Petrů *et al.* 2006). All sites share similar topographic conditions, they are all located on southern slopes of calcareous bedrock and at similar elevations (Harel *et al.* 2011).

Field experiments were complemented with a Botanical Garden experiment at the Botanical Garden at Tel Aviv University, Israel (N 32°11', E 34°81') in a climate similar to the Mediterranean field conditions, albeit with higher temperatures. The Botanical Garden is situated on sandy loam. There were strong seasonal variations of rainfall for the two years of the experiment. The first growing season (2010/2011) was relatively dry with only 84.3% (491.7 mm) rainfall of the long-term average (583 mm). Furthermore, with the first plant growth triggering rainfall in December the rainy season started very late. During the 2011/2012 season, rainfall exceeded the annual mean by 10%, with rainfall of 641.5 mm. However the rainfall season was concentrated over a relative short period of only five months (Nov.–Mar.). The experimental area of the Botanical Garden as well as the three field sites were fenced to exclude grazing (i.e. goats and cattle).

### *Study species*

*Sarcopoterium spinosum* (L.) Spach is a long-lived, thorny, dwarf shrub, which dominates large areas in the eastern Mediterranean region (Litav & Orshan 1971). The shrub is characterized by a cushion shaped canopy, 30-60 cm high, and a distinct deep branched taproot that enables the shrub to grow on infertile and dry soils (Litav *et al.* 1963; Litav & Orshan 1971; Henkin & Seligman 2011). The tap root reaches up to 40 cm and remains dominant throughout the first 2–4 years, until adventitious roots are formed (Litav & Orshan 1971). Particularly at this early life stage, seedlings are highly sensitive to drought and competition with neighbour plants (Litav *et al.* 1963; Reisman-Berman 2007; Seifan *et al.* 2010).

### *Field experiment*

To examine the effects of climate on shrub seedling dynamics and how climate affects the interaction with the neighbouring community, we performed a full factorial experiment along the natural rainfall gradient. For half of the plots the presence of herbaceous neighbours was manipulated by removing all herbaceous seedlings regularly with a scalpel at ground level, until no further seedlings emerged. The herbaceous neighbouring community present treatment was represented by a natural annual community, composed of site specific species (see Petruš *et al.* 2006).

We used seedlings of *S. spinosum* that originated in two opposing climatic conditions (i.e. semi-arid and mesic-Mediterranean). Just before the main rainy season started, shrub seeds from the two opposing origins were sown and germinated in the net house of the Botanical Garden in controlled conditions and were transferred to the experimental sites after two cotyledons leaves as well the first leaf were present (approx. January until early February). At the time shrub seedlings were transplanted into the experimental units the annual vegetation at the mesic-Mediterranean and Mediterranean site had an average height of 4-5 cm and 3-4 cm at the semi-arid site. Four seedlings each were transplanted into 50×20 cm plots (N=112 per site). During the first two weeks seedlings were closely monitored and dead seedlings were replaced until all seedlings were fully established.

### *Botanical Garden experiment*

The garden experiment enabled us to analyse abiotic and biotic factors affecting shrub seedlings under controlled conditions. Climate change manipulations were simulated by two different levels of rainfall availability – control and drought conditions. Control conditions were represented by plots which were subjected to the natural rainfall. Drought conditions were simulated by plots receiving 30% less natural rainfall which is in accordance with climate change predictions for the Mediterranean region (Smiatek *et al.* 2011). The exclusion of natural rainfall was achieved by rainout shelters (3.10×2.5×1.55 m; Yahdjian & Sala 2002). U-shaped clear plastic bands supported by a metal frame intercepted 30% of the natural rainfall. The rainout shelters were set up with a slight inclination draining excess water into ditches channelling the intercepted water away from the plots. Rainout shelters were open to the sides in order to allow wind movement and minimize microclimatic differences, such as temperature and relative humidity (Fay *et al.* 2000). From here on, we will refer to these treatments as control and drought.

The neighbour treatment had three levels: a) no annual neighbour's present, b) community of annuals from the semi-arid site and c) community of annuals from the mesic-Mediterranean site. By using herbaceous communities from the two origins (same as our focal species was collected from) we were able to control for the potential effect of species identity and common adaptations. Seeds of annual plants were collected prior to the experiment. In order to break the seed dormancy seeds were stored in the field where

they were subjected to natural conditions. The two artificially created communities had the same seed density and were composed of three species for each of the major functional groups (see Appendix A). Annual plant communities were sown directly into the experimental units just before the main rainy season started and their emergence density was monitored to maintain similar composition and density in all the plots. As for the field experiment, *S. spinosum* seedlings originated in semi-arid and mesic-Mediterranean conditions were transplanted into experimental units.

Seeds were sown simultaneously with seeds from the field experiment and transplanted to the experimental units (cotyledon stage). Prior to the transplantation of the shrub seedlings, the two annual neighbouring communities were thinned to the same seedling composition and density. At this stage the average height of the annual vegetation was 4-5 cm. Within each subplot (30×30 cm) three seedlings of *S. spinosum* were transplanted (total N=528). Here, too, seedling establishment was monitored carefully for two weeks and dead seedlings replaced if necessary.

#### *Environmental measurements*

Several biotic and abiotic factors involved in determining shrub seedling dynamics were measured to validate the treatment effects. We measured annual community biomass, soil moisture, soil temperature and light availability for each plot at the end of vegetation period (end of May). For measuring aboveground biomass of neighbouring annual community in the three field sites, we harvested three quadrats (20×20 cm) from areas adjacent to each plot. Afterwards, samples were oven dried at 75°C for 48h, and weighed. Annual community biomass in the Botanical Garden was harvested at about the same time. To mimic potential habitat amelioration by the neighbouring community during the summer months we immediately covered plots with mulch. Samples were processed the same way as mentioned above.

Soil samples (5 cm depth) were collected with a 1.5 cm core for every plot and soil moisture was measured gravimetrically (oven-drying 48h at 105°C). Additionally, soil temperature (5 cm belowground) and light intensity (5 cm aboveground) was recorded for each treatment combination at noon. Soil temperature was measured with a digital thermometer and light intensity measurements were carried out by using a lux meter. Due to logistical problems, light availability and soil temperature were only measured in the first year of the experiment.

#### *Seedling survival monitoring*

After seedlings were transplanted to the field and garden and their establishment was secured, we monitored survival on a monthly basis. Seedlings which showed completely dry leaves were assigned as dead and seedlings damaged by external disturbing factors (e.g. herbivory) were excluded from further analysis.

## Data analysis

We used generalized linear models (GLM) with normal distribution and identity link function to test the differences in soil moisture, soil temperature, light availability and biomass at the three experimental field sites and neighbour treatments. The same analysis was applied to analyse differences in soil moisture among artificial water treatments and neighbouring community of the Botanical Garden experiment.

For analysing potential effects of climate change manipulation, neighbour treatment and shrub seedling origin on seedling survival, we conducted a GLM with a binominal distribution and a logit-link function. We tested different combinations of variables and their interactions to determine the best model based on the AIC (Akaike information criterion; Anderson 2008). As adding explanatory variables (e.g. soil moisture, herbaceous biomass) resulted in a higher AIC, these were excluded from the model in order to increase the goodness of fit of the model. The same procedure was applied to analyse seedling survival for the experimental field sites, with site, neighbour treatment and shrub seedling population origin as independent variables.

To test the hypothesis that interactions between our target species and the neighbouring annual community shift from competition to facilitation with increasing environmental stress we used the “relative interaction index” (RII) by Armas *et al.* (2004). The index is calculated as follows:  $RII = (X_{+N} - X_{-N}) / (X_{+N} + X_{-N})$ , where  $X_{+N}$  and  $X_{-N}$  describe the performance of the target species with and without neighbours, respectively. The index ranges between 1 and -1, indicating either a positive (facilitative) or negative (competitive) effect of the neighbours on shrub seedlings. We calculated index values for survival and then tested for differences among sites and shrub seedling population origin for the field experiment and climate change manipulations, neighbour treatment and shrub seedling population origin for the garden experiment. The analysis was run with a GLM with normal distribution and log link function.

For comparison of significant variables and their interactions of all models described above we performed post-hoc comparisons with the least significant differences multiple comparison method. All analyses were performed using SPSS software Version 21.

## Results

### Environmental measurements

*Field experiment:* In both years soil moisture content along the geographical gradient decreased significantly with decreasing rainfall (2011:  $\chi^2_2 = 580.14$ ,  $p < 0.001$ ; 2012:  $\chi^2_2 = 201.21$ ,  $p < 0.001$ ). No effect of neighbour treatment on soil moisture content was observed. Mean soil temperature declined significantly towards the wetter end of the gradient in the first year of the experiment ( $\chi^2_2 = 738.58$ ,  $p < 0.001$ ). Annual neighbours significantly decreased soil temperature ( $\chi^2_2 = 7.74$ ,  $p = 0.005$ ). Site, neighbour community as well as their interactions significantly influenced light availability 5 cm above ground level. The highest light

availability was measured in the semi-arid site ( $\chi^2_2 = 40.12$ ,  $p < 0.001$ ) and in general light availability was significantly reduced by the presence of annual neighbours ( $\chi^2_2 = 131.89$ ,  $p < 0.001$ ). The lowest levels of light availability were measured in the Mediterranean site in the neighbour treatment. In the first year there were significant differences in biomass production between the semi-arid and the two wetter sites ( $\chi^2_2 = 252.06$ ,  $p < 0.001$ ). In the second year of the experiment, the same trend of increasing biomass production with increasing rainfall could be observed, however biomass production at the semi-arid site was almost fourfold compared to the first year. Mean parameter values ( $\pm$  SE) for all explanatory variables are shown in Appendix B.

*Botanical Garden experiment:* In the garden experiment soil moisture was significantly wetter in the control than in drought treatment (2011:  $\chi^2_1 = 6.94$ ,  $p = 0.08$ ; 2012:  $\chi^2_1 = 4.07$ ,  $p = 0.044$ ) and also neighbouring annuals increased soil moisture significantly in both years (2011:  $\chi^2_1 = 12.74$ ,  $p < 0.001$ ; 2012:  $\chi^2_1 = 18.21$ ,  $p < 0.001$ ). A reduction of rainfall by 30 % was accompanied by significantly increased soil temperature ( $\chi^2_1 = 8.41$ ,  $p = 0.04$ ) and the presence of annual neighbours significantly reduced light availability ( $\chi^2_1 = 120.43$ ,  $p < 0.001$ ). The drought treatments influenced the biomass production of the neighbouring community (2011:  $\chi^2_1 = 4.28$ ,  $p = 0.039$ ; 2012:  $\chi^2_1 = 4.25$ ,  $p = 0.039$ ), with significantly increased biomass in the drought treatment. Mean parameter values ( $\pm$  SE) for all explanatory variables are shown in Appendix C.

### *Seedling survival*

*Field experiment:* In both years, there were significant differences between sites and neighbour treatments (Table 1). Seedling survival along the geographical gradient followed a u-shaped response (Figure 1a). The mesic-Mediterranean site showed the highest survival probability which decreased drastically at the Mediterranean site. However, survival probability increased again at the very dry end of the gradient. Despite higher rainfall in the second year of the experiment and a longer vegetation period, seedling survival probability was substantially lower than in the first year (2011:  $42 \pm 4.6\%$ ; 2012:  $14 \pm 3\%$ ). In general a similar trend of survival probability could be observed in the second year (Figure 1b), except that this year, survival probability was highest in the semi-arid site. At the Mediterranean site, the survival probability was extremely low and no seedling survived in plots with annual neighbours. In both years, seedling survival probability was always significantly higher in the plots without neighbouring community present. Neither the shrub seedling population origin nor any of the interactions of the various factors had any effect on the probability of shrub seedling survival.



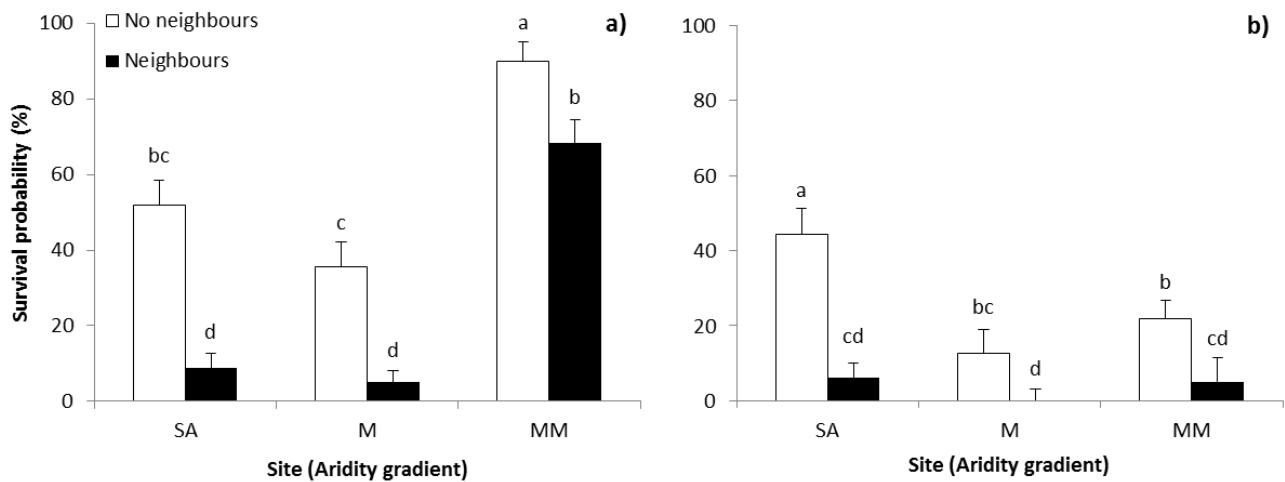


Figure 1. Seedling survival probability in a) 2011 and b) 2012 in the three sites along the natural rainfall gradient, indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) locations ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).

Table 1. Summary of separate GLMs used to quantify the effect of site, population origin and neighbour treatment, as well as their interaction on the survival probability of the transplanted *S. spinosum* seedlings.

Source	df	2011		2012	
		$\chi^2$	P	$\chi^2$	P
Site (S.)	2	58.92	< 0.001	674.74	< 0.001
Neighbour (N.)	1	32.86	< 0.001	420.00	< 0.001
Origin (O.)	1	1.24	0.265	0.23	0.631
S.*N.	2	1.59	0.451	0.73	0.393
S.*O.	2	4.53	0.104	3.07	0.215
N.*O.	1	0.59	0.444	0.49	0.485
S.*N.*O.	2	1.22	0.543	2.15	0.143

*Botanical Garden experiment:* Seedling survival probability of *S. spinosum* was notably different between the two consecutive years. Despite lower rainfall levels in the first year survival probability was significantly higher than in the second year. Analyses for both seasons separately showed that seedling survival had a negative response to the annual neighbouring community with significantly lower values (Figure 2a-b; Table 2). Climate change manipulations and the shrub seedling population origin had no effect on shrub seedling survival probability.

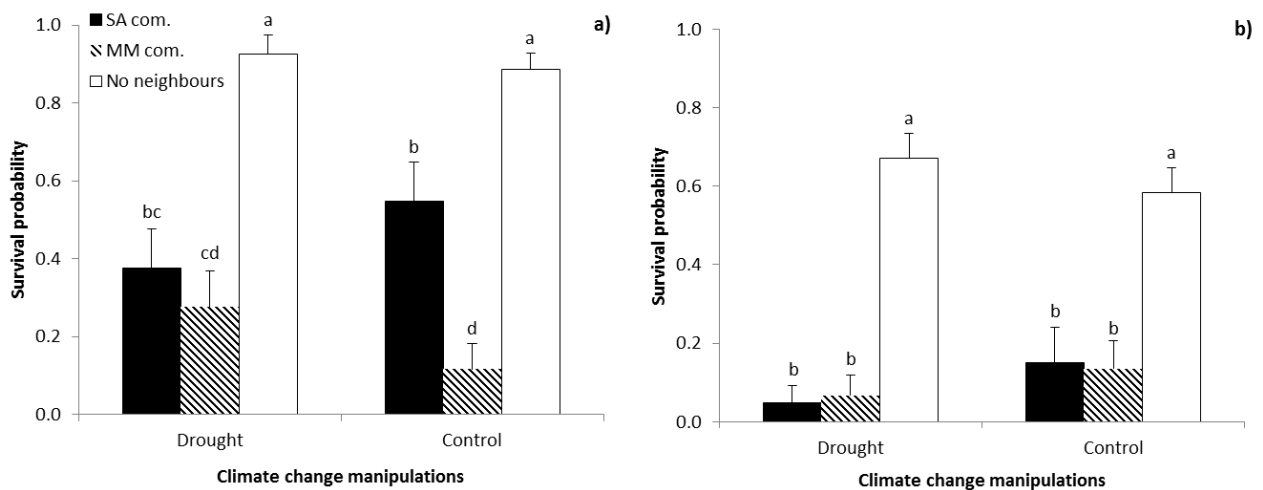


Figure 2. Seedling survival probability in a) 2011 and b) 2012 in the Botanical Garden for the diverse treatment combinations ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).

Table 2. Summary of separate GLMs used to quantify the effect of water treatment, neighbouring community and population origin, as well as their interaction on transplanted *S. spinosum* seedlings.

Source	df	2011		2012	
		$\chi^2$	P	$\chi^2$	P
Water (W.)	1	0.43	0.513	1.07	0.301
Neighbours (N.)	2	45.53	< 0.001	43.75	< 0.001
Origin (O.)	1	0.27	0.605	0.55	0.459
W.*N.	2	3.48	0.175	2.57	0.276
W.*O.	1	0.02	0.882	0.20	0.652
N.*O.	2	3.83	0.147	2.29	0.319
W.*N.*O.	2	3.28	0.149	2.31	0.315

### RII (Relative Interaction Index)

*Field experiment:* The analysis of the interaction index for survival showed a significant site effect in the first year of the experiment ( $\chi^2_2 = 15.23$ ,  $p < 0.001$ ; Figure 3a). Despite a negative interaction index for all treatment combinations, the post hoc test revealed less negative interactions at the wet end of the aridity gradient. Population origin and the interaction of site and origin had no effect on the interaction index and both shrub seedling origins showed the same trend. For the second year also only negative interactions were found, with none of the factors altering interaction intensity (Figure 3b).

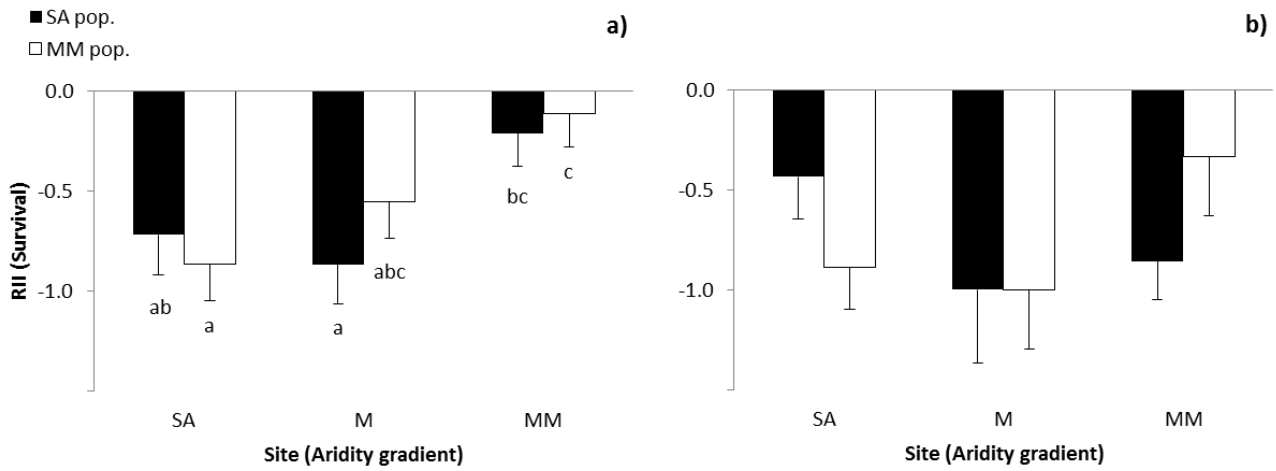


Figure 3. RII ( $\bar{X} \pm SE$ ) for a) 2011 and b) 2012 survival data for different sites along the natural aridity gradient. Letters represent significantly different groups ( $p < 0.05$ ).

*Garden experiment:* Our results show that at the end of the vegetative period only competitive interactions occurred as a response to annual neighbouring community (Figure 4a-b). Nevertheless, the magnitude of the negative effect changed among treatment combinations (Table 3). In the first year, the competitive effect of the mesic-Mediterranean neighbours was significantly more negative than of the semi-arid neighbours. Additionally, we found a marginally significant interaction between water treatment and neighbouring community. This interaction was caused by a significant release from competitive interactions in the control treatment and semi-arid neighbours. In the following year we could observe significant differences between the two water treatments with decreased negative interactions in the control treatment for both annual communities.

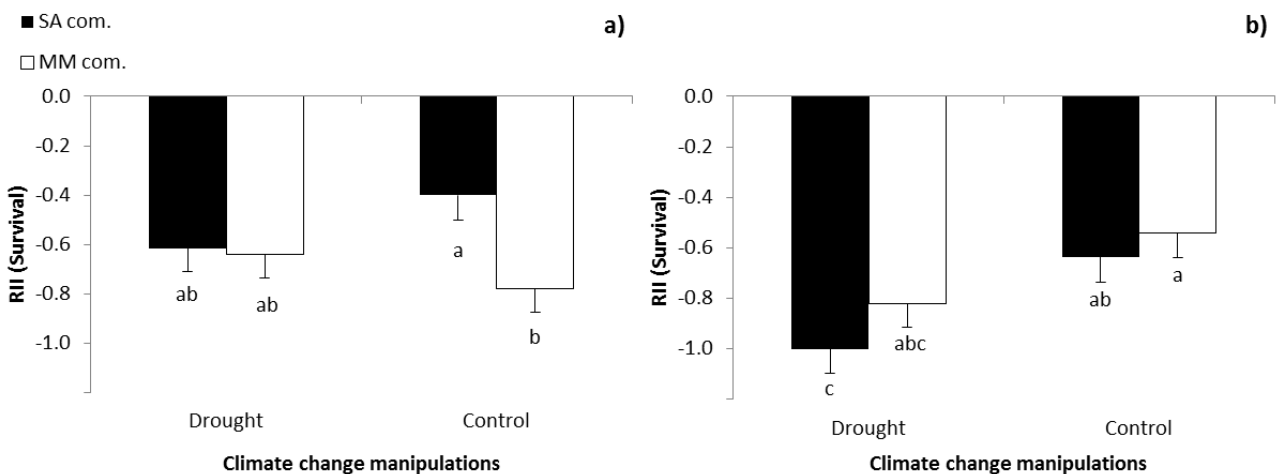


Figure 4. RII ( $\bar{X} \pm SE$ ) for a) 2011 and b) 2012 survival data for the different population origins and years. Letters represent significantly different groups ( $p < 0.05$ ).

Table 3. Summary of separate GLMs with RII as dependent variable and water treatment, neighbouring community and population origin, as well as their interaction as independent variables.

Source	df	2011		2012	
		$\chi^2$	P	$\chi^2$	P
Water (W.)	1	0.08	0.783	4.12	0.042
Neighbours (N.)	2	6.98	0.008	0.33	0.566
Origin (O.)	1	3.76	0.052	0.03	0.872
W.*N.	2	3.87	0.049	1.11	0.293
W.*O.	1	3.84	0.050	0.58	0.448
N.*O.	2	0.01	0.915	2.39	0.122
W.*N.*O.	2	0.70	0.403	0.39	0.531

## Discussion

Our overall results suggest that biotic interactions play a much more important role than abiotic interactions for shrub seedling establishment while both, direct or indirect effects of climate were negligible. Most strikingly, shrub seedling establishment was not affected by a reduction in water availability, suggesting that a reduction in rainfall due to climate change may not at all affect shrub seedling dynamics. However, competition with the neighbouring herbaceous community significantly decreased shrub seedling establishment.

Our field experiment provides contradictory evidence regarding the commonly observed patterns of survival along environmental stress gradients. Instead of the mostly observed linear decreasing trends in seedling survival along environmental stress gradients (Davis *et al.* 1999; Castro *et al.* 2004; Castro *et al.* 2005; Lloret *et al.* 2005; Lloret *et al.* 2009; Matías, Gómez-Aparicio, *et al.* 2011; Matías *et al.* 2012) our results revealed a u-shaped pattern, with shrub seedling survival being lowest under intermediate water availability. Also our results from the Botanical Garden experiment revealed counterintuitive results, because drought had no significant negative effect on shrub seedling survival in either of the years. These rather surprising results may have been due to the specific nature of our focal species. According to Evenari *et al.* (1986), *S. spinosum* successfully establishes at areas receiving between 250-1000 mm annual rainfall. Therefore, annual rainfall varying between 300-780 mm was above the species distribution margin and consequently, could have been well above the threshold needed for successful establishment. Miranda *et al.* (2009) observed in Mediterranean conditions comparable results where a rainfall reduction of 25% did not cause any shifts in perennial plant community composition. Miranda *et al.* (2009) suggested that this could be due to the high resilience and adaptation of these environments to inter- and intra-annual rainfall variability. Similar suggestions have been provided by Jump and Peñuelas (2005) dealing with adaptation and mechanisms of how species might handle a rapid changing climate. In our study system, rainfall varied by 22% at the mesic-Mediterranean site and 37% at the semi-arid site, respectively.

Since water availability was demonstrated as an unimportant factor directly influencing shrub seedling establishment, we attribute the observed increase in shrub seedling survival towards the two extreme ends of the natural gradient to the neighbouring community. Water availability directly influenced the biomass production of the neighbouring community which in return altered the abiotic environment, as well as direction and intensity of interactions.

In contrast to our expectation, the interaction with the neighbouring community was mostly competitive. We expected to see a change in the intensity and direction of interactions as a result of changing water availability, signifying that environment is a major factor. However, again the drought treatment had no evident effect, even not via a modification of neighbour effects, though neighbour competition alone was a large constraint for shrub seedling establishment. These findings contradict numerous other studies indicating clear facilitative effects under similar environmental conditions due to habitat amelioration via decreased evapotranspiration and protection from intensive radiation (Castro *et al.* 2004; Gómez-Aparicio, Gómez & Zamora 2005; Padilla & Pugnaire 2007). Similar trends were observed along the natural stress gradient. However, despite the overall negative interactions, we observed a change in the interaction intensity along the gradients. Just as shrub seedling survival, RII followed a u-shape with a reduction in competitive interactions towards the two extreme ends of the climatic gradient. The explanation for our observation could be based on two opposed resource gradients, namely the direct effect of decreasing water availability and the indirect effect of a decreasing neighbour density, therefore increasing light availability. Hence, shrub seedling establishment was not directly affected by decreasing water availability, however the annual neighbouring community responded with a significantly decreasing biomass production which led to alterations in the abiotic environment. Specifically, at the mesic-Mediterranean site sufficient belowground resources (here, soil moisture) for our target plants as well as herbaceous neighbours led to a slight release in competitive interactions (Ariza & Tielbörger 2011). However, aboveground competition for light may explain the yet only competitive interactions in plots with annual neighbours present. At the Mediterranean site shrub seedling survival was significantly inhibited by competition for above- and belowground resources, which is easily explained by a significantly decreased water and light availability in plots with annual neighbours present. At the semi-arid site which is characterized by extremely dry conditions, water is the limiting factor while light levels are sufficient due to relatively sparse annual vegetation. Sufficient light availability might therefore counteract limited water availability (Seifan *et al.* 2010). These findings are in accordance with the light-water model proposed by Holmgren *et al.* (1997) which predicts a complex trade-off between various factors (here, light and water) along natural gradients, where the response of plants to these trade-offs depends on the correlated changes. Thus, net effects of decreased soil moisture correlated with decreased light availability, such as at the intermediate site, provoked highly competitive interactions. Additionally, these findings are in accordance with several other

studies investigating the effect of light availability on shrub seedling establishment (Reisman-Berman *et al.* 2006; Seifan *et al.* 2010). The slight relieve in competitive interactions in the second year might be explained by habitat amelioration due to an increased neighbouring community (Castro *et al.* 2004; Gómez-Aparicio, Gómez & Zamora 2005), resulting in slightly higher soil moisture levels until the end of the vegetative period.

Interestingly, a previous study by Soliveres *et al.* (2013), in a similar environment, found similar results for plant-plant interactions in response to changes in the magnitude and frequency of rainfall. Soliveres *et al.* (2013) explained the prevalence of predominantly negative interactions by the rather favourable environmental conditions for a drought adapted species. Accordingly, an occurrence of facilitative interaction would be rather unlikely. As *S. spinosum* is also resistant to drought (Litav & Orshan 1971), stress levels along the natural rainfall gradient and drought treatments within the garden experiment might not have been sufficient enough to evoke facilitative interactions. However, more studies across a wider range of environmental stress are required to further test these assumptions.

Surprisingly, there was no evidence of seed origin influencing the response to either abiotic stress or competitive levels. Seeds from dry origin showed no advanced strategies under high abiotic stress and seeds from the mesic-Mediterranean origin revealed no adaptation to competition. The coherent and aligned response of both origins emphasizes the species overall resistance to abiotic stress but concurrent vulnerability to competition with herbaceous neighbours at young stages. Consistent with our findings, Ariza and Tielbörger (2011) found no evidence of local adaptation in two annual species growing within the same study system (but see Petrů *et al.* 2006; Petrů & Tielbörger 2008; Tielbörger & Petrů 2010).

Overall, we were able to show that climate change with its predicted decrease in water availability is only secondary to shrub seedling establishment. In contrast to prior studies predicting that climate change is the main future constraint, yet forget that organisms have a biotic environment, we emphasize the importance of biotic interactions which generally speaking appear to be far more important. We were able to show that ignoring biotic interactions may lead to falls conclusions about mechanisms influencing a species dynamics and distribution and my result in either over- or underestimating the future distribution of a species. Therefore, while experiments manipulating abiotic factors are useful systems to investigate how climate change can affect plant dynamics, more studies incorporating biotic interactions are needed.

# Chapter 3

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Who cares about climate change impact if you have neighbours to deal with?

## **Abstract**

Despite the co-occurrence of abiotic and biotic factors simultaneously influencing and shaping natural ecosystems, only a few studies so far investigated their joint effect. Even less attention has been given to long-lived, woody species and how these factors influence their seedling dynamics. However, as perennials form the main structural component in dryland ecosystems, and as they contribute considerably to ecosystem stability, understanding their future dynamics is of high importance. In accordance with two conceptual models describing shifts in interactions along natural stress gradients we predicted that 1) the effect of annuals on shrub seedlings will shift from competition to facilitation with decreasing water availability; 2) grazing, i.e. removal of competitive features displayed by the annual community, will reverse competitive interactions and, therefore, facilitate shrub seedling establishment; 3) facilitative effects created by grazing will be reversed with decreasing water availability; 4) due to a long life history under certain environmental conditions we expect shrub individuals originating from benign environments to be better competitors than individuals from harsh environments. To evaluate our predictions we conducted a field and Botanical Garden experiment where we manipulated water availability, neighbouring plant community (present, absent and clipped) as well as the individual's origin. In contrast to our expectations, water availability had no effect on shrub seedling establishment or the intensity and direction of biotic interactions. Despite habitat amelioration by the neighbouring community (e.g. increased soil moisture), interactions were always competitive, with competition for light being the main factor inhibiting shrub seedling establishment. As expected, clipping of the annual community counteracted this limiting factor by increasing light availability significantly. Overall, our findings show that in order to give realistic predictions about future shrub seedling dynamics, understanding only expected climatic shifts is insufficient; we additionally need to consider biotic interactions which may vary depending on the abiotic environment.



## Introduction

Understanding and predicting changes in plant population dynamics due to anthropogenic interferences, i.e. modifications in land-use and climate change has become one of the major challenges in ecology (Adler & HilleRisLambers 2008). Fuelled by a relentless change in species distribution and abundance, an increasing body of research attempts to describe plant-plant interactions, as they are the most important feature determining the structure and dynamics of populations and plant communities (Lortie *et al.* 2004; Maestre *et al.* 2005; Van der Putten *et al.* 2010). The main focus has been on understanding plant-plant interactions along abiotic stress or herbivory gradients (e.g. Holzapfel *et al.* 2006; Graff *et al.* 2007), but only few attempts have been done describing the joint effect of both stressors simultaneously (Smit *et al.* 2009; Soliveres *et al.* 2011). Even fewer attempts have been done on describing abiotic and biotic factors affecting and altering the dynamics of perennial species (but see Seifan *et al.* 2010). This is regrettable, as perennials form the main structural component in dryland ecosystems and particularly as they contribute considerably to ecosystem stability.

A common approach of describing plant-plant interactions is the stress gradient hypothesis (SGH), a conceptual model describing shifts in the strength and direction of plant interactions along a gradient of stress (Bertness & Callaway 1994). According to the stress gradient hypothesis competitive interactions between plants prevail in less stressful environments which shift to facilitative interactions with increasing environmental stress. However, this very simplistic approach was refined after several studies emphasized the importance of combining different stressors as they may alter the direction of interaction (Graff *et al.* 2007; Maestre *et al.* 2009; Smit *et al.* 2009). Maestre *et al.* (2009) proposed that in order to give reliable predictions about the facilitative and competitive interactions in plant communities there are two factors that need to be considered. First, abiotic stressors may either be resource (e.g. water availability) or non-resource related (e.g. temperature) and second, species involved may differ in their life history, i.e. may either be tolerant to stress or show competitive abilities. Within our study system annual neighbours impose a competitive effect on the predominantly stress tolerant beneficiary, thus facilitative interactions are expected to dominate at intermediate stress levels which again will shift to competition with increasing abiotic stress promoted by water shortage (Maestre *et al.* 2009). Further, Smit *et al.* (2009) proposed biotic interactions (here consumer pressure) to be one of the main drivers increasing the complexity and altering the direction and intensity of plant-plant interactions. More precisely, the presence of herbivory, and especially cattle which favour annual vegetation, is expected to alter plant-plant interactions. In highly productive environments grazing may reduce competitive effects between annuals and shrubs for the benefit of shrubs. In return, grazing may counteract facilitative effects by the annual neighbouring community under harsh climatic conditions. Interestingly, in a preliminary study in a similar ecosystem, Seifan *et al.* (2010) indicated positive drought effects mediated by interactions between annuals and shrub seedlings, i.e. annual

vegetation promoted shrub seedling establishment in stressful years. Therefore, increasing aridity is expected to increase shrub establishment at the expense of annual plants and thus decrease the fodder value of the land (Maestre *et al.* 2003). Grazing however may counteract the positive effect of the annual vegetation on shrub seedling establishment (Seifan & Kadmon 2006). According to these previous observations, it is clear that predictions concerning the combined effect of climate and land-use are complex, especially without particular knowledge of biotic interactions within the system.

Here, we present the results of a field and Botanical Garden experiment, designed to investigate the effect of abiotic stress, grazing and their interaction on shrub seedling establishment and their interaction with the neighbouring community. We combined both geographical gradient and local scale manipulations in order to encompass multiple influencing factors and to capture a wide natural variation. In particular we tested the following hypotheses: 1) Plant-plant interactions along the natural aridity gradient will shift in accordance with the stress gradient hypothesis, i.e. facilitation will predominate under harsh and competition under benign environmental conditions. We expected to find the same shift in interactions in the garden experiment, where we artificially manipulated water availability simulating future climate change scenario. 2) Grazing of the annual community will result in a release from competitive interactions, therefore facilitating shrub seedling establishment. 3) However, neighbour effects and grazing are expected to interact along the natural aridity gradient. Grazing is expected to counteract the facilitative effect of the neighbouring annual community with decreasing water availability and therefore reverse the direction of interactions. And 4), based on the hypothesis of different life histories (Maestre *et al.* 2009) we expect to see differences in the response depending on the origin of our focal species. In detail we predicted that individuals of a common dwarf shrub originated from dry sites will show better adaptation to abiotic stress whereas individuals originated from more predictable and benign environments will strike with their resilience to high competitive levels.

We chose to conduct our study in a Mediterranean ecosystem as this region has been under grazing for millennia, i.e. grazing is a regular feature in the dynamics of these ecosystems (Perevolotsky & Seligman 1998). Centuries of grazing contribute to the stability of these systems so that either overgrazing or abandonment of grazing could seriously threaten the balance of these ecosystems, i.e. the balance between perennial and annual species (Perevolotsky *et al.* 2001). Dry regions where grazing has not been managed properly have experienced an increase in perennial, unpalatable species occupying large areas at the expense of the annual herbaceous community (Asner *et al.* 2004) indicating the importance of studying shrub demography.

## Material and Methods

### *Study sites and species*

The study was conducted at three field sites spread over a steep climatic gradient in Israel, complemented by a garden experiment in the Botanical Garden of Tel Aviv University, Israel. The three field sites have similar characteristics, i.e. all sites are situated on southern slopes of calcareous bedrock, with stony and shallow soils (Harel *et al.* 2011). The mean annual temperature is approx. 17-19.1 °C. The climate is Mediterranean, with hot, dry summers and mild, rainy winters closely linked to the main growing season (Nov.-Mar.). The three experimental sites are located in the Galilee Mountains (mesic-Mediterranean site, N 33°0', E 35°14'), the Jerusalem Mountains (Mediterranean site, N 31°42', E 35°03') and at the border of the northern Negev desert (semi-arid site, N 31°23', E 34°54'). All sites differ greatly in their average and variance in annual rainfall with 780, 540 and 300 mm average annual rainfall at the mesic-Mediterranean, Mediterranean and semi-arid site, respectively. For more detailed information on the sites see Holzapfel *et al.* (2006) and Sternberg *et al.* (2011). The experiment was carried out during the two vegetative seasons 2010/11 and 2011/2012. Rainfall during our two experimental seasons was 832 and 808 mm at the mesic-Mediterranean site, 357 and 575 mm at the Mediterranean site and 178 and 318 mm at the semi-arid site for the first and the second season, respectively.

The vegetation at all sites is a typical scrub formation, locally referred to as *batha*, an assemblage of annual and perennial herbaceous plants associated with dwarf shrubs (for details see Holzapfel *et al.* 2006; Petrů *et al.* 2006). We chose *Sarcopoterium spinosum* (L.) Spach (Rosaceae) as the shrub species for our experiments because of its abundant occurrence at all three sites and its overall importance in the composition of the *batha*. *Sarcopoterium spinosum* is a long-lived, thorny, dwarf shrub with a cushion shaped canopy. Characteristic is its deep reaching taproot, enabling the shrub to grow on shallow, infertile and dry soils (Litav *et al.* 1963; Litav & Orshan 1971; Henkin & Seligman 2011) and its sensitivity at seedling stage to drought and competition with neighbour plants for resources (Litav *et al.* 1963; Reisman-Berman 2007; Seifan *et al.* 2010).

Main climatic features at the Botanical Garden (N 32°11', E 34°81') are consistent with the Mediterranean field site. Annual rainfall was 492 mm in the first year and 642 mm in the second year, thus first below and then above the long term annual average (583 mm). Grazing, e.g. by goats and cattle was avoided by fencing of all experimental areas.

### *Experimental design*

*Field experiment:* In June 2010, we established seven randomly-chosen plots (1,5x2 m) at each of the three experimental field sites along the steep climatic gradient. All plots were located in herbaceous annual communities, avoiding microclimatic interference by neighbouring shrubs. Within each plot we established

six experimental units of 40x20 cm which were subjected to one of the following treatments: two different origins of our target species *S. spinosum* and three different treatments of vegetative cover, i.e. annual neighbours left intact, annual neighbours manually clipped with scissors to a height of 2-3 cm or complete removal of annual neighbours by cutting the stems at ground level with a scalpel in order to avoid soil disturbance. Seeds of our target species were collected one season prior to the experiment, and stored under field conditions. Previous experiments conducted at the same field sites showed that storing seeds under field conditions is the optimal way for breaking conditional summer seed dormancy (Petrů & Tielbörger 2008). With the first winter rain seeds were sown in plastic trays and germinated in the net house at the Botanical Garden of Tel Aviv University. Approx. four weeks after germination (end of January until early February), seedlings were transplanted to the field, after emergence and establishment of herbaceous seedlings in the field. All seedlings appeared to have the same physical conditions, i.e. two cotyledon leaves as well as the first leaf were present. In each experimental unit four seedlings were planted in a quadrat at least 10 cm apart to avoid competition. Seedlings were closely monitored for the first two weeks and dead seedlings were replaced until all seedlings were fully established.

*Botanical Garden experiment:* The field experiment was complemented by a Botanical Garden experiment at Tel Aviv University, enabling us to control for confounding effects. We established nine 6x3.10 m plots; plots were divided in a control treatment receiving natural rainfall amount and a drought treatment. The drought treatment was created by rainout shelters, intercepting 30% of the natural rainfall. Each water treatment was further divided into twelve experimental units (30x30 cm) to which we randomly assigned five different neighbour removal treatments, i.e. annual neighbours from a semi-arid or mesic-Mediterranean origin left to grow, the same compositions of annual neighbours from the two opposing climatic origins clipped manually to a constant height of 2-3 cm, simulating grazing, and no annual neighbours present. Within each of the different neighbour treatments three seedlings of our target species from the two climatic extremes were transplanted. Overall, we had two water treatments x five neighbour treatments x two shrub seedling origins resulting in 20 plots that were replicated nine times for each treatment combination (a total of 180 plots).

Seeds of our target species were germinated simultaneously with the seeds of our field experiment and transplanted to the experimental units at the same morphological stage, i.e. two cotyledon leaves as well as the first leaf were present. In each plot three seedlings were regularly arrayed in a triangle, resulting in a total of 540 seedlings. For the two following weeks dead seedlings were replaced until all seedlings were established. The artificial annual neighbouring communities, however, were directly sown into the experimental units. For further information on seed handling, composition and density of the two annual communities see Appendix A.

### *Monitoring of seedling survival*

Once *S. spinosum* seedlings had established in the experimental units of the field and garden experiment we monitored seedling survival at one-monthly intervals. Seedlings were assigned dead when all leaves were dry; damaged seedlings were excluded from further analysis.

### *Environmental measurements*

We measured several abiotic and biotic factors in order to validate that all treatment manipulations showed an effect in the expected direction. This will allow us to draw conclusions about expected direct and indirect interactions of our target seedling.

In the three field sites as well as in the garden we measured various abiotic and biotic factors in order to validate that the treatments were performing as designed. We measured soil moisture, soil temperature and light availability in all experimental units. Measurements were taken once at the peak of vegetative growth, just before the onset of senescence (end of April) when most of the shade by the annual neighbouring community is created. Soil samples were taken early in the morning with a 1.5x5 cm core and weighted before and after drying (samples oven-dried 48h at 105°C). Soil temperature was measured 5 cm below the surface with a digital thermometer and light availability was measured 5 cm aboveground with a Lux meter. Both measurements were taken at noon.

The annual neighbouring community was harvested around the same time in both clipped and unclipped plots to determine biomass production. All samples were oven dried (48h at 75°C) and weighted. In the field we harvested biomass from areas adjacent to our experimental plots and analysed biomass production with the same method as mentioned above. Logistical problems allowed us to only retrieve data from the first year of the experiment.

### *Data Analysis*

*Survival:* To analyse differences in survival among the three field sites we conducted a generalized linear model (GLM) with binary error structure and log-link function with site and neighbour treatment as the main effects. The same model was applied to analyse the influence of water treatment and neighbour treatment on shrub seedling survival in the Botanical Garden experiment. The best fitted model was determined based on the AIC (Akaike information criterion; Anderson 2008). Additional explanatory variables (e.g. soil moisture) were removed from the model as they increased the AIC, hence decreased the goodness of fit of the model. Originally we also fitted target species origin to our model, but as no statistical differences could be observed and as AIC was increased, we excluded this factor from the model. Adding a scale weight variable accounted for overdispersion.

*Relative interaction index:* To analyse interactions between the target species and the neighbouring community and to test whether grazing implies a relief from competitive interactions we used the “relative interaction index” (RII) described by Armas *et al.* (2004). This index displays the following main features: it ranges from 1 to -1, with positive values describing facilitative interactions and negative values implying competitive interactions between the target and the neighbouring plants. The index is linear, continuous and is symmetrical around zero. The index is calculated as  $RII = (X_{+N} - X_{-N}) / (X_{+N} + X_{-N})$ , with  $X_{+N}$  and  $X_{-N}$  describing the performance (here probability of survival) of the target species with neighbours or without annual neighbours present. Index response to water treatment and site, respectively, as well as different neighbour treatments (clipped and unclipped) was analysed using a GLM with normal distribution and identity link. As for the survival data originally population origin as well as origin of the annual neighbouring community was fitted. But also for this analysis no differences could be observed, which is why we excluded these factors from the model.

*Environmental measurements:* Soil moisture, soil temperature, light availability and biomass were analysed with GLM, with normal distribution and identity-link function to check for statistical differences across treatments. Also here we first tested if our data meet the requirements of an analysis of variance, i.e. test for homocedasticity (Levene’s test) and normality (Kolmogorov–Smirnov test).

In all cases post hoc analyses were carried out using the Fisher’s least significant difference (LSD) method for comparing significant variables and interactions. In the following text all means are displayed with standard error (SE).

## Results

### *Environmental conditions*

*Field experiment:* In both years soil moisture decreased significantly towards the dry end of the natural rainfall gradient ( $\chi^2_2 = 518.95$   $p < 0.001$  and  $\chi^2_2 = 89.79$   $p < 0.001$ , for the years 2011 and 2012, respectively). The opposite trend was discovered for the soil temperature which increased with decreasing rainfall ( $\chi^2_2 = 515.65$   $p < 0.001$ ). Additionally an increasing soil temperature with decreasing soil cover in all three sites could be observed ( $\chi^2_2 = 8.03$   $p = 0.018$ ). In all three sites light availability decreased significantly with annual neighbours present ( $\chi^2_2 = 48.35$   $p < 0.001$ ). Except for the SA site, clipping resulted in an increase in light availability 5 cm above ground. Mean aboveground biomass in both years increased significantly with increasing water availability ( $\chi^2_2 = 252.06$   $p < 0.001$  and  $\chi^2_2 = 50.92$   $p < 0.001$ , for the years 2011 and 2012, respectively).

*Garden experiment:* Intercepting 30% of the rainfall reduced the soil moisture significantly ( $\chi^2_1 = 6.94$   $p < 0.008$  and  $\chi^2_1 = 10.97$   $p < 0.001$ , for the years 2011 and 2012, respectively). Soil moisture was also significantly affected by the different neighbour treatments ( $\chi^2_2 = 12.74$   $p < 0.001$  and  $\chi^2_2 = 18.39$   $p < 0.001$ , for the years 2011 and 2012, respectively) with significantly reduced soil moisture levels in the clipping treatment. Trends in soil temperature were in accordance with the soil moisture data, i.e. temperature was significantly decreased in control plots ( $\chi^2_1 = 8.02$   $p = 0.005$ ) and in plots with annual neighbours present ( $\chi^2_1 = 6.31$   $p = 0.037$ ). As for the field experiment we could observe that the presence of annual neighbours significantly decreased light availability ( $\chi^2_2 = 60.95$   $p < 0.001$ ). As expected, clipping significantly reduced biomass ( $\chi^2_1 = 276.06$   $p < 0.001$ ), however, water availability had no effect ( $\chi^2_1 = 0.92$   $p = 0.339$ ).

### Seedling survival

*Field experiment:* The survival probability of *S. spinosum* seedlings was significantly reduced with neighbouring community ( $\chi^2_2 = 20.2$ ,  $p < 0.001$  and  $\chi^2_2 = 396.75$ ,  $p < 0.001$ , in the year 2011 and 2012, respectively, Figure 1). Seedling survival probability was enhanced by clipping the annual neighbouring community. This phenomenon could be observed for both years and all three sites along the natural rainfall gradient. Additionally there were significant differences between sites ( $\chi^2_2 = 65.13$ ,  $p < 0.001$  and  $\chi^2_2 = 385.29$ ,  $p < 0.001$ , for the year 2011 and 2012, respectively). In 2011, seedling survival probability was highest in the mesic-Mediterranean site, whereas in 2012 the highest survival probability was measured for the semi-arid site. In both years survival probability was lowest at the Mediterranean site.

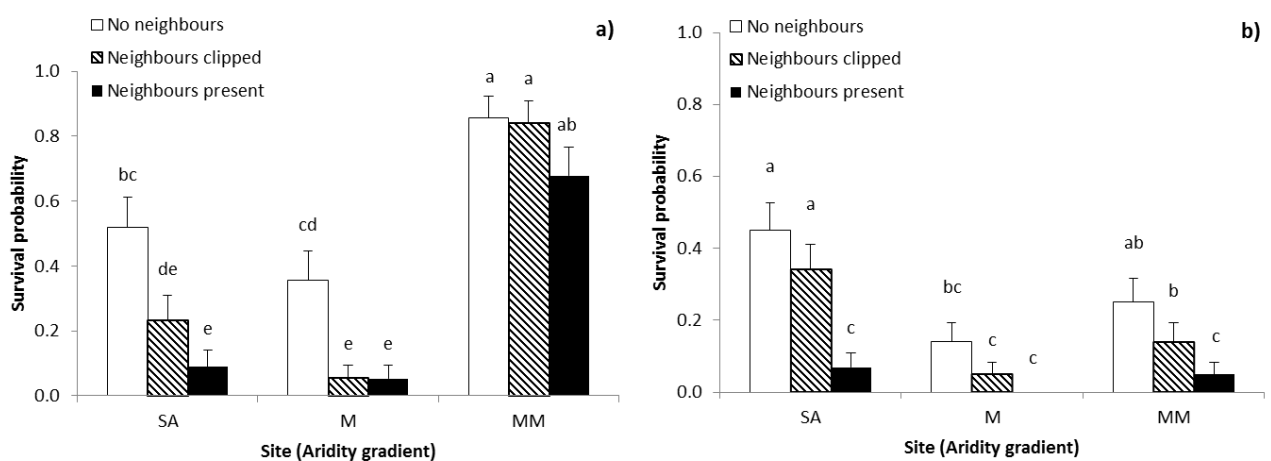


Figure 1. Seedling survival probability in a) 2011 and b) 2012 in the three sites along the natural rainfall gradient, indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) locations ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).

*Garden experiment:* Survival probability of the *S. spinosum* seedlings was highly affected by the various community treatments ( $\chi^2_4 = 53.61$   $p < 0.001$  and  $\chi^2_4 = 52.71$   $p < 0.001$ , for the years 2011 and 2012, respectively; Figure 2). Fisher's pairwise comparison revealed that this significant community effect was

caused by the significantly higher survival probability of seedlings grown in plots with no annual neighbours. For all other treatments with annual neighbours present, independently of the origin of the annual community and if neighbours have been clipped or left intact, seedling survival probability was significantly decreased. Although drought had no effect on shrub seedling survival probability ( $\chi^2_1 = 0.76$   $p = 0.384$  and  $\chi^2_1 = 1.31$   $p = 0.253$ , for the years 2011 and 2012, respectively), a slightly higher survival could be observed in plots receiving the natural amount of rainfall. This was particularly the case in plots with standing neighbouring biomass.

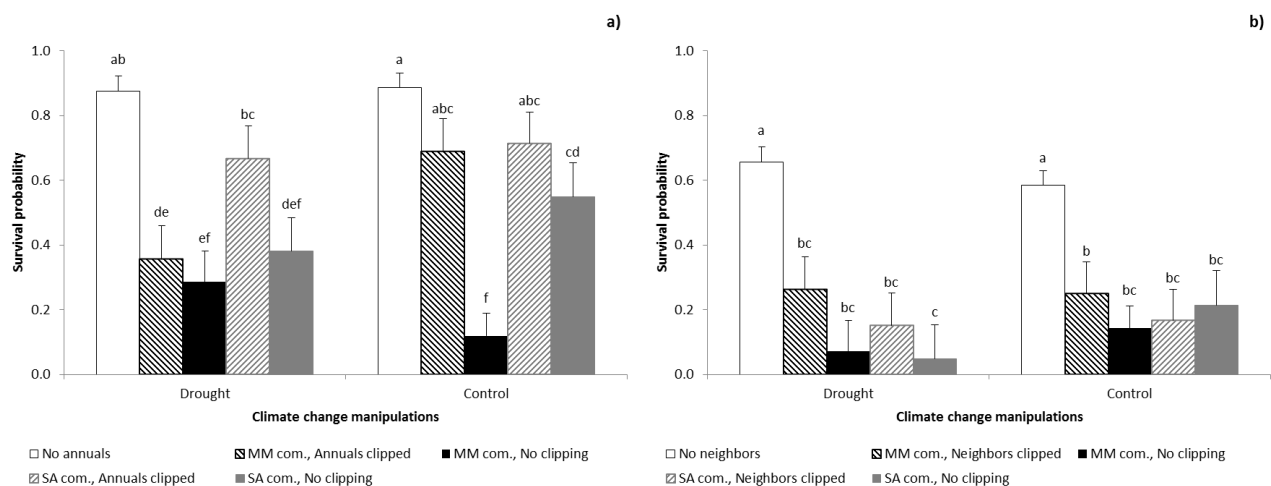


Figure 2. Seedling survival probability in a) 2011 and b) 2012 ( $\bar{X} \pm 1SE$ ). Treatments: two water treatments (drought vs. control) and five neighbour treatments. Letters represent significantly different groups ( $p < 0.05$ ).

### Relative interaction index

*Field experiment:* Analysis of the relative interaction intensity (RII) showed that for both years competition is the main process driving interactions between shrub seedlings and annual neighbours (Figure 3). In 2011, RII was significantly different between the three sites ( $\chi^2_2 = 26.82$   $p < 0.001$ ) and in 2012, clipping had a significant effect on RII ( $\chi^2_1 = 8.39$   $p = 0.004$ ). Despite entirely negative interactions for both years and all treatments we observed a release from competitive interactions in the most productive environment, particularly if the neighbouring community had been clipped. In the following year there were barely any site differences, instead clipping caused a release from competitive interactions in all sites, which was highly significant in the semi-arid site.



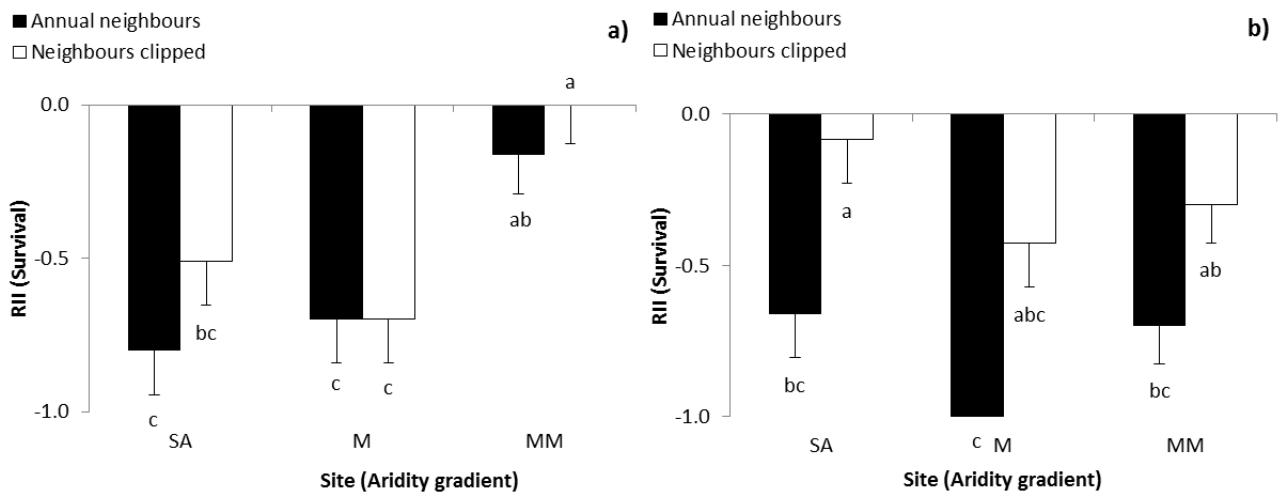


Figure 3. RII calculated using a) 2011 and b) 2012 seedling survival data in the three sites along the natural rainfall gradient, indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) locations ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).

*Garden experiment:* Although all treatment combinations showed competitive interactions to the presence of neighbouring community, we could observe that plots where annual neighbours were clipped experienced a significant release from competitive interactions ( $\chi^2_1 = 22.2$   $p < 0.001$  and  $\chi^2_1 = 4.82$   $p = 0.028$ , for the years 2011 and 2012, respectively; Figure 4).

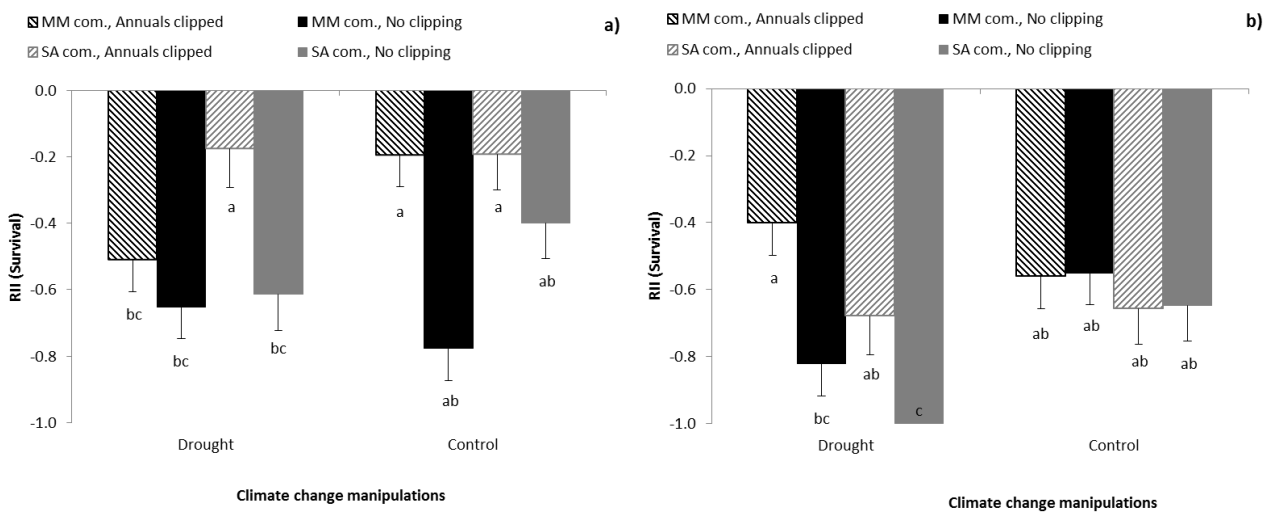


Figure 4. RII calculated using a) 2011 and b) 2012 seedling survival data ( $\bar{X} \pm SE$ ). Treatments: two water treatments (drought vs. control), two community origin treatments and neighbour treatment (grazed vs. annual neighbours present) Letters represent significantly different groups ( $p < 0.05$ ).

Origin of the annual neighbouring community had a significant and marginally significant effect on RII ( $\chi^2_1 = 6.63$   $p = 0.01$  and  $\chi^2_1 = 3.9$   $p = 0.048$ , for the years 2011 and 2012, respectively). In 2011 competitive interactions were slightly increased for seedlings grown in the mesic-Mediterranean originated annual community, whereas in 2012 we observed the opposite effect with increased competitive interactions in the

semi-arid originated annual community. Additionally, while the intensity of interactions did not show any differences in the two water treatments (drought and control, for the years 2011 and 2012, respectively) if the neighbouring community was clipped, negative interactions were significantly decreased in control plots with annual neighbours left intact for the year 2012 (significant water treatment x neighbour treatment interaction; Figure 4).

## Discussion

Overall, the results of the present experiment contradict the accepted theory of changing interactions along an environmental stress gradient in two ways. Firstly, contrary to the expected shift towards positive interactions with increasing environmental harshness all the interactions we observed were competitive. Secondly, whenever we were able to observe a shift in the intensity of interactions they followed no constant trend – indeed, while in one of the years the negativity of interactions decreased toward the harsh end of the gradient, thus corresponding to the predictions of the stress gradient hypothesis, in the second year of the same experiment interactions showed the reverse trend with notably mitigated interactions occurring at the benign end of the gradient, thus contradicting the predictions of the stress gradient hypothesis. Adding a second potential stress axis, here grazing simulated by clipping, contributed to the complexity of interactions. It mainly caused a release of the predominantly competitive interactions. No effect of decreasing water availability on shrub seedling establishment was detected, suggesting that the predicted decrease in rainfall may have no effect on shrub seedling dynamics. Additionally, individuals originated from the two opposing climatic conditions showed no differences in their response to either alteration in abiotic or biotic factors.

Based on the stress gradient hypothesis (Bertness & Callaway 1994) and the refined predictions by Maestre *et al.* (2009) we expected to find a shift towards facilitative interactions with decreasing water availability. However, our results obtained in the field and garden experiment contradicted the initial hypothesis and the results of several other studies investigating plant-plant interactions in a similar environment (Holzapfel *et al.* 2006; Schiffers & Tielbörger 2006; Seifan *et al.* 2010). Despite remarkably improved water availability in plots with annual neighbours we only observed competitive interactions and a significantly decreased shrub seedling survival. These findings indicate that shrub seedling survival might have been inhibited by competition for a different resource than water. Only recently a similar trend has been described by Soliveres *et al.* (2013) who observed a decrease in shrub seedling survival with decreased light availability. Soliveres *et al.* (2013) explained their findings by the fact that shade intolerant species are not able to benefit from habitat ameliorations and that under such circumstances facilitative interactions may not predominate. Nevertheless, these findings are in accordance with several other studies investigating the effect of decreased light availability on interactions in shrub seedling establishment (Pagès *et al.* 2003;

Vandenberghé *et al.* 2008; Seifan *et al.* 2010). This general negative response to decreased light availability and the slight decrease in competitive interactions observed at either extreme end of the gradient could be explained by two resource gradients working in opposite directions – namely, water and light (Holmgren *et al.* 1997). Water availability decreased significantly from the mesic-Mediterranean site to the semi-arid site whereas light availability followed the opposite direction. Hence, significantly decreased soil moisture levels and a decreased light availability by a still dense annual neighbouring community might explain the highly competitive interactions at the intermediate site. These findings are supported by several other studies showing that insufficient light levels combined with insufficient water availability evoked highly competitive interactions (Seifan *et al.* 2010; Soliveres *et al.* 2010; Soliveres *et al.* 2013). In summary, annual neighbours benefitting shrub seedling establishment by increased water availability were seemingly not able to compensate for the significantly decreased light availability, resulting in notably decreased shrub seedling survival.

Clipping of the neighbouring community significantly increased shrub seedling establishment and competitive interactions experienced by shrub seedlings were drastically decreased. These results reaffirm Smit *et al.* (2009) and their urgent proposition to include biotic stress in studies predicting species interactions along environmental stress gradients. Already several other studies delivered substantial evidence supporting that grazing is a major driver in altering plant-plant interactions along abiotic stress gradients (e.g. Seifan & Kadmon 2006; Eskelinen 2008; Graff & Aguiar 2011), with grazing reversing competitive and seedling establishment inhibiting effects. Abiotic measurements conducted in our study system revealed a significantly increased light availability in clipped plots. These results support our prior findings that *S. spinosum* shrub seedlings exhibited a probable inability to tolerate shade, especially if linked to decreasing water availability. The direct effects of clipping on shrub seedling establishment were disregarded from the beginning as Seifan (2005) demonstrated that damaged seedlings had a high resilience and showed no differences in performance from unclipped seedlings. Nevertheless, further studies involving different levels of grazing pressure are required to improve our understanding about the response of shrub seedling as well as plant-plant interactions to grazing along an abiotic stress gradient.

Based on the assumption of different life histories (Maestre *et al.* 2009) and a reciprocal transplant experiment conducted in the same environment (Liancourt & Tielbörger 2011) we expected to see origin dependent differences in the performance of our focal species. However, our results revealed no origin related differences contradicting our hypothesis and emphasizing once more the wide natural distribution range of our focal species (Evenari *et al.* 1986). In general, these results highlight that our focal species displays plastic features enabling shrub seedlings to easily cover a wide range of climatic conditions. Overall, our findings suggest that all individuals exhibit the same drought resistant and competition features. Additionally, we expected to find shifts in interactions depending on the type of stressors involved (Maestre

*et al.* 2009). Annual-shrub seedling interactions within our study system proved to be mainly non-resource driven, i.e. light limitations by the neighbouring community, inhibiting seedling establishment significantly. Incorporating biotic stress (Smit *et al.* 2009) reinforced these findings as clipping of the annual neighbouring decreased competitive interactions by increasing light availability.

In conclusion, anthropogenic interferences, such as climate change and overgrazing have been proposed to be the main reason for shrub invasion of abandoned or incorrectly managed crop- and grasslands (van Auken 2000), decreasing the fodder value of the land dramatically (Maestre *et al.* 2003). However, although numerous studies consider *S. spinosum* a common invader (Litav & Orshan 1971; Perevolotsky *et al.* 2001) our study revealed that seedling establishment was generally very low and additionally drastically inhibited by annual neighbours. Specifically, our experiment showed that biotic interactions play a more important role in influencing shrub seedling establishment regardless of climate, even if differences in climate are very large. These findings indicate, that management practices such as increasing the competitive effect of annuals on shrub seedlings appears to be a successful approach in regulating shrub seedling establishment (Henkin *et al.* 1998).

# General conclusions

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Anthropogenic interferences, such as climate change and land-use, afflict natural ecosystems and may compromise and change their species composition and productivity (Walther *et al.* 2002). According to several climate change models, the Eastern Mediterranean region is expected to face drier and hotter climatic conditions, therefore putting this region at high risk of potential alterations. The main objective of this thesis was to understand how abiotic and biotic factors as well as their interactions influence the shrub seedling dynamics of a common Mediterranean dwarf shrub in order to give reliable predictions about the future dynamics, especially concerning climate change predictions.

## **Climatic gradients as proxy for species shifts under climate change**

The first chapter was designed in order to test if climatic gradients can serve as a proxy for predicting species shifts under climate change. This space-for-time approach is the primary assumption of bioclimatic envelope models (e.g. Bakkenes *et al.* 2002; Thomas *et al.* 2004). To validate the precision and reliability of this approach we compared the response of shrub seedlings of a common Mediterranean dwarf shrub along natural and experimental rainfall gradients (Figure 1a). In our study, we focused on the seedling stage, which is considered a bottleneck for plant establishment (Leck *et al.* 2008) and has been proposed to be most vulnerable to environmental alterations (Howard & Goldberg 2001; Fay & Schultz 2009). The key finding of our study was that climatic gradients are a poor proxy for predicting species shifts under future climate change. We were able to show that ignoring biotic interactions is fatal and can result in misestimating the future distribution of a species. Specifically, biotic interactions and their direct effect on shrub seedling establishment (competition vs. facilitation) as well as the complex interplay of abiotic and biotic factors (here, decreasing water availability and decreasing neighbouring community) proved to be crucial elements influencing and regulating shrub seedling establishment. These findings are in line with previous studies on the same species suggesting complex trade-offs between various abiotic and biotic factors (Seligman & Henkin 2000; Reisman-Berman *et al.* 2006; Seifan *et al.* 2010). Overall, these findings fuel the debate about the reliability of bioclimatic envelope models for predicting the climate change impact on the future distribution of a species (Davis *et al.* 1998; Pearson & Dawson 2003; Huntley *et al.* 2010). In accordance with Pearson and Dawson (2003) we call for the consideration of biotic interaction in order to overcome bioclimatic envelope model shortcomings and have more realistic predictions of climate change impacts on the future distribution of species. For that purpose we propose the combination of both natural climatic gradients and local long-term climate manipulations (Fukami & Wardle 2005).

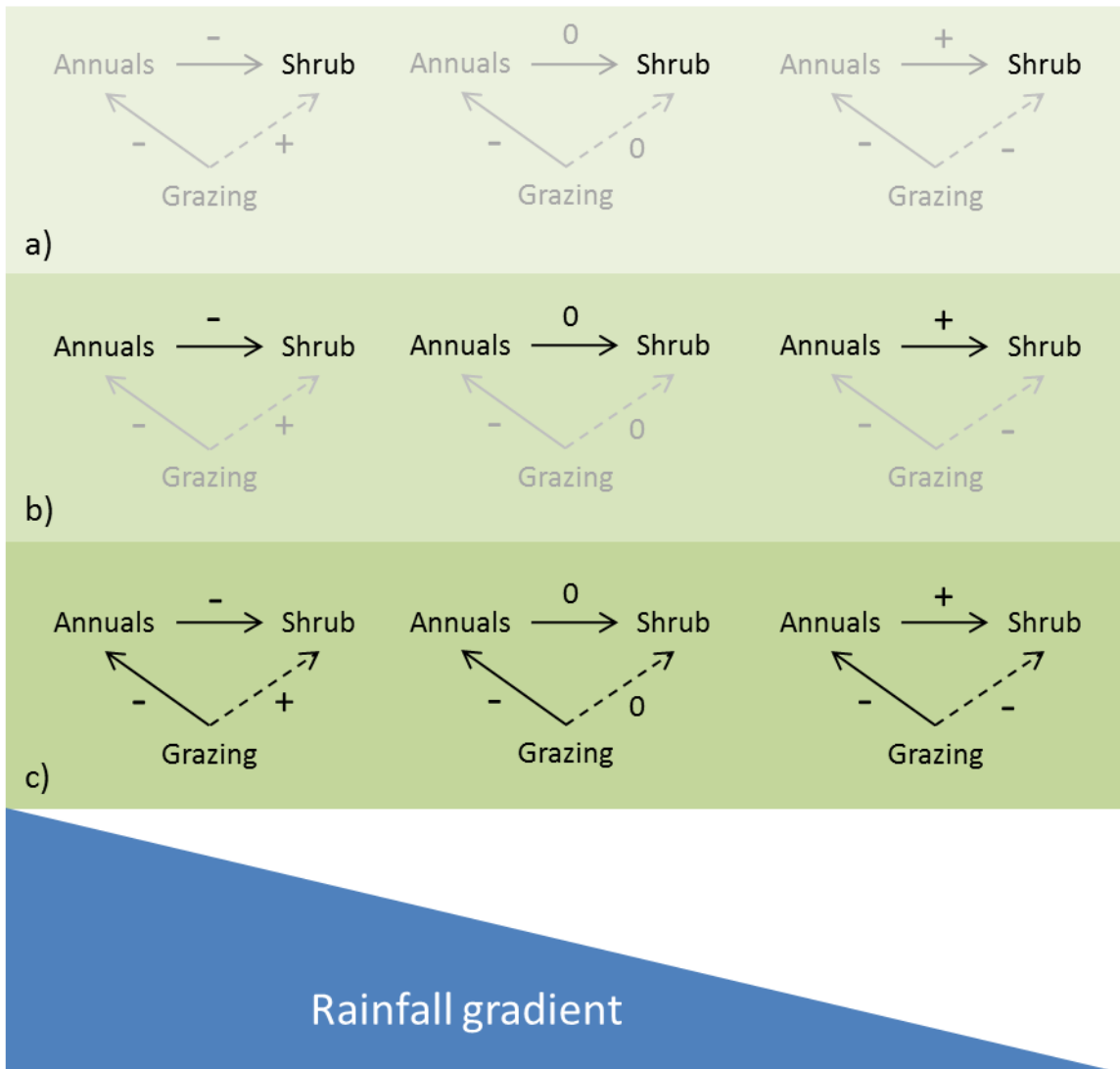


Figure 1. Conceptual model of the effect of rainfall on shrub seedling establishment and shrub seedling-annual interactions in a Mediterranean environment (rainfall gradient may be artificial or natural). a) Shrub seedling establishment is expected to decrease with decreasing rainfall. b) The effect of annual neighbours on shrub seedling establishment will shift from competition to facilitation towards the dry end of the gradient. c) Domestic grazing is expected to have an indirect effect on shrub seedling establishment by altering the interactions between annual neighbours and shrub seedlings. I.e. domestic grazing is expected to decrease competitive effects of the annual neighbouring community at the benign end of the gradient. However, at the harsh end of the gradient, domestic grazing might counteract potential habitat amelioration (i.e. facilitative effects) by the neighbouring community. The direction of the proposed relationship between various factors is indicated by plus, zero or minus indicating positive, neutral and negative relationships. Solid lines indicate the direct effect of one factor on the other. Dotted lines indicate indirect effects of a factor acting via a third factor. See text for further explanations. Adapted from Seifan and Kadmon (2006).

### Biotic interactions in contrasting environments

In the second chapter we overcame the shortcomings of the space-for-time approach by considering biotic interactions following the predictions of the stress gradient hypothesis (Figure 1b). In accordance with the stress gradient hypothesis we predicted that annual neighbours will facilitate shrub seedling establishment with decreasing water availability. However, we found that annual neighbours always negatively affected shrub seedling establishment, regardless of the water availability. Moreover, a decrease in

water availability, naturally as well as artificially, had no direct effect on seedling establishment. These findings even suggest that future climate change predictions are negligible and that shrub seedlings are relatively robust to a future decrease in available water resources. These findings contradict several other studies and theoretical models that predict that particularly ecosystems with already limited resources will be negatively affected by a further reduction in rainfall (Davis *et al.* 1999; Schröter *et al.* 2005; Thuiller *et al.* 2005). Consequently, these findings indicate that there were other factors than only water affecting shrub seedling establishment and interactions between annuals and shrubs.

Our results suggest that there are two key factors switching in their importance, determining shrub seedling establishment success as well as the direction and intensity of interactions, namely water and light. Generally, competition for water and light are expected to be the major interaction between plants, particularly in water limited ecosystems such as the Mediterranean (Vilà & Sardans 1999). Our study confirmed these expectations with water and light governing plant interactions, with limitation of both factors amplifying competitive interactions. Similar results regarding seedling establishment inhibition have been reported by Reisman-Berman *et al.* (2006) and Seifan *et al.* (2010).

Overall, this study showed that the predictions of the stress gradient hypothesis as originally proposed by Bertness and Callaway (1994) may be inadequate and that adjustments for predicting the outcome of future interactions under climate change will be needed (Maestre *et al.* 2009; Smit *et al.* 2009).

### **Interplay between abiotic stress and grazing on plant-plant interactions**

After the second chapter revealed that the prediction of the general stress gradient hypothesis does not apply to the shrub-annual interactions, I adopted a more specific approach which separates the potential factors affecting plant-plant interactions into resource- and non-resource related abiotic factors and biotic stressors, here grazing (Figure 1c; Holmgren *et al.* 1997; Maestre *et al.* 2009; Smit *et al.* 2009; Malkinson & Tielbörger 2010). Especially grazing (simulated by clipping) was predicted to have a strong effect on shrub seedling dynamics via its effect on the neighbouring community (Eskelinen 2008). Our findings suggest that annual-shrub seedling interactions within our study system are mainly non-resource driven, i.e. light limitations by the neighbouring community, inhibiting seedling establishment significantly. Incorporating biotic stress (Smit *et al.* 2009) reinforced these findings as clipping of the annual neighbouring community decreased competitive interactions by increasing light availability.

Overall, these findings support the request to further develop and improve the general model of the stress gradient hypothesis in order to give more reliable predictions about the dynamics of single species under future climate change.

## **Shrub population dynamics - general conclusions**

This study was designed in order to give reliable predictions about future shrub population dynamics in a Mediterranean scrub community. We tackled this problem by conducting a field experiment along a natural rainfall gradient, complemented by a Botanical Garden experiment, where we tested the precision and reliability of several theoretical models. Our work emphasizes that predicting future species dynamics by considering only abiotic factors (e.g. space-for-time approach) is fatal and that including biotic factors is crucial.

Many previous studies assumed that shrub encroachment of abandoned or incorrectly managed crop- and grasslands is unavoidable (Brown & Archer 1999; van Auken 2000), consequently decreasing the fodder value of the land dramatically (Maestre *et al.* 2003). Additionally, numerous studies consider our focal species *S. spinosum* a common invader (Litav & Orshan 1971; Perevolotsky *et al.* 2001) exposing an immediate threat to Mediterranean ecosystems. However, our study revealed that seedling establishment was generally very low and additionally drastically inhibited by annual neighbours regardless of the water availability. These findings indicate, that management practices such as increasing the competitive effect of annuals on shrub seedling appears to be a successful approach in regulating shrub seedling establishment (Henkin *et al.* 1998). However, grazing may counteract these processes regulating shrub establishment, resulting in an increased shrub establishment success, regardless of climate change.

## **Open questions**

As for any field in ecology we urge caution in making generalizations. E.g. even though manipulating water availability is a rather common approach for climate change experiments conducted in arid ecosystems, there are actually multiple other aspects changing with climate change that have not been tested (e.g. temperature). Nevertheless, aware of these shortcomings our results indicate that a decrease in water availability is negligible, indicating that shrub seedlings are relatively resistant to predicted climate change. However, after a comprehensive literature review indicating the potential for facilitative interactions and personal observational results, I strongly suggest that attention should be given to the following factor: our observed competitive effect of herbaceous neighbours on the interaction intensity may be explained by the fact that our environmental stress gradient as well as our Botanical Garden experiment are located within the natural distribution range of our focal species (Evenari *et al.* 1986). I.e. even though the chosen levels of water availability are based on modern climate change models (e.g. Smiatek *et al.* 2011) shrub seedlings of this specific shrub may not perceive these climatic conditions as extremely harsh. Therefore, only if seedlings might be exposed to environmental conditions below the natural distribution margin, facilitative interactions might be evoked. This would increase the accuracy of predicting future seedling dynamics.

Nevertheless, I strongly believe that the results of this study shed light on the complex interactions



between plant species and their environment, and significantly contributes to the constant strive to improve our understanding of plant dynamics which is ultimately linked to giving more accurate predictions about plant dynamics under global change.

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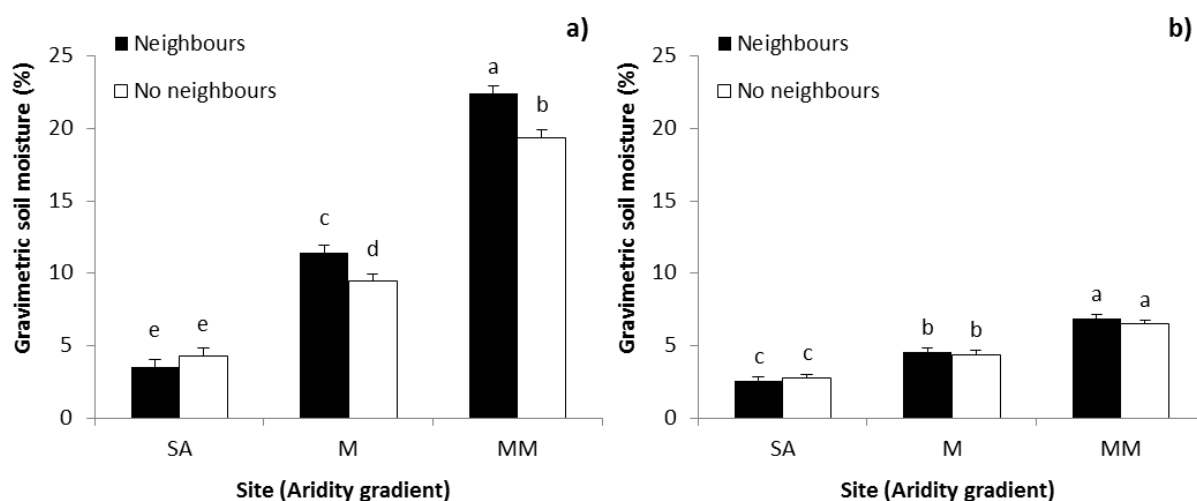
# Appendices

## Appendix A. Species list

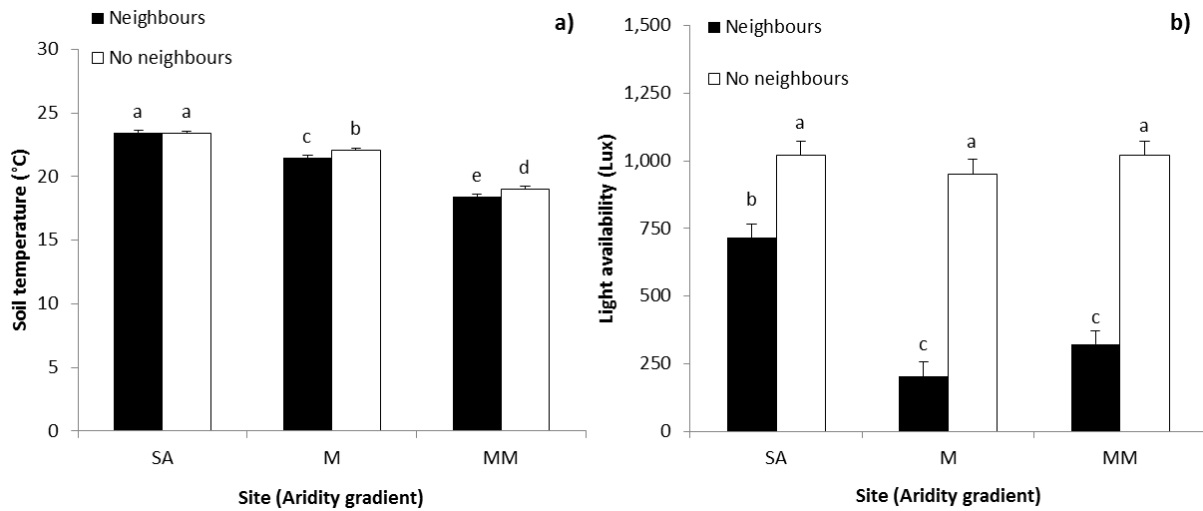
List of plant species of the experimental neighbouring community of semi-arid origin and mesic-Mediterranean origin.

Functional group	Family	Species	Semi-arid annual neighboring community	Mesic-Mediterranean annual neighboring community
Forb	Myrsinaceae	<i>Anagallis arvensis</i>	x	x
Forb	Apiaceae	<i>Daucus subsessilis</i>	x	
Forb	Asteraceae	<i>Hedypnois rhagadioloides</i>		x
Forb	Plantaginaceae	<i>Plantago cretica</i>		x
Forb	Apiaceae	<i>Torilis tenella</i>	x	
Grass	Poaceae	<i>Aegilops peregrina</i>	x	
Grass	Poaceae	<i>Brachypodium distachyon</i>	x	x
Grass	Poaceae	<i>Catapodium rigidum</i>		x
Grass	Poaceae	<i>Crithopsis delileana</i>	x	
Grass	Poaceae	<i>Lolium rigidum</i>		x
Legume	Fabaceae	<i>Hippocrepis unisiliquosa</i>	x	
Legume	Fabaceae	<i>Hymenocarpus circinnatus</i>	x	x
Legume	Fabaceae	<i>Onobrychis squarrosa</i>	x	
Legume	Fabaceae	<i>Trifolium campestre</i>		x
Legume	Fabaceae	<i>Trifolium stellatum</i>		x

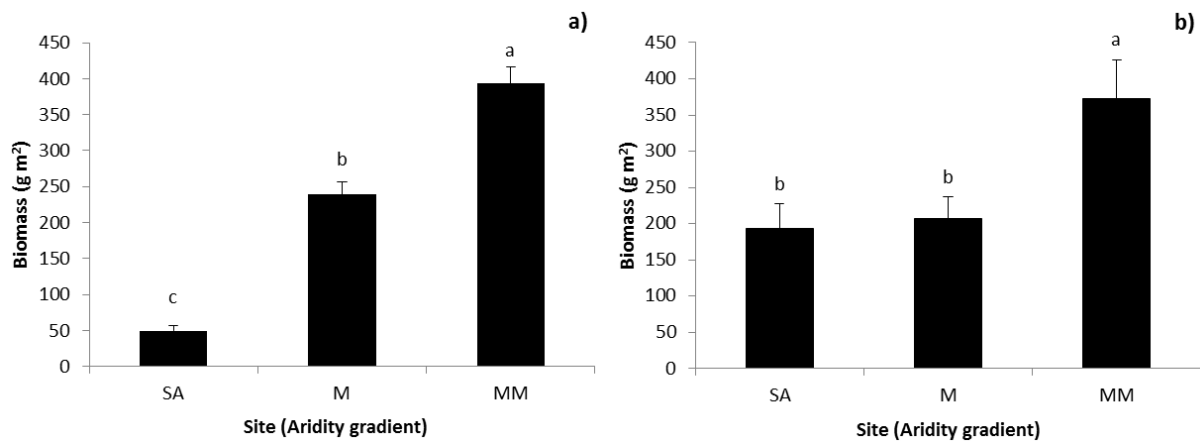
## Appendix B. Environmental measurements - field experiment



Mean values of soil moisture (%) in the three sites along the natural rainfall gradient, indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) locations ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ). a) and b) refer to the two experimental years, 2011 and 2012, respectively.

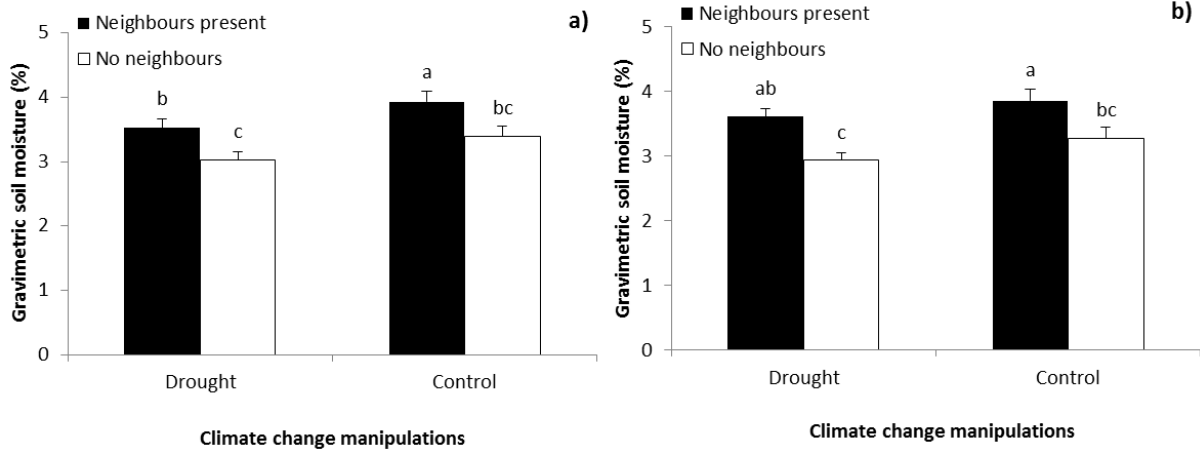


Mean values of a) soil temperature (°C) and b) light availability (Lux) in the three sites along the natural rainfall gradient, indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) locations ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).

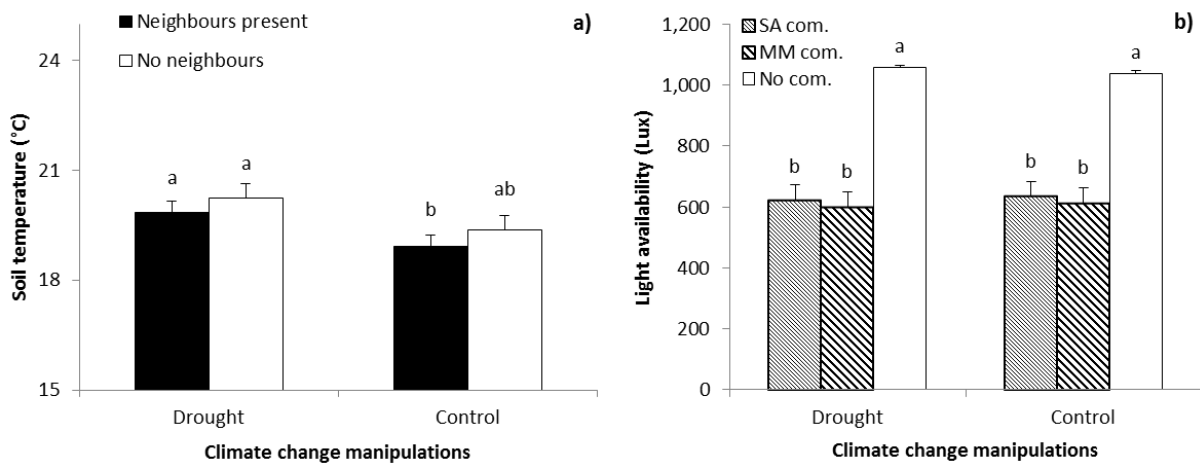


Mean values of herbaceous biomass (g m<sup>-2</sup>) in the three sites along the natural rainfall gradient, indicating semi-arid (SA), Mediterranean (M) and mesic-Mediterranean (MM) locations ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ). a) and b) refer to the two experimental years, 2011 and 2012, respectively.

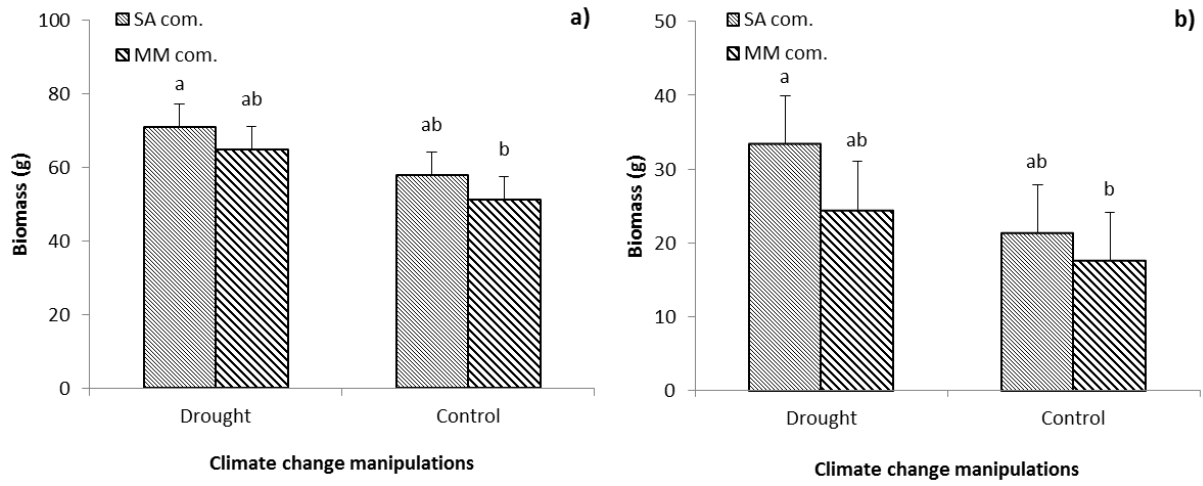
## Appendix C. Environmental measurements – Botanical Garden experiment



Mean values of soil moisture (%) in the Botanical Garden ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ). a) and b) refer to the two experimental years, 2011 and 2012, respectively.



Mean values of a) soil temperature (°C) and b) light availability (Lux) in the Botanical Garden ( $\bar{X} \pm 1SE$ ). Letters represent significantly different groups ( $p < 0.05$ ).



Mean values of herbaceous biomass ( $\text{g}\times\text{m}^2$ ) in the Botanical Garden ( $\bar{X} \pm 1\text{SE}$ ). Letters represent significantly different groups ( $p < 0.05$ ). a) and b) refer to the two experimental years, 2011 and 2012, respectively.

# Curriculum Vitae

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born 22.04.1982 in Filderstadt, Germany

2010 – 2013 Doctorate at the Department of Plant Ecology, University of Tübingen, Germany.

Thesis title: *Biotic and abiotic effects on species interactions in a water stressed ecosystem: the effect of precipitation and grazing on the survival and establishment of the Mediterranean dwarf shrub Sarcopoterium spinosum.*

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2006 – 2009 Master of Science in Agriculture at University Hohenheim, Stuttgart, Germany.

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