

Interspecific facilitation and critical transitions in arid ecosystems



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INTERSPECIFIC FACILITATION AND CRITICAL TRANSITIONS IN ARID ECOSYSTEMS

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INTERSPECIFIC FACILITATION AND CRITICAL TRANSITIONS IN ARID ECOSYSTEMS

Facilitatie tussen planten en kantelpunten in aride ecosystemen
(met een samenvatting in het Nederlands)

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CHAPTER

General introduction

1

ENVIRONMENTAL SCIENCES IN A TIME OF ACCELERATING GLOBAL CHANGE

Over the last three centuries, and especially since the 1950's, human impact on the climate system and on ecosystems has rapidly increased, both in the rate of change as in the spatial extent of changes (Steffen 2015). Due to rising global population levels (from approximately 600 million in 1700 to approximately 7.4 billion nowadays) and increasing affluence, more than half of the terrestrial ice free area has been transformed from natural areas to human shaped rangelands, croplands, villages and densely populated urban areas from 1700 till 2000 (Ellis et al. 2010, Klein Goldewijk et al. 2011). These land transformations have increasingly resulted in fragmentation of natural systems, pollution of environments, alteration of major biogeochemical cycles, and in reduced species diversity and functioning in most ecosystems (Vitousek et al. 1997, Adeel et al. 2005). As global population is expected to increase to 8.3 billion in 2030 (UN, 2015), even further increases in impacts are likely to occur, and because of that the need to develop tools to mitigate changes will increase as well.

The term Anthropocene has been coined (Crutzen 2002, Zalasiewicz et al. 2011) to highlight that we have entered an epoch in which human activities alter the climate system, ecosystems, and the way they interact at a global scale, with changes that occur at an unprecedented rate with long lasting, and hard to reverse impacts (Rockström et al. 2009). Although the exact onset of the Anthropocene is still debated, there is sufficient evidence that humanity has become a major environmental force, with long-lasting consequences at a global scale (Zalasiewicz et al. 2011). For example, a recent study showed that current anthropogenic emissions of carbon dioxide are occurring at an approximately 10 times higher rate as compared to the highest carbon release rate that has occurred over the past 66 million years (Zeebe et al. 2016), illustrating the astounding degree of the impact of human activities on the system earth.

Possibly the most important driver of current global change is the human induced rise in atmospheric CO₂. Since 1750, the atmospheric concentration of carbon dioxide has steadily increased by about 44% (from about 280 to 400 parts per million in 2016), primarily due to the combustion of fossil fuels and land use changes. This rise in atmospheric CO₂ concentration can be directly linked to already observable climate changes in many regions in the world and changes are

projected to further increase over the coming century, depending on the future CO₂ emission pathways (IPCC 2014). One main, already observed effect is a global increase in the earth's surface temperature. The globally averaged combined land and ocean surface temperature has risen with 0.85 °C over the period from 1880 to 2012 (IPCC 2014). In line with this trend, the period from 1983 to 2012 was very likely the warmest 30-year period of the last 1400 years in the Northern Hemisphere.

Next to changes in global temperature, another major component of climate change is a change in precipitation patterns over many regions across the globe. Due to climate change, extreme weather events such as droughts but also extreme rainfall events, have already increased in frequency and magnitude (IPCC 2012). Moreover, forecasts by global climate models predict that increasing temperatures and more frequent and longer drought periods will impose an increasing stress on ecosystems over the coming decades, and among the most impacted regions will be arid regions such as the Sahel, the Mediterranean and other semi-arid climate zones (IPCC 2014).

Many dryland (*see box 1*) climates are characterized by a high variability in rainfall amounts, meaning that there is a high risk for prolonged periods with very low rainfall (Adeel et al. 2005). Drylands might be vulnerable ecosystems to projected climate change, as in drylands a decline in precipitation can lead to a reduction in the vegetation productivity and cover (Tietjen et al. 2010, Cherwin and Knapp 2012, Vicente-Serrano et al. 2012, Vicente-Serrano et al. 2013), although the consequences of long-term reduced rainfall and extreme drought events on vegetation cover are still uncertain. Furthermore, grazing by large herbivores in drylands may further increase the risk of declines in vegetation cover and associated ecosystem degradation (Miehe et al. 2010). Under unfavourable conditions, like severe drought conditions or under high grazing pressure, theoretical model studies predict that dryland ecosystems may move from a vegetated to a non-vegetated state, and that recovery from this degraded state is very difficult to achieve (Rietkerk and Van De Koppel 1997, Kéfi et al. 2007, Kéfi et al. 2016).

This relatively high risk for degradation is especially troublesome because about 90% of dryland inhabitants are living in developing countries, with poor socioeconomic outlooks. Nearly 500 million people in drylands, mostly in Asia and Africa but also in regions of Mexico and northern Brazil, are living in rural areas and are very much dependent on the local environment for their livelihoods (UNCCD

2011). For example, currently most of the people living in rural dryland regions of East and West Africa, rely on herding and cropland farming and their income is thus highly dependent on rainfall amounts and related vegetation presence (Cervigni & Morris 2016, Martin et al. 2016). The combination of high rainfall variability and relatively high levels of poverty results in societies that may be very sensitive to climate driven ecosystem change. An important question is therefore what the impacts will be of projected climatological changes on dryland ecosystems. This is essential information for constructing effective management strategies that prevent future degradation of ecosystems.

Box 1. Drylands

Drylands are ecosystems where plant productivity is mainly limited by soil water availability (*figure 1*). Drylands are important environments from a global perspective as they cover roughly 41% of the Earth's land surface and are populated by more than 2 billion people (Adeel et al. 2005, Reynolds et al. 2007). Drylands contain a wide variety of ecosystem types, including sandy deserts, temperate grasslands and savanna woodlands, but they all are characterised by water limitation, given that precipitation is lower than the potential evapotranspiration. The main economic activities in drylands are pastoral grazing and cropland agriculture. Drylands support approximately 50% of the world's global livestock and croplands cover approximately 25% of global drylands (Adeel et al. 2005). Next to those economic uses, drylands form a habitat for 28% of endangered species worldwide and protecting drylands is thus crucial for upholding global biodiversity as well (Adeel et al. 2005).

The Sahel region is a classic historical example of how persistent drought conditions can have devastating effects on local communities. In the 1950s and 1960s higher than normal rainfall levels occurred, resulting in increased economic activity. But when in the 1970s normal low levels of rainfall conditions returned, these drought conditions resulted in high impacts on local communities. An estimated 250,000 people died because of water and food shortages, along with nearly all their cattle, sheep, and

goats (UNCCD 2011). Also in the 1980s and the 1990s low rainfall levels have resulted in agricultural drought and famine, and even currently (May 2016), many countries in sub Sahelian Africa are suffering from the worst drought conditions in decades. For increasing global equity levels and for reaching the global sustainability goals, understanding processes that can prevent the degradation of drylands has therefore been given a high priority by the United Nations Environmental Program (UNCCD 2011)

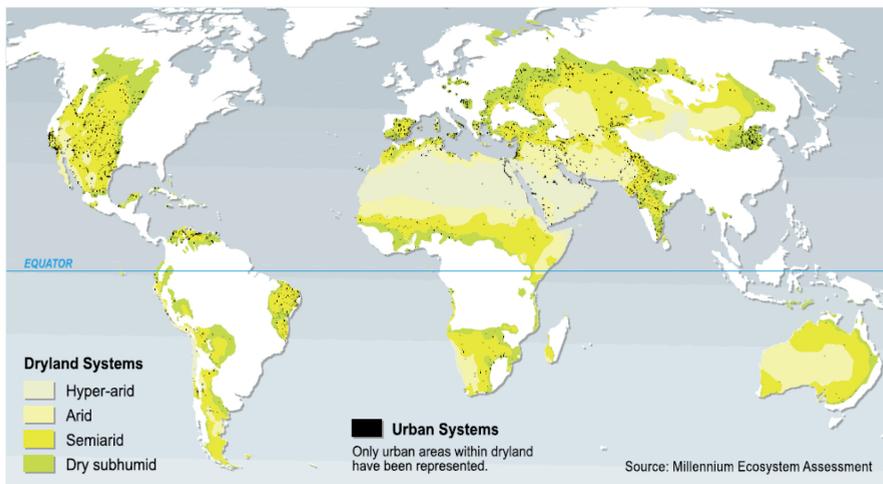


Figure 1. The above map shows drylands as defined by the U.N. Convention to Combat Desertification, defined as lands where the annual precipitation (P) is less than two thirds of potential evapotranspiration (PET). Dryland categories include dry sub humid areas ($P/PET = 0.50-0.65$), semi-arid areas ($P/PET = 0.20-0.50$), arid areas ($P/PET = 0.05-0.20$), and hyper arid areas ($P/PET < 0.05$), but excluding arid polar areas.

OBSERVED AND PREDICTED DROUGHT TRENDS IN DRYLANDS AND THE MEDITERRANEAN

A drought is a period of dry weather that lasts long enough to cause a hydrological imbalance, thereby causing related water shortages. Several types of droughts can be distinguished (IPCC 2014). A period with a below-normal precipitation is defined as a meteorological drought. A shortage of precipitation during the growing season, leading to below normal soil moisture levels that hamper the vegetation growth is defined as an agricultural drought (IPCC 2014). A drought that causes changes in runoff and groundwater percolation that primarily affect the

water supplies is defined as a hydrological drought (IPCC 2014). Changes in soil moisture content and groundwater levels are also affected by processes as the actual evapotranspiration, overland flow and groundwater extractions, in addition to changes in precipitation. Agricultural or hydrological droughts are therefore a result of both physical and societal processes (Van Loon et al. 2016), next to the rainfall deficiency (meteorological drought) that is the underlying cause. As we study the effects of rainfall deficiency on vegetation in semi-natural areas, with very low agricultural activity, we follow the definition of meteorological drought throughout this thesis.

Globally there has been an increase in the meteorological drought frequency, duration, and severity for the period 1951-2010, although the drought frequency decreased in the Northern Hemisphere. The increase in drought frequency, duration, and severity has been found to be most significant in Africa, Eastern Asia, the Mediterranean region, and in southern Australia (Spinoni et al. 2014). Moreover, global climate models predict that increasing temperatures and prolonged drought periods will impose an increasing stress on ecosystems over the coming decades (IPCC 2014). Increasing drought is likely over central America, southern Europe, southern and central Africa, Australia and southeast Asia as global warming continues in the twenty-first century (Dai 2013).

Climate-change projections for the Mediterranean Basin show an increased variability in weather conditions and an increase in extreme weather events, with longer and more frequent intense heat waves and droughts. The Mediterranean is widely considered a "climate change hot spot" (Giorgi 2006), meaning that the region has been very sensitive to changes that have already occurred, and is expected to be a sensitive responder to predicted changes. Beniston et al. (2007) found that the regional climate models that they analysed all simulated earlier and longer droughts in the Mediterranean. The main contributing factors are reduced rainfall intensity, and an earlier onset and longer duration of summer drought. Summer drought periods over southern Spain are projected to last over three weeks to a month longer than at present at the end of this century (Giannakopoulos et al. 2009), depending on the emission scenario pathway that were analysed.

STRESS AND ECOSYSTEM SHIFTS

How an ecosystem will respond to increased stress is a central theme in ecology and environmental sciences. Stress is here defined as conditions that hamper the vegetation growth, survival or reproduction compared to optimal conditions, with stress being a combination of abiotic stress (e.g. drought) and biotic stress by consumers (i.e. disturbance sensu Grime 1977). In response to increasing drought stress, arid ecosystems can either undergo gradual changes (*figure 2, panel a*), or respond in a nonlinear way, such that they undergo a more rapid change than expected after a certain threshold of stress has been reached (*figure 2, panel b*). Such critical transitions in ecosystems have been shown to occur in a wide variety of ecosystems (Scheffer et al. 2001, Scheffer et al. 2009, Kéfi et al. 2016) and are the consequence of positive feedbacks between organisms and the physical environment. If an ecosystem undergoes a critical transition and the ecosystem enters a degraded state under too high stress conditions, it may remain degraded for a long time as large changes in the environmental conditions are needed to reverse a shift to the original healthy state of the system (Scheffer et al. 2009). This difficulty of reversing is because a positive feedback can induce bistability in a system. This means that given a limited range of environmental conditions (*figure 2, panel b, green box*), the system can either be in the vegetated or the degraded state, depending on the initial value of the system. If a system starts in the healthy state, but stress increases and the system passes point 1 and reaches the degraded state, the stress level has to be reduced beyond point 2 to return the system to the vegetated state. Within the zone of bistability, if vegetation density is low (below the dotted line), the vegetation density will converge to the degraded state over time if the environmental conditions stay the same or become worse. If the initial vegetation density is high (above the dotted line), the system will converge to the vegetated state over time if the environmental conditions remain the same or improve.

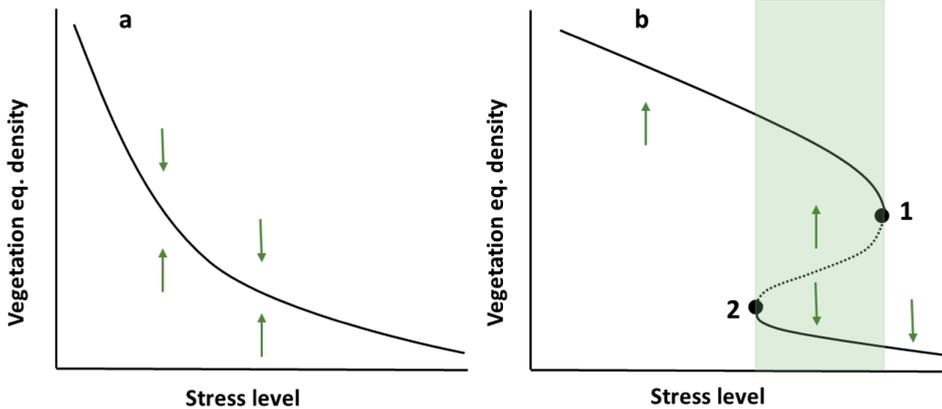


Figure 2. Examples of two different types of ecosystem responses to stress, being either a gradual transition (a) or a critical transition (b). The solid lines represent equilibrium vegetation densities, i.e. the density in biomass that an ecosystem will converge to over time. If an ecosystem is characterized by a critical transition and the system reaches a critical point – black dot 1– it will undergo a transition to a degraded state that is hard to reverse. To reverse the transition, the stress level has to be reduced below point 2. The green rectangle indicates the region of bistability. The dotted line within the rectangle indicates unstable equilibria, from which a system either diverges upward or downward, depending on the initial conditions.

Critical transitions in drylands can occur if an ecosystem is controlled by positive feedbacks between the vegetation and the physical environment, mainly related to water content of the soil or the soil quality (Rietkerk and Van De Koppel 1997, Kéfi et al. 2007). If the presence of plants increases the soil water infiltration rate or lowers the soil evaporation rate, the vegetation can enhance its own growth, thereby creating a positive feedback. The same process can occur the other way around though: when the vegetation density decreases, the positive effects of vegetation on its surroundings wanes, and vegetation might decrease even further. This positive feedback loop, which can work both ways, can lead to critical transitions in ecosystems, but it is still an open question under what conditions critical transitions occur in drylands.

Ecosystem models show that drought stress or grazing pressure in dryland ecosystems may lead to critical transitions from a vegetated to a non-vegetated state (Rietkerk et al. 1996, Rietkerk and Van De Koppel 1997, Scheffer et al. 2001, Kéfi et al. 2007, Xu et al. 2015). The former two studies have shown that increased infiltration around vegetation patches can be an explaining mechanism for induced bistability between a vegetated and a non-vegetated state. Whereas the latter two studies have shown that local facilitation can be a more general mechanism

resulting in bistability. Kéfi et al (2007) showed that local facilitation, defined by them as the process whereby vegetation is increasing the probability for vegetation establishment through improving soil conditions in their direct vicinity, can also lead to critical transitions under high drought or grazing stress. Critical transitions can also occur when positive interactions operate between woody plants, for example when mature plants protect seedlings in their direct vicinity, and thereby lower the mortality of seedlings growing close to neighbouring mature plants (Xu et al. 2015).

The possibility of critical transitions to a non-vegetated state further highlights the need to understand processes underlying desertification. As described above, feedbacks between water availability and vegetation density, and local facilitation between individual plants have been shown to lead to bistability in arid ecosystems, but the exact mechanism and under which conditions they lead to critical transitions remains uncertain (Kéfi et al. 2016). To better understand if critical transitions will occur in drylands it is important to gain more insight into how strong local facilitation is between plants along gradients consisting of drought and grazing stress, that are two important stressors in drylands.

PLANT-PLANT INTERACTIONS ALONG STRESS GRADIENTS

Ecosystems comprise of sets of complex interactions between the multiple species that they consist of and their environment, and those interactions can determine how stable an ecosystem is to increased stress. For this reason, investigating how species interactions change along environmental stress gradients is an important question in plant ecology. Plant species have long been known to compete for resources. How the importance of competition between plants evolves over stress gradients, and how competition relates to plant species diversity has been a classic debate in ecology during the 1970's and 1980's (Grime 1973, Tilman 1988). However, during the last two decades the opposite process, namely facilitation, has increasingly gained attention as a driving mechanism structuring plant communities.

Facilitation is the process by which a plant individual provides positive effects on the fitness of another individual within the same trophic level (Pakeman et al. 2009). Simply by growing, many plant species can provide positive effects by altering the environment, making their direct surroundings more hospitable for other

neighbouring individuals. Facilitation was long seen as a rare process in the field of plant ecology, as nearly all research on plant-plant interactions was focused on competition until the beginning of the 1990's (Michalet and Pugnaire 2016). However, over the last three decades a wide array of studies clearly indicated that facilitation is a common feature in plant communities, especially in harsh environments such as alpine or arid ecosystems (Callaway 2007, Pugnaire et al. 2011, Callaway 2013). Although researchers now widely agree that facilitation is an important process underlying ecosystem stability, diversity and functioning (Bruno et al. 2003, Michalet et al. 2006, Butterfield 2009, Cavieres and Badano 2009), they still disagree about what the different drivers of facilitative interactions are, and on how facilitation changes over environmental gradients.

Facilitation between plants can arise from a wide array of different mechanisms. For example, trees can shade the understory vegetation, thereby decreasing desiccation stress for less drought tolerant seedlings (e.g. Saccone et al. 2009). Similarly, spiny shrubs species can protect neighbouring individuals from being grazed by herbivores by providing them shelter with their canopies (e.g. Smit and Verwijmeren 2011). Also, many types of vegetation have been found to increase the nutrient levels or water status of soils underneath vegetation patches compared to the bare soil (for reviews see Callaway 2007, Brooker et al. 2008, Soliveres et al. 2015). Even though facilitation and competition can be driven by many different mechanisms, attempts have been made to conceptually predict how plant-plant interactions in general will change over stress gradients.

The study of this relationship between stress and facilitation has been a very active line of research over the past two decades. It was introduced by the Stress Gradient Hypothesis (hereafter SGH; Bertness and Callaway 1994), which predicts an increase in the frequency of positive interactions with increasing environmental stress. This stress can be either abiotic (e.g., drought stress, nutrient limitation) or biotic (e.g. herbivory). Following up on this it was hypothesized that facilitation strength would increase with increasing abiotic stress or consumer pressure (Callaway and Walker 1997). The reasoning behind the SGH is that both competition and facilitation act simultaneously upon species but shift from competition towards facilitation with increasing environmental stress, as less stress-tolerant species become dependent on amelioration of stress conditions provided by more stress tolerant species.

The SGH has been revisited multiple times. It has been argued that the shape of the relationship between the outcomes of plant–plant interactions and environmental stress gradients is unimodal (i.e. waning positive interactions can be expected at extreme stress) instead of linear (Michalet et al. 2006, Maestre et al. 2009). Also, over the years, a very wide array of factors have been identified that nuanced the SGH, showing that general predictions are not likely to hold. For example, different types of relationships between stress and positive interaction strength can be observed dependent on the type of stress and the number of stress types involved (Maestre et al. 2009, Smit et al. 2009). Despite the possible importance in determining ecosystem stability, it is thus still a challenge to predict how plant–plant interactions change along stress gradients (Soliveres et al. 2015). Although the combination of drought stress and grazing stress is common in arid ecosystems, studies that assessed changes in plant–plant interactions at combined drought and grazing gradients are still very scarce.

OUTLINE OF THE THESIS AND RESEARCH QUESTIONS

The main research questions of this thesis are: **how do plant–plant interactions change along combined stress gradients consisting of drought and grazing stress? And how do these changes relate to dryland ecosystem stability?** To answer these questions we combine in this thesis insights from a conceptual review study (**chapter two**), an observational study (**chapter three**), an experimental study (**chapter four**) and an ecohydrological modelling study (**chapter five**).

In **chapter two**, we present a literature review of studies on plant–plant interactions in general and in drylands in particular. By doing so, we made a conceptual model to predict how plant–plant interactions will change along a drought stress and grazing gradient, and discuss how changes in plant–plant interactions relate to the onset of critical transitions in drylands. In this review we combine insights from those two important ecological fields, the stress gradient hypothesis and critical transitions, to improve our ability to predict critical transitions in arid ecosystems.

To test the predictions from the second chapter we performed observational and experimental studies in the south east of Spain, which are described in **chapter three** and **chapter four** of this thesis respectively. There are several approaches to measure the changing nature of plant–plant interactions between plants along

stress gradients. The first method is to perform observational studies and measure the spatial associations between plants at sites along a stress gradient. Although spatial associations provide a good indicator for species interactions, they cannot distinguish between facilitation, and for example the characteristic of shrubs to act as a sink for seed deposition. Strong positive spatial associations, however, are important observations from which one can develop hypotheses about the direction and strength of plant-plant interactions along stress gradients, being either competitive, leading to segregation, or being facilitative leading to clustering of vegetation. Manipulative field experiments are required to test these hypotheses to further investigate the mechanisms responsible behind changes in spatial associations, and to separate plant interaction effects from microsite effects. Experimental work on plant-plant interactions often consists out of paired species experiments, where a 'protégé' plant species is planted either growing alone or growing in the direct vicinity of a neighbouring 'nurse' plant species, that might provide benefits to the 'protégé' plant through for example shading or grazing protection. To shed more light on how plant-plant interactions evolve along aridity and herbivory gradients we performed observational and experimental studies at a field site in the semi-arid south east of Spain (*see box 2*) that are the basis of chapter three and four of this thesis.

Box 2. Field site

We performed our field studies in field sites in the semi-arid south east of Spain near the city of Murcia (37°57'28.37"N - 1° 0'16.14"W). We choose this site as it is semi-arid and was grazed by a herd of approximately 200 goats. The local shepherd that collaborated in our studies, facilitated us to perform an observational study along a goat grazing gradient, and allowed us to perform an experimental study with a controlled amount of grazing by goats per plot. The study area has been grazed since April 2009 by a herd of approximately 200 goats of the Murciano Granadina breed. Besides grazing by goats, the site was grazed by rabbits (*Oryctolagus cuniculus*), enabling us to compare the effects of grazing by goats and rabbits on plant performance in our experimental design.

Average annual rainfall at the site is 301 mm and the climate is characterized by very low rainfall during the summer months. Highest rainfall occurs during spring and autumn and there is an extensive dry period in summer of approximately two months. The average rainfall in August and July is 10 and 5 mm, respectively. Mean monthly temperature ranges from 10.1 °C in January up till 26.7 °C in August (Agencia Estatal de Meteorología, AEMET). The soil texture is loamy sand and altitudes in our field site range from 175 to 302 m above sea level. The area consists of a mixture of relatively undisturbed slopes with slope inclinations ranging from 20 till 74 % (on our sampled slopes) and abandoned terraces in between the slopes.

Vegetation coverage ranges from 60 % at ungrazed north facing slopes down to 4 % on goat grazed south facing slopes. The vegetation occurs in patches (*figure 3*), meaning that patches of vegetation (ranging in diameter from 10 to 250+ cm) are interspersed with bare ground. The soil of bare ground is mostly covered with biological soil crusts enhancing stability of the soil. The vegetation consists of a mixture of tussock grasses, shrubs, chamaephytes, herbs and annuals. For both our studies we focussed on the perennial species. On the slopes, most abundant perennial species are *Teucrium polium* and *Fumana ericoides* (chamaephytes), *Rosmarinus officinalis* and *Anthyllis cytisoides* (medium-size shrubs), *Stipa tenacissima* (tussock grass), and *Brachypodium retusum* (short statured grass). On the

terraces, the vegetation coverage ranged from 40% to 60 % and the most dominant perennial vegetation consists of the shrub species *Artemisia herba-alba*, *A. cytisoides* and *Salsola genistoides*, and the herbaceous species *Marrubium vulgare*.



Figure 3. Overview of the field site showing the patchy nature of the vegetation.

In **chapter three** we performed an observational study to assess the combined effects of drought and grazing pressure on plant-plant interactions. Firstly, we assessed the relative effect of grazing pressure and slope aspect (as a proxy for drought stress) on vegetation cover and soil functioning. Secondly, to give an indication of the two stress effects on plant-plant interactions, we measured plant co-occurrence patterns by recording the presence of species in standardized quadrats. We assumed that co-occurrence of plants is an indirect measure of facilitation between plants and exclusion is an indirect measure for competition between plants.

In **chapter four** we describe the results of a study in which we experimentally investigated the combined impact of grazing pressure and drought stress on the interaction between unpalatable mature shrubs and palatable shrub saplings. We performed a manipulative experiment in which we planted 1280 saplings of the shrub species, *Anthyllis cytisoides* that is a preferred food source for both goats and rabbits. Saplings were planted either under the canopy of the protecting shrub species *Artemisia herba-alba* or in the open microsites in between shrub patches. If facilitation is occurring, we expected saplings that were planted underneath the canopy of *Artemisia herba-alba* shrubs to perform better than saplings that were growing alone. Over a period of 2 years, we followed growth and survival of saplings over the microsites in four treatments of grazing pressure: no grazing, low goat grazing pressure, high goat grazing pressure and rabbit grazing. Also, an extreme drought event occurred during the course of the experiment that enabled us to study the transient effect of a drought event on the treatment effects that we observed due to grazing.

Observational and experimental studies provide valuable insights into the nature of plant-plant interactions, but they can only show how plant-plant interactions evolve under the current, existing climatological circumstances. To predict the effect of projected decreases in rainfall amount and increased intermittency (the average time in between rainfall events) of rainfall, in **chapter five** we used a mathematical model to further investigate the effect of drought and grazing on plant-plant interactions. In this study, we used an ecohydrological model, to simulate the growth of two plant species interacting with each other under different scenarios of rainfall and grazing pressure. The modelled plants differed in their functional traits so that the unpalatable nurse species protected the palatable protégé species from grazing damage. Moreover, the nurse species was more drought resistant, i.e. it had a lower wilting point, whereas the protégé species had a higher optimal growth rate in return when soil water content reached field capacity. This way the modelled species represent the species we investigated in the experimental setup from chapter four of this thesis. By analysing the model we investigated the effect of rainfall intermittency on the long-term coexistence between the two plant species growing under a single limiting resource, namely water. Also we investigated how the plant-plant interactions evolved along the drought and grazing stress gradient.

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CHAPTER

2

Interspecific facilitation and critical transitions in arid ecosystems

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ABSTRACT

Climate change and intensified land-use impose severe stress on arid ecosystems, possibly resulting in relatively rapid degradation which is difficult to reverse. To prevent such critical transitions it is crucial to detect early warning signals. Increased 'patchiness' – smaller and fewer vegetated patches – is thought to be such a signal, but the underlying mechanisms are still poorly understood. Facilitation between plants is known to be an important mechanism driving the patchiness of the vegetation, but we lack understanding of how interactions between plants change in response to combined effects of drought and consumer pressure - the main stressors in many arid ecosystems. Over the last decade numerous experimental studies have tested how intensity of facilitation between plants changes with increasing stress. The most recent synthesis predicts a decline in facilitation intensity at the severe end of a drought stress gradient. Adding consumer pressure may result in even earlier and faster declines in facilitation intensity. So far, studies on critical transitions and plant-plant interactions have developed separately. The relationship between stress and facilitation intensity has been overlooked in critical transition theory, while facilitation intensity may determine the position of a critical transition threshold. In this study, we incorporate experimental studies on the relation between stress and facilitation intensity into the critical transition framework, to improve our ability to predict critical transitions. Moreover, we propose that a decline in facilitation intensity at the severe end of a stress gradient may occur prior to a critical transition. Inclusion of consumer pressure will speed up this process, leading to earlier and faster degradation. In-field monitoring of seedling-facilitator associations and declines in facilitator recruitment can indicate declines in facilitation intensity and may thus provide additional early warning signals for imminent critical transitions, besides increased patchiness.

INTRODUCTION

Human population growth, intensified land use and climate change are posing increasing stress on earth's ecosystems (Adeel, et al. 2005). Especially prone are arid ecosystems because gradual build up in stress by drought or grazing can result in a critical transition from a vegetated to a non-vegetated state (Rietkerk, et al. 1996, Rietkerk and Van De Koppel 1997, Scheffer, et al. 2001). Critical transitions can result from bistability meaning that two alternative stable states are possible given the same environmental conditions. After a threshold is reached vegetation cover will decline more rapidly than expected, which may have negative impacts on ecosystem multifunctionality through reduced plant diversity (Maestre, et al. 2012). Because critical transitions are hard to reverse there is a high need for early warning signals, indicating an imminent transition. Recent modelling and observational studies identified the rapid loss of relatively large vegetated patches with increased environmental stress as a possible warning signal prior to a critical transition (Kéfi, et al. 2007a, Kéfi, et al. 2007b), but the exact mechanisms remain so far unclear. More insight into the relevant mechanisms and a stronger link with experimental studies is currently highly needed to improve our ability to predict critical transitions (Scheffer, et al. 2009).

Interspecific facilitation is the mechanism whereby a 'facilitator' plant species ameliorates the environment for 'protégé' plant species. Facilitators can relieve abiotic stress for protégés by protecting against extreme temperatures and high irradiance, increasing water availability, improving nutrient availability or by reducing soil compaction and erosion (Callaway 2007). Next to that, facilitators can lower consumer pressure (disturbance, i.e. biotic stress *sensu* Smit et al. 2009) by protecting protégés against herbivores, a process known as associational resistance (*sensu* Hay 1986). These two types of facilitation (relief of abiotic stress and associational resistance) shape a wide range of ecosystems and the significance of facilitation in structuring plant communities is now fully recognized (Callaway 2007). However, there is much discussion on how the direction and intensity of facilitation respond to increased abiotic stress and consumer pressure and thus far, interspecific facilitation has not yet been explicitly considered in the framework of critical transitions in arid ecosystems.

Here we aim to combine insights from the theories on critical transition and on interspecific facilitation in plant communities along stress gradients (Stress Gradient Hypothesis framework) to improve our ability to predict the onset of critical transitions in arid ecosystems. These two important ecological theories have thus far developed separately and the relation between environmental stress and facilitation intensity has until now been overlooked in critical transition theory. Firstly, we discuss most recent insights in plant-plant interactions along abiotic and biotic stress gradients. Secondly, we link these insights to the framework of critical transitions, by emphasizing that changes in facilitation intensity can affect the position of a critical transition threshold. Finally, we propose a conceptual framework for critical transitions in arid ecosystems based on the notion that not only the density of facilitator species will decline with increasing abiotic stress and consumer pressure, but also the intensity of facilitation will change. We discuss the possibilities for ecological applications and give future directions for essential field and modelling studies.

CURRENT KNOWLEDGE ON SPECIES INTERACTIONS ALONG STRESS GRADIENTS

Classic conceptual models on direction and intensity of plant-plant interactions along stress gradients predict that the frequency and intensity of facilitative interactions increase with higher abiotic stress or consumer pressure (Bertness and Callaway 1994, Brooker and Callaghan 1998, Callaway 1995, Callaway and Walker 1997, Holmgren, et al. 1997). This conceptual framework was later called the Stress Gradient Hypothesis (Lortie and Callaway 2006, SGH). The reasoning behind the SGH (*figure 1, grey line*) is that both competition and facilitation act simultaneously upon species but shift from net competitive to facilitative with increasing environmental stress. Since its formulation, multiple studies have tested the SGH experimentally by assessing the interaction intensity for species pairs at several abiotic or biotic stress levels. Interaction intensity is typically measured as the performance of a target plant (protégé) close by a facilitator compared to its performance without a facilitator. Interaction intensity can range from negative (often called competition intensity) to positive (often called facilitation intensity). While several studies found supporting evidence for the SGH (Callaway, et al. 2002, Gómez-Aparicio, et al. 2004, Lortie and Callaway 2006) others found evidence against it (Armas and

Pugnaire 2005, Tielbörger and Kadmon 2000). These contrasting results have led to debate (Lortie and Callaway 2006, Maestre, et al. 2005, Maestre, et al. 2006) and calls for new studies, (Brooker and Callaway 2009, Brooker, et al. 2008, Michalet 2007), and as a consequence several refinements of the SGH have been proposed (Holmgren and Scheffer 2010, Kawai and Tokeshi 2007, le Roux and McGeoch 2010, Maestre, et al. 2009, Malkinson and Tielbörger 2010, Smit, et al. 2009, Soliveres, et al. 2011a). These proposals consider the length of the stress gradient studied, the type of stress imposed (resource or non-resource based, consumer pressure and their combinations), and the life-strategies (competitor vs. stress tolerant) and life-stages of facilitators and protégés. For example, in arid ecosystems where stress is mainly resource driven (stress tolerant facilitator, stress intolerant protégé), interaction intensity is predicted to show a hump shaped relation with stress (*figure 1, dashed line*). Plant-plant interactions first shift from competition to facilitation from high to low rainfall levels, but facilitation intensity wanes again at even lower rainfall levels (Maestre and Cortina 2004, Maestre, et al. 2005). The reason for a decline in facilitation intensity at high stress is that the level of resources become so low that positive effects of a facilitator on the microenvironment cannot outweigh competitive interactions by the canopy, such as rainwater interception or belowground root competition (Anthelme, et al. 2007, Holmgren and Scheffer 2010, Michalet, et al. 2006, Valladares, et al. 2008).

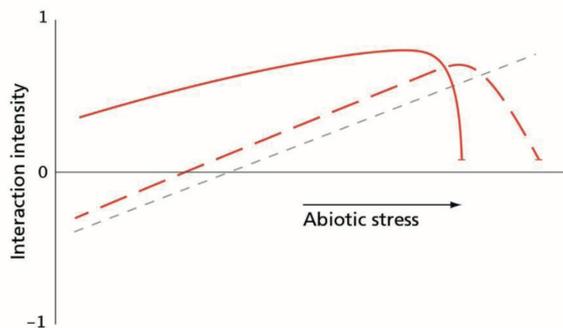


Figure 1: Possible shapes of the relation between stress and interaction intensity for arid ecosystems with (red solid line) and without (red dashed line) inclusion of consumer pressure along an abiotic stress gradient. Negative values represent competition and positive values facilitation. The original SGH (grey dash-dot line) predicted a monotonic increase from competition to facilitation. The revised SGH predicts a hump shaped relation. With inclusion of consumer pressure, we predict overall higher interaction intensity but an earlier and faster decline in interaction intensity at very high stress. These hypotheses predict the shape of the relationship between stress and interaction intensity in a qualitative way, we do not intend to make quantitative predictions.

Furthermore, abiotic stress and consumer pressure (e.g. grazing) will interact in driving plant-plant interactions (Smit, et al. 2009). It has even been suggested that consumer pressure is the primary driver of a decline in facilitation intensity at the severe end of a stress gradient consisting of both biotic and abiotic components (Forey, et al. 2009, Michalet, et al. 2006). Indeed, such declines in facilitation intensity at high consumer pressure have been observed in both terrestrial (Brooker, et al. 2006, Graff, et al. 2007, Smit, et al. 2007) and aquatic ecosystems (Le Bagousse-Pinguet, et al. 2012a, Levenbach 2009) where the facilitator was no longer able to protect protégés because it got damaged itself at very high consumer pressure. Hence for improved understanding, studies are needed that investigate the combined effects of both stress types on facilitation intensity in arid ecosystems. Studies attempting this are very scarce (but see Maalouf, et al. 2012, Soliveres, et al. 2011b) and are especially needed as consumer pressure has a large impact on the formation of spatial vegetation patterns and on the onset of critical transitions (Kéfi, et al. 2007a).

EXPANDING THEORY: COMBINING ABIOTIC AND BIOTIC STRESS GRADIENTS

Since studies that cross both biotic and abiotic gradients are still very scarce for arid ecosystems, we propose the following testable predictions on how plant-plant interactions may change if consumer pressure is superimposed on an aridity gradient. Adding consumer pressure may result in three important changes in the shape of the relation between facilitation intensity and abiotic stress (*figure 1, solid line*). Firstly, facilitation intensity may be higher over the whole aridity gradient (until a threshold is reached), because facilitators simultaneously protect against herbivory (i.e. associational resistance) and ameliorate the microenvironment (e.g. shading) (Smit, et al. 2009). With inclusion of consumer pressure, we hypothesize higher overall and a higher maximum in facilitation intensity as traits (e.g. spiny or waxy leaves) resulting in microenvironment amelioration often also result in protection against herbivores, leading to additive facilitative effects if both stress types operate simultaneously. Secondly, the importance of associational resistance relative to microenvironment amelioration may decrease with increasing abiotic stress (*figure 1, smaller distance between the solid and dashed line with increasing abiotic stress*). This is based on the idea that environments with low

abiotic stress are able to support more consumers than harsher environments (Grime 1977) and therefore associational resistance should be of higher importance at low abiotic stress. Two studies, one in rocky reef (Bulleri, et al. 2011) and one in salt marshes (Crain 2008) already have found support for this idea. Thirdly, the decline in facilitation intensity will occur at lower abiotic stress levels and will be faster when biotic and abiotic stresses operate simultaneously instead of separately (*figure 1*, earlier and faster drop of solid line): these interacting stress types will lower the facilitator's ability to ameliorate the microenvironment or effectively protect against herbivores.

CRITICAL TRANSITIONS IN ARID ECOSYSTEMS AND PATCH SIZES AS AN EARLY WARNING SIGNAL

Over the last decade much research has focused on providing warning systems for critical transitions to a degraded state in arid ecosystems (Kéfi, et al. 2007a, Rieterkerk, et al. 2004, Scheffer, et al. 2009). Spatial explicit modeling studies showed that when vegetation is regulated by local facilitation in combination with overall limitation of resources, the patch-size distribution of the vegetation is irregular and best described by a power law (Kéfi, et al. 2007a, Scanlon, et al. 2007). Local facilitation was modeled by inducing a positive effect of vegetated cells on neighboring bare soil cells, increasing the probability of vegetation recruitment close to a vegetated cell. A decrease in the intensity of local facilitation or an increase in the consumer pressure resulted in the disappearance of the largest patches from the ecosystem, prior to a critical transition to a bare state. This model outcome was confirmed by field observations of vegetation patchiness at field sites situated along a grazing gradient (Kéfi, et al. 2007a). Therefore, it was concluded that deviations in the power law – a deficiency in large patches – can be used as an early warning signal prior to critical transition to a desert state (Kéfi, et al. 2010, but see debate Maestre and Escudero 2009, Maestre and Escudero 2010). However, more recent analysis showed that models without local facilitation also show a similar disappearance of the largest patches during gradual transition from vegetated to a desert state (Kéfi, et al. 2011). So, more insight is still needed on how changes in plant-plant interactions along stress gradients relate to spatial patterns in vegetation, and in turn to the onset of critical transitions.

Kéfi et al. (2007b) also showed that whether a system will undergo a sudden collapse (i.e. discontinuous transition) or a gradual change (i.e. continuous transition) with increasing stress depends on the intensity of the local facilitative mechanism (Kéfi, et al. 2007b). Strong local facilitation intensity diminishes the risk of discontinuous transitions, because patches of vegetation can more easily form, spread and maintain themselves. The appearance of few vegetated patches in the system is already sufficient for vegetation recovery when facilitation intensity is very high. Hence, when facilitation intensity is very high, an ecosystem is predicted to undergo a continuous (i.e. gradual) transition with increasing stress. However, when facilitation intensity was decreased in their model, the system became bistable and the chance for critical transition from a vegetated to a desert state increased. With low facilitation intensity, the density of facilitators needs to be higher to effectively result in facilitative effects, as degraded sites need more than one neighbouring facilitator or need to be in closer proximity to the facilitator to receive the same benefit. So, a higher initial vegetation density is needed to lead the vegetation in the desired vegetated state, increasing the overall probability for a critical transition to occur.

INTEGRATING CHANGES IN SPECIES INTERACTIONS INTO THE CRITICAL TRANSITIONS FRAMEWORK

Facilitation intensity is predicted to change in a testable manner in response to multiple drivers of stress (*figure 1*). Moreover, facilitation intensity may determine the position of a critical transition threshold (Kéfi, et al. 2007b). From this, we will now describe how the shape of the relation between stress and facilitation intensity can be incorporated in critical transition theory in order to better predict the onset of critical transitions.

We propose that state transitions in arid ecosystems can be described by a plane that is very similar to a cusp catastrophe plane (*figure 2*). This way we can account for the notion that the nature of a state transition (gradual or discontinuous) and the position of a critical transition threshold may depend on the facilitation intensity. The use of a cusp catastrophe to describe vegetation dynamics in arid ecosystems has been previously proposed for transitions from perennial to annual vegetation types (Rietkerk, et al. 1996) and for transitions from grassland to shrubland (Turnbull, et al. 2008). The essential element of a cusp catastrophe

model is that it can explain both continuous and discontinuous transitions in vegetation states with increasing abiotic stress, dependent on a third variable, facilitation intensity in our case.

Changes in the facilitation intensity will result in a different nature of the transition (*figure 2*). With high facilitation intensity, the system will show continuous transitions, because even very low vegetation densities are able to exert strong enough positive effects for vegetation to recover. With intermediate facilitation intensity, the system will show discontinuous transitions, but with a relatively small sudden jump. With even lower facilitation intensity the system will show discontinuous transitions with relatively large sudden jumps. Whether a system will remain in a vegetated state or will converge to a bare state is thus dependent on both the density of vegetation and the intensity of interactions. Higher intensity may partly compensate for low density of vegetation, maintaining the system in a vegetated state. Conversely, lower facilitation intensity will only effectively maintain vegetation if vegetation is abundant at a higher density. This is reflected in the position of the critical transition threshold: with higher facilitation intensity the point of collapse occurs at higher abiotic stress levels (lower density), because higher facilitation intensity can compensate for a lower density of plants.

We thus hypothesize that the abiotic stress level at which a critical transition occurs is partly dependent on the shape of the relation between stress and facilitation intensity. We illustrate this by following two possible shapes of this relation, one with and one without inclusion of consumer pressure. Without inclusion of consumer pressure, the relation is hump shaped (*figure 1, red dashed line*). At low abiotic stress (*figure 2, red dashed line, panel A*) the ecosystem is in the upper part of the plane. The system is far from collapse, as while facilitation intensity is low, the vegetation density is still high enough to maintain itself. If the stress is moderate (*figure 2, red dashed line, panel B*), the system still has a low probability of collapse, because the decline in facilitator density is partly compensated by an increase in facilitation intensity. However, if stress becomes even higher (*figure 2, red dashed line, panel C*) and facilitation intensity begins to decline, the system will approach the threshold for critical transition very quickly, because now both the facilitator density and the facilitation intensity will decline with further increasing stress. With inclusion of consumer pressure (*figure 1, red solid line*), the relation between stress and facilitation intensity is also hump shaped, but the decline in facilitation intensity

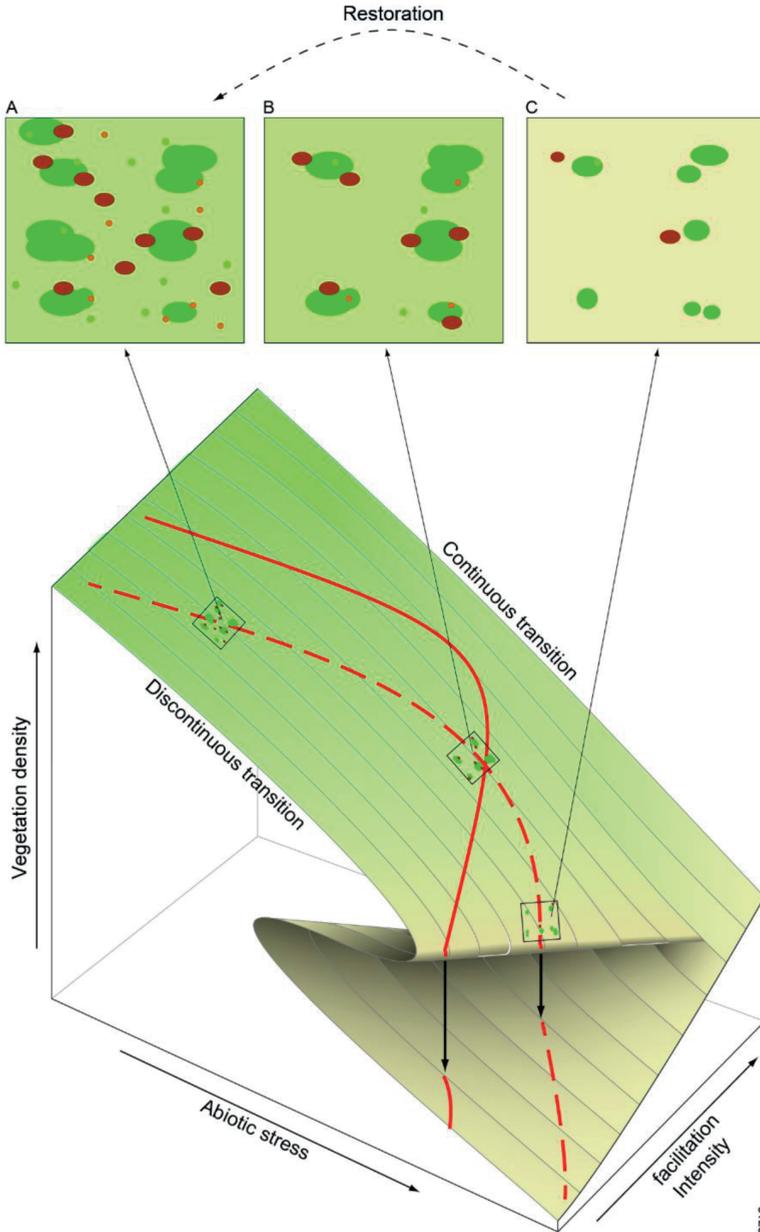


Figure 2. State transition from low to high facilitation intensity (i.e. positive interaction intensity) along a gradient of abiotic stress, with (red solid line) and without (red dashed line) inclusion of consumer pressure. Vegetation density is the amount of plants (both protégé and nurse species) per surface area. The downward arrows represent sudden jumps in the vegetation state. Panel A, B and C represent snapshots of the patch size distribution and coinciding changes in spatial association strength along a stress gradient. Dark green represents mature facilitator species, light green represents facilitator seedlings. Dark brown represents mature protégé species. Light brown represents protégé seedlings. The figure is adapted after Rietkerk et al. (1996) and Turnbull et al. (2008).

will occur at lower abiotic stress levels and will be faster. Therefore the collapse of vegetation will occur at lower abiotic stress levels and the sudden jump will be larger, as facilitation intensity will reach a lower level (*figure 2, red solid line*).

NEW EARLY WARNING SIGNALS? SPATIAL SPECIES ASSOCIATIONS AND FACILITATOR RECRUITMENT

As facilitation between plants plays a crucial role in the prevention of critical transitions, declines in positive interactions may provide an additional warning signal prior to critical transitions, in addition to a deficiency of large patches in the patch size distribution (Kéfi, et al. 2007a) or changes in cover (Maestre and Escudero 2009). In the following paragraphs we describe possible ways to monitor declines in facilitation intensity and predict how facilitator-protégé associations may change prior to the onset of a critical transition.

Previous studies showed that facilitation intensity is highest during recruitment and the earliest life stages of plants (Franks 2003, Lortie and Callaway 2006, Soliveres, et al. 2010), since tolerance and protection against stress have not fully developed yet. So, if shifts from facilitation to competition occur at very high stress, they will be most pronounced during the seedling and sapling stages of plants. Changes in spatial association strength (how often plants are co-occurring) between seedlings and nurse plants could thus effectively serve as a possible early warning sign that positive interactions are weakening.

Recent studies employing a network approach to map plant-plant interactions in arid ecosystems showed that community stability is determined by few very abundant species that have strong interspecific facilitative effects within the whole community (Saiz and Alados 2011, Verdú and Valiente-Banuet 2008). Given the key-role of these particular facilitator species, it is of crucial importance that viable populations of these species are maintained via consistent and frequent recruitment of new seedlings. Recruitment failure inevitably leads to local extinction of species which, in the case of a facilitator, may have dramatic consequences for the entire ecosystem. Seedlings of facilitators often still lack effective defense mechanisms against herbivores in their first life stage and can therefore be considered as protégés (Smit and Ruifrok 2011). So, if facilitation wanes this may result in recruitment failure of facilitator species, which over time will make a system converge to a state without facilitative effects. Recruitment failure of facilitator

species could thus indicate that a system is converging towards a degraded state. The speed at which this occurs is dependent upon the life span of the still present mature facilitator species, with fast degradation when facilitators are short-lived.

Therefore, we propose that a decline in nurse-seedling association strength and failure of facilitator recruitment can be used as additional warning signs for a nearby critical transition. If with increasing stress spatial associations strengthen, it may indicate that a system is not yet close to a critical transition or even that the system is not bistable due to high facilitation strength. However, if with increasing stress spatial association strength declines and facilitator recruitment declines, this might indicate that facilitative interactions are weakening and thus that an ecosystem might be approaching the critical transition threshold quickly. If facilitator recruitment fails this can indicate that a system has passed a threshold and is converging to a degraded state over time.

More specifically, we predict the following sequential change in spatial association strength when an ecosystem is approaching a critical transition, illustrated along the abiotic stress gradient presented in figure 2 (red dashed line). When stress is low, spatial association strength between facilitators and seedlings will be moderate and facilitator recruitment will be high enough to maintain the facilitative vegetation and to form new patches (*figure 2, panel A*). When stress becomes higher, the spatial association between mature facilitators and seedlings will become stronger, as sole standing seedlings from both protégés and facilitators species are no longer able to survive (*figure 2, panel B*). When stress becomes even higher and facilitation intensity starts to decline, the largest patches will disintegrate as facilitation intensity wanes (*figure 2, panel C*). Both protégé and facilitator recruitment will become very low and facilitator-seedling associations will become weaker, as a result of the waning facilitation intensity. This is the point where a deficiency in the largest patches occurs as observed by Kéfi et al. (2007a). We thus propose that the disintegration of largest patches is coincidental with a decline in spatial association strength between facilitators and seedlings and a decline in facilitator recruitment. With further increasing stress facilitator recruitment becomes too low to maintain the vegetation and a critical transition occurs. Moreover, we predict that with inclusion of consumer pressure this critical point is reached at lower abiotic stress as facilitation intensity might decline earlier (*figure 2, red solid line*).

Both changes in facilitation intensity and recruitment success of facilitator species can be obtained by field observations (transect or quadrat sampling of vegetation) to determine the spatial association strength and recruitment success of important facilitators. If facilitation intensity is observed to decline at increasing stress, tools to restore facilitative effects, such as revegetation with nurse plants (Pueyo, et al. 2009), should be applied in time to prevent further irreversible degradation. Moreover, by implementing removal or planting experiments, the stress level at which facilitation intensity wanes can be assessed more accurately. Experimentally assessing declines in facilitation intensity on crossed gradients of abiotic stress and consumer pressure could provide additional information for determining optimal land use under different drought scenarios.

SYNTHESIS AND FUTURE DIRECTIONS

In sum, many observational and experimental studies over the last decades showed that interactions between plants change along stress gradients. For arid ecosystems, most current syntheses predict a hump shaped relation between facilitation intensity and stress. This interplay between stress and facilitation intensity has been largely overlooked in critical transition theory, while the shape of the relationship between stress and facilitation intensity may importantly determine the position of a critical transition threshold. We therefore propose that assessing the shape between facilitation intensity and multiple drivers of stress (biotic and abiotic) may improve our ability to predict the onset of critical transitions. Furthermore, we propose that monitoring declines in facilitation intensity can be used to predict whether a system is approaching a critical transition threshold. More specifically, we propose that this decline in facilitation intensity can be observed in the field by monitoring facilitator-seedling associations and recruitment of facilitator species. A similar approach to predict critical transitions (focusing on declines in facilitation intensity) might be applicable to other systems as well. For example, indirect facilitation between macrophytes has been linked to a critical transition from a clear water state to a eutrophicated turbid state in freshwater ecosystems (Le Bagousse-Pinguet, et al. 2012b).

We now need experimental studies that test predictions on facilitation intensity for the dominant facilitator species at several levels of combined abiotic and biotic

stress. Next to that, observational studies are needed that monitor regeneration patterns of facilitator species to observe declines in facilitation intensity and to prevent chronic recruitment failure. Moreover, spatial explicit modeling studies are needed that integrate the interplay of facilitation intensity and multiple stress types in a mechanistic way, to better understand the implications of this interplay on spatial vegetation patterns and the onset of critical transitions. Such modeling studies could also add to the recent and ongoing discussion on facilitation importance (facilitation's relative impact, Brooker and Kikvidze 2008) by exploring the relationship between facilitation intensity and facilitation importance along stress gradients. Facilitation importance expresses the role of plant interactions compared to the role of other abiotic factors and may thus be a more direct indicator for declining positive interactions than facilitation intensity. While facilitation intensity and importance need not be related (Brooker et al. 2005), both indices have shown to be positively related along a complex stress gradient, consisting of both water stress and disturbance (Maalouf et al. 2012). More insight is needed on how facilitation intensity and importance change along combined stress gradients and how changes in both these indices relate to the onset of critical transitions.

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CHAPTER

3

Drought and grazing
combined: contrasting
shifts in plant
interactions at species
pair and community
level

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ABSTRACT

The combined effects of drought stress and grazing pressure on shaping plant-plant interactions are still poorly understood, while this combination is common in arid ecosystems. In this study we assessed the relative effect of grazing pressure and slope aspect (drought stress) on vegetation cover and soil functioning in semi-arid Mediterranean grassland-shrublands in southeastern Spain. Moreover, we linked these two stress factors to plant co-occurrence patterns at species-pair and community levels, by performing C-score analyses. Vegetation cover and soil functioning decreased with higher grazing pressure and more south-facing (drier) slopes. At the community level, plants at south-facing slopes were negatively associated at no grazing but positively associated at low grazing pressure and randomly associated at high grazing pressure. At north-facing slopes, grazing did not result in a shift in the direction of the association. In contrast, analysis of pairwise species co-occurrence patterns showed that the dominant species *Stipa tenacissima* and *Anthyllis cytisoides* shifted from excluding each other to co-occurring with increasing grazing pressure at north-facing slopes. Our findings highlight that for improved understanding of plant interactions along stress gradients, interactions between species pairs and interactions at the community level should be assessed, as these may reveal contrasting results.

INTRODUCTION

How plant interactions change along environmental gradients has been a central theme in plant ecology over the last few decades. First conceptual models focused on competition, hypothesising it would increase with increasing productivity (Grime 1979), or that it would be invariant along a productivity gradient (Tilman 1988). Over the last two decades an increasing number of studies have focused on facilitation, i.e. net positive interactions between plants (for review see: Callaway 2007, He et al. 2013, Pugnaire et al. 2011). Facilitator plant species (i.e. 'nurse' species) can relieve abiotic stress for other neighbouring 'protégé' plant species, for example by protecting them against extreme temperatures or by increasing the soil water availability (Callaway 2007). Next to that, facilitators can reduce grazing pressure by protecting other species against herbivores, a process known as associational resistance (*sensu* Hay 1986).

A popular conceptual model called the Stress Gradient Hypothesis (SGH) predicts that the frequency of positive interactions will increase with increasing abiotic stress or grazing pressure (Bertness and Callaway 1994, Callaway 1995, Callaway and Walker 1997). Many studies have attempted to test the SGH by experimentally investigating pairs of plant species and calculating the facilitation intensity (i.e. the performance of a plant with a protecting neighbour compared to a plant without) at several stress levels (Goldberg et al. 1999). However, studies from semi-arid ecosystems (e.g. Maestre and Cortina 2004, Maestre et al. 2005) showed contrasting patterns to the original SGH: plant interactions shifted from competition to facilitation and back to competition along a gradient of high to low rainfall. In parallel, other studies in drylands found temporal shifts from facilitation to competition during periods with very low rainfall (Armas and Pugnaire 2005, Tielbörger and Kadmon 2000). Also contradicting the predictions from the original SGH, studies in grazed ecosystems have reported an increase in facilitation intensity from low to high grazing pressure but a decrease again with further increases in grazing pressure (Smit et al. 2009). Such collapses in facilitation intensity at high grazing pressure have been observed in both terrestrial (Brooker et al. 2006, Graff et al. 2007, Saiz and Alados 2012, Smit et al. 2007) and aquatic ecosystems (Bulleri et al. 2011, Le Bagousse-Pinguet et al. 2012, Levenbach 2009). Plants can protect neighbours by physically sheltering them from herbivore damage, but

when grazing pressure becomes very high, facilitative interactions might wane, because the nurse itself gets damaged by grazing or trampling (Michalet et al. 2014), or because herbivores might start searching more intensively for resources (Soliveres et al. 2011a). Hence, from the examples above, it is clear that both abiotic conditions (resource and non-resource based, Maestre et al. 2009) and grazing pressure alter plant-plant interactions, but it remains unclear how multiple stressors combined, e.g. drought and grazing, shape plant interactions along combined gradients. Comparing the relative effects of both drought stress and grazing pressure on interaction intensity is important, because both may ultimately result in a decline in facilitative interactions, which may cause rapid degradation of arid ecosystems (Verwijmeren et al. 2013). One of the few available studies to date that tested combined effects of drought stress and herbivory is Soliveres et al. (2011a). This study showed that rabbit herbivory altered plant-plant interactions throughout the year, with positive interactions between *Stipa tenacissima* and *Retama sphaerocarpa* during winter and autumn, but with neutral interactions during summer. Other studies also showed that effects of grazing pressure on plant interactions can depend on water availability (Veblen 2008, Soliveres et al. 2012), but the interactive effects of drought and grazing on plant interactions is yet surprisingly understudied and therefore unclear. On the one hand, grazing may have a bigger effect on plant interactions under high drought stress, as plants will have lower ability to compensate for herbivory than in lower stressed environments (Gómez-Aparicio et al. 2008). On the other hand, herbivores may play a smaller role in higher drought stressed environments as herbivores density will be less abundant and plants are less palatable due to lower productivity (Smit et al. 2009, Verwijmeren et al. 2013).

Furthermore, the effect of stress on plant interactions is thus far mostly studied by observing one or multiple pairs of species (Soliveres et al. 2015). Studies that assess interactions at the entire community level, i.e. considering all species pairs within a community, are still scarce (but see; Saiz and Alados 2012, Soliveres et al. 2011b, Soliveres et al. 2012), particularly those that consider multiple (interacting) stress factors. Thus, it still remains unclear how species interact at the community level along an environmental gradient consisting of drought and grazing, and how this relates to changes at species level.

The aim of this study is to investigate how plant interactions shift along a combined gradient of grazing pressure and drought stress. We expected grazing to increase the amount of positive associations, because of increased importance of associational resistance (Saiz and Alados, 2012, Verwijmeren et al. 2013). We performed an observational study in semi-arid grassland-shrubland in south-eastern Spain, using aspect (north- vs south-facing slopes, reflecting a drought contrast) and grazing pressure (distance from stable). By performing transects we measured the grazing pressure, the vegetation cover and degradation level and also quantified species co-occurrence from species presence-absence data in quadrats along the same transects. We describe the effect of grazing pressure and aspect on vegetation cover and soil functioning, and indicate how these stressors interact in affecting aggregation and segregation patterns at plant community and at species-pair level.

METHODS

STUDY SITE

We performed our study at goat grazed semi-arid fields in southeastern Spain (Murcia region 37°57'28.37"N - 1° 0'16.14"W). Average annual rainfall here is 301 mm, with on average 38 rainy days with more than 1 mm of precipitation annually. Highest rainfall occurs during spring and autumn and there is an extensive dry period in summer; average rainfall in August and July is only 10 and 5 mm, respectively. Mean monthly temperature ranges from 10.1 °C in January up till 26.7 °C in August (Agencia Estatal de Meteorología, AEMET).

Altitudes in our field site range from 175 to 302 m above sea level. Soil type consists of loamy sand. We performed our observations on slopes where vegetation mainly consists of a mixture of woody shrubs, grasses and chamaephytes (dwarf shrubs). Most occurring species are (in order of abundance): *Teucrium polium* (chamaephyte), *Rosmarinus officinalis* (unpalatable shrub), *Anthyllis cytisoides* (palatable shrub), *Fumana ericoides* (chamaephyte), *Stipa tenacissima* (unpalatable tussock grass) and *Brachypodium retusum* (short grass).

The study area has been grazed since April 2009 by a herd of approximately 200 goats of the Murciano Granadina breed. We selected three large zones at increasing distance from the herd shelter; the furthest zone (800-1400 m from

shelter) was ungrazed by goats for decades, the middle zone (650 – 800 m from shelter) was grazed at low pressure since 2009, and the nearest zone (100 - 400 m from shelter) was grazed at high pressure since 2009. The nearest zone (high grazing pressure) was visited daily by the herd, while the middle zone (low grazing pressure) was visited weekly. The two grazed zones were grazed year-round except for the driest months July and August. We verified the assigned grazing pressure levels by counting droppings (described below). Besides grazing by goats, other - natural - herbivores in our site that may have a significant influence on the vegetation are rabbits (*Oryctolagus cuniculus*).

STUDY DESIGN AND DATA COLLECTION

To assess the impacts of grazing pressure and drought stress on degradation and plant-plant interactions, we established a study design with three levels of goat grazing along a range of slopes with aspect varying from north to south, thus differing in drought stress. This is because drought is more intense on sunnier and dryer south-facing slopes, and this is linked to decreased plant performance in semi-arid regions in previous studies (e.g. Gómez-Aparicio et al. 2004).

Within every grazing treatment we measured the perennial vegetation cover, soil functional status, and the species co-occurrence on replicated line transects. We selected 10-12 (1.5 x 30 m) transects within every grazing treatment, with transects following the maximum slope angle and being equally distributed over north-facing and south-facing slopes. The selected slopes varied in aspect and inclination. Aspect (expressed as degrees deviation from north) ranged from 0 (pure north) to 180 (pure south) and did not significantly differ between the three grazing levels (Kruskal-Wallis $\chi^2 = 0.252$; $p = 0.882$). Slope inclination ranged from 20 to 74 %, with an average of 42 % and did not significantly differ among the three grazing levels (Kruskal-Wallis $\chi^2 = 0.515$; $p = 0.773$). The minimum distance between selected slopes was 50 meter.

HERBIVORY

For every grazing level goat and rabbit droppings were counted. For this, we subdivided each transect in 20 quadrats of 1.5 by 1.5 m in which we recorded pellet presence. From this we calculated pellet density per transect, as a percentage of quadrats with droppings present. For rabbits we followed a likewise routine,

but instead of presence of individual droppings we counted presence of latrines along transects. At no grazing, we did not observe any goat droppings. A generalized linear model (normal errors, identity link function) with grazing as a factorial variable and aspect deviations from the north as a continuous covariable showed that goat droppings were more abundant at sites with high grazing pressure (Wald $\chi^2=124.148$, $df=2$, $p<0.001$), but aspect also had a significant effect with overall lower dropping counts at south-facing slopes (Wald $\chi^2=18.871$, $df=1$, $p<0.001$). Also, we found a significant interaction between grazing pressure and aspect (Wald $\chi^2=44.936$, $df=2$, $p<0.001$), with higher dropping count on highly grazed slopes that were more oriented to the north. Pairwise contrasts (at $p<0.001$) showed a significant higher dropping count at slopes with low grazing pressure compared to slopes with no grazing and also a significantly higher dropping count for slopes with high grazing pressure compared to slopes with low grazing pressure. A similar generalized linear model (normal errors, identity link function) showed that the number of rabbit latrines was not significantly different between the three goat grazing pressure levels (Wald $\chi^2=0.2626$, $df=2$, $p=0.269$), but we found significantly more rabbit droppings at north slopes than at south-facing slopes (Wald $\chi^2=37.444$, $df=1$, $p<0.001$). We found no significant interaction between goat grazing pressure and aspect (Wald $\chi^2=0.918$, $df=2$, $p<0.632$) for rabbit latrine number.

VEGETATION COVER AND SOIL FUNCTIONING (LFA)

Along all transects we recorded vegetation cover and soil functioning. In order to measure these two variables, we followed the guidelines from Landscape Functional Analysis (LFA; Tongway and Hindley 2004). Next to assessing vegetation cover along transects by point intersect transects, the LFA method provides indicators for soil functioning. These indicators were assessed by visual measurements of soil properties that were integrated into three indices representing infiltration ability, surface stability and nutrient cycling. For each transect, we assessed these three LFA indices in four quadrats of 50 x 50 centimetres, 2 located on bare soil and 2 underneath a plant patch (*figure 1*). This way we obtained one mean LFA value for soil under patches and one mean LFA value for bare soil.

SPECIES CO-OCCURRENCE (C-SCORE)

To assess species co-occurrence we sampled quadrats of 1.5 x 1.5 m along each transect (ca. 20 quadrats per transect) and we recorded perennial species presence per quadrat (*figure 1*). This information was used to create a presence/absence matrix for all observed species. We performed species co-occurrence analyses by using the Fortran based program PAIRS (Ulrich 2008) and used the C-score (Stone and Roberts 1990) as a metric of co-occurrence for each pair of plant species. The C-score is related to the competitive exclusion concept of "check-boardedness", i.e. how many of the possible species pairs in a given community

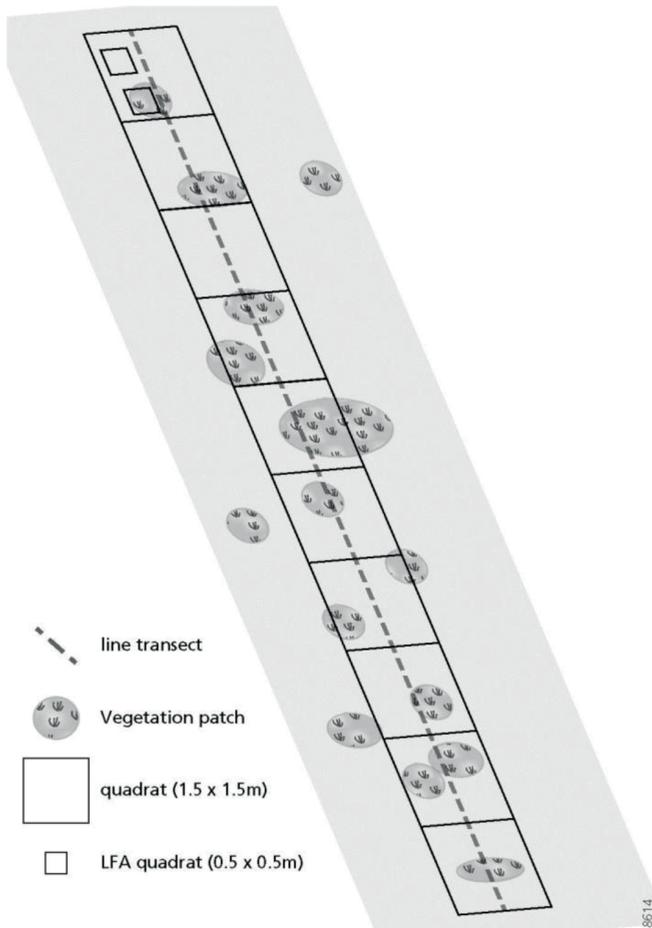


Figure 1. Sketch of a transect section used to measure vegetation cover (line transect), soil functioning (LFA quadrat) and species co-occurrence (quadrat of 1.5x1.5 m). Note that only a transect section is depicted; a full transect contained 20 quadrats, two LFA quadrats in bare soil and two LFA quadrats underneath a vegetation patch.

never appear together in the same quadrat. So, positive and large values of this index indicate that species are not co-occurring (exclusion) and that competition may be the driving mechanism determining the observed patterns (Gotelli 2000, Stone and Roberts 1990). We studied co-occurrence patterns at two levels: at the entire community level and for every pairwise species interaction.

COMMUNITY LEVEL

To estimate plant co-occurrence at the community level (the 'checkerboardedness' of all species together) we followed the approach used in similar transect-based studies on plant-plant co-occurrence (Maestre et al. 2010, López et al. 2013). We calculated co-occurrence of species by grouping all transects per treatment into one single matrix, because matrix sizes were too small per transect to detect any changes in co-occurrence at the community or the species pair level. For this analysis, aspect was made categorical by grouping deviations till 60 degrees in north-facing slopes and deviations higher than 120 degrees in south-facing slopes. Hence, we used six treatments obtained from three grazing levels crossed with two aspects (north and south). Observed *C*-scores per treatment were compared with an average for *C*-scores obtained by 1000 randomly assembled null matrices. Simulated matrices were assembled by Monte Carlo procedures using a fixed-equiprobable algorithm. This type of algorithm is recommended for absence-presence data from transect data (Gotelli 2000), as it does not constrain the number of plant species in one quadrat and assumes that every quadrat is equal in its probability of expected number of plant species, but maintains species abundance fixed (i.e., rare species remain rare while abundant species remain abundant). The degree of co-occurrence or exclusion was expressed as the deviation of our observed *C*-score from simulated *C*-scores. This deviation is expressed as the standardized effect size (SES). SES is calculated as $(I_{obs} - I_{sim}) / S_{sim}$, where I_{obs} is the observed value of the *C*-score, and I_{sim} and S_{sim} are the mean and standard deviation, respectively, of the *C*-score values that were obtained from the 1000 randomly created matrices. Positive *C*-score SES values indicate lower "checkerboardedness" (spatial segregation) than expected by chance, while negative values indicate higher "checkerboardedness" (spatial aggregation) than expected by chance. We calculated the *C*-score and corresponding SES for every transect at the community level, hence grouping all species together. In addition,

we calculated SES for the variance ratio (V ratio) and the number of species pairs, but since both indexes were highly correlated to the C-score SES ($r=0.96$ and $r=0.81$ respectively), we only presented the C-score effect sizes.

SPECIES PAIRS

As the number of species pairs in a matrix increases exponentially with the number of species (in our case, a matrix with 20 species holds 190 species pairs) many 'significant' species pairs can be expected by chance at the 1% or 5% error level. To reduce such high false detection error rates, we employed the conservative empirical Bayes mean criterion (Bayes M criterion) developed by Gotelli and Ulrich (2010). In this method, The C-score is calculated for all pairs of species, which are then grouped into 22 evenly spaced bins from 0.0 to 1.0. For each randomized matrix of the 1000 runs, the C-score values are calculated for all species pairs separately, which are grouped into the 22 bins. Then, the mean and confidence limits of the expected number of species pairs within each bin is calculated from these null matrices. Observed species pairs within each bin are ordered according to their observed C-scores and only pairs that fall above the mean (Bayes M criterion) for the expected number of species were considered for further analysis.

DATA ANALYSIS

We tested data of vegetation cover, LFA and C-score for normality using Kolmogorov-Smirnov tests and for homogeneity of variance using Levene's test. We found independence of grazing level and aspect (one way ANOVA: $F_{2, 36}=0.068$; $p=0.934$) and a correlation between vegetation cover and aspect ($R^2=0.406$), and between LFA values and aspect (R^2 ranging from 0.095 for infiltration at bare soil to $R^2=0.386$ for stability at bare soil). Therefore, to assess the relation between vegetation cover and grazing and the relation between the six separate LFA variables and grazing, we performed in these cases ANCOVA tests with aspect as a continuous covariable and grazing as a fixed categorical variable. We performed Tukey HSD post-hoc tests to further test for contrasts between the three grazing treatments. All analyses were performed in R, version 2.15.1 (R Core Team, 2013). For all linear models we calculated partial effect sizes (η^2), which is the partial variance explained by any significant factor.

RESULTS

VEGETATION COVER AND SOIL FUNCTIONING (LFA)

Vegetation cover ranged from 60 % at ungrazed north-facing slopes to 4 % on south-facing slopes with high grazing pressure. Full effects ANCOVA revealed significant effects of aspect ($F_{1,33}=31.111$; $p<0.001$, $\eta^2=0.373$) and grazing ($F_{2,33}=7.163$; $P=0.003$; $\eta^2=0.173$) on vegetation cover (*figure 2*). Vegetation cover significantly decreased with increasing aspect deviation from the north. Furthermore, vegetation cover was significantly lower at high grazing pressure than at ungrazed slopes, with an average decline of 14.5 % in cover from no grazing to high grazing pressure. There was no significant difference between no grazing and low grazing pressure (*figure 2*). Low grazing pressure only resulted in reduced vegetation cover for south-facing slopes, although the grazing-aspect interaction was non-significant ($F_{2,33}=2.033$, $p=0.147$).

Overall, LFA values significantly varied with both grazing pressure and aspect. For bare soils, aspect had a bigger effect on all three indices than grazing pressure (*table 1*). Higher aspect deviation from the north significantly reduced values for stability, infiltration, and nutrient cycling functions, while higher grazing significantly reduced LFA values for stability and nutrient cycling but not for infiltration. Tukey HSD tests for stability revealed significant contrasts between no grazing and low grazing ($\text{Mean}_{\text{no grazing}}=60.6$ $\text{Mean}_{\text{low grazing}} = 53.47$, $t = - 3.374$, $p<0.01$) but no contrast between low and high grazing. Nutrient cycling was significantly lower in high grazing pressure compared to no grazing ($\text{Mean}_{\text{no grazing}}=15.42$ $\text{Mean}_{\text{high grazing}} = 11.50$, $t = - 3.320$, $p<0.01$).

For soils underneath vegetation patches none of the three indices was significantly affected by grazing pressure, while higher aspect deviation from the north reduced the values for all the three indices (*table 1*). Also we found a significant interaction between grazing and aspect, with high grazing pressure exacerbating the negative impact of aspect within patches.

PLANT-PLANT INTERACTIONS AT THE COMMUNITY LEVEL

For north-facing slopes SES values were positively deviating from null models for both ungrazed slopes ($p<0.001$) and slopes with high grazing pressure ($p<0.001$), indicating exclusion between species being dominant. At north-facing slopes

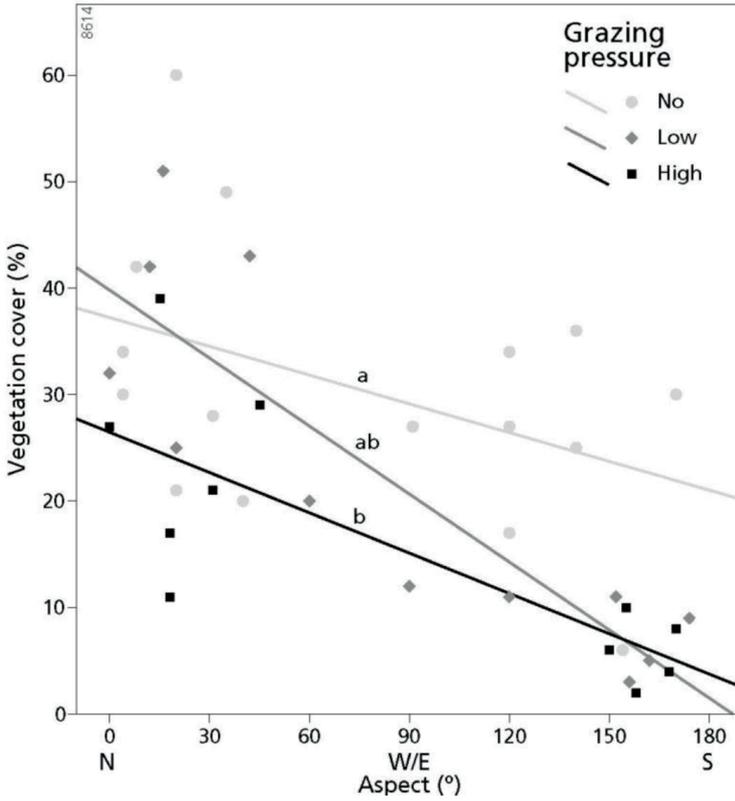


Figure 2. Mean vegetation cover along the sampled transects as a function of grazing pressure and aspect. Aspect is expressed as degrees of deviation from north. Different letters indicate significant differences obtained from Tukey HSD tests at $p < 0.01$.

	Grazing			Aspect			Grazing:Aspect		
	F	p	η^2	F	p	η^2	F	p	η^2
Bare soil									
Stability	7.511	0.002	0.207	27.062	<0.001	0.37	0.187	0.830	0.052
Infiltration	0.058	NS	0.030	3.878	0.0582	0.099	2.481	0.101	0.124
Nutrients	6.085	0.006	0.172	26.658	<0.001	0.371	1.333	0.278	0.037
Patch									
Stability	2.471	NS	0.087	8.562	0.006	0.152	6.445	0.005	0.229
Infiltration	1.791	NS	0.076	5.356	0.027	0.113	4.129	0.026	0.175
Nutrients	1.768	NS	0.063	6.373	0.017	0.117	7.17	0.002	0.264

Table 1. F, p and η^2 (partial effect sizes) values for the two main factors and their interactions for the three LFA indices for bare soils and soil underneath vegetation patches.

with low grazing pressure SES were not significantly deviating from null models, indicating neutral interactions to be dominant. At south-facing slopes SES were significantly positive at ungrazed slopes ($p=0.005$), indicating exclusion between species being dominant. However, SES shifted to being significantly negative at slopes with low grazing pressure ($p=0.030$), hence indicating a shift towards co-occurrence being dominant. At slopes with high grazing pressure SES were not significantly deviating from null models (*figure 3*).

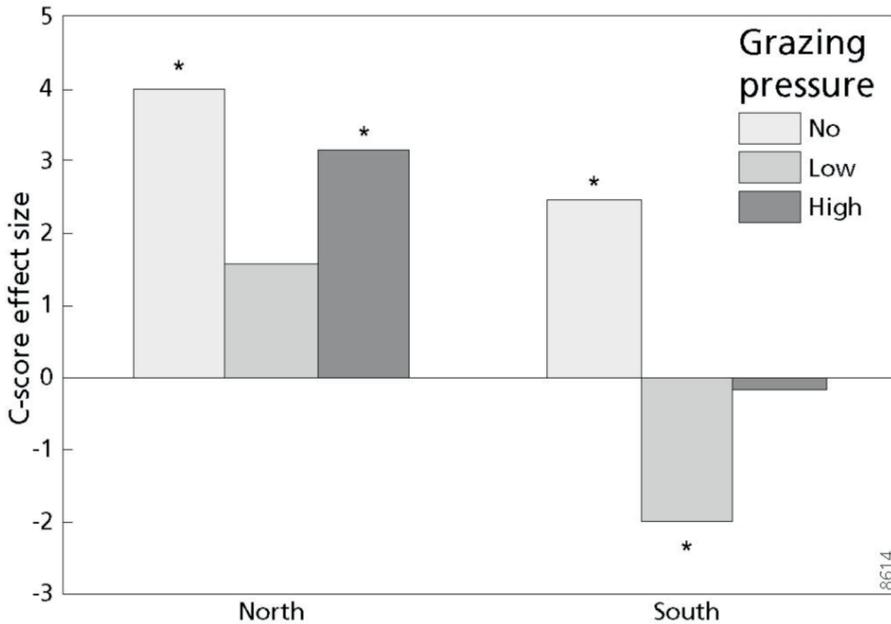


Figure 3. C-score standardized effect sizes (SES) along three levels of grazing pressure at north- and south-facing slopes (reflecting drought stress). Positive values indicate exclusion of species. Negative values indicate co-occurrence of species. * indicate significant deviation from the simulated null models at $p < 0.05$.

PLANT-PLANT INTERACTIONS AT THE SPECIES PAIR LEVEL

Overall, the patterns at species pair level were in line with the results from the community wide C-score analysis. At north-facing slopes that were either ungrazed or grazed at high pressure, we found more significantly excluding pairs than co-occurring pairs (*table 2, table 3*). On the slopes with low grazing pressure we found no significantly co-occurring or excluding species pairs and at north-facing slopes with high grazing pressure we found co-occurring pairs to

be dominant. Also at ungrazed south-facing slopes, most significant C-score SES indicated exclusion except for five species pairs. However, at the south-facing slopes with low grazing pressure we only found significantly co-occurring species pairs. At high grazed south-facing slopes we found one species pair to be co-occurring and an one pair to be excluding each other.

Treatment	Nr of species	Nr of poss. sp. comb.	Sign. co-occurring species (%)	Sign. excluding species (%)
North slopes				
No grazing	17	136	2.94	6.61
Low grazing	13	78	-	-
High grazing	11	55	1.89	10.91
South slopes				
No grazing	15	105	4.76	7.61
Low grazing	11	55	7.27	0
High grazing	8	28	3.57	3.57

Table 2. Percentage of significant co-occurring species pairs and percentage of significant excluding species pairs expressed as the total of nr. of possible species pairs used in the pairwise C-score analysis.

The analysis of pairs revealed two abundant species pairs that made shifts from being significantly positively associated to significantly negatively associated, or the other way around, with increased grazing pressure or drought stress associated to aspect. On north-facing slopes, *Stipa tenacissima* and *Anthyllis cytisoides* were significantly excluding each other under low grazing pressure (table 3), whereas they were significantly co-occurring under high grazing pressure. Furthermore, at the ungrazed slopes, *Stipa tenacissima* and *Salsola genistoides* switched from excluding each other at south-facing slopes to co-occurring at north-facing slopes. These three species were all highly abundant with occurrences of 38% (*Anthyllis cytisoides*), 31% (*Stipa tenacissima*) and 19% (*Salsola genistoides*) of the sampled quadrats.

Treatment	Species 1	Species 2	Obs. C-score	Exp. C-score	C-score SES	P value
North slopes						
No grazing	RoOf	ArBa	0.415	0.219	4.84	0.000
	RoOf	HeAp	0.866	0.338	3.20	0.001
	AnCi	StTe	0.465	0.291	2.90	0.003
	AnCi	SaGe	0.483	0.300	2.94	0.003
	TePo	FuEr	0.353	0.227	3.72	0.000
	BrRe	ArBa	0.496	0.287	4.30	0.000
	BrRe	HeAp	0.496	0.295	4.02	0.000
	FuEr	ArBa	0.622	0.347	4.76	0.000
	FuEr	CiCl	0.685	0.446	3.31	0.000
	<i>StTe</i>	<i>FuTh</i>	<i>0.258</i>	<i>0.415</i>	<i>-2.65</i>	<i>0.007</i>
	StTe	SaGe	0.289	0.544	-2.91	0.003
	<i>AnCi</i>	<i>HeAp</i>	<i>0.125</i>	<i>0.243</i>	<i>-2.72</i>	<i>0.006</i>
	<i>RoOf</i>	<i>FuEr</i>	<i>0.113</i>	<i>0.210</i>	<i>-2.85</i>	<i>0.004</i>
	High grazing	RoOf	AnCi	0.285	0.167	3.55
RoOf		StTe	0.420	0.219	4.74	0.000
RoOf		HeSt	0.712	0.328	3.50	0.000
AnCi		FuEr	0.517	0.299	2.85	0.004
StTe		BrRe	0.600	0.350	3.52	0.000
BrRe		FuEr	0.658	0.425	2.73	0.006
AnCi		StTe	0.156	0.406	-3.00	0.002
South slopes						
No grazing	StTe	ThVu	0.541	0.320	2.78	0.005
	StTe	HeAp	0.776	0.362	3.80	0.000
	StTe	TePo	0.658	0.286	6.04	0.000
	StTe	SaGe	0.584	0.323	3.14	0.002
	StTe	ArBa	0.559	0.244	6.87	0.000
	RoOf	ArBa	0.675	0.431	3.37	0.000
	ArBa	CiCl	0.812	0.538	3.10	0.002

Table 3. P-values for all significant species pairs that are either excluding or co-occurring in the matrix. Co-occurring species (negative SES) are highlighted in italics. In bold we highlight species pairs that made a shift in association direction with either grazing or slope aspect. Species acronyms: AnCi: Anthyllis cytisoides, ArBa: Artemisia barrelieri, BrRe: Brachypodium retusum, CiAl: Cistus albidus, CiCl: Cistus clusii, FuEr: Fumana ericoides, FuTh: Fumana thymifolia, GlCo: Globularia alypum, HeSt: Helichrysum stoechas, HeAp: Helianthemum apenninum, OnTr: Ononis tridentata, RoOF: Rosmarinus officinalis, SaGe: Salsola genistoides, StTe: Stipa tenacissima, TePo: Teucrium polium, TePs: Teucrium pseudochamaepitys, ThHi: Thymelaea hirsuta, ThVu: Thymus vulgaris.

(continued)

Treatment	Species 1	Species 2	Obs. C-score	Exp. C-score	C-score SES	P value
	ArBa	TePo	1.000	0.613	3.06	0.002
	<i>AnCi</i>	<i>HeAp</i>	0.203	0.663	-3.49	0.000
	<i>AnCi</i>	<i>TePo</i>	0.312	0.530	-2.71	0.000
	ArBa	<i>HeAp</i>	0.157	0.600	-3.27	0.000
	ArBa	<i>FuEr</i>	0.250	0.576	-3.07	0.002
	ArBa	<i>TePo</i>	0.133	0.497	-4.81	0.001
Low grazing	<i>TePo</i>	<i>AnCi</i>	0.156	0.393	-2.63	0.008
	ArBa	<i>AnCi</i>	0.156	0.403	-2.76	0.005
	<i>BrRe</i>	<i>ThVu</i>	0.337	0.712	-2.93	0.004
	<i>AnCi</i>	<i>GlCo</i>	0.156	0.403	-2.76	0.005
High grazing	RoOf	SaGe	0.792	0.409	3.90	0.000
	<i>FuEr</i>	<i>SaGe</i>	0.792	0.404	-2.68	0.007

Table 3. P-values for all significant species pairs that are either excluding or co-occurring in the matrix. Co-occurring species (negative SES) are highlighted in italics. In bold we highlight species pairs that made a shift in association direction with either grazing or slope aspect. Species acronyms: AnCi: Anthyllis cytisoides, ArBa: Artemisia barrelieri, BrRe: Brachypodium retusum, CiAl: Cistus albidus, CiCl: Cistus clusii, FuEr: Fumana ericoides, FuTh: Fumana thymifolia, GlCo: Globularia alypum, HeSt: Helichrysum stoechas, HeAp: Helianthemum apenninum, OnTr: Ononis tridentata, RoOf: Rosmarinus officinalis, SaGe: Salsola genistoides, StTe: Stipa tenacissima, TePo: Teucrium polium, TePs: Teucrium pseudochamaepitys, ThHi: Thymelaea hirsuta, ThVu: Thymus vulgaris.

DISCUSSION

The aim of this study was to investigate how plant-plant interactions shift along a combined environmental gradient of drought stress (slope aspect) and grazing pressure, and to assess how changes at species level are related to changes at the community level. Grazing pressure and aspect significantly affected vegetation cover and soil functioning, with overall decreasing cover and soil functioning with either an increase in grazing pressure or an increase in aspect deviation from the north. Together, the variation in vegetation cover and LFA indices showed a clear two-factor gradient in the functional/degradation status of the site, with stress increasing from no grazing on north-facing slopes to high grazing pressure on south-facing slopes. However, different patterns arose on this gradient, depending on whether species interactions were studied at the community level or at the species pair level. At the community level, on north-facing slopes, species were

excluding each other mostly over the whole grazing gradient. However, for one abundant species pair (*Stipa tenacissima* and *Anthyllis cytisoides*) we found a shift from exclusion to co-occurrence along the same grazing gradient from ungrazed to highly grazed north-facing slopes, highlighting the importance of assessing pairwise interactions next to changes in interactions at the community level. At south-facing slopes, species interactions at the community level shifted from being excluding at ungrazed slopes, to co-occurring at slopes with low grazing pressure, while interactions were neutral at south-facing slopes with high grazing pressure.

VEGETATION COVER AND SOIL DEGRADATION

We found that vegetation cover was affected by grazing and aspect, but aspect appeared to be the stronger driver of decreases in cover. Our grazing-driven decline in vegetation cover (14.4%) is in line with other studies on plant interactions along a livestock gradient in semi-arid ecosystems, with reported declines in vegetation cover of 10 percent (Alados, et al. 2004, Saiz and Alados 2012) and 12 percent (Bisigato and Bertiller 1997, Bisigato, et al. 2005). Aspect had a bigger impact on vegetation cover in our study, with an average decline of 17.2% from north- to south-facing slopes. The impact of low grazing pressure appears to have a bigger effect on south-facing slopes compared to north-facing slopes, but this interactive effect was not statistically significant.

Aspect influenced all soil functional indices (stability, infiltration, nutrients) for both bare soils and soils underneath vegetation patches, with soil functioning indices decreasing on slopes more oriented to the south. Grazing only influenced the stability and nutrient cycling indices at bare soil, probably because processes such as trampling may have a bigger effect in bare soils than in vegetation patches. This higher sensitivity of degradation processes in bare soil interpatches compared to vegetation patches is in line with previous studies (Mayor and Bautista 2012). The lack of an effect of grazing on the infiltration index is surprising, as this index has been reported to be particularly sensitive to small changes in soil condition (Mayor and Bautista 2012). Overall, increasing drought stress associated to higher aspect deviation from the north seems the biggest driver of a decrease in vegetation cover and soil functioning in our system.

PLANT INTERACTIONS AT THE COMMUNITY LEVEL

At both ungrazed north- and south-facing slopes we found exclusion between plant species to be the dominant interaction. This finding is in line with a similar field study performed in the south east of Spain where the plant community had an overall negative pattern of association (Saiz and Alados 2012). At slopes with intermediate grazing pressure plants shifted to being more positively associated on south-facing slopes, while on north-facing slopes they were not significantly deviating from the null model. On both aspects we thus found an increase in nestedness in the plant community (or a decrease in exclusion) from no grazing pressure to low grazing pressure. At high grazing pressure, species associations shifted back to neutral for south-facing slopes and to negative associations at north-facing slopes. These patterns partly support idea that intermediate grazing pressure can increase the amount of positive associations (or decrease the amount of negative associations in the case of north-facing slopes), due to a higher importance of associational resistance, while positive associations might disappear again with even higher grazing pressure (Saiz and Alados 2012, Verwijmeren et al. 2013). Moreover, we did only observe a shift in the direction of species association from negative to positive with grazing pressure at the south-facing slopes, while at the north-facing slopes we did not observe such a shift. This observation is in contrast with the idea that the effect of grazing on plant interactions is lower at higher drought stressed slopes. Our findings more support the idea that grazing particularly affects plant-plant interactions when drought stress is already limiting plant growth to a larger extent (Gómez-Aparicio et al. 2008, Soliveres et al. 2012).

PLANT INTERACTIONS AT SPECIES PAIR LEVEL

The percentages of co-occurring species pairs over our different treatments are in line with the results from the community wide analysis: we found the highest percentage of co-occurring species pairs at south-facing slopes with low grazing pressure. The total percentages of co-occurring species showed a slight increase from north-facing slopes compared to more drought stressed south-facing slopes and thus partly support predictions from the original SGH (Bertness and Callaway 1994, Callaway 1995, Callaway and Walker 1997). However, with higher grazing pressure we did not observe a straightforward increase in frequency of positive interactions, which is contrasting predictions from the original SGH. The observed

percentages of positive associations are lower than reported by similar studies in semi-arid grazed ecosystems of 18% (Soliveres et al. 2012) and 10-25% (Saiz and Alados 2012). This is probably due to the lower number of species observed in our study, but may also have to do with the different sampling techniques between studies.

We found that two abundant species pairs (*Stipa tenacissima* - *Anthyllis cytisoides* and *Stipa tenacissima* - *Salsola genistoides*) did show a shift in the direction of interactions, highlighting the importance of focusing also on pairwise interactions next to interactions at the community level. *Anthyllis cytisoides* was negatively associated to *Stipa tenacissima* at ungrazed north-facing slopes. This may indicate competition from *Stipa tenacissima* on recruitment of this species, which is in line with earlier studies showing that *Stipa tenacissima* is a strong competitor for resources resulting in competitive exclusion of shrub species (Alados et al. 2003). Likewise, studies from semi-arid ecosystems in Spain showed increasing negative effects of *Stipa tenacissima* on planted shrub seedlings (*Pistacia lentiscus*) in semi-arid ecosystems with an increase in drought stress (Maestre and Cortina 2004). However, at high grazing pressure the association between *Stipa tenacissima* and *Anthyllis cytisoides* shifted to being significantly positive in our study. As *Anthyllis cytisoides* is preferred by goats (pers. obs.), probably due to high nutrient (N) content, preferential feeding by goats outside the patches may thus explain this outcome. In agreement with this, results from another experimental study showed more feeding on planted palatable shrubs by rabbits outside *Stipa tenacissima* patches (Soliveres et al. 2011a).

The species pair *Stipa tenacissima* and *Salsola genistoides* also showed a significant shift, but not with increased grazing pressure but rather with a change in aspect. At ungrazed north-facing slopes both species were co-occurring, while at ungrazed south-facing slopes both species were significantly excluding each other. At north-facing slopes *Stipa tenacissima* might increase recruitment of *Salsola genistoides*, while with increasing drought stress this interaction might wane again. As *Salsola genistoides* is an important species in retaining soil shear strength and thus preventing erosion in degraded areas (De Baets et al. 2007), a lack of recruitment of *Salsola genistoides* could result in further degradation by increased gully erosion.

Our pairwise interaction analysis showed the special importance of the tussock grass *Stipa tenacissima* as a facilitator of shrubs. Both at increased grazing pressure and at relatively low drought stress, this tussock grass seems to act as a facilitator for recruitment. The highly abundant *Stipa tenacissima* proved to shift towards co-occurring with the very abundant shrub *Anthyllis cytisoides* with increased grazing pressure.

CONCLUSION AND PERSPECTIVES

Although changes in aspect were the biggest driver of reduced vegetation cover and soil functioning, grazing had a large effect on plant interactions at the community level, causing a significant shift from negative to positive species associations at south-facing slopes from no grazing to low grazing pressure. At north-facing slopes we did not find a significant shift in the direction of species association along the grazing gradient. However, our study showed that results at the species-pair level contrasted these findings from the community level approach, depending on the species studied. This is because species pairs that may be negatively associated to almost all other species may still be an important facilitator to a single, but very abundant species. This may have important implications in certain stages of succession in which a facilitated species is a weak competitor or is non-resistant to grazing. Hence, our results show the importance of assessing species association strength at both the species pair level and the community level. For understanding the impacts of stress at plant-plant interactions, both levels (community wide changes and species dependent changes) should be assessed simultaneously. Follow-up experimental studies can then disentangle the underlying mechanisms behind the shifts from positive to negative associations for key species along gradients consisting of multiple stressors. Such work is important for building knowledge that may help to prevent further degradation of semi-arid ecosystems under current ongoing global environmental change (Verwijmeren et al. 2013).

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CHAPTER

4

Waning positive
plant-plant
interactions under
combined drought and
grazing stress.

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ABSTRACT

Positive interspecific plant-plant interactions in arid ecosystems are crucial for upholding ecosystem diversity and stability, and therefore it is important to understand how plant-plant interactions may change under increasing drought and grazing pressure. In this study, we performed a manipulative experiment in semi-arid southeastern Spain where we planted 1280 saplings of the palatable shrub species, *Anthyllis cytisoides* (protégé species), either under the canopy of the unpalatable shrub species *Artemisia herba-alba* (nurse species) or in the open microsites between shrub patches. Over 2 years, we followed height and survival of saplings in four grazing treatments: no grazing, low goat grazing pressure, high goat grazing pressure and rabbit grazing. An extreme drought event during the course of the experiment enabled us to study the combined effects of drought and grazing pressure on possible shifts in the direction of plant-plant interactions. Prior to the extreme drought event, we found neutral interactions between the nurse and protégé in absence of grazing, but we found facilitative interactions at plots grazed by rabbits, with higher sapling survival under nurse shrubs. However, after the extreme drought event, at ungrazed plots, we found a higher survival of saplings in the open microsites, indicating prevalent negative interactions. At rabbit grazed plots, interactions shifted from positive to neutral. Our findings support the idea that positive plant-plant interactions may wane under the combination of high drought stress and grazing pressure. Such a waning of facilitative interactions may undermine ecosystem resilience to environmental change, due to increased drought and overgrazing.

INTRODUCTION

Over the last two decades an increasing number of studies showed the importance of interspecific facilitation (i.e. net positive interactions between plants) in plant communities (for reviews see: Callaway 2007, Brooker et al. 2008, Pugnaire et al. 2011, He et al. 2013) and in recent years the importance of interspecific facilitation for structuring ecosystems (Bruno et al. 2003) and for upholding of biodiversity (Michalet et al. 2006, McIntire and Fajardo 2014) has been widely recognised. In arid ecosystems, positive plant-plant interactions importantly determine ecosystem stability (Kéfi et al. 2007, Verwijmeren et al. 2013, Xu et al. 2015) and therefore it is crucial to understand how plant-plant interactions may change under changing environmental conditions.

Woody shrubs in arid ecosystems often act as nurse (i.e. facilitator) species by relieving abiotic stress for less stress tolerant neighbouring plants, for example by protecting them against extreme temperatures or by increasing water or nutrient availability within their direct vicinity (Gómez-Aparicio 2008, Pugnaire et al. 2011). Next to that, nurse species can lower consumer pressure (i.e. grazing) by physically protecting other neighbouring species against herbivores or by concealing them, a process known as associational resistance (Hay 1986). A central question to answer is how a combination of different stressors (e.g. drought and grazing) may influence the direction and strength of plant-plant interactions in arid ecosystems (He and Bertness 2014, Soliveres et al. 2015).

The Stress Gradient Hypothesis (SGH) predicts that facilitative interactions between plants become more common with an increase in abiotic stress or consumer pressure (Bertness and Callaway 1994, Callaway and Walker 1997). Many studies found support for the SGH by showing that plant-plant interactions switch from competition to facilitation with increased stress (e.g. Callaway et al. 2002, He et al. 2013, Molina-Montenegro et al. 2013). However, others proposed that under resource driven stress (e.g. aridity) nonlinear relations should be expected; i.e. facilitation between plant species may wane at the extreme end of a stress gradient (Michalet 2007, Maestre et al. 2009). Indeed, in arid ecosystems, observational studies showed that plant-plant interactions may shift back from facilitation to competition at sites or during periods with very low rainfall (Tielbörger and Kadmon 2000, Maestre and Cortina 2004, Armas and Pugnaire 2005, Saccone et

al. 2009, Butterfield et al. 2016). Because of this discrepancy, there is still ongoing debate on how facilitation and competition may vary over aridity gradients (Maestre et al. 2009, Michalet et al. 2014). In addition, the predictions from the original SGH were falsified by studies from grazed ecosystems showing that facilitation intensity increased from low to high grazing pressure, but decreased again with very high grazing pressure (Smit et al. 2007, Graff and Aguiar 2011, Saiz and Alados 2012). This may occur when under high grazing pressure the nurse plants themselves get damaged by grazing or trampling (Smit et al. 2009, Michalet et al. 2014), or when herbivores start searching more intensively for resources under low resource availability, effectively removing the protective effects of the nurse plant (Soliveres et al. 2011). Adding consumer pressure to aridity stress may thus possibly act as an accelerator in the waning of positive interactions at the extreme end of an aridity gradient, and pinpointing at what stress level positive interactions may wane is important to predict how ecosystems will respond to future climate change or to the introduction of herbivores (Verwijmeren et al. 2013).

Studies investigating the combined effect of drought and grazing stress on plant-plant interactions in arid ecosystems are still surprisingly scarce (but see, Soliveres et al. 2011, Verwijmeren et al. 2014, Noumi et al. 2015). Moreover, previous results on the combined effects of drought and grazing on the outcome of plant-plant interactions are not conclusive. Soliveres et al. (2011) showed that rabbit herbivory altered plant-plant interactions throughout the year, with positive interactions between the bunch grass *Stipa tenacissima* and saplings of the shrub *Retama sphaerocarpa* during winter and autumn, shifting to neutral interactions during summer, as rabbits grazed more intensively during dry summer conditions when food becomes scarcer. Contradicting this finding, Noumi et al. (2015) showed that competitive effects (competition for light and water) of shrubs on *Acacia tortilis* tree seedlings were strongest in relatively wet and ungrazed conditions, and that net competition decreased in importance with increased grazing stress. This was because the seedlings were too shade intolerant and drought tolerant to profit from a shading neighbour, but the seedlings did profit from grazing protection with increased herbivory, even at high drought stress.

It is thus still unclear, how grazing pressure and drought stress interact in shaping plant-plant interactions. On the one hand, it could be expected that grazing results in increased positive plant interactions under high drought stress, as plants

will have lower ability to compensate for herbivory than in less stressed conditions (Gómez-Aparicio 2008) and will depend more on a protecting neighbour. Following this reasoning, one might expect an increase in facilitation due to the combination of stressors. On the other hand, herbivores may cancel out positive plant interactions in highly drought-stressed environments as they search for food more intensively at reduced productivity during periods of high drought stress. This may result in a vanishing of positive interactions between plants due to the combination of both stressors (Smit et al. 2009, Verwijmeren et al. 2013, Michalet et al. 2014). Following this reasoning, one might expect positive plant-plant interactions to occur only at intermediate drought stress in combination with grazing, but a waning of facilitation at high drought stress in combination with grazing.

In this study we experimentally tested how plant-plant interactions shift along a combined gradient of grazing pressure and drought stress. We set up a controlled planting experiment with four grazing treatments crossed with two watering treatments, and measured the interaction between a mature unpalatable nurse shrub and saplings of a palatable protégé shrub. An extreme drought event during our experiment, enabled us to evaluate how the extreme drought event affected the outcome of plant-plant interactions under the four different grazing treatments. We expected (i) grazing to increase facilitative interactions between the two species at relatively low drought stress, and (ii) the interaction to shift back to neutral or competition at the combination of high drought stress and grazing, as protégé plants will be unable to cope with both stressors simultaneously.

METHODS

STUDY SITE

We performed our study at a semi-arid field site in southeastern Spain (Murcia region 37°57'28.37"N - 1° 0'16.14"W). Average annual rainfall is 301 mm, with on average 38 rainy days with more than 1 mm of precipitation annually. Highest rainfall occurs during spring and autumn and there is an extensive dry period in summer; average rainfall in August and July is 10 and 5 mm, respectively. Mean monthly temperature ranges from 10.1 °C in January up till 26.7 °C in August (Agencia Estatal de Meteorología, AEMET). Altitudes in our field site range from 175 to 302 m above sea level. Soil texture is loamy sand. The area consists of a

mixture of relatively undisturbed slopes and abandoned terraces. On the terraces, the dominant perennial vegetation consists of the shrub species *Artemisia herba-alba*, *A. cytisoides* and *Salsola genistoides*, and the herbaceous species *Marrubium vulgare*. The area has a history of shepherded goat grazing (during winter and spring) while the European rabbit (*Oryctolagus cuniculus*) is the dominant native herbivore in this system.

EXPERIMENTAL DESIGN

We set up a planting experiment in a blocked factorial design, with three factors: grazing (four treatments: no grazing, low goat grazing pressure, high goat grazing pressure, rabbit grazing), watering (two treatments: watered, unwatered), and microsite (two treatments: open, under shrub). As replicated blocks, we selected 4 terraces with a vegetation type consisting mainly of *A. herba-alba* shrubs intermixed with few individuals of *S. genistoides* and *M. vulgare*, with a vegetation cover of 40-60 percent. On all 4 selected terraces, we build fences of ca. 20 by 80 meter in December 2012. We subdivided each fence in four fenced plots of ca. 20 x 20 m; each being randomly assigned to one of the four grazing treatments. At the goat grazing and the ungrazed plots, rabbits were excluded by installing iron chicken mesh of 1 meter height (mesh size of 3 to 3 cm) dug into the soil until 50 cm depth. Only on one occasion (December 2013) rabbits broke through into two plots, and we dismissed plants that were damaged by rabbits on those plots for further analysis.

On 17 - 19 December 2012, we planted 1280 saplings of *A. cytisoides* (protégé species) over the four terraces, distributed equally over the four grazing treatments and the two microsities (n=40 per grazing treatment x microsite combination). We chose *A. cytisoides*, a drought-deciduous shrub from the *Fabaceae* family, as a protégé, because it is highly palatable for both goat and rabbits (personal observation). *A. cytisoides* was found to constitute 41 % of livestock goat diet and is thus considered as highly preferred food source for goats (Barroso et al. 1995). Half of the protégé saplings was planted underneath mature individuals of the shrub *A. herba-alba* (nurse species), and the other half was planted in the open microsities in between shrubs. We choose mature individuals of *A. herba-alba*, a perennial woody shrub from the *Asteraceae* family, as a nurse species because *Artemisia spp.* is not preferred by goats and has been found to be spatially associ-

ated with *A. cytisoides* in previous studies (Haase et al. 1996, Verwijmeren et al. 2014). *Artemisia spp.* is reported to be a highly unpreferred food source for goats as it only constitutes 3% of their diet (Barroso et al. 1995). Selected nurse patch sizes ranged from 50 centimeter to 2 meter (measured as the maximum width of each patch). Before planting, round planting holes (diameter 20 cm, depth 20 cm) were dug with a mechanical drilling device. All saplings that were planted underneath a shrub were planted north of the nurse patch underneath its canopy, 25-50 cm away from the nurse stem, and underneath the canopy of the mature shrub. All saplings that were planted in the open were minimally at 0.5 meter distance from the canopy of the nurse patch (or any other perennial plant species). After the planting of the saplings we removed all annuals in the vicinity of the planting holes (20 centimeter or closer) in order to remove potential competition and standardize initial conditions. In order to reduce the risk of mortality due to transplant shock, we watered the plants during the first week after planting and again 33 days after planting, by pouring 1 liter of water at every planting hole. All saplings were labelled with a metal tag placed on the soil, with unique numbers. Saplings were 1 year old and were obtained from a local nursery where they were grown in turf filled containers of 10 cm deep. Sapling heights were equalized before planting by cutting them at a standard height of 12 centimeters and were planted including their complete root system and the turf.

GRAZING EVENTS

In line with the normal practice of local shepherded goat grazing, we implemented grazing events only during winter and spring, outside the drought period. As *A. cytisoides* loses its leaves during the drought period in summer, this ensured palatability of the saplings. We randomly selected 36 lactating, mature, female goats from the herd of a local shepherd and implemented 3 grazing events: 14 May 2013, 31 January 2014 and 1 May 2014. For the low goat grazing pressure treatment, 3 adult goats were placed inside the enclosures, whereas for the high goat grazing pressure treatment 6 adult goats were placed within the enclosures. Goats were left in the enclosure from 10:00 till 17:00. These grazing events mimicked relatively low ($0.41 \text{ animals ha}^{-1} \text{ yr}^{-1}$) and high ($0.82 \text{ animals ha}^{-1} \text{ yr}^{-1}$) grazing pressures. The rabbit plots were not grazed by goats during the course of the experiment. To check for rabbit presence in the four rabbit plots, we qualitatively

assessed rabbit density by counting active latrine (with fresh droppings/pellets) numbers on each plot. We defined a latrine as an accumulation of 20 or more pellets on a surface of 20 x 30 cm (Virgós et al. 2003). Latrine abundance has been shown to be correlated with rabbit densities in Mediterranean scrublands (Virgós et al. 2003, Calvete et al. 2006). We found rabbit latrines on all four plots, ranging from 0.015 latrines per square meter to 0.019 latrines per square meter.

DROUGHT EVENT AND WATERING TREATMENT

During the ca. 2 years that we ran the experiment (Jan 2013 till October 2014), a total of 375.1 mm of rainfall was recorded by a nearby rainfall gauge from the SIAM (Sistema de Información Agrario de Murcia), weather station MU52. In 2014, from January till September, an extreme drought event occurred in which only 47.3 mm of rainfall was recorded. This enabled us to study the effect of an extreme drought event along our four different grazing treatments.

Combined with natural variability in rainfall over time, we watered half of the plants at three moments in time during our experiment to create two levels of watering. Plants to be watered were randomly selected with equal numbers in patch and open. We watered them by pouring 1.5 liters of water in the planting hole. As the planting hole makes a slight depression, this ensured the water to infiltrate in the plant's vicinity, thereby mimicking a rainfall event of ca. 12 mm. We watered plants at evenings to decrease direct evaporation. We repeated the watering at three events (25-28 July 2013, 13-16 April 2014 and 1-4 May 2014). These dates were chosen to simulate a shortening of the dry season. To test the effect of the watering events we measured Relative Water Content (RWC) from ten harvested leaves of 128 *A. cytisoides* saplings (distributed evenly over the microsites) before watering the saplings, and at three days after the watering event took place in May 2014. RWC of harvested leaves prior to the watering ranged from 30 % to 45 %, without significant differences between the microsites. After watering we measured RWC values ranging from 60% to 75 %, again without significant differences between the microsites.

SAPLING MEASUREMENTS

We surveyed the saplings after 32, 77, 123, 146, 396, 408, 489, 498 and 655 days after starting the experiment. As the primary fitness measure at every survey we

recorded sapling survival. Saplings were considered dead if they had a brownish colour under their bark and did not resprout during later measurements in the course of the experiment. As a secondary fitness measure we measured sapling height before and after every grazing event to assess the direct impact of goats. To obtain a non-destructive measure for the ability of the saplings to resprout after the first rains (75.5 mm 22-24 September 2014) after the extreme drought event, we harvested all fresh leaves of the saplings that were still alive at the final survey (October 6, 2014). After sampling, leaves were oven dried at 60 °C for 48 hours and weighed to determine the dry weight leaf biomass.

SOIL MOISTURE MEASUREMENTS

To compare the two microsites (open, under shrub) in their effect on soil moisture after rainfall events, we measured soil water content using Time Domain Reflectometry (TDR). TDR probes of 20 cm long were installed in the planting holes, 5 centimetres apart from the stem of *A. cytisoides* saplings, at twelve points per plot (six under a shrub, and six at the open microsite). Following a single rainfall event of 10.2 mm on the 28th of February 2013, we measured soil moisture on the 1st, 7th and 27th of March 2013, which allowed investigating the differential drying between the two microsites. Additionally, we measured soil moisture on the 5th of May 2013 (after two rainfall events of 27 mm and 24 mm on the 27th and 28th of April 2013), and on the 1st of October 2014 (after two rainfall events of 61.5 and 7.7 on the 22nd and 29th of September 2014).

DATA ANALYSIS

All analyses were performed in R 3.2.3 (R Development Core Team 2015). We calculated relative height by $\ln(\text{Height}_{\text{end}}) - \ln(\text{Height}_{\text{start}})$ as recommended by Paine et al. (2012) for cases where only one life stage of relative growth is modelled. We performed General(ized) Linear Mixed Effect models depending on the data distribution of the response variable (Zuur, 2009). For survival data we ran a generalized linear mixed effect model with binominal distribution and logit link. For height data we ran a general linear mixed effect model and only included plants that survived until the last measurement round. For both the survival data and the height data, we used two measurements in time; May 2014 (pre drought) and October 2014 (post drought), and regarded these as repeated measures,

subsequently indicated by 'drought event' (pre drought and post drought). We choose these two moments in time to explore the effect of the extreme drought event over time on the overall mortality during the summer months. To analyze the effects of grazing, watering, microsite and the drought event on the survival or the relative height, we ran General(ized) Linear Models with the factor grazing within terrace, and plant-ID as random factors (lme 4 package, Bates et al. 2014). We ran models with the factors grazing, watering, microsite and drought event and their interactions as the main fixed effects. To check for contrasts between microsites per grazing treatment we ran separate models for each grazing treatment - drought event combination, again with terrace as a random blocking factor, and applied a Bonferonni correction on the p-values. For the height data we did not include rabbit plots as survival (and thus observation nr.) was very low on these plots. Data on leaf biomass were analyzed using a Generalized Linear model with a Poisson distribution and a log link. For soil moisture content data we ran a General Linear model with only microsite as a fixed effect, as we found no effects of grazing. As we performed three soil moisture measurements after the single rainfall event in March 2013, we regarded the three measurement moments in March as repeated measurements by adding plant-ID as an extra variable in the random error structure and by adding time as a fixed factor.

RESULTS

SURVIVAL

Overall, we found significant effects of grazing treatment and drought event on survival of saplings, and a significant interaction between grazing treatment and microsite, as well as a significant interaction between grazing treatment and drought event (*table 1*). The watering treatment did not have any significant effect on sapling survival either alone or in interactions. Before the drought period we found equal survival rates for the ungrazed, low goat grazing and high goat grazing plots, but lower survival rates for the rabbit grazed plots (*figure 1*). During the drought event sapling survival dropped in all four plots, but less on the low goat grazing plots compared to the other three grazing treatments. Post-hoc comparison for pairwise contrasts between microsites within the four grazing treatments showed that in May 2014, survival rates were not significantly different between the two microsites

for no grazing ($\chi^2_{1,191} = 0.116$, $p = 0.732$), low goat grazing ($\chi^2_{1,257} = 0.249$, $p = 0.617$), and high goat grazing plots ($\chi^2_{1,285} = 0.007$, $p = 0.932$). For rabbit grazed plots however, sapling survival was significantly lower in the open microsite compared to under shrubs ($\chi^2_{1,323} = 16.826$, $p < 0.001$). In October 2014, pairwise contrasts showed no significant differences between the two microsites for the low goat grazing plots ($\chi^2_{1,257} = 0.393$, $p = 0.530$), high goat grazing plots ($\chi^2_{1,285} = 0.162$, $p = 0.684$) and rabbit grazing plots ($\chi^2_{1,323} = 0.178$, $p = 0.184$). On the ungrazed plots, however, we found a significant lower sapling survival under shrubs ($\chi^2_{1,191} = 5.74$, $p = 0.016$).

Factor	χ^2 value	df	p value
Grazing	176.686	3	<0.001
Microsite	10.455	1	NS
Watering	29.025	1	NS
Drought event	1145.190	1	<0.001
Grazing:Microsite	115.738	3	0.009
Grazing:Watering	26.749	3	NS
Microsite:Watering	0.0195	1	NS
Grazing:Drought event	130.223	3	0.005
Microsite: Drought event	14.283	1	NS
Watering: Drought event	24.110	1	NS
Grazing:Microsite:Watering	25.236	3	NS
Grazing:Microsite: Drought event	23.731	3	NS
Grazing:Watering: Drought event	16.766	3	NS
Microsite:Watering: Drought event	0.1611	1	NS
Grazing:Microsite:Watering: Drought event	10.698	3	NS

Table 1. Summary statistics of Generalized Linear Mixed effect Model with binomial distributions for survival data. For cases $p > 0.1$ we presented p values as NS (non-significant)

RELATIVE HEIGHT

Overall, we found significant effects of grazing treatment, microsite and drought event on the relative sapling height (*table 2*). Again, watering did not significantly affect the relative sapling height either alone or in interactions. In May 2014 (*figure 2, upper panel*), for the ungrazed plots, relative height was not different between the two microsites ($F_{1,61} = 1.478$, $p = 0.456$). For low goat grazing, however, relative height was significantly higher under shrubs than in the open ($F_{1,140} = 8.2811$, $p = 0.008$). For

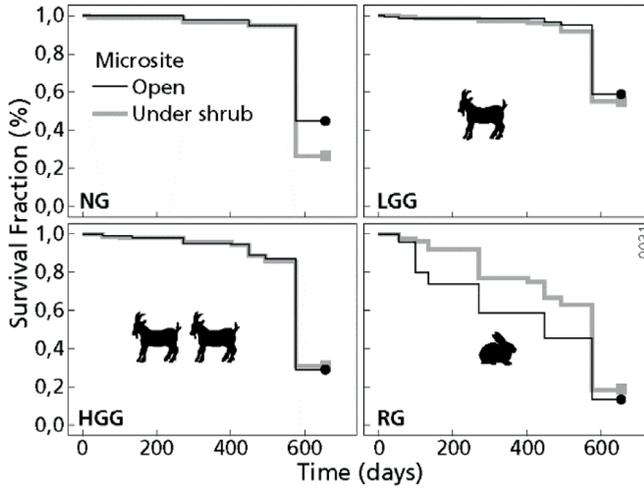


Figure 1. Survival fraction of planted saplings over time over the two microsites and the four grazing treatments. NG=No Grazing, LGG=Low Goat Grazing pressure, HGG=High Goat Grazing pressure, RG=Rabbit Grazing.

high goat grazing we similarly found significantly higher saplings under shrubs ($F_{1,81}=4.861$, $p=0.030$). At the final measurement in October 2014, after the severe drought (*figure 2, lower panel*) for ungrazed plots, saplings in both microsites had equal relative height ($F_{1,61}=2.842$, $p=0.192$). For low goat grazing we found significantly higher relative sapling height under shrubs ($F_{1,140}=6.086$, $p=0.031$), but for high goat grazing we found equal relative sapling height in the two microsites ($F_{1,81}=3.378$, $p=0.280$).

LEAF BIOMASS

Leaf biomass was not significantly different between the four grazing treatments (*table 3*). Microsite, however, did significantly affect leaf biomass, with higher leaf biomass for saplings growing in the open microsite (*table 3, figure 3*).

SOIL MOISTURE MEASUREMENTS

After a rainfall event of 10.2 mm in March 2013, we found significantly higher soil moisture in the open than in the shrub microsite (*table 4, figure 4 first three pairs*) and a significant interaction between microsite and time, indicating a decrease in the difference between the two microsites over time. After rainfall events of 27 mm and 24 mm in May 2013 soil moisture was no longer different between the

two microsites (*table 4, figure 4*). In October 2014, after rainfall events of 61.5 and 7.7 mm, we found the opposite: soil moisture was higher under the shrub than in the open (*table 4, figure 4*).

Factor	df _{num} :df _{den}	F value	p value
Grazing	2:6	90.277	0.017
Microsite	1:281	111.146	<0.001
Watering	1:281	0.3542	NS
Drought event	1:285	184.772	<0.001
Grazing:Microsite	2:281	0.2679	NS
Grazing:Watering	2:281	13.477	NS
Microsite:Watering	1:281	0.2085	NS
Grazing:Drought event	2:285	13.268	NS
Microsite:Drought event	1:285	0.1063	NS
Watering:Drought event	1:285	0.2743	NS
Grazing:Microsite:Watering	2:281	18.524	NS
Grazing:Microsite:Drought event	2:285	10.086	NS
Grazing:Watering:Drought event	2:285	0.6912	NS
Microsite:Watering:Drought event	1:285	0.8150	NS
Grazing:Microsite:Watering:Drought event	2:285	0.7802	NS

Table 2. Summary statistics of General Linear Mixed effect Model with normal distribution for relative height data for May 2014 and October 2014. For cases $p > 0.1$ we presented p values as NS (non-significant)

Factor	df	χ^2 value	p value
Grazing	3	1.586	NS
Microsite	1	13.263	<0.001
Watering	1	0.136	NS
Grazing X Microsite	3	2.876	NS
Watering X Microsite	1	0.411	NS
Grazing X Watering	3	0.946	NS

Table 3. Summary statistics of Generalized Linear Mixed effect Models with Poisson distribution for leaf biomass. For cases $p > 0.1$ we presented p values as NS (non-significant)

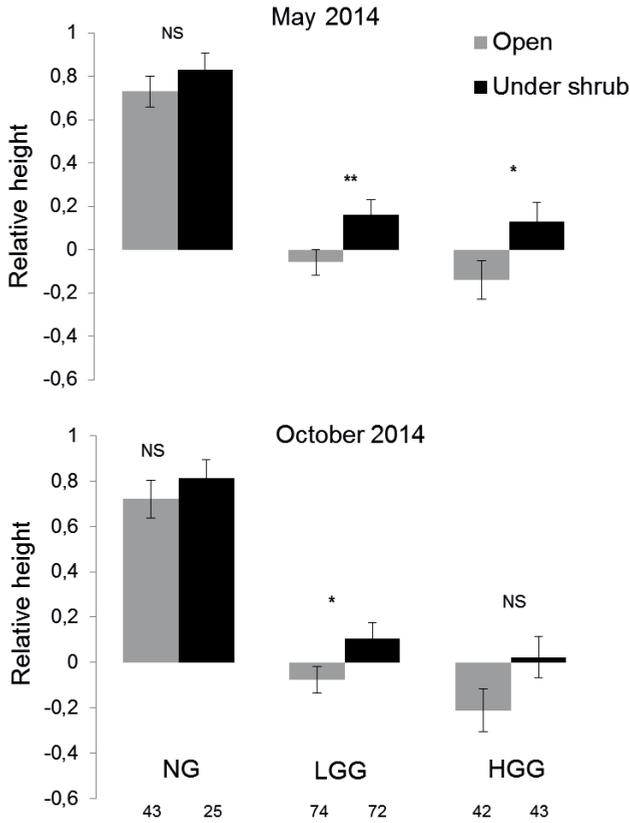


Figure 2. Relative height over the two microsites and the four grazing treatments before the severe drought in May 2014 (upper panel) and after the severe drought in October 2014 (lower panel). NG=No Grazing, LGG=Low Goat Grazing pressure, HGG=High Goat Grazing pressure. * and ** indicate a significant difference at $p < 0.05$ and $p < 0.01$ resp. between the two microsites. The numbers below the bars at the lower panel indicate the number of observations per treatment level.

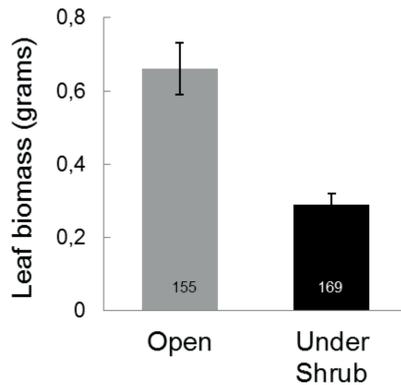


Figure 3. Dry weight biomass of leaves per sapling for the two microsites. The numbers within bars indicate the number of harvested saplings per treatment level.

	Factor	df _{nom.}	df _{denom.}	F value	p value
March '13	Microsite	1	227	30.618	< 0.001
	Time	1	247	3643.628	< 0.001
	Microsite X Time	1	248	29.760	< 0.001
May '13	Microsite	1	210	2.897	NS
Oct '14	Microsite	1	175	19.587	<0.001

Table 4. Summary statistics of General Linear Mixed effect Models with normal distribution for volumetric water content at three moments in time. For cases $p > 0.1$ we presented p values as NS (non-significant)

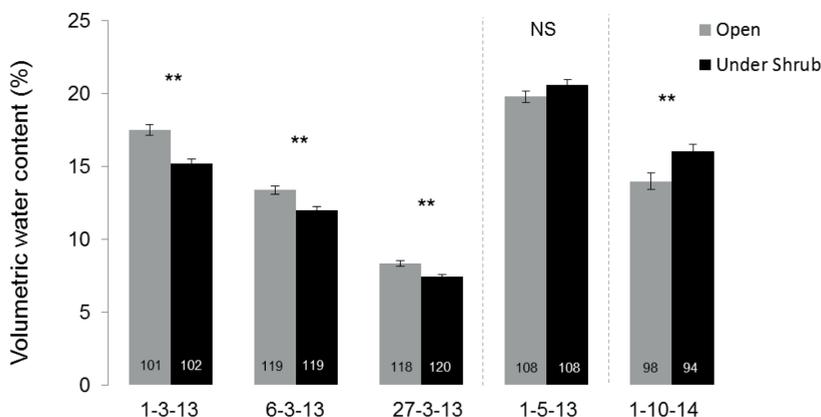


Figure 4. Volumetric water content at five moments in time. The first three measurements are treated as repeated measures after a rainfall event of 10.2 mm at February 28 2013. Soil moisture at 1-5-13 was measured after rainfall events of 27 mm and 24 mm at 27th and 28th April 2013 respectively. Soil moisture at 1-10-14 was measured after a rainfall event of 61.5 mm at 22 September and 7.7 mm at 29 September 2014. ** indicates a significant difference at $p < 0.01$. NS indicates non-significance. The numbers within bars indicate the number of observations per treatment level.

DISCUSSION

The aim of this study was to assess how interspecific plant-plant interactions respond to grazing pressure and drought stress, to test if facilitation wanes when drought and grazing stress operate simultaneously. While our watering treatment did not have any significant effects on sapling survival and height, we found contrasting responses to grazing pressure before and after the extreme drought event that occurred in the study area over the course of the experiment. Watering

effects were likely to operate only at a short term and were overridden by the effects of the extreme drought event and grazing treatments at the longer term. Before the drought event we found neutral interactions at ungrazed plots (survival and height), but this swapped to competitive interactions after the drought event (survival). Before the drought event, rabbit grazing resulted in higher survival of saplings under the nurse shrubs, and low goat grazing resulted in relatively higher growth under shrubs than in the open. This shows that associational resistance is still an important process at moderate drought stress. However, these positive interactions waned after the extreme drought event. This suggests that a combination of stressors may result in disappearance of positive interactions, confirming recent observations along a combined drought stress and mowing disturbance gradient (Le Bagousse-Pinguet et al. 2014). In addition, our findings reinforce the little empirical evidence to date that under drought stress associational resistance may be a more important mechanisms resulting in positive plant-plant interactions than microhabitat amelioration (Louthan et al. 2014, Perea and Gil 2014, Tálamo et al. 2015).

PLANT-PLANT INTERACTIONS UNDER UNGRAZED CONDITIONS

At the ungrazed plots, and before the drought event occurred, we found neutral interactions (no difference between open microsite and shrub microsites) between the nurse and protégé species for both survival and height. These neutral interactions between plants at moderate drought conditions (240 mm of rainfall in 2013) are consistent with previous studies that showed slightly positive or neutral plant-plant interactions in semi-arid ecosystems (Maestre and Cortina 2004). Neutral interactions can be explained by the contrasting results we found for soil moisture: higher soil moisture in the open microsite after a low rainfall event, equal soil moisture between the two microsites after moderate rainfall, and higher soil moisture under shrubs after a heavy rainfall. During dry conditions or at very low rainfall events, soil moisture might be slightly lower under a shrub canopy, due to rainwater interception by the nurse canopy (Tielbörger and Kadmon 2000) and due to water uptake by the nurse plant, resulting in lower water availability for saplings growing under the shrub (and thus relative better sapling performance in the open). However, after relatively high rainfall amounts, soil moisture can be higher under shrubs due to enhanced water infiltration (Mayor et al. 2009), result-

ing in higher sapling performance in this microsite. Considering the resprouting response to the first rains after the extreme drought event, our results showed that leaf biomass was higher in the open microsite. Hence, even if high rainfall results in higher soil moisture in the shrub microsite, this positive effect might be partly counteracted by other factors such as increased light availability in the open microsite. These short term trade-offs from single climatic extremes leading to shifts from competition to facilitation have also been observed by Grant et al. (2014), who found species specific shifts in grassland species in response to extreme weather events.

At the ungrazed plots, the extreme drought event resulted in a shift from neutral interactions towards negative interactions, with higher final survival of saplings in the open microsite. Although an increase in positive interactions is predicted by the SGH (Callaway and Walker 1997, Brooker and Callaghan 1998) and recent meta-analysis (He, Bertness & Altieri 2013), we thus found opposing patterns with an increase in competition at extreme drought stress. The meta-analysis by He et al. (2013) reported an overall shift towards more positive interactions with increasing drought stress in semi-arid ecosystems, but as they only used two drought stress levels (low and high) and did not capture very extreme drought events, this meta-analysis is not capable of detecting a shift back to more negative interactions from moderate to high drought stress. We propose that rainwater interception by the nurse canopy under extreme low rainfall is one likely explanation for the better performance of saplings in the open microsite; we recorded several rainfall events during the drought period of less than 2 mm which offer little opportunity for stem flow and through fall under nurse plants, but can be critical inputs in open microsities. Our volumetric soil moisture measurements showed that at these low rainfall events, higher soil moisture in the open microsite can be expected. Our observation is in line with previous studies from semi-arid ecosystems that showed that at the dry end of an aridity gradient, or during extended drought periods, negative interactions between plants may prevail (Tielbörger and Kadmon 2000, Maestre and Cortina 2004, Castanho et al. 2015, Butterfield et al. 2016). These and our results fit the notion that facilitation is only expected under intermediate stress along a resource driven stress gradients as only then the positive effects of neighbours exceed their negative effects (Maestre et al. 2009, Holmgren and Scheffer 2010).

PLANT-PLANT INTERACTIONS UNDER GOAT AND RABBIT GRAZING

Rabbit grazing led to higher sapling mortality than goat grazing. Overall, before the drought event, goat grazing did not significantly affect sapling survival nor resulted in differential survival rates between the two microsites, indicating neutral interactions between nurse and protégé. Rabbit grazing did however result in a significant decrease in sapling survival, with a higher survival rate of saplings under shrubs, indicating facilitative effects by the nurse shrubs. This difference between goat and rabbit treatments can be explained by the browsing behaviour of goats and rabbits. Goats only partly browsed the fresh shoots while rabbit grazing resulted in removal of all the aboveground biomass (*personal observation*), therefore having a bigger impact on survival. This result shows that rabbits can have a bigger inhibiting role on recruitment of shrubs than larger herbivores as goats, which is in agreement with studies from temperate ecosystems where rabbits rather than cattle were the limiting factor for clonal shrub expansion (Smit et al. 2010). As rabbits are not physically hindered to enter nurse patches, it is most likely that the higher survival under shrubs is caused by concealment of the sapling, as also observed by Louthan et al. (2014). We found an 18 percent higher survival of saplings under shrubs on rabbit plots. This is in line with a previous study in a semi-arid system that found a 22 % higher survival of saplings of *Retama sphaerocarpa* protected by the perennial tussock grass *Stipa tenacissima* two months after initialising the experiment (Soliveres et al. 2011). These and our results confirm that both shrubs and tussock grasses can be effective nurse species under moderate drought stress (before the drought event occurred) in combination with grazing by rabbits.

For both low and high goat grazing, saplings under shrubs got higher than saplings in open microsites. This confirmed the hypothesis that grazing can enhance positive interactions under drought conditions and that protection from grazing may be a more important facilitation mechanism than microhabitat amelioration. This is in line with Louthan et al. (2014) who showed that protégé forb concealment by nurse shrubs is a more important driver of facilitative interactions than microhabitat amelioration in African savannahs. In our study, the positive effect of shrubs on relative sapling height was highest at low goat grazing. This is consistent with other studies that showed that plant-plant interactions can shift from

neutral to positive when changing from ungrazed to grazed conditions (Brooker et al. 2006, Graff et al. 2007).

After the extreme drought event, we found a waning of the positive interaction for sapling survival at the rabbit grazed plots. A recent review by Michalet et al. (2014) proposed two possible mechanisms for a collapse in facilitation at the extreme end of environmental severity gradients; either a decrease in the functioning of the protecting nurse shrub under a disturbance (e.g., grazing) gradient, or an increase in competition for resources under extreme resource driven stress (e.g., drought). As in our case both grazing pressure and drought are interacting, it is hard to distinguish these potential explanations. We suggest that for the rabbit grazing plots, a combination of both competition for water as well as a decrease in protective effects could explain the waning of interactions, as the nurse species lost most of its leaves during the extreme drought period, resulting in decreased concealment of the saplings under their canopy.

SYNTHESIS

Overall, our results show that grazing can result in a shift from neutral to positive plant-plant interactions under moderate drought stress. Also we showed that an extreme drought event can result in competitive interactions between plants at ungrazed conditions while a combination of severe drought and herbivory can result in a waning of positive plant-plant interactions. These findings support recent theoretical frameworks predicting that a combination of stressors might result in the waning of positive interactions (Verwijmeren et al. 2013, Michalet et al. 2014), which may lead to more rapid degradation of arid ecosystems than would be expected when assuming increased positive interactions with increasing environmental severity. Our observations that facilitation wanes after an extreme drought event has thus important implications for the management of arid ecosystems: it shows that restoration measures using nurse species can only be successful under relatively benign environmental settings. Moreover, our results show the potentially large impacts of rabbit grazing on plant-plant interactions and plant recruitment. Most studies on plant-plant interactions in arid ecosystems so far focussed on the effects of larger herbivores, such as sheep and goat (e.g. Saiz and Alados 2011, Perea and Gil 2014), whereas our results underline the importance of rabbit grazing on plant recruitment and vegetation dynamics. As seedling and

sapling survival is the most critical stage in the life cycle of dryland plants, and because woody shrubs are considered important nurse plants for sapling survival (Bonanomi et al. 2011, Pugnaire et al. 2011), a decrease in positive interactions may lead to a lack of recruitment and hard-to-reverse degradation of arid ecosystems.

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CHAPTER

5

How rainfall and
grazing determine
the coexistence and
interaction outcome of
two plant species in a
semi-arid ecosystem

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ABSTRACT

Increases in drought frequency in combination with overgrazing may result in degradation of (semi-) arid ecosystems. Facilitative interactions between plants are a key mechanism in preventing degradation, but it is poorly understood how they respond to increased stress by combined drought and grazing. In this study, we used an ecohydrological model, to simulate the plant growth of two plant species interacting with each other under different rainfall and grazing pressure scenarios. The functional traits of the two modelled plants were based on a prior field experiment in southeastern Spain, in which an unpalatable 'nurse' species protected a palatable protégé' species from grazing. Moreover, the nurse species was more drought resistant, i.e. it had a lower wilting point, whereas the protégé species had a higher optimal growth rate. Firstly, we investigated the effect of rainfall intermittency on the coexistence of the two plant species growing under a single limiting resource. We found that increased rainfall intermittency (i.e. longer periods without rainfall) resulted in stable coexistence, whereas nearly constant rainfall led to competitive exclusion of the protégé by the nurse species. Secondly, we investigated how the nurse effect intensity and importance varied along our studied gradients. We found that competitive effects increased in intensity with drought stress. Moreover, higher grazing rates resulted in increased facilitative effects of the nurse on the protégé species, but facilitative effects could only prevail over competitive effects under currently observed or higher rainfall intermittency conditions. This study highlights the relevance of rainfall intermittency in explaining coexistence of species in dryland ecosystems and shows that increasing rainfall intermittency or grazing pressure can result in more facilitative effects from a nurse species. This information is crucial to obtain a better insight into the long-term coexistence of species, and the resulting stability of dryland ecosystems in response to future climate change.

INTRODUCTION

For the semi-arid Mediterranean area, climate models project an increase in high temperature extremes (high confidence), an increase in meteorological drought frequency (medium confidence), and an increase in heavy precipitation events (high confidence) (IPCC 2014). Increased drought stress in combination with overgrazing can result in rapid degradation of (semi-) arid ecosystems (Kéfi, et al. 2007), and facilitation between plants is suggested to be an important process keeping an ecosystems in a vegetated state (Kéfi, et al. 2016, Verwijmeren, et al. 2013, Xu, et al. 2015). Therefore there is an urgent need to understand how increased drought stress and grazing pressure differentially and simultaneously impact changes in species interactions and coexistence.

In semi-arid ecosystems, shrubs, trees and annual plant species compete for water, which is considered the main growth-limiting resource. Shrubs, however, often also provide positive (i.e. facilitative) effects by relieving drought stress for less drought-tolerant neighbouring plants, for example by shading or by increasing water availability (via increased infiltration or hydraulic lift) within their direct vicinity (Pugnaire, et al. 2011). Next to that, shrubs can lower grazing pressure by protecting neighbouring individuals against herbivore damage, a process known as associational resistance (Hay 1986). The total net effect of one plant (nurse plant) on the other plant (protégé plant) is a trade-off between competitive and facilitative effects, and a crucial question to be answered is how a combination of different stressors (e.g. drought and grazing) influences the direction and strength of plant-plant interactions in dryland ecosystems (Soliveres, et al. 2015).

Early conceptual models of plant-plant interactions hypothesized that the net outcome of plant-plant interactions shifts from competition towards facilitation with increasing drought stress or grazing pressure (Bertness and Callaway 1994, Callaway 1995, Callaway and Walker 1997). Recent meta-analyses indeed showed that at the global scale a shift towards more facilitative plant-plant interactions is observed as stress levels increase (Cavieres, et al. 2014, He, et al. 2013). However, recent studies question if positive species interactions can be expected under very severe drought stress (Butterfield, et al. 2016, Metz and Tielbörger 2016), as competitive effects may become more intense during severe dry periods. In addition, studies from grazed ecosystems show that under severe grazing stress,

plant-plant interaction wane from facilitation to neutral, as plants that provide benefits for neighbours lose their ability to do so under very high grazing pressure (Graff and Aguiar 2011, Smit, et al. 2007). So far, only very few studies empirically tested the impact of combined effects of drought and grazing (but see Maalouf, et al. 2012, Verwijmeren, et al. 2014), and changes in plant-plant interactions along combined stress gradients are still not well understood (Grant, et al. 2014, Soliveres, et al. 2015). So far, there are no two-species mechanistic ecohydrologic model studies that investigated the simultaneous impact of these two stressors on changes in plant-plant interactions. A mechanistic modelling approach can be very insightful as experimental or observation studies are often not able to assess plant-plant interactions along wide controlled gradients consisting of multiple stressors, or for extreme stress situations that are expected in the near future.

Previous ecohydrological models investigated the balance between positive and negative interactions between two plant species (e.g. Diaz-Sierra, et al. 2010, Gilad, et al. 2007), by modelling the competitive water uptake by plants in combination with an increased infiltration of water in the soil due to increased biomass. These studies assessed which factors tilt the balance between the facilitative and competitive effects along a drought gradient. Positive interactions between plants have also been studied in a mechanistic modelling study (Gross 2008), in which grazing mortality was made dependent on the biomass of a neighbouring species, thus representing grazing protection. This mechanism can result in coexistence of multiple species that are all limited by the same single resource. However, it has not yet been studied with mechanistic models how the joint effects of grazing and drought - highly realistic in (semi-) arid ecosystems - shape the net interactions between plants. Moreover, previous models used constant rainfall, ignoring the important aspect that rainfall in semi-arid ecosystems is highly intermittent, i.e. it occurs in pulses (Chesson, et al. 2004).

In the Mediterranean region rainfall shows a high temporal variability, i.e. it is characterized by pulse events. Several ecohydrological model studies addressed the role of stochastic and intermittent rainfall on the coupled dynamics of vegetation and soil moisture in dryland ecosystems (Baudena and Provenzale 2008, Baudena, et al. 2013, D'Onofrio, et al. 2015, Kletter, et al. 2009, Rodriguez-Iturbe 2000, Siteur, et al. 2014). Most of these studies, however, considered only one type of vegetation, and so far only a few studies have considered the role of rainfall intermittency on

species coexistence (D'Onofrio, et al. 2015). Conceptually, it has been hypothesised that the temporal stochasticity in rainfall pulses is an important driver that can explain the coexistence of plants competing for a single resource by the so-called storage effect, or by relative non-linearity in growth response to a limiting resource (Adler, et al. 2013, Chesson 2000, Chesson, et al. 2004). Under the storage effect, species may have equal responses to a single limiting resource, but each species is favoured at a different period of time. The storage effect can for example explain coexistence between perennial plant species by their different timing of germination, so that two perennial plants that have different phenology in germination can coexist on the long term if rainfall patterns vary inter annually, favouring one species in one year and the other species in the subsequent year (Chesson 2000, Chesson, et al. 2004). Relative non-linearity implies that plants have differences in growth rate responses to a single limiting fluctuating resource. This process allows one species to benefit from temporarily high resource availability, while another plant species is benefitting in times of temporarily lower resource availability, thus favouring long-term coexistence. It promotes coexistence if perennial species, when abundant, drive the resource fluctuation in the direction that favours their competitor. Recent model studies showed that relative non-linearity can be a strong factor contributing to coexistence, next to the storage effect (Yuan and Chesson 2015), but the role of relative non-linearity under realistic rainfall scenarios without the storage effect at play has not been previously investigated. It is thus still unknown how rainfall intermittency (temporal variability in rainfall) can affect the coexistence between species and the direction of plant-plant interactions.

In this study we used a mechanistic two-species ecohydrological model to investigate how plant coexistence and plant-plant interactions are dependent on different rainfall amounts, rainfall intermittency and grazing pressure scenarios. We made model assumptions and parameter settings based on the experimental outcomes of a plant-plant interactions setup at a field site in southeastern Spain (Murcia region 37°57'28.37"N - 1° 0'16.14"W) where a relatively drought resistant nurse plant protected a protégé plant - that had a higher optimal growth rate - from being grazed. We modelled realistic (stochastic) rainfall scenarios to explore the effect of rainfall intermittency on the coexistence of the two species that differed in their optimal growth rate and wilting points. Also we explored the shifts in

nurse effect intensity and importance along gradients of combined mean annual rainfall and grazing under different intermittency scenarios.

METHODS

MODEL DESCRIPTION

In order to study the interacting effects of annual rainfall amount, rainfall intermittency and grazing on plant coexistence and on the competitive or facilitative effects of a nurse species on a protégé species, we introduced a mechanistic two-species-model coupled to a hydrological model of a single soil layer. The model adopted here describes the coupled dynamics of vegetation and soil moisture, and it is a combination and extension of the models presented by Baudena et al. (2007), Laio et al. (2001) and Díaz-Sierra et al. (2010). We used a one layer bucket model, as in our field site we observed relatively shallow soil depths (20-30 cm) accessible for both plant types root systems (personal observation). Water input in the model consisted of stochastic rainfall events based on statistics of historical data for the yearly amount and timing of rainfall. Parameter settings for rainfall and plant growth were chosen from field data as described in more detail below. The system dynamics was modelled by using three coupled ordinary differential equations (ODEs), for the soil water (s) dynamics, and for the nurse (N) and the protégé (P) plant growth dynamics.

SOIL WATER DYNAMICS

The soil water (s) dynamics was a function of the infiltration, I , transpiration by the nurse species, T_n , transpiration by the protégé species, T_p , evaporation, E , and leakage, L (*equation 1, table 1*).

$$\frac{ds}{dt} = \frac{1}{nz} [I(N, P, r, t) - E(s) - T_n(N, s) - T_p(P, s) - L(s)] \quad (\text{Eq. 1})$$

Where r is the rainfall, n is the soil porosity and z is the soil depth.

Infiltration, I , was modelled as a function of the rainfall amount over time, and the protégé and nurse biomasses:

Parameter	Value	Units	Description
s	(0-1)	-	Volumetric water content
N	(0-inf)	g m ⁻²	Biomass Nurse
P	(0-inf)	g m ⁻²	Biomass Protégé
g _{maxn}	0.15	g m ⁻² mm ⁻¹	Conv. Water - plant density nurse
g _{maxp}	0.20	g m ⁻² mm ⁻¹	Conv. Water- plant density protégé
C _n	0.01	mm d ⁻¹ m ² g ⁻¹	Maximum water uptake nurse
C _p	0.01	mm d ⁻¹ m ² g ⁻¹	Maximum water uptake protégé
m _n	1/25/365	d ⁻¹	Baseline mortality nurse
m _p	1/30/365	d ⁻¹	Baseline mortality protégé
g	(0-0.6)/365	d ⁻¹	Grazing rate
a	1	-	Half saturation constant grazing protections
n	0.42	-	Porosity
z	200	mm	Soil depth
k _s	500	mm d ⁻¹	Saturated hydraulic conductivity.
i _n	0.01	m ² g ⁻¹	Infiltrative effect nurse
i _p	0.01	m ² g ⁻¹	Infiltrative effect nurse
s ₀	0.5	-	Infiltration constant bare soil
E ₀	1.61	mm d ⁻¹	Maximum evaporation at saturation
S _{wn}	0.06	-	Wilting point of nurse
S _{wp}	0.07	-	Wilting point of protégé
S* _n	0.15	-	Soil water content at which stomata fully open, nurse
S* _p	0.15	-	Soil water content at which stomata fully open, protégé
S _n	0.04	-	Hygroscopic point
IM	1-8-11	d	Rainfall inter-arrival time
RY	50-600	mm year ⁻¹	Rainfall per year

Table 1. Default parameter settings and units.

$$I = r(t) \left(\frac{i_p P + i_n N + s_0}{i_p P + i_n N + 1} \right) \quad (Eq. 2)$$

The parameters i_p and i_n determined the positive effect of the protégé and nurse species, respectively, on the water infiltration in the soil layer. They represent the inverse of the half saturation constant, implying that at our chosen values of $i_p = i_n = 0.01$, the biomass dependent increase in infiltration is half of the maximum increase in biomass dependent infiltration at a value of 100 g m^{-2} . For s_0 we used a value of 0.5, implying that half of the rainwater infiltrates regardless of the positive

effect of biomass on increased infiltration (Díaz-Sierra, et al. 2010). If the soil layer was saturated, and the infiltration from Eq. 2 would exceed the available water storage in the soil, the excess was converted into surface runoff (Baudena, et al. 2007).

Rainfall, $r(t)$, was a stochastic function over time, in which a rainfall value was generated per day given a certain amount of annual rainfall and the inter-arrival time between rainfall events. We modelled rainfall as stochastic Poisson events, with exponential distributions for inter-arrival time (i.e., time in between rainfall events), and for mean daily rainfall intensity (calculated from the mean annual rainfall) (Laio, et al. 2001). We also included a dry season without any rain, occurring once every year, to simulate the summer dry season that is characteristic for the climate of the field site. We calculated realistic values for mean annual rainfall, rainfall inter-arrival time and length of drought season, based on 72 years of rainfall records for the Alcantarilla weather station nearby our field site (Agencia Estatal de Meteorología, AEMET). The annual average rainfall was 300 mm per year. The average duration of the dry season was 61 days. The average time interval between rainfall events outside the dry season was 7.8 days (hereafter referred to as the current rainfall intermittency, $IM=8$ days). We varied rainfall values and intermittency values in subsequent model runs, with a higher intermittency resulting in higher rainfall inter-arrival time and thus also increased mean daily rainfall intensity (see figure A1 for an overview of the stochastic rainfall, soil water and plant growth dynamics).

Evaporation, E , was modelled as a function of soil moisture content:

$$E = \begin{cases} E_0 \frac{s-s_h}{1-s_h} & \text{if } s \geq s_h \\ 0 & \text{if } s < s_h \end{cases} \quad (\text{Eq. 3})$$

Evaporation from the soil layer was zero if the volumetric soil water content dropped below the hygroscopic point (s_h) of the soil (Baudena, et al. 2012). When soil water content was above the hygroscopic point it increased linearly as a function of the soil water content up to a maximum value at saturation, E_0 (Baudena and Provenzale 2008, Kim, et al. 1996).

Plant transpiration (T_n , T_p) was modelled as a function of the soil moisture content, and includes plant specific wilting point (s_{wi}), optimum uptake point (s_{*}),

and maximum water uptake rate C_i , with $i = p$ or n for the protégé or nurse plant respectively (as in Baudena, et al. 2012, Laio, et al. 2001, Rodriguez-Iturbe 2000).

$$T_n = \begin{cases} 0 & \text{if } s \leq s_{wn} \\ C_n N \frac{s - s_{wn}}{s_{*n} - s_{wn}} & \text{if } s_{wn} < s \leq s_{*n} \\ C_n N & \text{if } s > s_{*n} \end{cases} \quad (Eq. 4)$$

$$T_p = \begin{cases} 0 & \text{if } s \leq s_{wp} \\ C_p P \frac{s - s_{wp}}{s_{*p} - s_{wp}} & \text{if } s_{wp} < s \leq s_{*p} \\ C_p P & \text{if } s > s_{*p} \end{cases} \quad (Eq. 5)$$

When the water content was below the wilting point, transpiration was assumed to be zero, as plants fully close their stomata under below wilting point conditions. When soil moisture was above the wilting point, transpiration increased linearly as a function of the soil water content as plants open their stomata. When the soil water content was above s_{*i} , transpiration reached an optimum (plants are assumed to have fully open stomata) at a constant rate $C_i N$.

Leakage losses from the soil layer were modelled using a power law:

$$L = k_s s^4 \quad (Eq. 6)$$

Where k_s is the saturated hydraulic conductivity (Kim, Stricker & Torfs 1996; Baudena et al. 2012). The exponent is related to the pore size distribution index and we used a factor 4, as recommended for loamy soils (Kim, et al. 1996).

PLANT DYNAMICS

We modelled two plant types. Based on previous models (Díaz-Sierra, et al. 2010, Rietkerk and Van De Koppel 1997), plant growth was modelled as proportional to transpiration, a function of the soil water content, with a proportionality constant that would determine the maximum growth rate, $g_{max\ i}$. The 'nurse' species (N) suffered a baseline mortality rate, m_n , but did not suffer a grazing mortality:

$$\frac{dN}{dt} = g_{max\ n} T_n(N, s) - m_n N \quad (Eq. 7)$$

The second plant type, the 'protégé' species (P) also suffered a baseline mortality, m_p , on top of which a grazing mortality was implemented (third term on the

right hand side of equation 8). The biomass removal per year was proportional to its own biomass with grazing rate g . Grazing damage was reduced by a function depending on the ratio of nurse biomass over protégé biomass:

$$\frac{dP}{dt} = g_{\max} T_p(P, s) - m_p P - g P \left(1 - \frac{\left(\frac{N}{P}\right)^2}{a + \left(\frac{N}{P}\right)^2} \right) \quad (\text{Eq. 8})$$

The nurse species decreased the amount of grazing-induced mortality for the protégé species. We followed the approach by Gross (2008), but while that model uses the grazing protection as a function of the neighboring species alone, we choose to model grazing protection as a function of the ratio of nurse biomass over protégé biomass to account for size dependence (a small nurse plant cannot protect a larger protégé plant). Grazing protection was modelled using a Holling type III function, where the ratio of the nurse biomass over the protégé biomass determined the amount of reduction in grazing mortality. The parameter a determines how effectively the nurse can protect the protégé. More specifically, the square root of a is the half saturation constant for grazing protection along an axis of the ratio of nurse biomass over protégé biomass. For a , we choose a value of 1, so that the protective effect is 0.5 when both plants have a similar size (i.e. the ratio of N over P is one), as we assumed that half of the protégé plant in that case is protected by the nurse. When the ratio of N over P becomes higher than 4, the Holling type III function will approach 1, assuming that a nurse of >5 times the biomass of the protégé will provide almost full protection, and the grazing will not affect the protégé biomass.

SELECTION OF PARAMETERS FOR THE DIFFERENT PLANT TYPES

In our model study we distinguished two distinct plant functional types, a nurse (N) and a protégé (P) species. Both are woody perennial species that were selected in a parallel experiment in which plant growth of protected and unprotected planted saplings of *Anthyllis cytisoides* was monitored (Verwijmeren et al. in preparation). In line with this experiment, we used *Artemisia herba-alba* as nurse plant in this study and modelled its growth. *Artemisia spp.* is not preferred by goats and has been found to be spatially associated with *A. cytisoides* in previous studies (Haase, et al. 1996, Verwijmeren, et al. 2014).

Also in line with field observations and parallel experiments, we used *Anthyllis cytisoides* as protégé species in our model. *A. cytisoides* is a drought-deciduous shrub from the *Fabaceae* family, and it is highly palatable for both goat and rabbits. *A. cytisoides* has been found to constitute 41 % of livestock goat diet and is thus considered as highly preferred food source for goats (Barroso, et al. 1995). As an optimum growth rate, $C_p g_{\max p}$, for *A. cytisoides* we used 5 times the average yearly growth rate as measured in our experimental setup (*personal observation*) and for *A. herba-alba* we used a lower optimum growth rate (*personal observation*). For wilting points we used parameter settings based on values that we found in scientific literature: *Artemisia spp.* has been observed to reach wilting point at -1630 kpa (Kappen, et al. 1972) and *A. cytisoides* at - 1500 kPa (Archer, et al. 2012). We, thus used a difference of around 10 % between the wilting points for the two species in our model, namely a wilting point of 0.06 (as percentage of volumetric water content) for *A. herba-Alba* and a wilting point of 0.07 for *A. cytisoides*. Moreover, the effect of varying parameter settings for the two species wilting points and growth rates is explored in the appendix (*figure A2, A3, A4*). In the default parameter setting, the two species are characterised by a trade-off: although *A. herba-alba* has the benefit of being able to grow under lower soil moisture levels because of its lower wilting point, *A. cytisoides* has a higher growth rate under more benign moist conditions. A similar trade-off between drought tolerance and optimal growth rate has been reported in several studies in dryland ecosystems and has been proposed as a possible mechanisms promoting plant coexistence (Angert, et al. 2009, Chesson, et al. 2004). As *A. cytisoides* is drought deciduous, and the study site was not grazed during the dry season, the grazing rate on the protégé was set to zero in cases of a dry season that lasted for 30 days or more. Average lifespan was set to 30 years for the protégé species and 25 years for the nurse species, as *A. cytisoides* has been reported to have a longer lifespan than *A. herba-alba* (Haase, et al. 1997).

CALCULATING NURSE EFFECTS ON THE PROTÉGÉ

To measure the nurse effect on the protégé along gradients, we calculated the nurse effect intensity and the nurse effect importance, formerly called interaction intensity and importance (Armas, et al. 2004, Brooker and Kikvidze 2008). The

nurse effect intensity calculates the absolute effect of the nurse on the protégé biomass, whereas the nurse effect importance calculates the relative effect of the nurse on the protégé biomass, with respect to the effect that stress has on the protégé's biomass. As recent studies showed that the widely used indices RII (Armas, et al. 2004) and I_{imp} (Seifan, et al. 2010) are both not well defined to evenly weight facilitative and competitive effects (Díaz-Sierra et al. in review, Mingo 2014, Seifan and Seifan 2015), we calculated the nurse effect intensity and the nurse effect importance using two newly defined indices by Díaz-Sierra et al. (in review) from the Neighbour Effect Index family, as explained below.

MULTIPLICATIVE INTENSITY INDEX

For intensity we used the Neighbour Effect Intensity index with multiplicative symmetry, $NInt_M$ (equation 9). This index weights the difference between the protégé's biomass with and without a nurse, with respect to the sum of biomasses of the protégé with and without a nurse. Being an intensity index, it standardizes the effect that the nurse exerts on the protégé's biomass at any point of the stress gradient, with respect to the sum of the protégé with and without a nurse at that same point of the stress gradient. This index has equal boundaries ranging from -1, indicating maximal competitive effects, to +1, indicating maximal facilitative effects. The index shows multiplicative symmetry, meaning that a twofold increase or decrease in the protégé biomass due to the nurse presence will result in equal deviations from 0 in the index value. This index is recommended for cases where facilitative effects and competitive effects are not in the same order of magnitude (Díaz-Sierra et al. (in review)). We also calculated Neighbour Effect Intensity index with additive symmetry, $NInt_A$, and the widely used Relative Interaction Intensity index (RII, Armas, et al. 2004) as provided in the appendix. Both intensity indices resulted in very similar patterns across our investigated gradients (figure A5, A6, A7, A8).

$$NInt_M = 2 \frac{\Delta P}{P_{sum} + |\Delta P|} \quad (Eq. 9)$$

Where, $P_{sum} = P_{-N} + P_{+N}$ is the sum of the performances of the protégé species without, P_{-N} , and with a neighbouring nurse, P_{+N} , that is used to standardize for the protégé size, and where $\Delta P = P_{+N} - P_{-N}$ is the absolute impact of the nurse species on the protégé species.

MULTIPLICATIVE IMPORTANCE INDEX

For importance, we used the Neighbour Effect Importance index with multiplicative symmetry: $NImp_M$ (equation 10). This index weights the difference between the protégé's biomass with and without a nurse, both with respect to the size of the sum of the protégé with and without a nurse species, and with respect to the effect that stress has on the protégé's biomass over the entire stress gradient. $NImp_M$ thus reflects the ratio between the neighbour-driven change in performance, standardized for size, and weighted for the change in performance driven by "all the factors in the environment that influence plant success" following the definition of interaction importance by Brooker et al. (2008), but corrected to include standardization for size. This index has the same boundaries and symmetry as the above described interaction index $NInt_M$.

$$NImp_M = 2 \frac{\Delta P}{2 \cdot MP_{sum} - P_{sum} + |\Delta P|} \quad (Eq. 10)$$

Where P_{sum} and ΔP are the same as defined above, and MP_{sum} is defined as the maximum value of the sum of the performances of the protégé species with and without neighbours at any point along the combined gradient. We also calculated the Neighbour Effect Importance index with additive symmetry, $NImp_A$, as provided in the appendix. Both importance indices resulted in very similar patterns across our investigated gradients (figure A5, A6).

SCENARIOS

To assess changes in nurse effect intensity and nurse effect importance along our grazing, annual rainfall, intermittency scenarios, we ran different model runs of 1000 years varying the annual rainfall, grazing rate and intermittency respectively. We varied annual rainfall over a gradient ranging from 50 mm yr⁻¹ up to rainfall of 600 mm yr⁻¹ with steps of 50 mm yr⁻¹. Moreover we varied the grazing rate ranging from no grazing ($g=0$ yr⁻¹) to high grazing ($g=0.6$ yr⁻¹) with steps of 0.1 yr⁻¹. We varied the intermittency from 1 day, as a nearly constant control treatment, to 8 days, as based on current observed climatological values, to 11 days, as a high intermittency treatment, to test a possible increase in the intermittency as future climate change scenarios predict.

In order to simulate our experimental setup that consisted of planted protégé sapling either sole standing or growing under the canopy of a *A. herba-alba* shrub,

we ran all scenario's one time with the nurse species and the protégé species coexisting (both at initial values of 100 g m^{-2}), and one time with the nurse biomass set to zero (keeping the initial protégé biomass at 100 g m^{-2}). To avoid considering transient dynamics, we ran the simulations for 1000 years and averaged the biomass density values over the final 200 years, and used these for our comparisons. We also varied initial biomass of both the nurse and protégé species to start with either 1 or 1000 g m^{-2} to check for possible multistability in the nurse or protégé biomass.

RESULTS

Nurse species average biomass values ranged from 0 till about 2100 g/m^2 at 600 mm of yearly rainfall. Protégé species average biomass values ranged from 0 till about 3400 g/m^2 at 600 mm of yearly rainfall, because of its higher growth rate under optimal conditions. Biomass of nurse and protégé species and the competi-

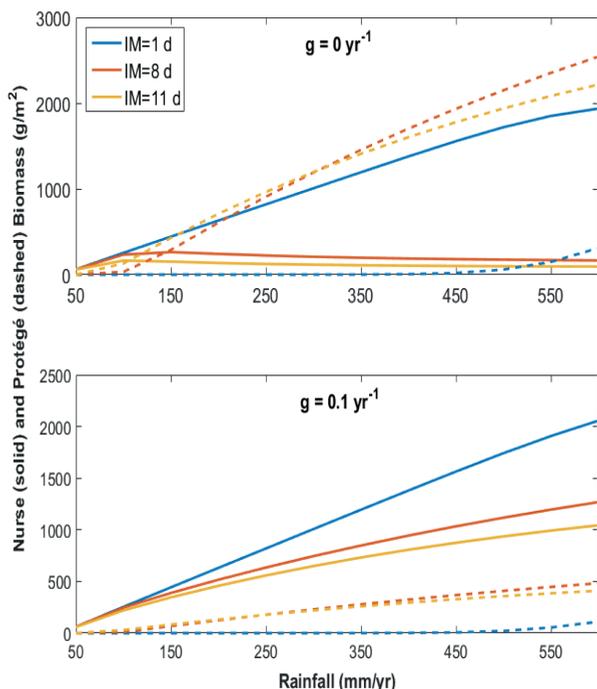


Figure 1. Averaged biomass after 1000 years for the nurse species (solid lines) and the protégé species (dotted lines) for three intermency scenarios (IM=1 day, IM=8 days, IM=11 days) and two grazing rate scenarios ($g=0$, $g=0.1 \text{ yr}^{-1}$). The Coexistence range along the rainfall gradient widened with an increase in intermency.

tive outcome were heavily dependent on the rainfall intermittency, annual rainfall amount and grazing rate variations (*figure 1, figure 2*). We did not find any effects of varying the initial conditions of nurse or protégé biomass on the final biomass values, implying the modeled system did not display any multistability.

EFFECT OF INTERMITTENCY ON COMPETITIVE OUTCOME

In the low rainfall intermittency scenario and without grazing (*figure 1, upper panel*), the nurse species outcompeted the protégé species. The protégé species could persist however at high rainfall levels ($>500 \text{ mm yr}^{-1}$), but remained at low values of biomass in comparison to the nurse species. With currently observed rainfall intermittency ($\text{IM}=8$ days), the competitive outcome along the rainfall gradient shifted. The protégé species was only outcompeted by the nurse at very low rainfall levels ($<100 \text{ mm yr}^{-1}$). For higher rainfall levels we found stable coexistence between the nurse and the protégé species, with the protégé becoming more dominant at the higher end of the rainfall gradient. With a further increase in rainfall intermittency ($\text{IM}=11$ days) the same pattern arose, but with slightly higher protégé biomass at low values of rainfall and a slightly lower protégé biomass at high rainfall levels,

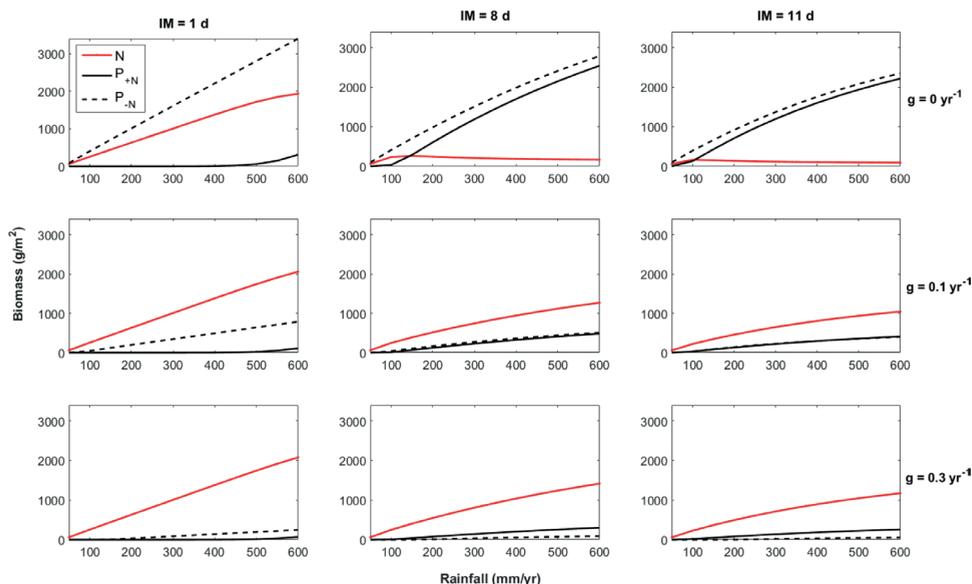


Figure 2. Averaged biomass after 1000 years for the Nurse species (N, red line), the Protégé species with a neighboring nurse species (P_{+N} , solid black line) and the Protégé species without a neighboring nurse species (P_{-N} , dashed black line). The columns represent different intermittency scenarios ($\text{IM}=1, 8$ or 11 days). The rows represent different grazing rate scenarios ($g=0, 0.1, 0.3 \text{ yr}^{-1}$).

compared to protégé biomass at the currently observed intermittency. With an increase in grazing to 0.1 yr^{-1} (*figure 1, lower panel*), the protégé did not become dominant at any point along the rainfall gradient, as the higher growth rate under optimal conditions of the protégé was counteracted by the biomass removal due to grazing. With low intermittency the protégé species was outcompeted, except for high rainfall values ($> 500 \text{ mm/year}$). With an increase in intermittency, the protégé could persist, but at relatively low biomass values.

We varied the wilting point and optimal growth rate of the protégé species to investigate the effect on coexistence under different intermittency scenarios (*figure A2, A3, A4*). This showed that coexistence could only occur when there is a trade-off between a lower wilting point (more drought resistance) and a lower optimal growth rate. We found one limiting case for this (*figure A3 third panel*): when the optimal growth rates for the nurse and protégé were equal but the wilting point of the protégé was higher, the protégé could still coexist with the nurse at high rainfall and intermittency conditions, because the mortality rate of the nurse is higher than the mortality rate of the protégé ($m_n > m_p$). Overall, an increase in intermittency widened the rainfall range at which coexistence could occur, shifting down the minimum value of rainfall needed to obtain co-occurrence.

NURSE EFFECT INTENSITY AND IMPORTANCE ALONG A RAINFALL GRADIENT

In the low intermittency scenario (*figure 2, left column*) and without grazing ($g=0$) the protégé species without a nurse performed better than the protégé species with a nurse along the entire rainfall gradient. This indicates that with low intermittency, competition for water was the dominant driver of the interaction outcome. With increased grazing rates the protégé biomass decreased. For currently observed rainfall intermittency (*figure 2, middle panel*) and without grazing ($g=0$), the interaction outcome changed: only at low rainfall ($<150 \text{ mm yr}^{-1}$) the nurse could remain dominant over the protégé species. However, the protégé without a neighbour remained more successful along the whole rainfall gradient for ungrazed conditions. With an increase in grazing to low or high grazing the protégé species with a neighbour could perform equally well or better than the protégé without a neighbour over the entire rainfall gradient. With high intermittency (*figure 2, right panel*)

we found similar results, but with a slightly better performance of the protected protégé as grazing increased.

In the low intermittency scenario (*figure 3, left panel*) and without grazing, both nurse effect intensity, $NInt_M$, and nurse effect importance, $NImp_M$, were negative along the whole rainfall gradient, indicating competition to be dominant. Moreover, we found an increase in competition with increasing rainfall. Overall, with currently observed intermittency (*figure 3, middle panel*) nurse effect intensity and nurse effect importance increased compared to the low intermittency case, indicating competition to become less prevalent. With no grazing and with low grazing ($g=0.1 \text{ yr}^{-1}$) the nurse effect intensity remained negative along the whole gradient, but competition intensity decreased with an increase in rainfall. With high grazing ($g=0.3 \text{ yr}^{-1}$) we found positive values over the whole gradient for nurse effect intensity and nurse effect importance, indicating facilitation to be prevalent. A further increase in intermittency (*figure 3, right panel*) resulted in similar patterns, but with lower negative intensity at low rainfall levels, especially under low grazing ($g=0.1 \text{ yr}^{-1}$).

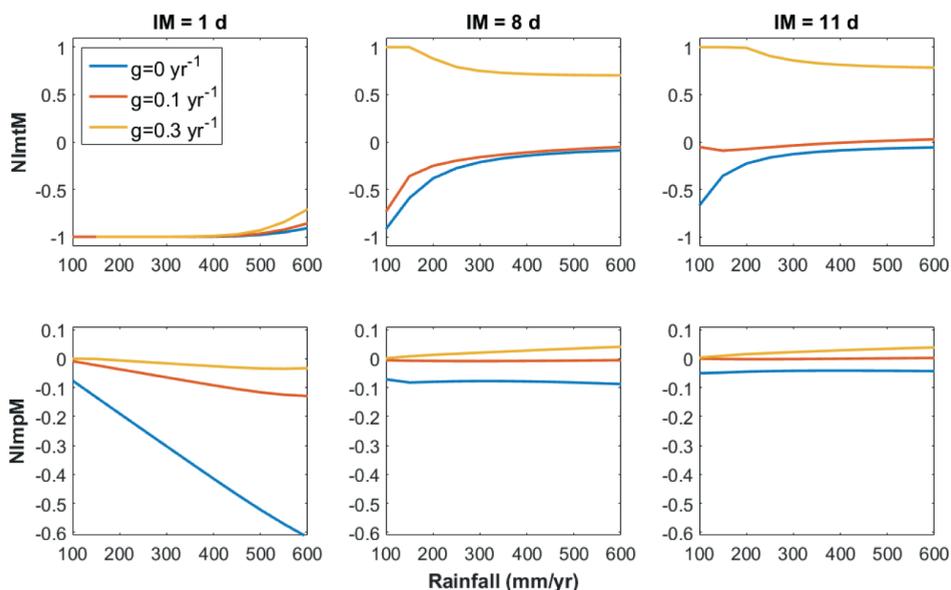


Figure 3: The effect intensity and the effect importance of the nurse on the protégé biomass along a rainfall gradient for three intermittency scenarios (IM=1, 8 or, 11 days) and three grazing scenarios ($g=0$, 0.1 , 0.3 yr^{-1}). A value of 1 represents maximum facilitative effects, a value of -1 represents maximum competitive effects. An increase in intermittency resulted in a decrease in competitive effects. A combination of high grazing and currently observed or high intermittency resulted in facilitative effects.

NURSE EFFECT INTENSITY AND IMPORTANCE ALONG A GRAZING GRADIENT

In the low intermittency scenario (*figure 4, left panel*) the nurse species was constant over the grazing gradient, as the protégé was fully outcompeted. The protégé without a nurse could persist when being grazed, but only for high rainfall values or low grazing rates. The protégé without a nurse could only obtain biomass values higher than the nurse under ungrazed conditions. For the currently observed intermittency scenario (*figure 4, middle panel*), the protégé with a nurse could persist at higher rainfall values, and became dominant over the nurse when there was no grazing. The biomass of the protégé without a nurse only exceeded the biomass of the protégé with a nurse if there was very low grazing ($g=0.1$) or no grazing at all. For the high intermittency scenario (*figure 4, right panel*) biomass values showed a similar pattern, but again with a slight increase in the performance of the protégé with a nurse.

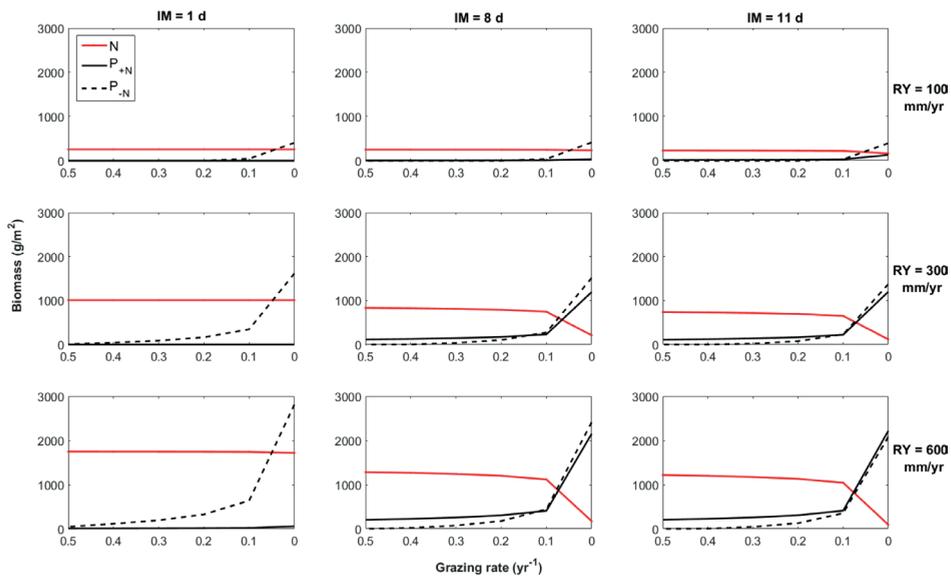


Figure 4. Averaged biomass after 1000 years for the Nurse species (N, red line), the Protégé species with a neighboring nurse species (P_{+N} , solid black line) and the Protégé species without a neighboring nurse species (P_{-N} , dashed black line). The columns represent different intermittency scenarios ($IM=1, 8$ or 11 days). The rows represent different rainfall per year scenarios ($RY=100, 300$ or 500 mm yr^{-1}).

Overall, increased grazing resulted in a shift from negative to positive values both for the nurse effect intensity and the nurse effect importance (*figure 5*), indicating

a shift from competition to facilitation. In the low intermittency scenario (*figure 5, left panel*) the intensity shifted from -1 to 1 with increased grazing, but this shift only occurred at very high grazing rates ($>0.5 \text{ year}^{-1}$). In the currently observed intermittency scenario (*figure 5, middle panel*), with low values of grazing ($g=0.1$) we observed negative values for the nurse effect intensity, whereas with an increase in grazing the intensity shifted to positive and gradually increased towards 1, indicating that the protégé could only survive when it was protected by a nurse species. In the high intermittency scenario (*figure 5, right panel*) we observed a similar trend but with slightly higher values, indicating increased facilitation. The trends in importance were roughly equal for the three intermittency scenarios. The nurse effect importance shifted from negative to positive with increased grazing, but stayed at very low positive values for high grazing scenarios.

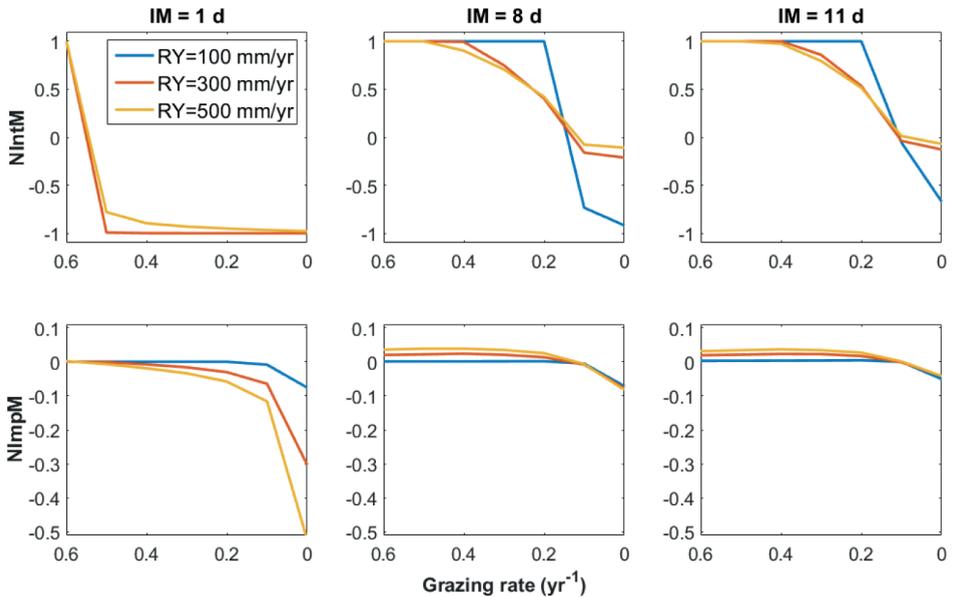


Figure 5. The effect intensity and the effect importance of the nurse on the protégé biomass along a grazing gradient for three intermittency scenarios (IM=1, 8 or, 11 days) and three rainfall scenarios (RY=100, 300 or 500 mm yr⁻¹). A value of 1 represents maximum facilitative effects, a value of -1 represents maximum competitive effects. An increase in grazing resulted in higher nurse effect intensity or importance.

DISCUSSION

RAINFALL INTERMITTENCY AND COEXISTENCE BETWEEN PLANTS

Our mechanistic two-species ecohydrological model results show that coexistence between plant species growing under a single limiting resource can arise under stochastic rainfall due to differences in growth rates as a function of the temporal variability in soil moisture. The two species could coexist if the wilting point was lower for a plant species that also had a lower optimal growth rate under high resource availability. Our study shows that stochastic, realistic rainfall can lead to coexistence under one single resource due to relative non-linearity in growth rates (and without including the storage effect on germination). Also, our results show that relative non-linearity in combination with a change in rainfall intermittency can shift the interaction outcome, i.e. which species is dominant. Due to its lower wilting point, the nurse species will take up water during the moments in time when soil water content exceeds its own wilting point. This way, with low intermittency or (nearly) constant rainfall, the nurse species prevents soil water content to exceed the wilting point of the protégé species, thereby preventing the protégé species from increasing in biomass. For this reason, under more constant rainfall scenarios the nurse is dominant as it has strong competitive effects on the protégé species. For higher intermittency or higher annual rainfall, stable coexistence between the nurse and the protégé species is possible. Under more intermittent rainfall, rainfall is accumulated in fewer events, resulting in increased mean daily rainfall intensity. Because of this, the soil water will reach more often above the wilting point of the protégé, enabling the protégé to coexist next to the nurse species. With high intermittency and rainfall combinations the higher optimal growth rate of the protégé species even causes it to become dominant. However, under these conditions, the nurse species is not excluded by competition, as it can still take up water during the periods in time that soil moisture is momentarily lower than the wilting point of the protégé species. An increase in rainfall intermittency results in a wider range of coexistence as rainfall events are more intense, pushing soil water levels above the wilting point of the protégé more often.

COMPETITION AND FACILITATION ALONG STRESS GRADIENTS

Our results highlight the relevance of taking intermittency in rainfall into account as a factor influencing the competition and facilitation intensity and importance, which was not yet considered in most recent conceptual models on species interactions along stress gradients (Maestre, et al. 2009, Soliveres, et al. 2015). Our results show that considering rainfall intermittency is essential to make mechanistically sound predictions on how plant-plant interactions may vary along drought gradients, as not only the total annual rainfall amount affects the competitive outcome, but also the temporal distribution in rainfall events.

As the nurse species was a better competitor under low resource availability due to its lower wilting point, the competitive effect was most pronounced under low intermittency in rainfall. The protégé could only effectively survive under higher intermittency scenarios because in those cases the soil water level would more often be higher than the wilting point of the protégé, allowing that species to grow. For the same reason we also found a decrease in competitive effects with an increase in rainfall, which is opposing classic literature on plant-plant interactions along stress gradients (Bertness and Callaway 1994, Callaway and Walker 1997) and a recent review on plant-plant interactions (He, et al. 2013). Our observation of increased competition with drought stress is in line with previous studies from semi-arid ecosystems that showed that at the dry end of an aridity gradient negative interactions might prevail (Tielbörger & Kadmon 2000; Maestre & Cortina 2004; Castanho, Oliveira & Prado 2015). We note that this result of our model study is due to the specific trait combination of the nurse species having a lower wilting point and the protégé having a higher optimal growth rate, but such trade-offs between drought tolerance and growth capacity may be common (Reich 2014), and can even occur within plant species that are considered of the same functional type (Angert, et al. 2009). This underlines the importance of taking into account explicitly the plant traits related to the mechanisms that can result into competitive or facilitative effects, when making predictions on plant-plant interactions along stress gradients.

The protégé is not benefited by nurse biomass, and competition for resources is the driving mechanism when there is no grazing, as both species in our model increased the infiltration rate equally. The protégé is only being facilitated effectively when it is being grazed, and with increased grazing it receives more facilitative

effects from the nurse. Under very severe herbivore pressure, plant-plant interactions have been observed to wane (Graff and Aguiar 2011, Smit, et al. 2009, Smit, et al. 2007). The loss of net facilitation under extreme levels of grazing pressure can occur either if the nurse species reaches a limit in the ability to reduce grazing damage, or because the positive effects from grazing damage do not compensate the costs of sharing resources between neighbouring plants (Michalet, et al. 2006, Verwijmeren, et al. 2013). Our results did not show a waning of interactions as grazing in our model only affected the protégé species and not the nurse species. Also, the protégé received almost full protection against grazing damage if the nurse biomass was more than 5 times the protégé biomass. Further exploration of the ability of nurse shrubs to give either full or partial protection against grazing related to their size and traits, is needed to make modelling studies on plant-plant interactions more realistic.

CONTRASTS IN INTERACTION INTENSITY AND IMPORTANCE

Previous studies showed that interaction intensity and importance do not need to be correlated along a stress gradient (Brooker, et al. 2005, Kunstler, et al. 2011). Our results even showed that nurse effect intensity and nurse effect importance can show contrasting trends along the same stress gradient. In the low intermittency case, the nurse effect intensity increased with rainfall, whereas the nurse effect importance decreased along the rainfall gradient. This difference in trend can be explained by the different standardization for intensity and importance indexes. As the intensity index is standardized for size, the standardized difference between P_{+N} and P_{-N} decreases for larger plants at the more productive, wet part of the gradient, i.e. the impact of standardizing for size increases with increased rainfall. As the importance index is standardized for size, but is also weighted for the effect of stress over the total gradient, this index increases with stress because the standardized difference between P_{+N} and P_{-N} increases with respect to the effect of stress when moving to the more productive, wet end of the gradient, i.e. the impact of standardizing for stress decreases with increased rainfall.

Along the grazing gradient, the trends in importance were roughly equal for the three intermittency scenarios and $NImp_M$ remained at very low positive values for high grazing scenarios. This low importance is due to the very low biomass at high rates of grazing, compared to the optimum biomass at no grazing. This shows

that when the most pronounced facilitative effects of the nurse on the protégé biomass occur at the most stressed point of the gradient, the importance will show very low values. For this reason, the $NImp_M$ shows an opposite trend along the grazing gradient than along the rainfall gradient, where the positive effects of the nurse are largest at the least stressed, high rainfall end of the gradient.

SYNTHESIS

Our model study shows the importance of rainfall intermittency in driving coexistence between species, and shows that intermittency can largely determine the plant-plant interaction outcome between two species. Moreover, our results show that if a nurse species has a lower wilting point than the coexisting protégé species, competitive effects can be expected to increase with drought stress, especially when rainfall intermittency is low. Facilitative effects from the nurse did increase with increased grazing stress or increased intermittency. This work shows the value of using mechanistic two-species ecohydrological models to evaluate the joint effects of different stressors on plant-plant interactions, which can be useful for making future predictions of plant-plant interactions and stability of semi-arid ecosystems.

ACKNOWLEDGEMENTS

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APPENDIX FIGURES

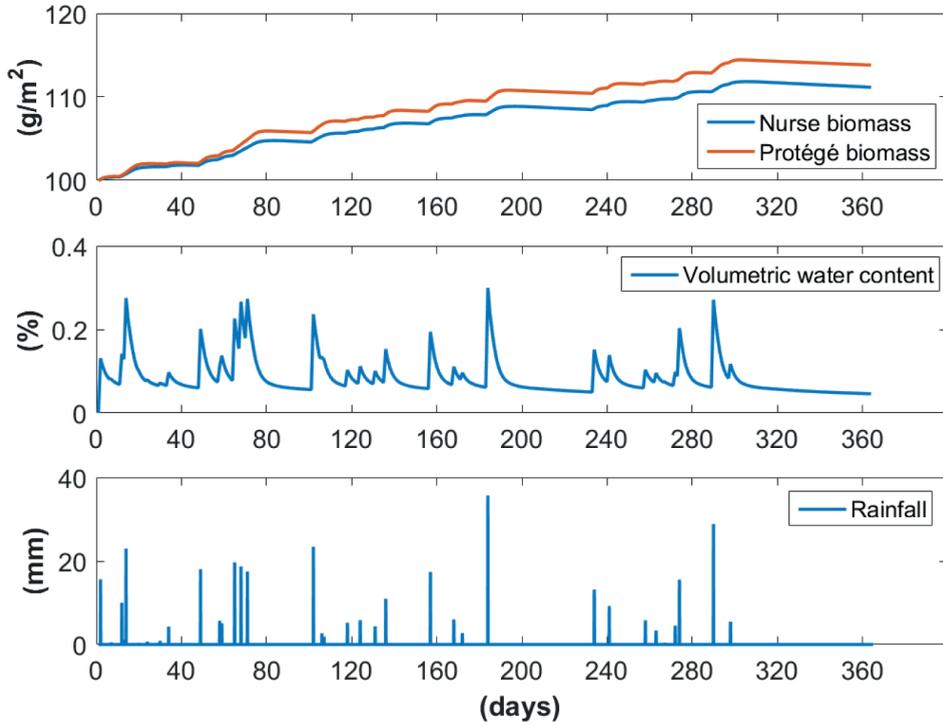


Figure A1. Example of the yearly variation in biomass of the nurse and protégé species (upper panel), soil moisture dynamics (middle panel) and stochastic rainfall (lower panel). For this example the annual rainfall was 300 mm/year, intermittency was 8 days, and grazing was 0. The rest of the parameters were default, as displayed in table 1.

VARIATIONS IN WILTING POINTS AND GROWTH RATES

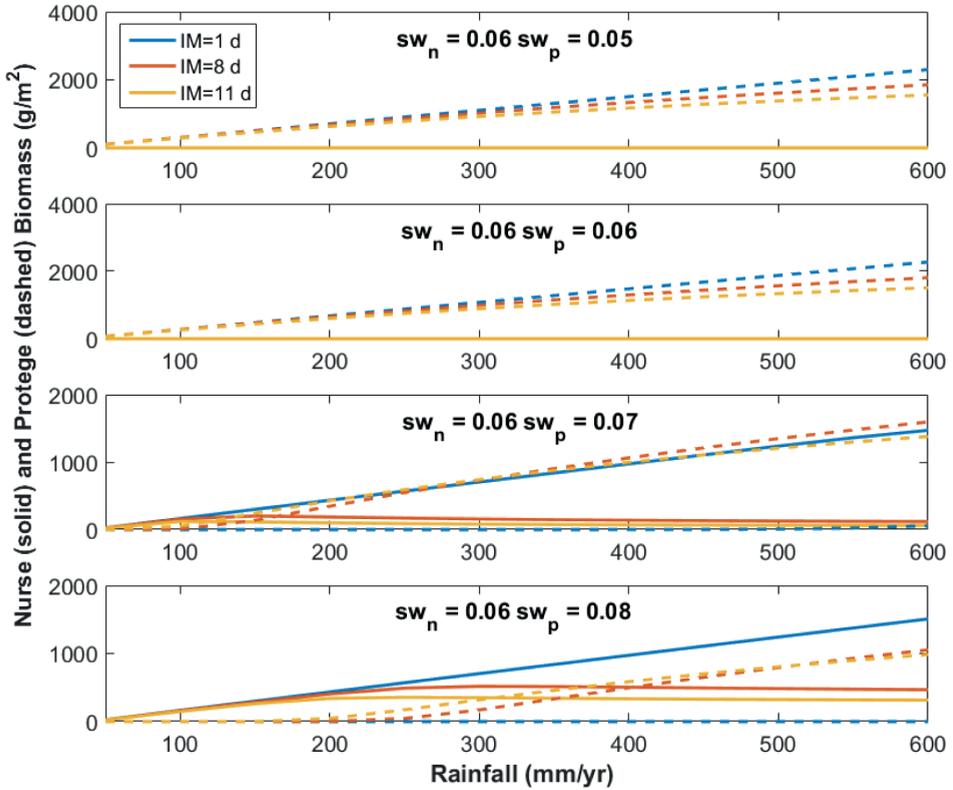


Figure A2. Equilibrium biomass after 1000 years for the Nurse species (N, solid line) and the Protégé species (dotted lines) with the nurse having a lower optimal growth rate. $g_{maxn}=0.15 \text{ g m}^{-2} \text{ mm}^{-1}$, $g_{maxp}=0.2 \text{ g m}^{-2} \text{ mm}^{-1}$. The rows represent different wilting points for the protégé species, increasing from top to down. The three colours represent three intermittency scenarios (IM=1, 8 or, 11 days). sw_n and sw_p represent the wilting points of the nurse and the protégé respectively. If the nurse had a higher or equal wilting point than the protégé there was never coexistence (upper two panels). Coexistence only occurred if the wilting point of the protégé was higher than the nurse's (lower two panels).

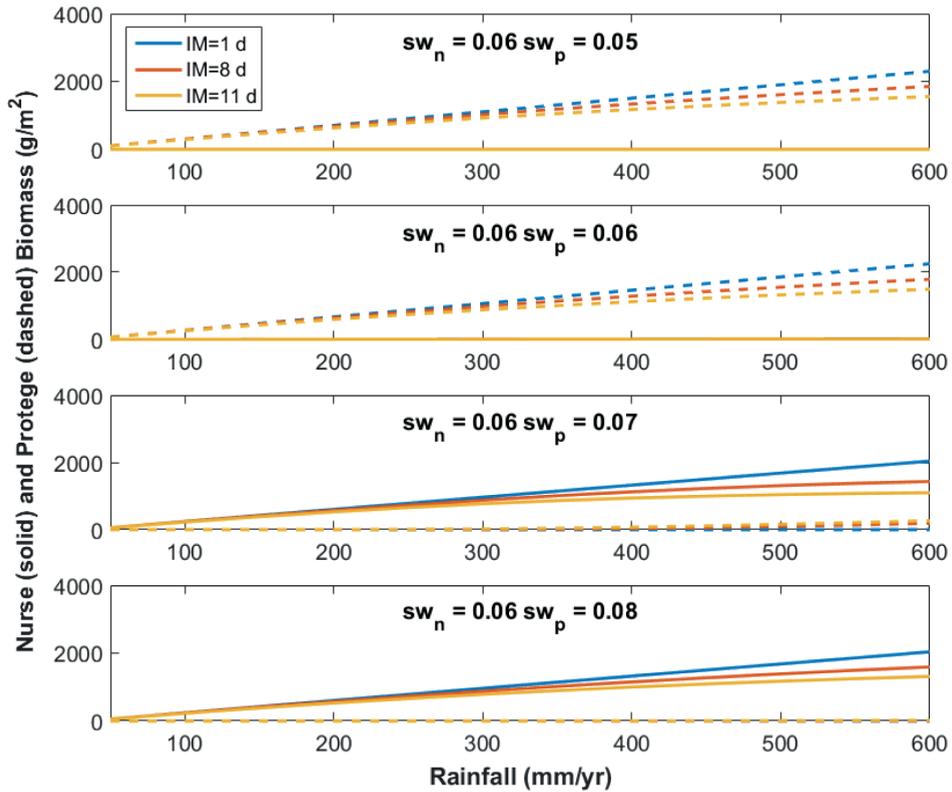


Figure A3. Equilibrium biomass after 1000 years for the Nurse species (N, solid line) and the Protégé species (dotted lines) with the two species having equal optimal growth rates, $g_{\max n}=0.2 \text{ g m}^{-2} \text{ mm}^{-1}$, $g_{\max p}=0.2 \text{ g m}^{-2} \text{ mm}^{-1}$. The rows represent different wilting points for the protégé species, increasing from top to down. The three colours represent three intermittency scenarios (IM=1, 8 or, 11 days). sw_n and sw_p represent the wilting points of the nurse and the protégé respectively. With equal optimal growth rates, and a higher wilting point for the nurse species or equal wilting points for the two species, we did not find coexistence as the protégé species outcompeted the nurse (upper two panels). With a higher wilting point for the protégé, the nurse became dominant, but the protégé could still persist at low biomass under high intermittency/rainfall conditions (third panel). If the difference in wilting points increased, the nurse outcompeted the protégé (lower panel).

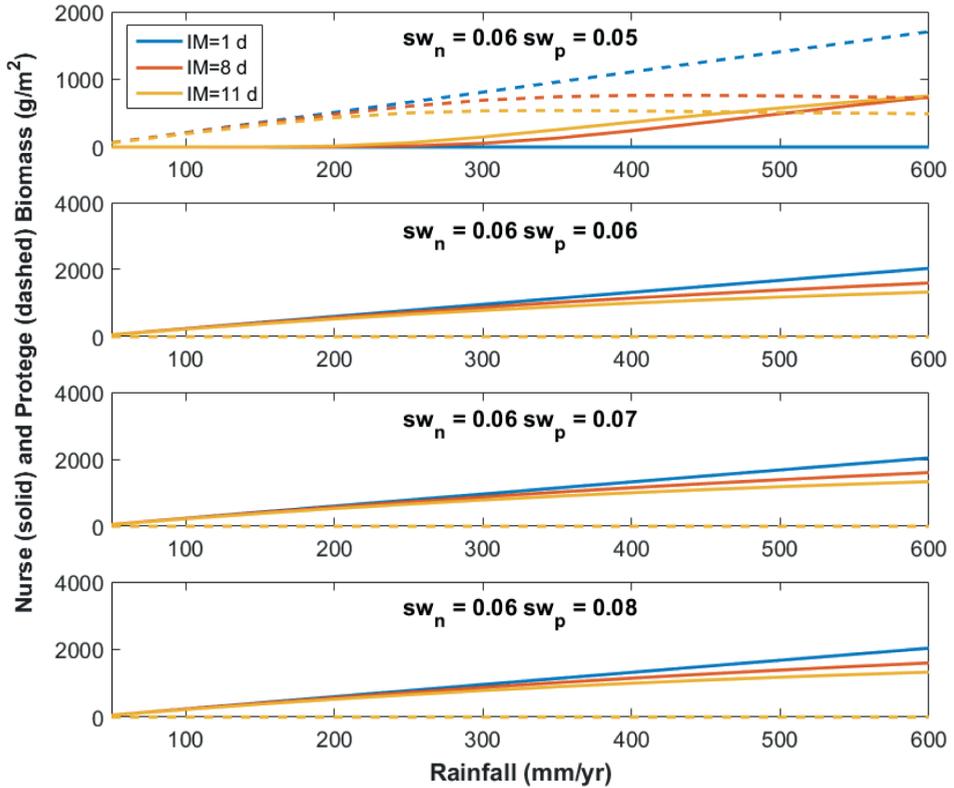


Figure A4. Equilibrium biomass after 1000 years for the Nurse species (N, solid line) and the Protégé species (dotted lines) with the nurse having a higher optimal growth rate, $g_{maxn}=0.2 \text{ g m}^{-2} \text{ mm}^{-1}$, $g_{maxp}=0.15 \text{ g m}^{-2} \text{ mm}^{-1}$. The rows represent different wilting points for the protégé species, increasing from top to down. The three colours represent three intermittency scenarios (IM=1, 8 or, 11 days). sw_n and sw_p represent the wilting points of the nurse and the protégé respectively. If the protégé had a lower wilting point than the nurse, the nurse and protégé species co-existed (upper panel). If the wilting points were equal or if the nurse had a lower wilting point, the protégé was outcompeted (lower three panels).

MEASURING NURSE EFFECTS ON THE PROTÉGÉ

We obtained very similar results using the Neighbour Effect Intensity index with additive symmetry, $NInt_A$, the Neighbour Effect Importance with additive symmetry, $NImp_A$, (figure A5, A6) or the Relative Interaction Intensity index, RII. (figure A7, A8).

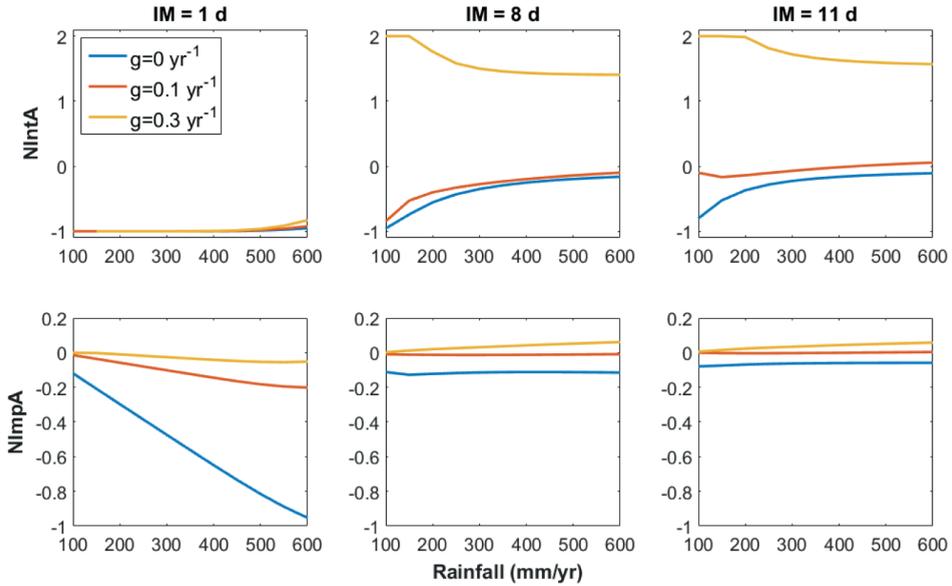


Figure A5. The effect intensity and the effect importance of the nurse on the protégé biomass along a rainfall gradient for three intermimency scenarios (IM=1, 8 or, 11 days) and three grazing scenarios ($g=0, 0.1, 0.3 \text{ yr}^{-1}$). A value of 2 represents maximum facilitative effects, a value of -1 represents maximum competitive effects. An increase in intermimency resulted in a decrease in competitive effects. A combination of high grazing and currently observed or high intermimency resulted in facilitative effects.

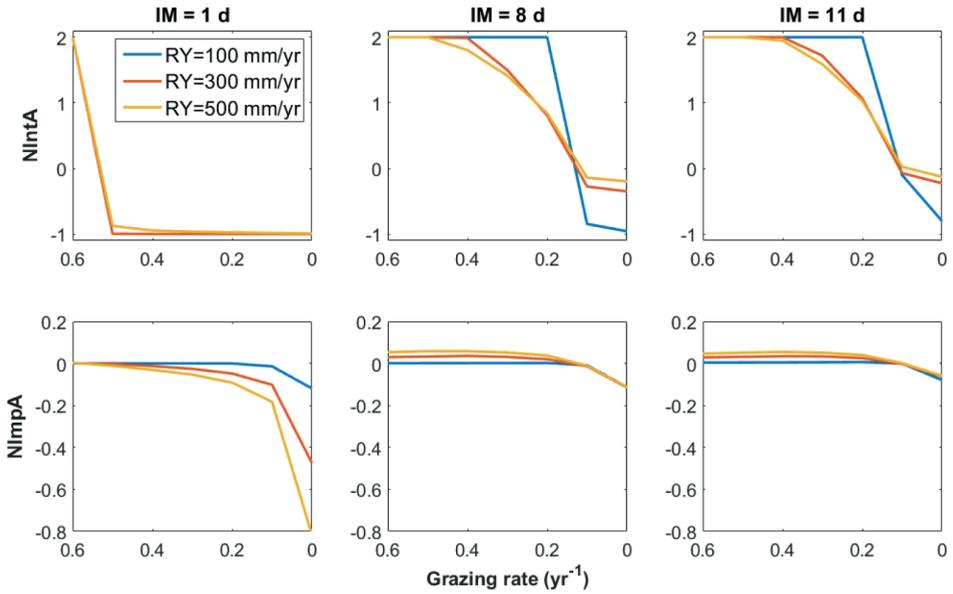


Figure A6. The effect intensity and the effect importance of the nurse on the protégé biomass along a grazing gradient for three intermency scenarios (IM=1, 8 or, 11 days) and three rainfall scenarios (RY=100, 300 or 500 mm yr⁻¹). A value of 2 represents maximum facilitative effects, a value of -1 represents maximum competitive effects. An increase in grazing resulted in higher intensity or importance of facilitative effects.

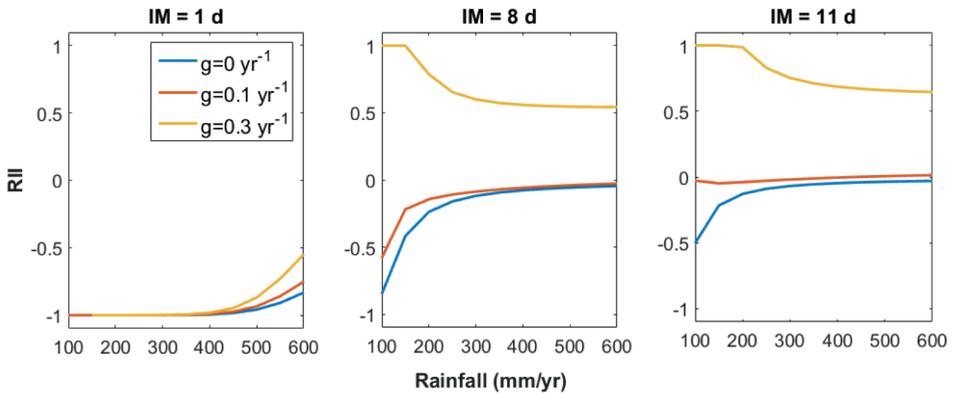


Figure A7. The Relative Interaction Intensity index (RII) along a rainfall gradient for three intermency scenarios (IM=1, 8 or, 11 days) and three grazing scenarios ($g=0, 0.1, 0.3 \text{ yr}^{-1}$). A value of 1 represents maximum facilitative effects, a value of -1 represents maximum competitive effects. An increase in intermency resulted in a decrease in competitive effects. A combination of high grazing and currently observed or high intermency resulted in facilitative effects.

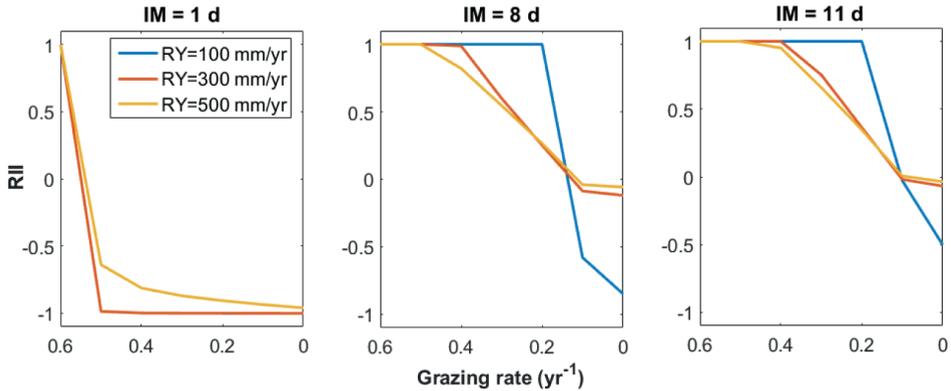


Figure A8. The Relative Interaction Intensity index (RII) along a grazing gradient for three intermimacy scenarios (IM=1, 8 or, 11 days) and three rainfall scenarios (RY=100, 300 or 500 mm yr⁻¹). A value of 1 represents maximum facilitative effects, a value of -1 represents maximum competitive effects. An increase in grazing resulted in higher interaction intensity.

ADDITIVE INTENSITY INDEX

The Neighbour Effect Intensity index with additive symmetry, $NInt_A$ (equation A1), standardizes the effect of the nurse on the protégé's biomass on any point of the stress gradient, with respect to the size of the protégé without a nurse, at that same point of the stress gradient. This index has unequal boundaries ranging from -1, indicating maximal competitive effects, to +2, indicating maximal facilitative effects. The index shows additive symmetry, meaning that an equivalent increase or decrease in biomass of the protégé biomass, due to the presence of a nurse, will result in equal deviations from 0 in the index value. This index is recommended for cases where facilitative effects and competitive effects are in the same order of magnitude Díaz-Sierra et al. (in review).

$$NInt_A = 2 \frac{\Delta P}{P_{-N} + |\Delta P|} \quad (Eq. A1)$$

Where, P_{-N} is the performances of the protégé species without a neighbour, that is used to normalise for the protégé size, and $\Delta P = P_{+N} - P_{-N}$ is the absolute impact of the nurse species on the protégé species.

ADDITIVE IMPORTANCE INDEX

The Neighbour Effect Importance index with additive symmetry, $NImp_A$ (equation A2), standardizes the effect of the nurse species on the protégé species, both with respect to the size of the protégé without a nurse species, and with respect to the effect that stress has on the biomass of the protégé without a nurse over the total stress gradient. $NImp_A$ thus reflects the ratio between the neighbour-driven change in performance, normalized for size, and normalized for the change in performance driven by “all the factors in the environment that influence plant success” following the definition of importance by Brooker et al. (2008), but corrected to include standardization for size. This index has the additive symmetry and ranges from -1 for maximal competitive effects up to +2 for maximal facilitative effects.

$$NImp_A = 2 \frac{\Delta P}{2 \cdot MP_{-N} - P_{-N} + |\Delta P|} \quad (Eq. A2)$$

Where $\Delta P = P_{+N} - P_{-N}$ is the absolute impact of the nurse species on the protégé species, and MP_{-N} is defined as the maximum performance of a protégé without a nurse at any point along the gradient.

RELATIVE INTERACTION INTENSITY INDEX

The relative interaction intensity index (RII , equation A3) is a widely used index to calculate the intensity of facilitative or competitive effects of a nurse species on a protégé species. It has similar properties as the $NIInt_m$ with multiplicative symmetry and equal boundaries ranging from -1, indicating maximal competitive effects, to +1, indicating maximal facilitative effects.

$$RII = \frac{P_{+N} - P_{-N}}{P_{+N} + P_{-N}} \quad (Eq. A3)$$

CHAPTER

Synthesis and
perspectives

6

Mart Verwijmeren

Drylands may be vulnerable ecosystems to climate change as global climate models project profound changes in precipitation patterns in several drylands across the globe, with increasing drought stress as a result (IPCC 2014). Positive plant-plant interactions are suggested to play an important role in maintaining ecosystems in a vegetated state, but the exact mechanisms behind positive interactions between plants and how interactions change along drought stress gradients remains disputed (He and Bertness 2014, Michalet and Pugnaire 2016). Moreover, it is uncertain how a combination of multiple stressors affects plant-plant interactions (Soliveres et al. 2015). In this thesis I shed light on (i) how interspecific plant-plant interactions change along combined stress gradients consisting of drought and grazing stress, and (ii) how interspecific plant-plant interactions relate to dryland ecosystem stability.

To answer these questions I used four distinct methods for unravelling the changing nature of plant-plant interactions. In chapter two I presented a conceptual model based on literature review. In chapter three I presented the results of an observational study on plant-plant interactions derived from plant spatial associations measured in the field. In chapter four I described the results from an experimental study to further test the joint effects of drought stress and grazing stress on plant-plant interactions. In chapter five I presented the results from a two-species ecohydrological model study to investigate the change in interaction intensity and importance along wider environmental gradients. This enabled me to make predictive statements about plant-plant interactions under dryer and more intermittent rainfall scenarios. In the following I will highlight the most important results, link the outcomes of the four chapters, and I will identify main knowledge gaps for future studies to focus on.

In **chapter two** I proposed that increased abiotic stress may result in a decline in facilitation intensity and in a failure of facilitator recruitment, prior to a critical transition. Also I hypothesized that a combination of drought stress and herbivory will speed up the waning of facilitation, leading to earlier and faster degradation of arid ecosystems. Furthermore I suggested that in-field monitoring of nurse species recruitment and declines in interaction intensity could possibly provide us with additional early warning signals for imminent transitions from a vegetated to a non-vegetated state. Also, I proposed that the changing nature of facilitation intensity should be incorporated into critical transition theory to improve our

understanding of the underlying mechanisms that will result in the onset of a critical transition from a vegetated to a non-vegetated state, which is important for in-time interventions to prevent critical transitions, or restoration of already degraded sites.

In **chapter three** I showed that vegetation cover and soil functioning decreased with higher grazing pressure and more south-facing (drier) slopes. Along this combined stress gradient, at the community level it appeared that plants at south-facing slopes were negatively associated at ungrazed conditions, but were positively associated at low grazing pressure and were randomly associated at high grazing pressure. At north-facing slopes, grazing did not result in a shift in the direction of the association. However, analysis of pairwise species co-occurrence patterns showed that the dominant species *Stipa tenacissima* and *Anthyllis cytisoides* shifted from excluding each other to co-occurring with each other with increasing grazing pressure at north-facing slopes. This possibly indicates that the highly palatable *Anthyllis cytisoides* is dependent on protection from the tussock grass *Stipa tenacissima* when grazing intensity by goats increases. These findings highlight that for improved understanding of plant interactions along stress gradients, interactions between species pairs and interactions at the community level may reveal contrasting results, and should thus both be assessed.

In **chapter four** I showed that grazing by rabbits or goats can result in a shift from neutral interactions to positive interactions, but that severe drought stress may result in a waning of positive interactions. Both grazing and an extreme drought event changed plant-plant interactions significantly during the course of our experiment. Prior to the extreme drought event, neutral interactions prevailed between the nurse and protégé in absence of grazing, as saplings performed equally under shrubs and in the open microsites. However, at plots that were grazed by rabbits I measured higher sapling survival if they were growing under shrubs, indicating positive interactions. Contrarily, I found a higher survival of saplings in the open microsites after the extreme drought event at ungrazed plots, indicating negative interactions to prevail. At rabbit grazed plots, interactions shifted from positive to neutral during the extreme drought event. These findings support the idea that grazing can shift plant interactions from neutral to positive, but also show that positive plant-plant interactions return to neutral at

the extreme end of the multiple stress gradient under the combination of high drought stress and grazing pressure.

In **chapter five** I showed that increased intermittency in rainfall (i.e. longer periods without rainfall) resulted in stable coexistence between two competing species, whereas nearly constant rainfall led to competitive exclusion of the protégé by the nurse species. Also I showed that competitive effects increased in intensity with drought stress. Moreover, higher grazing rates resulted in increased facilitative effects of the nurse on the protégé species, but only under currently observed or higher rainfall intermittency conditions, and with increasing intensity of facilitation with rainfall. This study highlights the importance of rainfall intermittency in explaining coexistence of species in semi-arid ecosystems and shows that an increase in rainfall intermittency or grazing pressure can result in more facilitative interactions between plants. This information is crucial to obtain a better insight into the long-term coexistence of species in semi-arid ecosystems in response to future climate change.

PLANT-PLANT INTERACTIONS ALONG COMBINED DROUGHT STRESS AND GRAZING GRADIENTS

The hypotheses I posed in chapter two on plant-plant interactions build upon the classic predictions from the Stress Gradient Hypothesis (SGH, Bertness and Callaway 1994, Callaway and Walker 1997), and on the refinements that were formulated afterwards. Whereas the original SGH predicts a general increase in frequency and strength of positive interactions if either abiotic stress or consumer pressure increases, the refinements that were proposed more recently predict that the relationship between stress and species interactions are dependent on species traits and on the stress types involved (Maestre et al. 2009), being either resource driven stress (e.g. temperature), non-resource driven stress (e.g. drought) or consumer pressure (Smit et al. 2009).

In chapter two I expanded on these theories and hypothesized a waning in positive plant-plant interactions under extreme drought stress, and an accelerated waning of positive interactions if herbivory is stressing plants next to drought stress. In chapter three I indeed showed shifts in spatial patterns of plants with most co-occurrence of plants under intermediate grazing stress, but neutral patterns at high drought and grazing stress. In chapter four I showed that grazing may

result in a shift from neutral to positive interactions, but that under severe drought stress interactions may shift to neutral again. These findings suggest that facilitative interactions indeed may shift back to neutral when stressors are combined at the high end of a stress gradient, which adds to the body of recent studies proposing a waning of facilitation under severe drought conditions (Holmgren and Scheffer 2010, Michalet et al. 2014, Butterfield et al. 2016, Metz and Tielbörger 2016).

Chapter four showed that our focal nurse species *Artemisia herba-alba* did not exert clear positive effects on the protégé species at ungrazed conditions. One possible explanation for the lack of positive effects in our semi-arid study system is the temporal switch from positive to negative effects of vegetation on soil moisture content. Although vegetation can improve soil moisture content by increasing the infiltration rate, during low intensity rainfall events, vegetation-induced positive effects on soil moisture cannot be expected, due to rainfall interception and competition for water (Grant et al. 2014, Butterfield et al. 2016). In line with this, several recent studies showed that positive effects of vegetation on soil moisture wane when aridity increased (Butterfield et al. 2016, Noumi et al. 2016).

The third and fourth chapter of this thesis underlined the importance of grazing in explaining observed positive interactions or spatial associations between plants in a semi-arid ecosystem. As suggested by recent studies in arid ecosystems, the origin of so called islands of productivity might be primarily grazing protection from nurse vegetation rather than drought amelioration from nurse vegetation (Allington and Valone 2014, Louthan et al. 2014). I emphasize that for observational studies the grazing history should be incorporated, and the function of small herbivores such as rabbits should not be overlooked, as chapter four of this thesis showed that rabbit grazing had higher impacts than goat grazing on mortality of saplings. Thus, although large herbivores are considered as the main driving force behind vegetation fragmentation in dryland ecosystem models (Kéfi et al. 2007a, Schneider and Kéfi 2016), I stress the importance of analysing the impact of smaller herbivores as well, as they might limit plant recruitment in greater extent than large herbivores.

A limitation of chapter three and four is that it still remains uncertain where the threshold lies between extreme or moderate stress, i.e. it is still uncertain at what drought stress levels positive interactions begin to wane. Defining the range of

drought stress under which positive interactions can be expected should be the focus of future studies. Such studies should focus on the distance that a plant species is from its optimal growth conditions to standardize the stress that plants experience (He and Bertness 2014, Soliveres et al. 2015), instead of using proxies for stress like slope aspect or the amount of rainfall. A related challenge for future studies on plant-plant interactions is to include stress gradients that comprise a larger range of a plant's niche, and especially to include more than two or three stress levels. As roughly 95% of studies on plant-plant interactions only assessed two or three stress levels (He et al. 2013), complex hypotheses as posed in chapter two remain hard to verify, and linear changes in interactions, such as found by He et al. (2013) are the only simplified patterns that can be detected by meta-analyses. We should move forward by assessing plant-plant interaction in at least four stress levels to gain better insight into how plant-plant interactions might wane (or not) along drought stress gradients. Also, future studies would benefit from a more exact determination of stress that plants actually experience. He and Bertness (2014) suggest a classification in which low, medium, high or extreme stress corresponds with a less than 20%, 30-50%, 50-80%, or a more than 80 % reduction in the optimal species performance. Such exact characterisation of stress treatments would allow for better comparison between studies performed along different types of stress gradients.

Another challenge for future studies is the issue of the methodological dependencies determining the outcome of studies on pairwise plant-plant interactions. For example, the way how plant performance is measured (survival, growth or reproduction) has been shown to lead to different outcomes in plant-plant interaction studies (chapter four, Goldberg et al. 1999, Gómez-Aparicio 2009, He et al. 2013). Also, the methodological approach (being either observational or experimental) has been shown to lead to contrasting outcomes, even if applied along the same stress gradient (Metz and Tielbörger 2016). In chapter three I added to these methodological dependencies that also the scale of investigation matters for the observed plant spatial aggregation or segregation. I propose to focus more on experimental approaches, as they are most suitable in separating plant-plant interactions from other factors, and will be more suitable in creating the fixed levels of stress as proposed by He and Bertness (2014) than observational studies.

Next to methodological dependencies, individual species traits have been shown to drive the direction of plant-plant interactions. For example, the growth form under investigation determines the direction of plant-plant interactions, with shrubs having more facilitative effect and grasses having more competitive effects (Gómez-Aparicio 2009, He et al. 2013). Another important trait determining the direction of positive interactions is the stress tolerance of species. Stress-tolerant species are more likely to be nurse plants, while stress-intolerant species are more likely to be protégé plants (Liancourt et al. 2005). Related to this, facilitation has been found to be more common for species that have a low tolerance to stress but have a high ability to take up resources (Liancourt et al. 2005, Gross et al. 2010). In chapter five I showed however that a protégé species with lower drought tolerance (i.e. higher wilting point) and higher optimal resource uptake than the nurse species, will receive increasing competitive effects from the nurse species with higher drought stress. This is because competitive effects from the nurse become more severe during lower intensity rainfall events, because volumetric soil water content cannot rise above the wilting point of the protégé. To test these model results, we need field studies that investigate how facilitation and resource competition varies along controlled drought stress gradients, for multiple species pairs with differing wilting point and optimal growth rate combinations.

The effect of rainfall intermittency on plant-plant interactions, as shown in chapter five, has not been previously considered as a driving factor altering the direction of interactions between plants. In chapter five I showed that a more constant input of resources makes it more likely for a drought tolerant species to keep resource level under a threshold, thereby preventing other less drought tolerant species to persist. This finding could be a possible new explanation why in temperate ecosystems, where rainfall is generally more constant, plant-plant interactions are more frequently competitive than facilitative (Callaway 2007). To test the proposed effect of rainfall intermittency on plant-coexistence and plant-plant interactions, long-term rainfall exclusion experiments should incorporate differences in the rainfall intermittency, besides rainfall amount.

A final factor that we should consider to improve our ability to make general predictions on plant-plant interactions is the metric that is currently being widely used by plant ecologists to quantify the intensity of facilitation in pairwise plant-plant interaction studies. A recent study showed that the commonly used metric

to quantify interaction intensity, RII, is underestimating facilitation compared to equal cases of competition under certain circumstances (Díaz-Sierra et al., under review). This is due to the fact that RII has a multiplicative symmetry, meaning that a twofold increase in biomass due to facilitation is weighted evenly with respect to a twofold decrease in biomass due to competition. This can lead to an underestimation of facilitation when negative and positive values for RII are compared or averaged. I advocate former studies using the RII metric to be re-evaluated using the newly defined family of indices by Díaz-Sierra et al. (under review) to quantify interaction intensity (or interaction importance) along stress gradients or between several stress treatments.

The results from this thesis and the above mentioned recent insights underline that we should avoid making premature general predictions on how plant-plant interactions will change with increasing drought and grazing stress, as plant-plant interactions are species specific and dependent on the above mentioned factors. I agree with the recent statement by Metz and Tielbörger (2016) that we need a new conceptual basis for the SGH, especially to predict plant-plant interactions in drylands. Positive interactions, and an increase in facilitation with increasing aridity should not be expected a priori. What is especially needed now is (i) identification of the particular species trait combinations that lead to positive interactions between plant species pairs in drylands, and (ii) defining the environmental settings under which positive interactions remain functional under drought stress. More specifically, we need to assess how plant species traits interact with rainfall amount in driving vegetation induced increases or decreases in volumetric soil moisture content. In order to do so, we need more efforts to measure soil moisture in a continuous manner at vegetated and non-vegetated sites, using species with varying optimal water uptake rates and drought tolerance traits. To make general predictions on plant-plant interactions along drought stress gradients, we first need to deepen our knowledge on the mechanisms behind facilitation between plants. This will also improve our ability to predict the implications that facilitation may have on species coexistence and ecosystem stability in drylands.

FACILITATION AND CRITICAL TRANSITIONS

Although model studies showed that local (intraspecific) facilitation can lead to bistability in dryland ecosystems (Kéfi et al. 2007a, Kéfi et al. 2007b, Xu et al.

2015b), it is still uncertain at what strength of positive interactions bistability is expected to occur. On the one hand, cellular automata modelling studies (Kéfi et al. 2007a, 2007b) showed that with very strong local (intraspecific) facilitation between plants, the system should be able to recover even at very low densities of vegetation under very severe stress. In this model facilitation consisted of plants improving the soil conditions in their direct vicinity, thereby increasing colonisation probability of vegetation. This model outcome would suggest to find bistability in a system only for intermediate intraspecific facilitation intensity, and gradual transitions with very strong intraspecific facilitation intensity. A decline in facilitation along a stress gradient may then thus also result in an earlier onset of a critical transitions along the same stress gradient. However, an individual based model study, in which woody plants increased the survival of their own offspring in their vicinity, found a contrasting result, namely that critical transitions can only be expected under strong (intraspecific) plant-plant interactions (Xu et al. 2015b). Although Xu et al. (2015b) did not explore the changing nature of facilitation intensity along the stress gradient, they suggested that stronger facilitation may cause the ecosystem in more stressful environments to have a larger range of conditions where bistability occurs. If critical transitions only occur under strong facilitation as suggested by (Xu et al. 2015b), our results from chapter four – waning positive interactions with severe stress - would suggest the opposite. Namely that critical transitions should not be expected as facilitative interactions might wane when a system moves to the high end of a stress gradient.

To solve these apparent contradictory conclusions, and to further test the predictions we made in chapter two on interspecific facilitation and the onset of critical transitions, we need two-species model studies incorporating interspecific facilitation. Such model studies should investigate how changes in facilitation intensity along a stress gradient may influence the stability of a system moving along that same stress gradient. We should move forward by modelling mechanistically how plants affect the resources they depend on, and by investigating how this in turn affects the growth and survival of neighbouring species (e.g. Diaz-Sierra et al. 2010), without a priori assuming facilitation to occur between plants, or assuming increased facilitation with increasing stress. Next to this, insights from two-species ecohydrological model studies as presented in chapter five, may enable us to analyse further (i) how different variations in species traits

will result in different predictions on how plant-plant interactions change along drought gradients, and (ii) under what range of drought stress, bistability can be expected to occur given the before mentioned change in interactions along the stress gradient. Such modelling studies may provide new hypotheses on which plant functional types are most likely to induce bistability in arid ecosystems.

The model study as presented in chapter five did not allow for testing of the hypothesis that were posed in chapter two, as I did not observe bistability in the model, given the used parameter setting. The stochastic nature of the model might explain the absence of bistability, as recent model studies showed that stochasticity increases the likelihood for continuous transitions (Realpe-Gomez et al. 2013, Villa Martín et al. 2015). Developing a spatial explicit version of our model would allow for further, more realistic exploration of how varying species traits and interspecific facilitation relate to bistability in the system. A spatial explicit component might increase the likelihood for critical transition as resource-concentration (i.e., increased infiltration near the plants and depletion of resources further away) will occur, which is a well-known mechanism leading to bistability as shown by differential equation models of arid ecosystems (Rietkerk et al. 2002, von Hardenberg et al. 2010). Also, increasing rainfall intensity has been shown to further increase the risk for a critical transition towards a desert state in a differential equation model with one vegetation type inducing resource concentration (Siteur et al. 2014). Developing spatial explicit models with multiple species interacting under multiple stress types would improve our ability to predict if critical transitions can be expected to occur. Moreover, to further investigate under which conditions positive interactions can cause critical transitions, we need to investigate how stochasticity in resource availability interacts with different types of positive interactions (e.g. resource concentration, local (intraspecific) facilitation and interspecific facilitation).

Another improvement in the model presented in chapter five would be to model the grazing rate as stochastic and spatially explicit events. This might also increase the likelihood for critical transitions, especially if grazing would be incorporated as a spatially heterogeneous pressure, because the effects of grazing would become more profound on a more fragmented vegetation structure, accelerating ecosystem degradation (Schneider and Kéfi 2016). Making a spatially explicit version of the model presented in chapter five, in combination with more realistic, stochastic

grazing events, would allow for making more efficient and precise grazing management strategies that could reduce the risk for future land degradation in the light of future reductions in rainfall or increases in rainfall intermittency.

In chapter two I build upon studies that predict a possible critical transition towards a desert state with increasing grazing or drought stress. Although ample model studies predict such a hard to reverse transition from a vegetated to a non-vegetated state (e.g. Rietkerk et al. 2002, Kéfi et al. 2007a, Mayor et al. 2013), up till now there is no evidence from long-term field experiments that shows rapid, hard-to-reverse degradation of dryland ecosystems under increased drought stress or grazing pressure. Recent insights from a nine year rainfall manipulation experiment in Eastern Mediterranean shrublands indicated that there may be very little effect of reduced rainfall on biomass, species composition, species richness and vegetation density (Tielbörger et al. 2014). These results challenge the notion to classify dryland ecosystems as very vulnerable to projected global change. Tielbörger et al. (2014) attributed this resistance to increased drought stress to the high temporal and spatial heterogeneity under which plants in drylands have evolved over time. A second factor that might explain why ecosystems may be resilient along drought stress gradients, is the stochasticity of rainfall events in real systems (chapter five, Realpe-Gomez et al. 2013, Villa Martín et al. 2015). These apparent contrasts between model studies and this long-term experiment should be reconciled by applying more realistic, stochastic modelling approaches, to further investigate the dryland stability to ongoing environmental change. Also these contradictions call for more long-term experiments that investigate the effect of increased drought conditions in arid ecosystems, to better understand when the conditions for bistability are expected to apply.

In order to upscale predictions from plant-plant interactions research in arid ecosystems, and to link these interactions to predictions from spatial explicit model studies, a very useful approach could be to couple high resolution remote sensing data to field measured spatial patterns of the vegetation. A recent study assessed plant-plant interactions from remote sensing images by linking remote sensing derived, spatial patterns of the vegetation (features of the plant patch-size distribution of the vegetation) to plant co-occurrence patterns as measured in field studies (Xu et al. 2015a). This approach suffers however from the same limitation as the observational methods as presented in chapter three, i.e. spatial

patterns in vegetation cannot be directly linked to plant-plant interactions, without assessing the mechanisms behind the observed spatial patterns. To elucidate the mechanism behind the observed spatial vegetation patterns, high resolution satellite imagery could be used to compare the soil moisture dynamics in bare soil and vegetated soils after rainfall events. Remote sensing methods such as SAR (Synthetic Aperture Radar) can provide spatially explicit surface soil moisture measurements over both bare and vegetated soils at a large scale with high spatial and temporal resolution (e.g. Santi et al. 2013, El Hajj et al. 2016). Linking such satellite derived measures for soil moisture to field derived measures on plant traits and plant-plant interaction metrics would greatly improve our understanding of which plant traits are crucial for vegetation induced soil moisture improvements, and under which drought stress levels such improvements can still be expected.

CONCLUDING REMARKS

Currently it is still a challenge to predict how interspecific facilitation will change with increased drought and grazing pressure. In this thesis, I showed that linear increases in facilitation intensity with stress cannot a priori be expected, as positive interactions may wane under severe stress by a combination of drought and grazing. Future model studies should incorporate the more complex nature of interspecific plant-plant interactions to improve our ability to predict how (semi-) arid ecosystems will respond to projected increases in drought conditions or changes in grazing regimes. In this era of accelerating changes in the environment, a surge of research effort is necessary to elucidate how different growth forms of plants interact with their environment along combined stress gradients. And even more importantly, to investigate how changes in interspecific plant-plant interactions along such gradients relate to the stability of (semi-) arid ecosystems. Combining long-term experimental outcomes, to dynamic model studies will be essential to expand on these topics, in order to progress our ability to indicate which areas of (semi-) arid ecosystems are most at risk to future degradation.

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Summary &
Samenvatting

Acknowledgements

Curriculum Vitae

List of publications

SUMMARY

The human impact on the climate system and on ecosystems has rapidly increased over the last century. Global climate change and intensified land-use have already caused an increasing pressure on the functioning of natural systems on a global scale. Moreover, forecasts by global climate models predict that increasing temperatures and more frequent and longer drought periods will impose an increasing pressure on ecosystems over the coming decades, with arid regions such as the Sahel, the Mediterranean and other (semi-)arid climate zones, among the most impacted regions.

How a dryland ecosystem will respond to increased drought stress is a central theme in ecology and environmental sciences, as drylands cover around 40 percent of the earth's surface, and sustain livelihoods to roughly 2 billion people. Theoretical ecosystem models show that increasing drought stress or grazing pressure in dryland ecosystems may lead to a critical transition from a vegetated to a non-vegetated state. Such transitions are difficult to reverse and it is therefore of utmost importance to increase our understanding of under what conditions critical transition may occur. Previous ecosystem model studies showed that critical transitions in drylands can occur if an ecosystem is controlled by positive feedbacks between the vegetation and the physical environment, or if plants provide facilitative effects, for example when mature 'nurse' plants protect neighbouring 'protégé' seedlings from grazing damage.

Over the last three decades a wide array of experimental field studies clearly showed that interspecific plant facilitation, i.e. positive interactions between different plant species, is a common feature in plant communities, especially in harsh environments such as alpine or arid ecosystems. Although facilitation is now recognised as an important process underlying ecosystem stability, diversity and functioning, it is still uncertain how facilitation changes over environmental gradients. In particular, although the combination of drought stress and grazing stress is common in arid ecosystems, it is unclear how facilitative interactions between plants may change along combined drought and grazing gradients.

The two main research questions of this thesis are: how do plant-plant interactions change along combined stress gradients consisting of drought and grazing stress? And how do these changes relate to dryland ecosystem stability? To shed

light on these questions, in this thesis I combined insights from a conceptual review study (chapter two), an observational study (chapter three), an experimental study (chapter four) and an ecohydrological modelling study (chapter five).

In **chapter two**, I developed a conceptual model on how interspecific plant-plant interactions may change along a drought stress and grazing gradient, and discussed how changes in plant-plant interactions relate to the onset of critical transitions in drylands. I proposed that increased drought stress may result in a decline in facilitation intensity and in a failure of nurse plant recruitment, prior to a critical transition. Also I hypothesized that a combination of drought stress and herbivory will speed up the waning of facilitation, leading to earlier and faster degradation of arid ecosystems. Furthermore, I suggested that in-field monitoring of nurse species recruitment and declines in interaction intensity could provide us with additional early warning signals for imminent transitions from a vegetated to a non-vegetated state. Moreover, I proposed that the changing nature of facilitation intensity should be incorporated into critical transition theory to improve our understanding of the conditions that will result in the onset of a critical transition from a vegetated to a non-vegetated state.

In **chapter three** I assessed the combined effects of drought and grazing pressure on plant-plant interactions by measuring plant co-occurrence patterns along a combined grazing and drought gradient. I showed that along this combined stress gradient, at the community level, plants at south-facing slopes were negatively associated at ungrazed conditions, but were positively associated at low grazing pressure and were randomly associated at high grazing pressure. At north-facing slopes, grazing did not result in a shift in the direction of the spatial association. However, analysis of pairwise species co-occurrence patterns showed that the dominant species *Stipa tenacissima* and *Anthyllis cytisoides* shifted from excluding each other to co-occurring with each other with increasing grazing pressure at north-facing slopes. These findings highlight that plant-plant interactions between species pairs and interactions at the community level may reveal contrasting results, and should thus both be assessed.

In **chapter four** I described the results of an experiment in which 1280 saplings of *Anthyllis cytisoides*, a preferred food source for both goats and rabbits, were planted either under the canopy of the protecting shrub species *Artemisia herba-alba* or in the open microsites in between shrub patches. Over a period

of 2 years, I followed growth and survival of saplings over the two microsites in four treatments of grazing pressure: no grazing, low goat grazing pressure, high goat grazing pressure and rabbit grazing. Moreover, an extreme drought event occurred during the course of the experiment. Prior to this drought event, neutral effects prevailed from the nurse on the protégé at ungrazed plots, as saplings performed equally under shrubs and in the open microsites. However, at plots that were grazed by rabbits I measured higher survival for saplings growing under shrubs, indicating a facilitative effect from the nurse shrub. Contrarily, I found a higher survival of saplings in the open microsites after the extreme drought event at ungrazed plots, indicating competitive effects to prevail. At rabbit grazed plots, the nurse effect shifted from positive to neutral during the extreme drought event. These findings support the idea that grazing can shift plant interactions from neutral to positive, but also show that positive plant-plant interactions may return to neutral at the extreme end of the multiple stress gradient under the combination of high drought stress and grazing pressure.

To further investigate the effect of drought and grazing on plant-plant interactions, in **chapter five** I analysed an ecohydrological model, in which I simulated the growth of two plant species interacting with each other under different scenarios of rainfall and grazing pressure. Both species were competing for water, but the unpalatable nurse species protected the palatable protégé species from grazing damage. Moreover, the nurse species was more drought resistant, i.e. it had a lower wilting point, whereas the protégé species had a higher optimal growth rate when soil water content reached field capacity. I showed that increased intermittency in rainfall (i.e. longer periods without rainfall) resulted in stable coexistence between the two competing species, whereas nearly constant rainfall led to competitive exclusion of the protégé by the nurse species. Also I showed that competitive effects increased in intensity with drought stress. Moreover, higher grazing rates resulted in increased facilitative effects of the nurse on the protégé species. This chapter highlights the importance of rainfall intermittency in explaining coexistence of species in semi-arid ecosystems and shows that an increase in rainfall intermittency or grazing pressure can result in more facilitative interactions between plants.

Overall, my findings of the third and fourth chapter of this thesis underlined the importance of grazing in explaining observed positive interactions or spatial

associations between plants in a semi-arid ecosystem. Also I showed that facilitative interactions may shift back to neutral when stressors are combined at the high end of a stress gradient. This thesis also shows that positive interactions in drylands, and an increase in facilitation with increasing aridity should not be expected a priori. Our model results showed that intermittency is highly important in driving plant coexistence ranges and in determining the intensity and direction of plant-plant interactions. A future increase in rainfall intermittency in drylands may result in increased positive interactions between plants, thereby possibly partly counteracting the negative effects of increased prolonged dry spells in arid ecosystems. To test these model results, we need field studies that investigate how facilitation and resource competition vary along controlled drought stress gradients, for multiple species pairs with differing wilting point and optimal growth rate combinations. Developing spatial explicit models with multiple species interacting, under combined multiple stochastic stressors would further improve our ability to predict under what range of environmental conditions and for what species trait combinations, critical transitions can be expected to occur. Future model studies should incorporate the more complex nature of interspecific plant-plant interactions to improve our ability to predict how (semi-) arid ecosystems will respond to projected increases in drought conditions. Combining long-term experimental outcomes, to mechanistic model studies will be essential in order to progress our ability to predict which areas of (semi-) arid ecosystems are most vulnerable to future degradation.

NEDERLANDSTALIGE SAMENVATTING

De menselijke invloed op het klimaatsysteem en op ecosystemen is drastisch toegenomen over de laatste decennia. Klimaatverandering en intensief landgebruik hebben tot een toenemende druk geleid op het functioneren van ecosystemen op een mondiale schaal. Daarbovenop voorspellen klimaatmodellen dat klimaatverandering niet alleen zal leiden tot een verder toenemende temperatuur, maar óók tot het nog vaker voorkomen van droogtes, die bovendien langer zullen duren. Vooral droge ecosystemen zoals in de Sahel regio, in het Middellandse zeegebied en in andere (semi-)aride gebieden, zullen hierdoor de komende decennia steeds meer onder druk komen te staan.

Hoe aride ecosystemen zullen reageren op toenemende droogtestress is daarom een centraal thema in de milieuwetenschappen en de ecologie. Vooral ook omdat droge ecosystemen ongeveer 40 procent van het aardoppervlak beslaan en 2 miljard mensen afhankelijk zijn van deze gebieden. Wiskundige ecosysteemmodellen laten zien dat een toenemende droogtestress of begrazingsdruk kan leiden tot een kritiek kantelpunt in een ecosysteem. Als het ecosysteem over dit kantelpunt 'geduwd' wordt, kan het ecosysteem omslaan van een begroeide tot een gedegradeerde, onbegroeide toestand. Het herstellen van het ecosysteem is dan moeilijk en kostbaar en het is daarom belangrijk om te begrijpen onder welke condities zulke kantelpunten verwacht kunnen worden. Theoretische modelstudies hebben laten zien dat kantelpunten in ecosystemen verwacht worden als positieve interacties tussen de vegetatie en het fysieke milieu een rol spelen, of als er facilitatie optreedt tussen plantensoorten, bijvoorbeeld wanneer een volwassen 'beschermende' struik bescherming biedt tegen begrazing voor een 'protegé' zaailing.

De laatste decennia is er veel ecologisch veldonderzoek verricht naar facilitatie tussen verschillende plantensoorten, waaruit blijkt dat facilitatie inderdaad een belangrijk proces is voor de stabiliteit, diversiteit en het functioneren van ecosystemen. Facilitatie tussen planten blijkt vooral een belangrijke rol te spelen onder barre omstandigheden, zoals in aride ecosystemen of in hoog-alpiene ecosystemen. Het is echter nog steeds niet duidelijk of facilitatie tussen planten toeneemt als ook de droogtestress in een ecosysteem toeneemt, of dat facilitatie juist weer zal afnemen onder extreme droogte. Ondanks dat de combinatie van

droogte stress en begrazingsdruk veel voorkomend is in aride ecosystemen, is het vooralsnog niet goed onderzocht hoe deze twee stressoren de facilitatie tussen planten tezamen beïnvloeden.

De twee onderzoeksvragen van deze thesis luiden daarom als volgt: hoe veranderen positieve plant-plant interacties over een gecombineerde droogte en begrazingsgradiënt? En hoe relateren deze veranderingen tot de stabiliteit van aride ecosystemen? Om licht te werpen op deze twee vragen heb ik in deze thesis de inzichten gecombineerd van een conceptuele reviewstudie (hoofdstuk twee), een observationele veldstudie (hoofdstuk drie), een experimentele veldstudie (hoofdstuk vier) en een ecohydrologische modelstudie (hoofdstuk vijf).

In **hoofdstuk twee** heb ik een conceptueel model ontwikkeld om te voorspellen hoe interacties tussen planten veranderen over een droogte en begrazingsgradiënt. Ook bediscussieer ik hierin hoe deze veranderingen relateren tot het vóórkomen van kantelpunten in het ecosysteem. Ik stelde hierbij voor dat een toenemende droogte stress kan leiden tot een afname in de facilitatie-intensiteit onder extreme droogte en tot een gebrek aan regeneratie van 'beschermende' struiken, voordat een kantelpunt bereikt wordt. Ook stelde ik voor dat een combinatie van droogtestress en begrazingstress tot een versnelde afname van de facilitatie intensiteit zal leiden, wat tot een versnelde degradatie van een aride ecosysteem kan leiden. Daarnaast stelde ik voor dat het gebrek aan regeneratie van 'beschermende' struiken of een afname in facilitatie-intensiteit, kunnen dienen als additionele vroegtijdige waarschuwingssignalen voor het plaatsvinden van kantelpunten van een begroeide naar een onbegroeide toestand. Ook stelde ik voor om de veranderingen in plant-plant interacties over stress gradiënten te integreren in de theorievorming van kantelpunten in aride ecosystemen, om beter te begrijpen wanneer kantelpunten zullen optreden en ze daarmee ook beter te kunnen voorspellen.

In **hoofdstuk drie** heb ik de effecten van droogte en begrazingsstress onderzocht op plant-plant interacties door de ruimtelijke associaties tussen verschillende plantensoorten te meten. Ik liet zien dat over deze gecombineerde gradiënt, op het schaalniveau van de gehele plantengemeenschap, planten negatief geassocieerd waren op onbegraasde zuidhellingen, maar positief geassocieerd waren op laag begraasde zuidhellingen en willekeurig geassocieerd waren op hoog begraasde zuidhellingen. Op noordhellingen had begrazing geen effect op de

mate van associatie tussen verschillende soorten. Op paarsgewijs schaalniveau vond ik echter tegengestelde effecten van begrazing op de associatie. De veelvoorkomende soorten *Stipa tenacissima* en *Anthyllis cytisoides* waren negatief geassocieerd op onbegraste noordhellingen, maar positief geassocieerd op hoog begraste noordhellingen. Deze bevindingen laten zien dat interacties op het schaalniveau van de plantengemeenschap contrasterend kunnen zijn met interacties op paarsgewijs schaalniveau, en dat ze dus beiden onderzocht moeten worden voor een beter begrip van het effect van stress op plant-plant interacties.

In **hoofdstuk vier** beschrijf ik de resultaten van een experiment waarin 1280 *Anthyllis cytisoides* protegé zaailingen geplant zijn, onder of buiten de beschermende struik *Artemisia herba-alba*. Over een periode van 2 jaar volgde ik de groei en de overleving van de zaailingen over deze twee microsites over vier begrazingsbehandelingen: niet begrast, lage begrazing door geiten, hoge begrazing door geiten, of begrazing door konijnen. Tevens vond tijdens het experiment een extreme droogteperiode plaats. Voorafgaand aan deze droogteperiode vond ik op niet begraste plots vooral een neutraal effect van de beschermende struik op de protegé zaailingen; zaailingen onder en buiten de struik presteerden even goed. Op door konijnen begraste plots vond ik echter een hogere overleving van zaailingen binnen de bescherming van de struik, wat op een facilitatief effect duidt. Na de droogteperiode vond ik een concurrerend effect van de struik op niet begraste plots. Op door konijnen begraste plots vond ik een neutraal effect. Deze bevindingen laten zien dat plant-plant interacties door begrazing positief kunnen worden, maar ze laten ook zien dat facilitatieve interacties kunnen terugkeren naar neutrale interacties onder een combinatie van begrazings- en extreme droogtestress.

In **hoofdstuk vijf** heb ik een ecohydrologisch model geanalyseerd om de effecten van droogte en begrazing op plant-plant interacties verder te onderzoeken. In dit model heb ik de groei en interactie van twee plantensoorten gemodelleerd onder verschillende regenval en begrazingsscenario's. De twee soorten concurreerden om water opname, maar een niet-eetbare 'beschermende' soort beschermde de andere wel-eetbare 'protegé' soort tegen begrazing. Daarnaast was de 'beschermende' soort meer droogtebestendig, aangezien deze plant een lager verwelkingspunt had. De 'protegé' soort had echter een hogere optimale groeisnelheid onder hogere bodemwaterstanden. De modelanalyse liet zien dat

intermitterende regenvalpatronen (regenval met droge tussenpozen) leidde tot co-existentie van deze twee soorten, maar dat meer constante regenval leidde tot het wegconcurreren van de protegé soort door de beschermende soort. Daarnaast liet de modelanalyse zien dat concurrentie toenam onder hogere droogtestress, maar dat begrazing tot een toename van faciliterende effecten leidde. Dit hoofdstuk benadrukt het belang van intermitterende regenval in het verklaren van het samen voorkomen van soorten en laat zien dat een toename in begrazing of de intermittentie in regenval leidt tot meer facilitatieve interacties tussen plantensoorten.

De bevindingen uit het derde en vierde hoofdstuk onderstrepen het belang van begrazing in het verklaren van positieve interacties of positieve ruimtelijke associaties tussen planten in droge ecosystemen. Daarnaast heb ik laten zien dat facilitatieve interacties kunnen omslaan naar neutrale interacties als stressoren gecombineerd worden, met name onder extreme stress. Dit kan leiden tot een verhoogde kans op ecosysteemdegradatie. Deze thesis laat zien dat positieve interacties in droge ecosystemen, en een toename in facilitatie over een stressgradiënt, niet a priori verwacht kunnen worden. Onze modelresultaten laten daarnaast zien dat intermitterende regen van groot belang is in het verklaren van co-existentie van soorten én in het bepalen van de richting van plant-plant interacties. Een toekomstige toename in de intermittentie in regenval kan ertoe leiden dat interacties tussen planten meer facilitatief worden, wat de mogelijke negatieve effecten van langer durende droge periodes mogelijk deels kan opheffen. Om deze model resultaten te testen hebben we meer lange termijn veldstudies nodig die onderzoeken hoe facilitatie en concurrentie variëren over stressgradiënten, voor verschillende soorten met variërende groeistrategieën. Het ontwikkelen van ruimtelijke modellen waarin meerdere soorten interacteren onder meerdere stressoren kan ons verder helpen in het voorspellen van onder welke droogtecondities en groeistrategieën van planten, er kantelpunten in ecosystemen te verwachten zijn. Zulke modelstudies moeten de meer complexe werking van plant-plant interacties incorporeren, om beter te kunnen voorspellen hoe droge ecosystemen zullen reageren op de toekomstige veranderingen in het klimaat. Het combineren van experimentele lange termijn veldstudies met mechanistische modelstudies zal essentieel zijn om te voorspellen welke aride gebieden het meest kwetsbaar zijn onder toekomstige klimaatverandering.

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CURRICULUM VITAE

Mart Verwijmeren was born in Tilburg in 1986. He finished secondary school in 2004, and followed the bachelor program Earth Sciences at Utrecht University with a specialisation in Physical Geography from 2004 to 2008. After this he followed the master program Sustainable Development at Utrecht University with a specialisation in Environmental Sciences from 2008 to 2010. As a first research project he performed field work in a grazed woodland in the Netherlands on the relation between acorn dispersal by wood mice and spatial associations between oaks and shrubs. For his master thesis project he did an internship at the Mammal Research Institute in the Białowieża Primeval Forest in Poland under supervision of Dries Kuijper and Chris Smit. In this project he investigated how forest habitat structure and wolf scat presence relate to red deer behaviour and forest regeneration. After finishing his master degree (Cum Laude), Mart worked as a postman for several months, and then began his PhD project at the Copernicus Institute of Sustainable Development in March 2011 under supervision of Chris Smit, Max Rietkerk and Martin Wassen, which this thesis is the result of. For his PhD thesis he combined ecohydrological modelling and ecological field studies that he performed in the south east of Spain in collaboration with the Universidad de Alicante. Currently Mart is working as a lecturer in the bachelor program Global Sustainability Science at Utrecht University.

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