

The impact of rabbit grazing and soil moisture variation on sapling growth and survival in a semi arid Mediterranean ecosystem

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July, 2013 - University Utrecht
Master: Sustainable Development; Global Change and Ecosystems
Thesis (45 ECTS)
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Abstract

Plant-plant interactions have been proposed as fundamental mechanisms in semiarid ecosystems. Yet little is known on how increased consumer pressure effects plant-plant interactions. Therefore this study aimed to investigate how facilitative effects changed under grazing regimes. We examined the effect of rabbit grazing and microsite variation on: 1) soil moisture 2) sapling growth and survival. Moreover we measured how sapling growth and survival changed along a rabbit grazing gradient. This was tested through a sapling transplant experiment where measurements of soil moisture, survival and height of saplings (*Anthyllis cytisoides*) were taken under the canopy of shrubs (*Artemisia barrelieri*) and in interpatches in a semiarid Mediterranean ecosystem. As we found that replicates were submitted to differences in rabbit activity we were able to mimic a grazing gradient. Results showed that rabbit grazing had a negative effect on saplings, decreasing height and survival. Microclimate amelioration and associational resistance, reduced negative effects of grazing, causing microsite variation of growth and survival. Along our rabbit herbivory gradient there was an increase in facilitation. However we found that under our highest grazing pressure, in contrast to the stress gradient hypothesis (SGH), facilitation declined. Although this decline, there remained a positive net effect for both growth and survival. We conclude that grazing by rabbits is a large driver affecting the spatial and temporal variation of sapling growth and survival and with increasing biotic stress facilitation is more likely to follow a humped-shape pattern than monotonically increase as predicted by the SGH.

Key words

Plant-plant interactions • consumer pressure • rabbit grazing • facilitation theory • grazing gradient • microclimate amelioration • associational resistance

Introduction

Increasing environmental stress by human activities or climate change could lead to desertification, affecting the livelihood of more than 25% of the world's population (MEA, 2005). Added knowledge on natural processes is needed to be able to properly manage natural habitats, prevent environmental risks and secure the continued supply of ecosystem services (Pugnaire, 2011). Moreover, added knowledge can help the development of suitable strategies for nature restoration in the Mediterranean (Castro et al., 2004).

Semiarid ecosystems are especially vulnerable to degradation as even small changes in natural processes can have large impacts on ecosystem functioning (Ramírez et al., 2007). Therefore, it is crucial to determine controlling factors. A mechanism playing a dominant role in ecosystem functioning is local fa-

cilitation through plant-plant interactions (Schlesinger et al., 1990; Callaway & Walker, 1997; Aguiar & Sala, 1999; Alados et al., 2006). Hence, in semiarid environments, like the Mediterranean, plant-plant interactions are at the base of ecosystem functioning and knowing them in detail can substantially improve understanding of its responses to increasing environmental stress (Pugnaire, 2011).

Overgrazing has been identified as one of the main stress factors leading to desertification (Geist & Lambin, 2004; Bisigato et al., 2005), as grazing causes a reduction of plant cover and fragmentation (Holm et al., 2002). Rietkerk and van de Koppel (1997) showed in model studies that there is a positive feedback between reduced size and/or number of vegetation patches and reduced water and nutrient availability in semiarid ecosystems. Increased grazing may af-

fect plant-plant interactions as it causes a reduction of plant cover and fragmentation, reducing the water and nutrient availability. In that sense, plant-plant interactions have been proposed as fundamental mechanisms in semiarid ecosystems. However, little is known on how increased consumer pressure affects plant-plant interactions.

Plant-plant interactions

Plant-plant interactions may include facilitation (microhabitat amelioration and associational resistance) and competition for resources. Stress may change the balance between facilitation and competition, affecting germination and sapling growth under perennial plants (Escudero, 2000). According to the stress gradient hypothesis (SGH) both types of interactions act simultaneously upon species but shift from net disadvantageous towards beneficial with increasing environmental stress (Bertness & Callaway, 1994; Callaway, 1995; Holmgren et al., 1996; Callaway & Walker, 1997; Brooker & Callaghan 1998).

Maestre and Cortina (2004a,b) evaluated how the net effect of *Stipa tenacissima* (facilitator) on *Pistacia lentiscus* (protégé) varied across a gradient of abiotic stress in ten semiarid Mediterranean steppes from SE Spain. In contrast to the SGH, they found that the outcome of this plant-plant interaction followed a hump-shaped pattern, with competitive interactions dominating at both extremes of the gradient and facilitation in the middle. These results suggest a shift in plant-plant interactions from facilitation to competition under high abiotic stress conditions. However, effects of abiotic stress on plant-plant interactions are hard to generalize. Microclimate amelioration may depend on factors like plant architecture, physiology and the magnitude of rainfall events (Pugnaire, 2011). Associational resistance may depend on palatability of species (Graff & Aguiar, 2011).

Microhabitat amelioration

Microhabitat amelioration by perennial plants may reduce abiotic stress on saplings by increased infiltration, shade and improvement of water distribution for protégé plants (Pugnaire, 2011). It is widely acknowledged that improved soil physical properties under vegetated patches (increased soil organic matter and aggregate stability, and decreased bulk density and penetration resistance) are generally translated into higher infiltration rates and lower runoff and erosion rates compared with adjacent interpatches (Green, 1992; Bochet et al., 1999; Eldridge & Rosentreter, 2004; Puigdefábregas, 2005).

Bhark and Small (2003) showed that infiltrati-

on is typically higher in vegetated soils, in both grassland and shrubland, than in interpatches. However, this does not hold true when rainfall is low, interception by the canopy prevented low rainfall to reach the vegetated soils. On the other hand, high rainfall increased infiltration, as runoff from interpatches flowed to patches creating a source-sink dynamics. This is an important process in the distribution of soil moisture influencing microsite variations. Interpatches act as sources for overland flow while vegetated patches act as sinks (Rietkerk et al., 2000; Bhark & Small, 2003; Mayor et al., 2009). Plant components in contact with the soil surface (litter, stems, branches and leaves) can slow overland flow and decrease losses due to runoff (Abrahams et al., 2006). With large overland flow microsite variation of soil moisture gain will be larger (Bhark & Small, 2003).

Previous studies in arid and semi-arid ecosystems assumed that greater canopy shading of the soil surface and increased plant litter under vegetation may reduce soil temperatures, which can decrease soil evaporation causing less drying in vegetated soils compared to the interpatches (Breshears et al., 1998; Domingo et al., 2001, and others). Research by Gomez-Aparicio et al. (2004) shows that pioneer shrubs generally have a positive effect on woody sapling survival and initial growth in Mediterranean mountains and on shady slopes. In addition, saplings planted under shrubs grew more than those in the interpatches. However, on sunny slopes where radiation intensity and temperatures were higher, this positive relation was not found.

In contrast, other studies found consistently drier conditions under shrubs despite lower soil evaporation rates (Hennessy et al., 1985; Breshears et al., 1997, 1998; Duniway et al., 2010). Transpiration losses could explain the significantly lower soil moisture in vegetated patches during the dry periods. According to Duniway et al. (2010) it is likely that patches have greater rooting densities and thus more soil water loss to transpiration causing microsite variation of soil moisture.

Through hydraulic lift during dry periods, *Artemisia tridentata* plants could redistribute up to 19% of ground water used for transpiration (Ryel et al., 2002). Hydraulic redistribution has been proposed as a mechanism that buffers soil moisture under shrubs and trees by decreasing the rate at which water is depleted during the drying period. Prieto et al. (2010) showed that *A. barrelieri* shrubs can keep redistributing water from deep wet layers into shallow soil layers during suppressed transpiration at least for short periods (72 h).

In sum, on the one hand decreased bulk density, shade, source-sink dynamics, increased plant litter and hydraulic lift might result in an increased water content in patches compared to bare soil. On the other hand canopy interception and transpiration losses might lead to reduced water content in vegetated soils. Hence, net facilitation effects mentioned above occur only if benefits from enhanced nutrient availability, shade and/or protection from grazing exceed competition for water (Holmgren et al., 1997) or if the benefactor species can increase the availability of water (Maestre et al., 2009).

Associational resistance

Associational resistance is the process whereby a unpalatable species protects a palatable species from being grazed (Hay, 1986). Nurse plants may protect their understory vegetation from herbivore damage by hiding protégé plants under their canopies, or by sharing their defences (spines or toxins) against herbivores (Baraza et al., 2006; Veblen, 2008). These two processes lower consumer pressure and are likely to increase sapling survival and growth. Protection against herbivores may thus be a controlling factor in vegetation dynamics and biodiversity in grazed systems (Veblen, 2008). Associational resistance is especially important for plant recruitment as saplings or seedlings generally lack defensive mechanism (Smit & Ruifrok, 2011). Graff and Aguiar (2011) found that associational resistance (biotic refuge) was the dominant process in grazing situations, although the strength of it depended on the difference in palatability between benefactor and beneficiary species.

Little is known on how facilitative interactions between plants change when subjected to biotic stress, such as that caused by consumer pressure or herbivory (Smit et al., 2009; Van den Berghe et al., 2009). Smit et al. (2007) proposed that associational resistance would show a hump-shaped curve rather than increasing monotonically with increasing biotic stress levels. It has been suggested that at high grazing pressures herbivores may be forced to become less selective and feed upon low-quality food plants, therefore the protective role of the benefactor species on their protégés is expected to decrease (Graff et al., 2007; Smit et al., 2007).

Next to associational resistance, the improvement of water availability under facilitator plants may also positively affect protégé plants recovery from herbivore damage (Rand, 2004; Acuña-Rodríguez et al., 2006). However, even in dry environments shade casted by nurse plants can lead to light limitation for the protégé plants (Seifan et al., 2010; Soliveres et

al., 2010). The joint effect that both shade and an improved water availability have on the recovery of the protégé plants from herbivore damage will depend on the relative importance of water and light as limiting factors for plant performance and if consumer pressure affects their uptake (Wise & Abrahamson 2005, 2007).

Hence, the final response of protégé plants to consumer pressure is difficult to generalize. More studies along these lines are needed to refine predictions on how plant communities respond to different levels of abiotic and biotic stressors (Graff et al., 2007; Smit et al., 2009).

Adding consumer pressure to the SGH

So far it has been suggested that consumer pressure is the primary driver of a decline in microhabitat amelioration at the severe end of a stress gradient consisting of both biotic and abiotic components (Michalet et al., 2006; Forey et al., 2009). Adding consumer pressure to the SGH has had little attention: over the past 15 years plant-plant interactions and the impact of consumer pressure have been considered separately by numerous studies in semiarid ecosystems. An improved understanding of plant-plant interactions as drivers of ecosystem structure and functioning under consumer pressure may improve the development of conceptual and mathematical models of land degradation.

It was found that associational resistance tends to have the strongest effect on spatial aggregation of species at intermediate grazing pressure (Graff et al., 2007; Smit et al., 2007; Graff and Aguiar, 2011). Positive interactions did not increase with increased biotic stress (grazing intensity) as expected from the SGH. The results of co-occurrence analysis supported the proposed hump-shaped curve, with that associational resistance being more important at intermediate grazing pressures (Smit et al., 2007; Graff & Aguiar, 2011).

Modelling and experimental research on the effects of increasing consumer pressure have mainly focused on the impact of larger herbivores (i.e. goats and sheep) (Rietkerk et al., 2004; Alejandro et al., 2005; Bisigato et al., 2005; Kefi et al., 2007; Smit et al., 2007; Graff & Aguiar, 2011; Zhang et al., 2012). Few studies include the impact of rabbit grazing (but see Walker et al., 2005; Soliveres et al., 2011). Therefore, the effect of grazing by livestock may be overestimated as part of the impact is caused by rabbits. Added knowledge may help to properly manage natural habitats, prevent environmental risks, and secure the continued supply of ecosystem services.

Aim of study

The aim of this study is to investigate how facilitative effects of *Artimisia barrelieri* changes under two grazing regimes, answering if: 1) soil moisture variations were affected by rabbit grazing and microsite variation, 2) sapling growth and survival were affected by rabbit grazing and microsite variation, 3) microsite variation of growth and survival changed along a rabbit grazing (biotic stress) gradient. We assume that grazing by rabbits is a large driver affecting the spatial and temporal variation of sapling growth and survival in a semi arid Mediterranean ecosystem. We test this through a sapling transplant experiment where measurements of soil moisture, survival and height of *Anthyllis cytisoides* saplings were taken under patches of *Artimisia barrelieri* and in interpatches in a semiarid Mediterranean ecosystem. We mimicked a rabbit grazing gradient as replicates are submitted to differences in rabbit activity.

We hypothesize that: 1) Soil moisture will not be affected by rabbit grazing, as the facilitator species is not grazed. Microclimate amelioration by *A. barrelieri* will have a positive effect on soil moisture in the patches, causing microsite variation in soil moisture. 2) Rabbit grazing will have a negative effect on saplings, decreasing their growth and survival. Associational resistance by *A. barrelieri* will decrease grazing by rabbits, thereby increasing growth and survival of *A. cytisoides* sapling in patches compared to interpatches. Through microclimate amelioration *A. cytisoides* sapling growth and survival in patches is increased compared to interpatches. In sum, facilitation, microclimate amelioration and associational resistance, will have a positive effect on the growth and survival of saplings, causing microsite variation of sapling survival and growth. 3) Under increasing grazing pressure, in contrast to the SGH, microsite variation caused by associational resistance on growth and survival will first increase microsite variations and then decline, as under high grazing stress saplings in the patches are also grazed. Microclimate amelioration will not be affected by an increasing grazing gradient as rabbits do not graze the facilitator species *A. barrelieri*.

Methods

Study area and experimental setup

This study was conducted in South-East Spain near Murcia on a set of terraces. The area is characterized by a semiarid Mediterranean climate. Here rainfall variability between years is very common, the average rainfall is ± 301 millimetre per year (Aemet, 2012). Four terraces were selected with an vegetation cover of 40-60 percent, *Artimisia barrelieri* being the most occurring species at all the selected terraces.

We conducted a sapling transplant experiment to examine the spatial and temporal variation of sapling growth and survival under no grazing and rabbit grazing. On every terrace fences were build, subdivided in four plots of 20 by 20 meter, three in which rabbits are excluded and one were rabbits can freely enter. Large herbivores were excluded from all plots. In December 2012 in each plot 40 *Anthyllis cytisoides* saplings were planted under the canopy of *A. barrelieri* shrubs and 40 in the interpatches. Planting holes were 20 centimetres in diameter and 20 centimetres deep and made using a mechanised drill. At every planting hole one sapling was planted. Plants that died due to the transplant shock were replaced after four weeks (<5%). With the absence of rainfall, to increase survival, all saplings were watered two times by pouring 1 litre of water on the planting hole at the beginning of January and the beginning of February. To reduce the effects of competition with other plants, we removed all annual species within 10 centimetres of the planting hole during the course of the experiment.

Measurements

Soil moisture variability may affect sapling survival and growth. To measure soil moisture TDR probes were placed within 5 centimetres from the *A. cytisoides* saplings in the planting holes. We measured soil moisture for the top 20 centimetres of the soil. In each plot twelve patches of *A. barrelieri* were selected for soil moisture measurements, the same amount of soil moisture measurements were taken in the interpatches. Rainfall was recorded with pluviometers located on two terraces. Measurements of soil moisture were taken on the 1e, 7e and 27e of March after a rainfall event (17mm) at the end of February.

In this sapling transplant experiment measurements of survival and height of *A. cytisoides* saplings (protégé) were made under the canopy of *A. barrelieri* (facilitator) and interpatches. Measurements were taken during a two month period (January-March 2013, on the 20th January, 1th February and 1th March). During each visit we recorded survival and height of each individual sapling. Saplings were recorded as

dead if there was no presence of green leaves. Height was measured from a mark, set at the first measurements day, up to the top of the fresh leading shoot. Saplings that were found to be dead were dismissed from the height measurements.

We were able to mimic a rabbit grazing gradient as replicates of grazing are submitted to differences in rabbit activity. As we placed three quadrates of one square meter per rabbit grazed plot and measured rabbit droppings over the period of one month (February to March), we were able to scale rabbit activity along a grazing gradient. As plots were situated more than 150 meters from each other, there was independence of rabbit activity between the plots (Eldridge & Koen, 2008). In one rabbit grazed plot there was a high count of rabbit droppings, however there was little impact. Due to high cover of thorny annuals (*Carduus*) it was likely that saplings were sheltered, resulting in a low rabbit activity. As there were differences between the grazed plots due to differences in rabbit activity, we mimic a grazing gradient. Our grazing gradient consisted of 5 different levels, which scaled out gradient from 0 to 4 (0 = no activity, 1=lowest activity, 2=low activity, 3=high activity and 4=highest activity). This enabled us to link sapling growth and survival to rabbit activity, a biotic stress gradient.

Statistical design

We used General Linear Models (or Generalized Linear Mixed Models if data were not normally distributed) to test for differences in: soil moisture, sapling growth and sapling survival. The statistical design consists of two fixed factors: GRAZING (grazed vs. not grazed) and MICROSITE (patch vs. interpatch). TERRACE was set as random factor. We tested all data sets for normality by looking at the Skewness and Kurtosis and for homogeneity of variance using Levene's test. Significant levels were set at .05/n to reduce type 1 errors. All statistical analyses were performed in R software (version 3.0.0, 2013).

Soil moisture measurements were analysed for three measurement days (1-03, 7-03 and 27-03). Analyses were performed through a GLM. To ensure normality, we excluded outliers of soil moisture contents (soil moisture content >22). We tested if grazing affected the soil moisture content within the terraces and if there was microsite variation of soil moisture within the plots. Furthermore we analysed the effect of grazing and microsite on the total drying of the soil between the 1st of March and the 27th of March. The effect of different factors on the drying of the soil could only be compared when there was similar initial soil moisture, as the initial soil water content is a key

factor affecting soil surface hydrology (Mayor et al., 2009).

Sapling growth was analysed over a one month period (1-02 and 1-03) using a GLM. We tested for differences between the grazed and control plots and for microsite variation of growth. Measurements made on the 20th of January were used to analyse if there were no initial differences in height within the control plots due to differences that could have occurred during the planting of the saplings. To analyse sapling growth on our mimic grazing gradient we compared microsite variation of the five grazing levels through a GLM.

Sapling survival was analysed on two measuring days (20-01 and 1-03). As data was not normally distributed, we used a GLMM to test for differences between grazing levels and microsite variation of survival. In the same way as with the growth analyses, differences in rabbit activity on the grazed plots enabled us to mimic a grazing gradient, linking sapling survival to rabbit activity. This was done for measurements on the 1st of March through a GLMM.

Results

Soil moisture

Within the terraces there were no differences between the rabbit and the control plots (1-03 $F=0.445$ $p=.573$ $df=1$, 7-03 $F=0.048$ $p=.846$ $df=1$ and 27-03 $F=0.033$ $p=.872$ $df=1$). There was microsite variation of soil moisture, the interpatches were wetter than the patches over the three measurement days (1-03 $F=15.883$ $p=.007$ $df=1$, 7-03 $F=10.721$ $p=.017$ $df=1$ and 27-03 $F=10.118$ $p=.019$ $df=1$) (fig. 1).

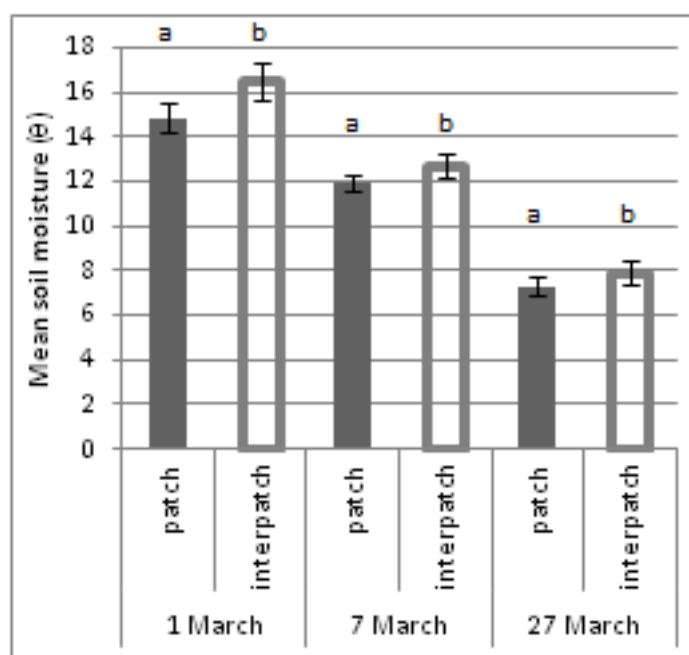


Figure 1: Mean soil moisture contents per measuring day divided per microsite. Error bars indicate a 95 confidence interval. Overall the interpatches are wetter than the patches.

Over the drying period there was microsite variation of soil moisture, the interpatches dried more than the patches ($F=17.059$ $p=.006$ $df=1$). Although the interpatches remained wetter than the patches. As the interpatches were wetter to start with, it is not surprising that they dried more. Grazing by rabbits did not affect the drying of the soil ($F=2.181$ $p=.278$ $df=1$).

Saplings growth and survival

Growth

To start off, on the first measuring day (20-01) saplings height did not differ between the microsites of the control plots ($F=4.975$ $p=.112$ $df=1$). With microsites grouped, growth differed significantly between the grazed and the control plots ($F=217.006$ $p=.0431$ $df=1$). While the saplings in the control plots grew, the saplings in the grazed plots decreased in height. Overall there was significant microsite variation for both rabbit and control plots ($F=20.607$ $p=.004$ $df=1$), patches grew more/decreased less in height than interpatches (fig.2).

Survival

For sapling survival on the first measuring day (20-01) we found no differences between the grazed and the control plots with microsites grouped ($z=-0.933$ $p=.351$). On the second measuring day (1-03) we found a significant difference ($z=-2.227$ $p=.026$). In the grazed plots overall survival was lower than in the control plots (fig. 3).

Survival analysis for the first and second measuring day showed significant microsite variation between the patches and interpatches of the grazed plots ($z=4.282$ $p=.000$ and $z=4.371$ $p=.000$). In the control plots there was no microsite variation in survival, on both measuring days survival was high (>95%).

On both measuring days we found a significant difference between the survival of the interpatches of the grazed and control plots ($z=-2.970$ $p=.003$ and $z=-2.997$ $p=.003$), clearly indicating the negative effect of rabbit grazing. For the patches there was no significant difference between the grazed and control plots ($z=-0.979$ $p=.328$ and $z=-1.284$ $p=.199$), indicating the positive effect of associational resistance.

Saplings growth and survival on a grazing gradient

Growth

An increase in grazing pressure had a negative effect on the growth of saplings. We found differences between the patches and interpatches, indicating that there was a facilitative effect (fig 3). In the control

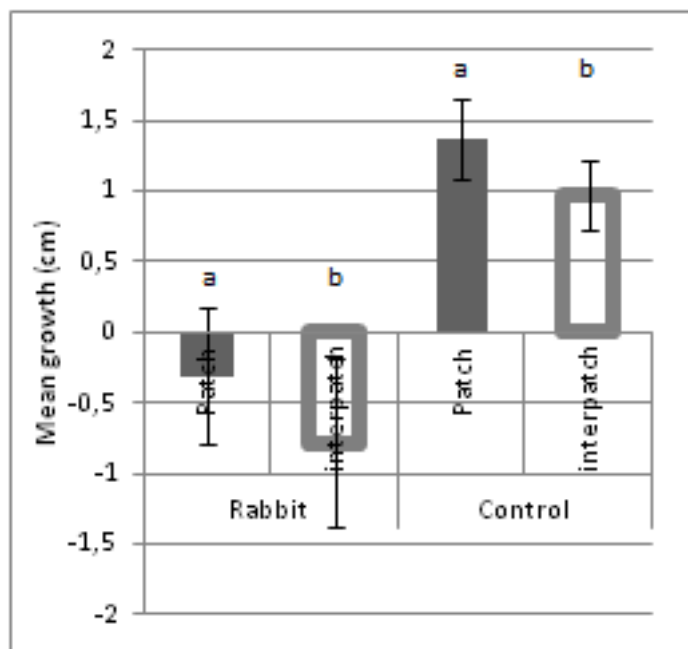


Figure 2: Mean growth in cm for grazed and control plots per microsite. Error bars indicate a 95 confidence interval.

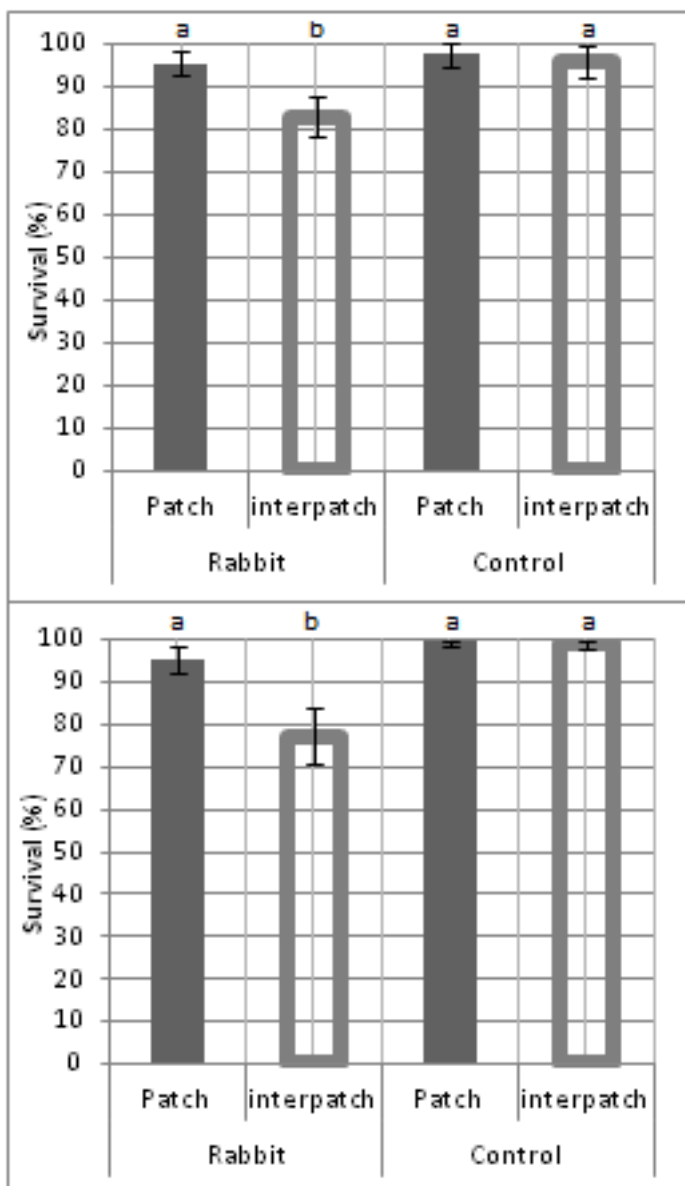


Figure 3: Survival percentage of saplings under grazing by rabbits and in the control per microsite on the 20st January (left) and the 1st March (right). Error bars indicate a 95 confidence interval.

plots (level 0) there was overall the largest growth. Moreover, under no and low consumer pressure the difference in growth between the patches and interpatches was small, indicating a low facilitative effect of *A. barrelieri*. In the control plots (level 0) there was overall the largest growth. Moreover, under no and low consumer pressure the difference in growth between the patches and interpatches was small, indicating a low facilitative effect of *A. barrelieri*.

With the lowest stress level (activity 1), in accordance to dropping counting, there was a slight growth of saplings. Also, under this low grazing pressure the growth between the microsites increased slightly. A small increase in rabbit activity (activity 2) showed that in the patches there was still an increase in saplings height while in the interpatches sapling height decreased. Overall a slight increase in facilitative effect could be seen.

A high rabbit activity (activity 3) showed a high decrease in height of mainly the saplings in the open as they were eaten first. In contrast, saplings in the patches only decreased a little in height. Here there was the largest microsite variation, indicating the largest facilitative effect. With even higher rabbit activity (activity 4), patches and interpatches both declined largely in height. This indicated that there was lower facilitation by *A. barrelieri* to *A. cytisoides* saplings under the highest grazing pressure, however there was still a net positive facilitative effect.

There was a clear trend shown by these results, with increasing grazing pressure facilitation first increased and then at the highest grazing level decreased. However the differences between the microsites were not significant for the different grazing pressures. Values for microsite variation per activity level for 0-4 were: $F= 4.343$ $p=.0378^*$ $df=1$, $F= 0.559$ $p=.457$ $df=1$, $F= 1.008$ $p=.319$ $df=1$, $F= 2.351$ $p=.131$ $df=1$, $F= 0.222$ $p=.640$ $df=1$.

Survival

With no grazing (activity 0) almost all saplings survived and there was no microsite variation (fig. 5). Low grazing pressure (activity 1) showed a clear decrease in the survival of saplings in the open, while in the patches survival remained above 99 percent. With increasing grazing pressure (activity 2) survival in the open showed a constant large decline, in the patches there was a slight decline of survival. With high grazing pressure, (activity 3) there was a sharp decrease in the survival of saplings in the open, in the patches there was a constant slight decrease in survival. Here the facilitative effect of *A. barrelieri* to the saplings is most obvious. At the highest grazing pressure (activi-

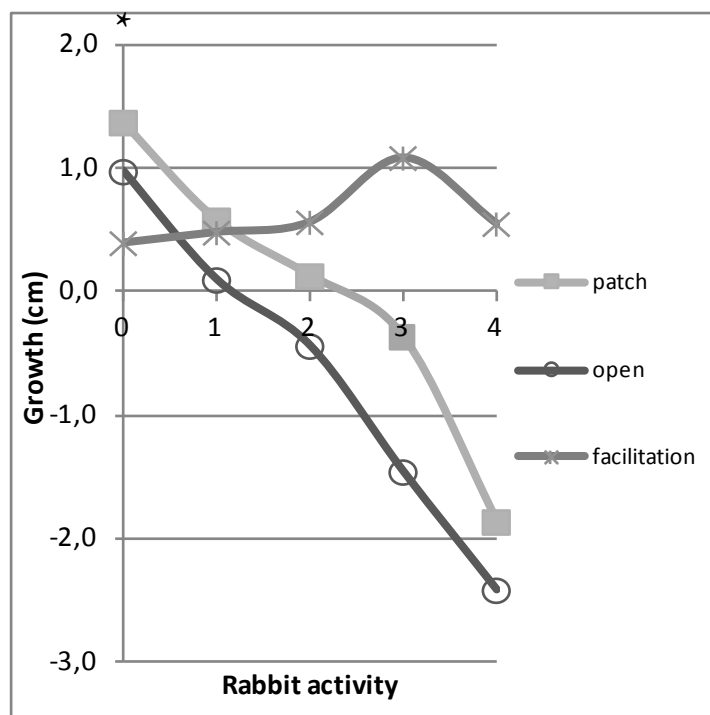


Figure 4: Sapling growth per microsite on a rabbit grazing gradient. The gradient was estimated through rabbit droppings counting (0 = no activity, 1=lowest activity, 2=low activity, 3=high activity and 4=highest activity). The facilitative effect represents the difference in growth between the microsites (patch-interpatch). Overall the facilitative effect remained positive.

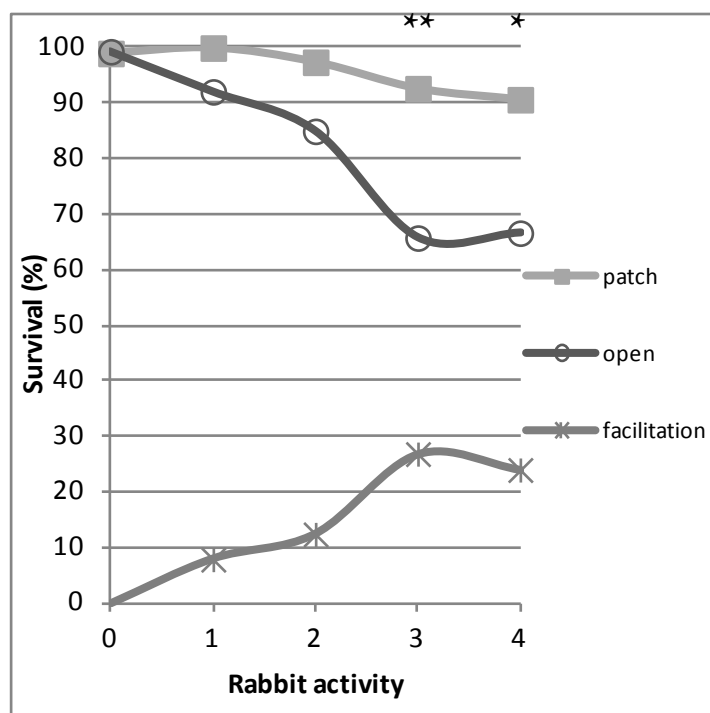


Figure 5: The percentage of saplings that survived 10 weeks after planting (1st of March) over a rabbit grazing gradient (0 = no activity, 1=lowest activity, 2=low activity, 3=high activity and 4=highest activity). The facilitative effect represents the difference in survival between the microsites (patch-interpatch).

ty 4) there was a slight decrease in facilitative effect. Survival in the open seemed to stabilize, as in the patches there was still a slight decrease in survival. With increasing grazing pressure, from 0 to 4, microsite variation became a significant factor ($z = 0.572$ $p = .567$, $z = 0.003$ $p = .997$, $z = 1.287$ $p = .198$, $z = 2.734$ $p = .006^{**}$, $z = 2.421$ $p = .015^*$).

Discussion

The aim of this study was to investigate how facilitative effects of *Artimisia barrelieri* changed under grazing regimes. We showed that with no grazing microclimate amelioration in patches increased the growth of saplings, causing microsite variation of growth. As patches were found to be consistently drier than interpatches ($p < .05$), these differences were not caused by soil moisture variation. During the time period of this experiment water was probably not the main stressor. It is therefore likely that other benefits of microclimate amelioration, like enhanced nutrient availability and/or shade, exceeded the competition for water. Moreover, with no grazing we found no microsite variation in survival as almost all saplings survived (>97%), indicating that overall there was little stress when grazing was excluded.

Rabbit grazing had a negative effect on saplings, decreasing sapling height (-1,7 cm) and survival (-11%). Facilitation in the patches, microclimate amelioration and associational resistance, reduced the negative effects of grazing, causing microsite variation of both growth and survival. As a result, the impact of grazing was larger in the interpatches than in the patches. Differences were not caused by soil moisture variation, as the facilitator species was not grazed, soil moisture variation was not affected by rabbit grazing.

Along the rabbit grazing (biotic stress) gradient microsite variation of survival differed. Along our biotic stress gradient the positive effects the facilitator species had on the survival of the protégé species increased with increasing grazing pressure. However, in contrast to the SGH, we found that under high grazing pressure there was a decline in facilitation. Growth showed almost no microsite variation on a grazing gradient as differences hardly increased with increasing biotic stress. There was however a clear trend, indicating first an increase in facilitation with increasing grazing pressure and decreased facilitation at the highest grazing level. Although we found a decline in facilitation for both growth and survival, there was still a net positive effect of the plant-plant interaction.

We conclude that grazing by rabbits is a large driver affecting the spatial and temporal variation of

sapling growth and survival in a semi arid Mediterranean ecosystem and that facilitation is more likely to follow a humped-shape with increasing biotic stress than monotonically increase as predicted by the SGH.

Plant-plant interactions

The plant-plant interactions between *A. barrelieri* (facilitator) and *A. cytisoides* (protégé) had a positive effect on both the growth and survival of the protégé species. Through microclimate amelioration and associational resistance sapling survival and growth was increased in patches. Adding consumer pressure, a biotic stressor, influenced spatial variation of growth and survival. Results suggest a shift in plant-plant interactions with increasing facilitation under increasing stress, to a decrease under high biotic stress conditions.

Microhabitat amelioration

Our results indicate that spatial variation of soil moisture is not a driving factor affecting sapling growth nor survival during winter in the SE of Spain. We conclude that there was microclimate amelioration by *A. barrelieri* as growth of *A. cytisoides* saplings was increased in patches in ungrazed plots. However, soil moisture was higher in the interpatches, therefore it was not found to be a driving factor. Survival was not affected by soil moisture variation as most saplings (>95%) survived the first 10 week. As measurements were taken during a three month period in winter, water is not as limiting as in summer. Moreover, we watered the saplings during the first two months after planting reducing abiotic stress. This caused low mortality within the control plots probably eliminating microsite variations of survival.

Rabbit grazing did not affect soil moisture variations as the facilitator (*A. barrelieri*) was not grazed. Results show that within terraces there were no significant differences in soil moisture between the control plots and the rabbit grazed plots.

Other studies found that greater canopy shading of the soil surface and increased plant litter under shrubs may reduce soil temperatures, decreasing soil evaporation and causing higher soil moisture in vegetated soils (Breshears et al., 1998; Domingo et al., 2001). Transpiration losses could explain the fact that we found significantly lower soil moisture in vegetated. According to Duniway et al. (2010) it is likely that patches have greater rooting densities and thus more soil water loss to transpiration causing microsite variation of soil moisture. Different studies found that canopy interception lowered infiltration during rainfall events causing patches to be drier than interpatches (Duniway et al., 2010; Hennessy et al., 1985; Breshears

et al., 1997, 1998, and others).

Moreover, due to the creation of planting holes the positive effects (aggregate stability, and decrease bulk density and penetration resistance) that saplings in patches have over saplings in the interpatches is removed. Furthermore, as the time resolution of the soil moisture measurements was too large, we were unable to see slight increases in water content. This may have caused us to measure wetter conditions in the interpatches.

On microsite scale, we found consistent drier conditions in the vegetated soils compared to the interpatches. In contrast, there were significant differences in growth between the microsites of the control plots ($p=0.004$). Saplings under the patches grew more than those in the interpatches, despite the lower soil moisture. We conclude that not soil moisture but other factors, like shade, could have facilitated the growth of the saplings.

Research by Gomez-Aparicio et al. (2004) showed that saplings under pioneer shrubs have a higher survival and larger growth than those in the interpatches. This was true in mountains and on shady slopes. However, on sunny slopes where radiation intensity and temperatures were higher, this positive relation was not found. To their results we can add that on terraces, when there is low abiotic stress, there is a facilitative effect of shrubs on the growth of saplings.

Associational resistance

We found that protection against herbivores was a controlling factor in vegetation dynamics in grazed plots. Rabbit grazing significantly decreased both growth and survival of saplings. This decrease was larger in the interpatches than in the patches causing microsite variations. Through associational resistance *A. barrelieri* had a positive effect on both the growth and the survival of *A. cytisoides* saplings. This is in line with other studies (i.e. Graff et al., 2007; Smit et al., 2007; Graff & Aguiar, 2011) who found similar results for different palatability species under grazing pressure of large herbivores.

Saplings in the patches were more protected from grazing than saplings in the interpatches, increasing their survival. It was clear that with increasing grazing pressure seedlings in the open are eaten first. Only under high grazing pressure, sapling survival in the patches decreased, however remained high (91%), while in the open microsite survival was much lower (66%). As these measurements of survival were taken during a small time period (10 weeks), longer monitoring is required to verify if these observed differences sustain over a longer period.

Smit et al. (2007) found similar survival percentages of tree saplings after one grazing season in a continental climate: no grazing 95% in the open to 98% in the patches with grazing pressure 61% in the open to 81% in the patches. Similar to Smit et al. (2007) abiotic stress was low during the course of our experiment. While grazing stress used by Smit et al. (2007) was limited to a few events (as grazing by large herbivores often is), rabbit grazing can be seen as a constant stressor, resulting in higher biotic stress levels. Moreover, as rabbit grazing is a constant stressor, recovery of saplings was likely lower than saplings exposed to grazing events. Hence, as we found similar survival percentages in a much shorter time period, we conclude that rabbit grazing has a larger impact on sapling survival than grazing by larger herbivores.

Adding consumer pressure to the SGH

Under increasing grazing pressure facilitation sUnder increasing grazing pressure facilitation seems to first increase the spatial variation, however, at the highest grazing level facilitation declines, decreasing spatial variation. We conclude that this is in contrast to the SGH and more like a hump-shaped pattern. This is in line with results of Smit et al. (2007), namely increasing microsite variation under increasing grazing pressure followed by a decline in microsite variation under the highest grazing pressure.

Even though we found decreasing facilitation at the severe end of our stress gradient our study does not confirm findings of and Cortina (2004a,b). While Maestre and Cortina (2004a,b) found competition at the severe end of a stress gradient, still we found a net facilitative effect. As in our experiment the facilitator species was not affected by our stressor (rabbit grazing) there remained a net positive effect. Maestre and Cortina (2004a,b) found this effect on a abiotic (water) stress gradient, we found a similar trend on a biotic stress gradient. We show that there was an increasing difference between the survival of saplings in the patches and interpatches with a decline at the end. With increasing stress associational resistance by *A. barrelieri* protects the saplings from grazing. However with high stress, this protection is less evident.

It is likely that at high grazing pressures rabbit became less selective, while saplings survival in the interpatches stabilized at high grazing pressure, sapling survival in the patches decreased. The protective role of the benefactor species on their protégés decreased, confirming results of previous studies (Graff et al., 2007; Smit et al., 2007; Graff & Aguiar, 2011). Possibly a threshold is past, causing facilitation to decrease.

Significant microsite variation was only found

for the survival of saplings. For growth there is a similar trend, however not significant. Due to the short time period of measurements saplings did not grow much, increasing this time period would probably have resulted in more differences in microsite variation of growth. Moreover, as our stressor, rabbit grazing, was naturally present in the area we could only exclude rabbits, control over the level of rabbit grazing was not possible. Therefore we could not increase the stress causing us to see little differences in the growth of saplings.

Recommendations

Added knowledge on natural processes is needed to be able to properly manage natural habitats, prevent environmental risks and secure the continued supply of ecosystem services (Pugnaire, 2011). Moreover, added knowledge can also help the development of suitable strategies for nature restoration in the Mediterranean (Castro et al., 2004). We showed that a rabbit grazing had a negative effect on both the growth and survival of saplings. Plant-plant interactions reduce these negative effects through microclimate amelioration and associational resistance. As in this study the facilitator species was not affected by the stressor there remained a net positive facilitative effect. We recommend further studies that combine both biotic and abiotic stressors. Hereby the effect of a facilitator species on a protégé can be tested for a combination of declining associational resistance and changing microclimate amelioration.

We have shown that the presence of rabbit grazing, a natural biotic stressor, is a large factor affecting the spatial variation of sapling growth and survival. This is important knowledge that can help the development of suitable strategies for nature restoration in the Mediterranean. Plant-plant interactions have shown to increase both growth and survival of saplings. Therefore, in repopulation projects, the combination of controlling rabbit populations and planting saplings in patches of a facilitator species will significantly increase effectiveness.

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