UNIVERSIDAD AUTONOMA DE MADRID

INSTITUTO PIRENAICO DE ECOLOGÍA
IPE CSIC

Efecto de las condiciones ambientales en la dinámica sucesional y en la estructura de la vegetación de montaña mediterránea

---

Effect of environmental conditions on the successional dynamics and structure of Mediterranean mountain vegetation

Paloma Nuche Gálvez

Tesis Doctoral

Zaragoza 2015

A mis padres Luis y Paloma
y a mi hermana Margarita

Presentación

Los motores de cambio que afectan a los ecosistemas a nivel global en la actualidad incluyen cambio climático, deposición de nitrógeno atmosférico, elevada concentración de CO^2, cambio en usos del suelo e invasiones biológicas (Sala 2000). En los ecosistemas mediterráneos son los cambios en usos del suelo y el cambio climático los que tienen un mayor impacto (Sala 2000). Por ello, en la presente tesis, se abordan los posibles efectos del cambio climático y del cambio en las actividades humanas en los ecosistemas de montaña mediterránea. Hemos evaluado el efecto del clima y el efecto de las actividades antrópicas en la vegetación de montaña separadamente y también la interacción de estos dos motores de cambio. Nos hemos centrado en el estudio del papel de las interacciones entre plantas como mecanismo que articula la respuesta de los ecosistemas al cambio. Específicamente hemos estudiado la interacción entre dos especies arbustivas del pirineo, *Echinospartum horridum* (Vahl.) Rothm y *Buxus sempervirens* L. (Capítulo 1). Estas especies están implicadas en el proceso de revegetación de tierras abandonadas. Hemos observado que la interacción entre ambas especies puede variar con las condiciones climáticas, y por tanto, demostramos cómo el cambio climático puede determinar el proceso de sucesión secundaria del ecosistema tras el abandono mediante la modificación de las interacciones entre especies (Capítulo 3). El efecto directo de las actividades humanas ha sido investigado en el contexto de conservación de pastos, se han evaluado posibles estrategias de control de la expansión del matorral en pastos subalpinos abandonados del pirineo (Capítulo 2). El efecto del cambio climático se ha estudiado también en un roble del territorio sub-Mediterráneo, en el Pre-Pirineo Central para evaluar la capacidad adaptativa de las especies al cambio (Capítulo 4). A escala de comunidad se ha investigado el efecto del clima en el patrón espacial de la vegetación y las interacciones entre plantas (Capítulo 5). Los resultados muestran que las interacciones entre plantas son un mecanismo fundamental en el proceso de sucesión de la vegetación y en la estructura de la comunidad (patrón espacial y diversidad), y que éstos pueden verse afectados por un cambio en las condiciones climáticas. Además, observamos como el roble del territorio sub-Mediterráneo es capaz de desarrollar una respuesta adaptativa a un cambio en las condiciones climáticas. Es interesante tener en cuenta la capacidad de adaptación de las especies al cambio. Concluimos por tanto que las interacciones entre especies que forman la base de procesos que estructuran la vegetación pueden verse afectadas por el cambio climático y las actividades antrópicas, así como por la interacción de estos dos motores de cambio.

Table of contents

Introduction

Since humans are on Earth they have induced changes on ecosystems, which have been specially accused in the last century. Our activities have transformed land surface and its impacts might be accentuated by climate change. The affected ecosystems provide for essential goods and services, which includes regulating of soil erosion, purification of air and water, sustainable ways of rural development, conservation of genetic resources, etc. Is therefore of vital importance to respond the question of how ecosystems will respond to changes in human pressure and climate.

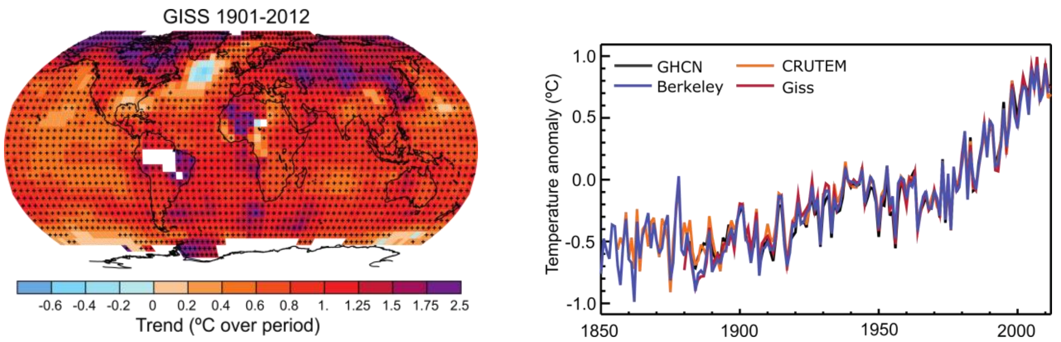
How do ecosystems will respond to changes in human pressure and climate?

Photo 1. El Collado de las Sabinas, at the Sierra Nevada National Park (by Nuche, P.). Photo 2. El Cebollar, Ordesa and Monte Perdido National Park (by Nuche, P.). Photo 3. Tella, Valle de Ainsa, Central Pyrenees (ceded by Fillat, F.).

- Main drivers of change

The main drivers of global change on ecosystems include human land uses, climate change, nitrogen deposition, elevated CO_2 atmospheric concentration and biological invasions (Sala 2000). On Mediterranean ecosystems are changes in human land uses and climate change the ones with a highest impact (Sala 2000).

Climate change has occurred over the Earths' history but the recent rate of warming far exceeds that of any previous warming episode in the past 10,000 years (Sakun et al. 2012, Marcott et al. 2013) (Figure 1). The predicted trends are an increase in global mean air temperature of 0.3-0.7 ºC in the 21st century (IPCC 2013).

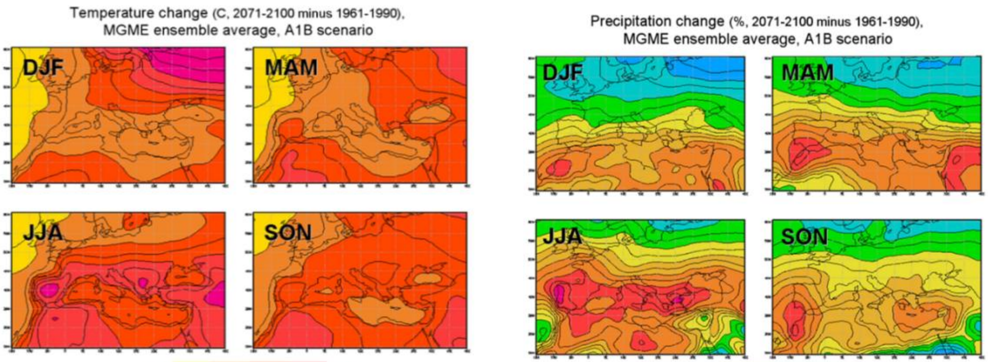


a.

b.

Figure 1. a. Trends in surface temperature from the Giss data set for 1901-2012. White areas indicate missing data. Black plus signs (+) indicate gird boxes where trends are significant. b. Global annual average land-surface air temperature (LSAT) anomalies relative to the period 1961-1990 climatology from the latest versions of four different data sets (Berkely, CRUTEM, GHCN and Giss). Source: Chapter 2, IPCC 2013.

Specifically, on the Mediterranean region climate models have predicted increases in air temperature and frequency of drought events (Figure 2) (Giorgi and Lionello 2008, Nogués-Bravo et al. 2008).



a) b)

Figure 2. a) Average change in surface air temperature. Units ºC. b) Precipitation change. Units % 1961-1990 value. DJF: December – January - February, MAM: March – April – May, JJA: Jun – July – April, SON: September - October – November. Source: Giorgi and Lionello 2008.

The effect of climate on species and on ecosystems has broadly studied since the first ecologists were interested in how organism arrange through the globe (Humboldt and Bonpland 1807, De Candolle 1855). In the last decades, with the change of global climatic conditions, scientists were then interested in the effect that this might cause on the functioning of species and ecosystems (Post 2013). The effects of climate change on ecosystems are diverse and could include: 1) the direct effect on species via physiological requirements, such as variation in organism phenology, e.g.: short vegetative period can cause faster annual shoot development (Montserrat-Martí et al. 2009), which might promote species adaptive responses to climate, migration or otherwise species extinction (Jump and Peñuelas 2005); 2) the effect on a population processes such as survival or recruitment (Batllori and Gutierrez 2008), i.e., the effect of climate on a species have a consequence at population processes; and 3) also climate change can have effects at community scale due to the directs effects on species and populations have secondary indirect effects on the other coexisting species. For that reason changes in community structure, such as spatial patterns or patterns of biodiversity could be affected by a change in climatic conditions (Bakkenes et al. 2002, Rietkerk et al. 2004, Thuiller et al. 2005).

Ecosystems over the world were used by humans to fuel supply (Foley et al. 2005, Millennium Ecosystem Assessment 2005). Many areas were converted to arable land or pastures for foraging animals (Goldewijk 2001; Ove et al. 2002, Zomeni et al. 2008, Yan et al. 2008). Especially, the Mediterranean basin has been constantly subjected to changes in land uses (Quezel and Barbero 1990, Hoùerou 1981). Productive lands were created mainly by deforestation (Barbero et al. 1990). During second half of the 20st century, this dynamics switched due to socioeconomic changes (Margaris et al. 1996, Lasanta and Vicente-Serrano 2007). Land abandonment together with the reduction of pastoral pressure caused a significant change in the landscape, in particular on the revegetation of abandoned lands (Margaris et al. 1996).

Given the high rate of climate change, is of vital importance to evaluate how a change in climatic conditions would affect this reforestation processes and the species involved.

Scientifics efforts during the recent decades were focused on the effects of climate and humans as drivers of change on ecosystems (Barrio et al. 2013). However, given the ecosystems complexity and the several direct and indirect effects of each driver on species and processes, several drivers are seldom investigated together and studies working on a specific driver are the norm. The combined effect of these two drivers of change, change in human land uses and climate change, might lead to complex and unexpected ecosystems responses. The common features of all those process are interactions. Whatever it is the direct effect that humans or climate has on ecological process, it would have secondary and indirect effects on other ecosystem processes due to biological interactions. In a broad sense interactions can be between organisms, such as plant-pollinator, plant-plant, prey-predator; and interactions can also refer to interactions between ecological processes, e.g.: interactions between drivers of ecological change.

Ecosystems responses to climate and anthropogenic activities fail to be additive and linear due to biotic interactions might have secondary indirect effects on several species.

- Species interactions in plant communities

Species interactions in plant communities are the basis for most important ecosystem properties and processes. Biotic interactions are involved in evolutionary processes e.g. coevolution of pollinator-floral traits (Ehrlich et al. 1964, Rathcke 1983, Torpe et al. 2011), structuring food webs (Colwell et al. 2012), generating vegetation spatial patterns (Saiz and Alados 2012), demarcating species range distribution (Wisz et al. 2012), and primary and secondary succession (Connell and Slatyer 1977, Walker and Del Moral 2003)**.**

At the coarsest level species interactions can be considered negative or positive. Competition has gathered up scientific efforts over the last century disregarding the importance of positive interactions (Connell 1983), although the relevance of positive interactions is now broadly recognized (Bruno et al. 2003). Negative and positive interactions might operate simultaneously but there is a net effect of the balance of both competition and facilitation, which can be either positive or negative (Callaway et al. 2002). A positive interaction such as facilitation occur when one species enhances the survivorship, growth or fitness of another (Callaway 1997). There are several mechanisms in positive plant interactions, such as attraction of shared pollinators (Ghazoul 2006), protection from herbivores, especially from larger mammals (Jaksič and Fuentes 1980, Bullock 1991), beneficial exchange of soil microbial communities (Newman and Ritz 1986), as well as an amelioration of local environment by increasing nutrient availability (Jackson and Caldwell 1993), improving soil conditions and moisture retention (Joffre and Rambal 1988), and protecting by extremely low temperatures and harmful sun radiation (Callaway 2002). Based on the fact that harsh environmental conditions, might limit the acquisition of resources by plants, an amelioration of severe environmental stress by neighbors might be likely to favor growth or fitness (Callaway and Walker 1997, Brooker and Callaghan 1998). Therefore, interactions between plants might vary under differing levels of stress. Conceptual models have been developed to predict how these plant interactions will vary along stress gradients). *Stress gradient hypothesis* (SGH) predicts that the relative importance of positive to negative interactions inversely vary along environmental stress gradients, being facilitation dominant under high levels of environmental stress (Bertness and Callaway 1994, Callaway and Walker 1997, Brooker and Callaghan 1998). This hypothesis has been tested in numerous ecosystems, such as salt marshes (Bertness and Ewanchuk 2002), arid ecosystems (Tielbörger and Kadmon 2000, Maestre et al. 2005) and alpine ecosystems (Choler et al. 2001, Callaway et al. 2002). Numerous field experiments supported this hypothesis (Bertness and Shumway 1993, Maestre et al. 2003, He et al. 2013, del Río et al. 2014), but others did not (Tielbödrger and Kadmon 2000, Pennings et al. 2003, Maestre and Cortina 2004). These departures from theoretical predictions are not surprising given that some factors were not taken into account in the original SGH, such as the variability associated with species growth rate and life-history stages and local adaptation to stress (Goldberg et al. 1999, Hastwell and Facelli 2003, Tielbödrger and Kadmon 2000, Pennings et al. 2003). Therefore, progress in understanding the relationship between plant-plant interactions and levels of environmental stress is needed. The effect of the facilitative and competitive effects to neighboring plants can vary with plant size or age (Figure 3), therefore in vegetation successional processes where species turnover implies different species vital stages is a suitable context to evaluate SGH.

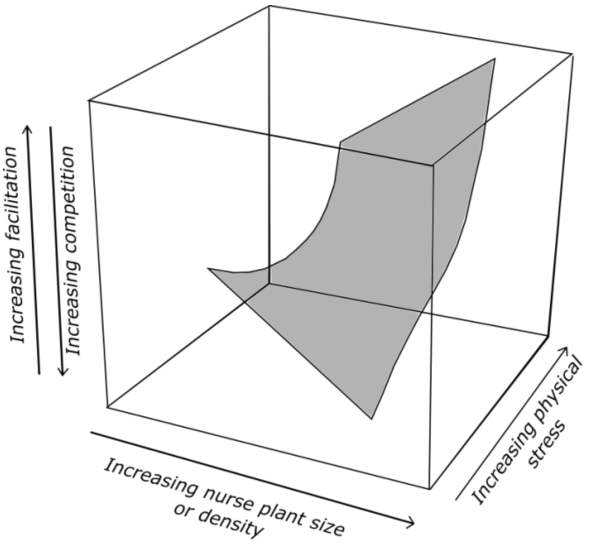


Figure 3. Conceptual model of the effects of size or density of nurse plant(s) and physical stress gradients on the relative importance of facilitation and competition. Source: modified from Walker and Del Moral (2003) and Callaway and Walker (1997). Under high physical stress, increasing nurse plant size, age or density increases the relative strength of facilitation, under more favorable physical conditions, increasing nurse plant size increases the relative strength of competition.

Drivers of global change, such as climate change and human lad uses, are changing the environmental stress experienced by plant species, therefore plant species interactions are bound to be affected too, which will ultimately affect all ecosystems process where they take part.

- Secondary vegetation succession after land abandonment

Nowadays, several causes mainly related to human activities, including changes in fire regimes (Sankaran et al. 2008, Collins and Callabrese 2012), grazing intensification (Archer 1989, Van Auken et al. 2000) and land abandonment (MacDonald et al. 2000, Lasanta et al. 2005) are leading to the invasion of grasslands by woody species. This process is named woody encroachment and is ubiquitous around the globe (MacDonald et al. 2000, Van Auken et al. 2000, Sankaran et al. 2008, Price and Morgan 2008, Brandt et al. 2013). Climatic variables such as changes in rainfall events (Fensham et al. 2005, Sankaran et al. 2008) and enhanced temperatures (Callaway 2007) have also shown to affect grasslands – woodlands dynamics. Therefore, woody expansion might be affected by current climate change (Theurillat and Guisan 2001).

When first colonizing shrubs enter the grasslands vegetation succession take place due to positive feedbacks and reversion to the prevalent grasslands is not straightforward (Gunderson 2000, D'Odorico et al. 2010) (Figure 4). Landscape changes at regional scale are reducing grasslands areas for the broadening of woody systems which entail a loss of natural resources and ecosystem services (Millennium Ecosystem Assessment 2005). This process is determinant in mountainous ecosystems, which have reduced area with increasing altitude. In addition, mountains hold more biodiversity than the global average and have high levels of endemism (Körner 2003, WallisDeVries et al. 2002), especially in the Mediterranean region where the Eurosiberian and Mediterranean species coexist (Mayers 2000).

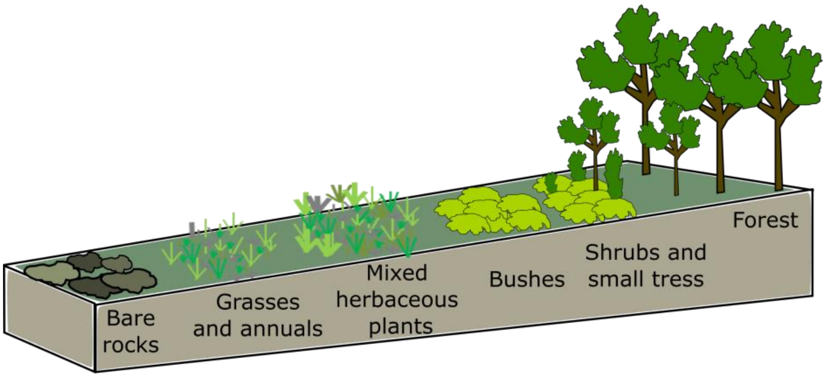


Figure 4. Plant succession. Is the species change over time in an ecosystem after a perturbation (Walker and del Moral 2003).

Species interactions are crucial in succession because species turnover is not simply the independent response of each species to abiotic changes in its environment (Walker and Del Moral 2003). Biological interactions determine the rate of succession and also it might affect the final outcome (Walker and Chapin 1987, Walker and Del Moral 2003). Plants selectively concentrate soil nutrients and add organic matter when they decay, modify the microclimate (e.g. shading), remove soil resources and impact their neighbors that often need the same limiting resources. These effects will affect plant species turn over.

The importance of plant interactions vary during succession, where facilitation tend to be more determinant at the initial successional phase and competition at advanced successional phase (Figure 5). In addition, the relative importance of both interactions, facilitation and competition, in the successional processes can change with environmental stress (Figure 5), consequently, a change in climatic conditions might affect the successional process by modifying the intensity of species interactions.

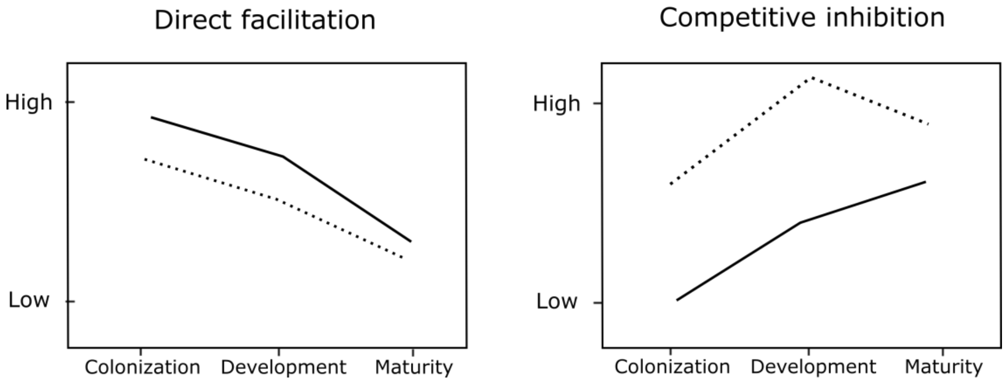


Figure 5. Importance of several processes to species change during three generalized stages of succession (colonization, development and maturity) at two levels of environmental stress (low stress, dotted lines; high stress, solid lines). Source: Modification from Walker and Del Moral (2003).

Mountainous ecosystems

Mountains include all regions above sea level 300 – 1000 meters (depending on latitude) and with steep slope; excluding plateaus, mountains occupy about one fifth of the terrestrial surface. Twenty percent (1.2 billion) of the world's human population lives in mountains or at their edges, and half of humankind depends in one way or the other on mountain resources (largely water).

Mountains are characterized by high biodiversity because of the compression of climatic life zones with altitude and small-scale habitat diversity caused by different topoclimates, mountain regions are commonly more diverse than lowlands and are thus of prime conservation value. They support about one quarter of terrestrial biodiversity, with nearly half of the world's biodiversity hot spots concentrated in mountains.

Mountains are ecosystems extremely fragile because are subjected to both natural and anthropogenic drivers of change. Mountain biota are adapted to relatively narrow ranges of temperature (and hence altitude) and precipitation. Because of the sloping terrain and the relatively thin soils, the recovery of mountain ecosystems from disturbances is typically slow or does not occur.

Source: Millennium Ecosystem Assessment.

• Vegetation spatial pattern and plant interactions

The net outcome of plant interactions can be inferred from the species co-occurrences in space and time (Tirado and Pugnaire 2005). If two species appear together more than it could be expected by hazard then it is assumed that there might be a beneficial effect and the interaction between both species is considered positive, in the opposite way, if the two species appear segregated more than it could be expected hazardously, then a negative interaction between the two species is assumed (Kikvidze et al. 2005).

The abundance of positive or negative plant interactions in a community scale might vary under deferring environmental conditions, being positive interactions more common under stressful environments. In addition, positive plant interactions have been shown to favor plant diversity in stressful alpine environments because nurse plants often create microenvironments where species can establish (Cavieres et al. 2013). Following stress gradient hypothesis there might be more positive plant interactions under high environmental stress, i.e.: low temperatures at alpine ecosystems, therefore plant diversity might be favored grace to plant interactions.

Vegetation spatial patterns can theoretically come-out from self-organizing processes (Rietkerk et al. 2004) in which plant facilitation is one the main mechanisms (Kéfi et al. 2007b). Therefore, changing environmental conditions might affect the vegetation spatial pattern through the modification of the abundance of positive and negative plant interactions at a community scale.

- Plant performance indicators: Developmental instability (DI)

Developmental stability is the ability of an organism to produce a specific phenotype genetically determined under a certain environmental conditions (Moller and Shykoff 1999). Developmental instability is therefore the disruption of the correct development during ontogenesis produced by environmental stress. Traditionally DI has been used as index of stress (Moller and Swadel 1997, Polak 2003), due to being correlated to several biotic and abiotic stressors, including environmental factors such as interspecific competition (Komac and Alados, 2012), drought (Escós et al. 2000, Fair and Breshears 2005), high temperature (Llorens et al. 2002), elevation (Hagen et al. 2008), and anthropogenic activities, such as urbanization and pollution (Cuevas-Reyes et al. 2013, Velickovic and Savic 2012, respectively). DI is based on the hypothesis that as stress increases the ability of the plant developmental program to resist perturbations decreases

(Freeman et al. 2004). Under stressful conditions the mechanisms that are intended to insure the correct development are interrupted leading to developmental errors (Freeman et al. 2003). For those reasons, DI can be used to assess plant responses to a change in climatic conditions. DI can be used as well as index of species interactions given that competition or the lack of facilitation could suppose elevated stress for coexisting species.

The mechanisms of developmental stability are not well known yet. Developmental instability has some advantages over other performance indices such as growth, because gives information of the general state of the plant (Alados et al. 2006). While growth, focus on the organism ability of producing biomass, developmental instability inform about the environmental stress experienced by the plant (Polak 2003) . On the contrary, DI might have also some disadvantages too. DI is not expressed in every organism traits in a similar way. It depends upon the type of environmental stress and also on the relation of trait to plant fitness (Palmer 1994). Traits that are very important to organism fitness such as floral symmetry, might not vary under stress conditions, are much canalized. The opposite also occurs; traits that are not related at all to fitness might vary even under no stressful conditions. In this work we used several indices of DI: fluctuating asymmetry (FA), based on bilateral symmetry and translational asymmetry (TA), based on allometric relationship on plant traits (Graham et al. 2010).

• Introduction to study area and species

In this thesis plant interactions are studied as mechanism in vegetation succession of abandoned subalpine grasslands and the management strategies to control shrub expansion at the Central Pyrenees, Spain (Figure 3). The first shrub entering the abandoned subalpine grasslands is *Echinospartum horridum* (Vahl.) Rothm and the following shrubs is *Buxus sempervirens* L. Plant interactions as mechanism of vegetation pattern formation were studied at community scale in Sierra Nevada Mountain Range, Spain (Figure 3). The effect of a change in climatic conditions was evaluated in *Q. faginea*, *E. horridum* and *B. sempervirens* due to they are the mains actors of revegetation after land abandonment at the Central Pyrenees. All study sites are showed in the following map (Figure 3).

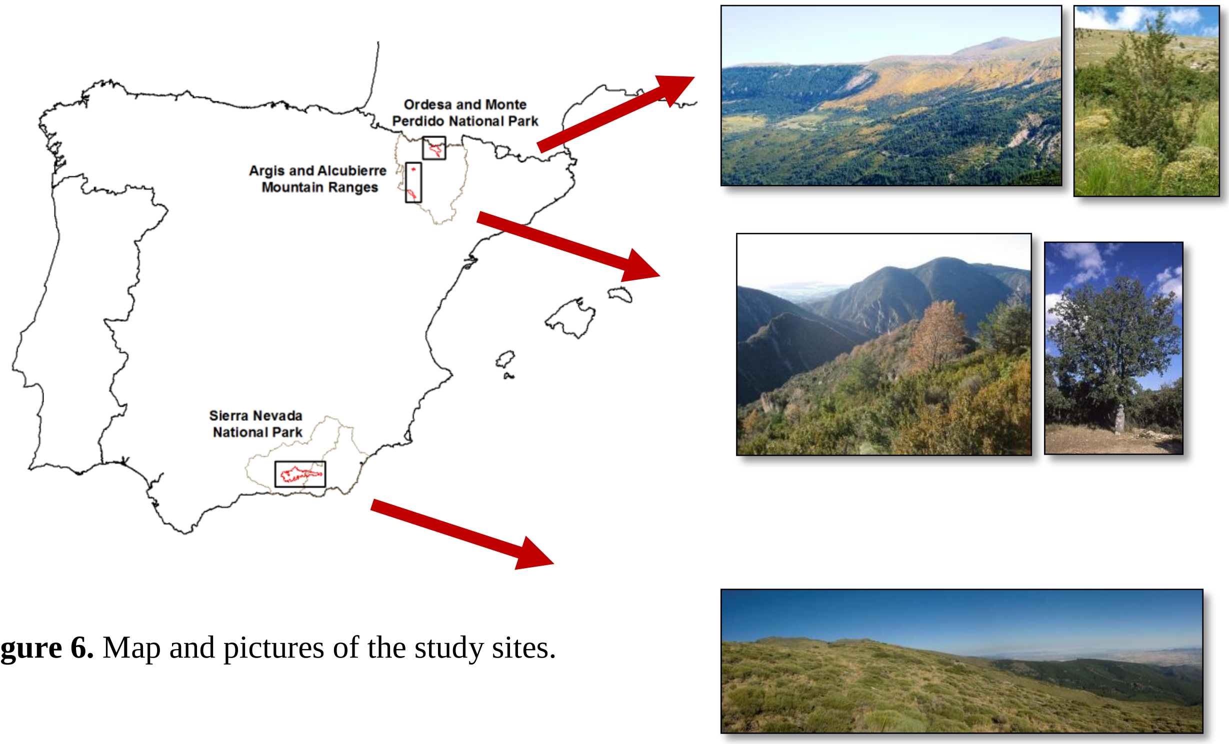


Figure 6. Map and pictures of the study sites.

At the Central Pyrenees, woody areas were deforested to being converted into productive lands over a wide range of altitude. At the peripheral mountain ranges on the Pyrenees, i.e. the Pre-Pyrenees, mixed forests, mainly formed by *Quercus faginea* Lam., were replaced by farming areas, especially for pastures and cereal farming in the years XX (Lasanta-Martínez 1989). At the subalpine belt similar process occurred, were original woodlands were replaced by farming lands. During second half of the 20st century, land use abandonment together with the reduction of pastoral pressure caused the revegetation of those lands (Lasanta and Vicente-Serrano 2007). At the Pre-Pyrenees, *Q. faginea* is now recolonizing old farming areas due to decrease in human density and land use abandonment but *Q. faginea* forests still have low extension (Kouba et al. 2010, Kouba and Alados 2014); and at high altitude at the subalpine belt at the Central Pyrenees, those lands are mainly colonized by *E. horridum* and *B. sempervirens* in calcicolous locations (Benito Alonso 2010), other shrubs such *Juniperus communis* L. colonizes preferentially the acid soils (Livinstone 1972).

The Mediterranean region is considered a hotspot of biodiversity (Meyers et al. 2000). Populations of *Q. faginea* are as well of special value of conservation because they host high plant diversity (Kouba et al. 2012, Maltez-Mouro et al. 2009, Rey Benayas et al. 2005). *Q. faginea* spread in the sub-Mediterranean region between the Mediterranean climate and the Eurosiberian climate, hence they might suffer the increased temperatures and drought events observed and predicted for the Mediterranean region (Giorgi and Lionello 2008). Their ability of occurs in this climate between Atlantic and Mediterranean might confer them the ability of adapt to changing climate conditions.

Quercus faginea Lam. is a winter-deciduous Mediterranean oak widely distributed in sub-Mediterranean areas of North Africa and the Iberian Peninsula, being dominant in NE Spain (Loidi and Herrera 1990). It is usually found in sites with base-rich soils with some precipitation during the summer period. Towards the north it tends to hybridize or be replaced by similar taxa such as *Q. cerrioides* Willk. and Costa and *Q. humilis* Miller. These three taxa co-exist in many sites in the N Pre-Pyrenees at mid to high elevations (Atlas de Flora de Aragón, 2005).

E. horridum and *B. sempervirens* coexistence is studied since many years (Montserrat and Montserrat 1984) because they are engineering species that from a mature ecosystem quite abundant in the Iberian Peninsula (Tena 2009), harboring several herbaceous plant species (Montserrat and Montserrat-Martí 1984). *E. horridum* can be also considered a foundation species because it define much of the structure of the ecosystem by creating local stable conditions for other species and by modulating fundamental ecosystem processes (Ellison et al. 2005). For those reasons, is of great importance to evaluate the possible impacts of current climate change on this ecosystem.

E. horridum is an evergreen thorny cushion plant (Palacio and Montserrat-Martí 2006), that develops at the Pyrenees and southern France (Villar et al.

1990). Is a strictly calcicolous (Aparicio et al. 2002) from relatively cold and humid sites with a short vegetative period (Camarero et al. 2012). It spread preferentially from 100 to 1700 meter a.s.l. although it can be found also from 390 to 2400 m a.s.l. (Marinas et al. 2004). *E. horridum* is usually located in south facing slopes and stony soils where there is not a lot of water availability (Montserrat and Montserrat-Martí 1984); water availability is not a limiting factor for it (Palacio 2006). *E. horridum* is highly invasive on the southern slopes, where it colonizes subalpine grasslands, one of the important ecosystems for plant diversity (Benito Alonso 2010). The dense *E. horridum* crown host many species of dry grasslands such as *Arenaria grandiflora* and many grasses, such as *Bromus erectus*, *Brachipodium pinnatum* and *Festuca rubra* (Montserrat and Montserrat-Martí 1984).

B. sempervirens is a broadleaf evergreen phanerophyte that can reach 5 m height, broadly distributed in the Mediterranean region and central Europe (Quézel and Médail 2003). In the Iberian Peninsula it spread preferentially from 400 meter a.s.l. to 1700 m a.s.l. (Tena 2009) although it can establish near the sea level (Iparragirre et al. 1985) and up to 2000 m.a.s.l. at the Pyrenees (according to P. Chouard in Lenoble and Boyer 1945).

Alpine vegetation in Sierra Nevada Mountain Range is subjected to two types of environmental constraints, one is the low temperatures and harsh climate with strong winds and high sun radiation characteristic of High Mountain and the second is the characteristic drought of Mediterranean climate. Therefore plant communities might respond to those combined stress effects in unexpected ways. The vegetation typically forms a patchy system were cushion shrubs are compact and prostrated. This morphology, also present in *E. horridum*, is characteristic of low temperature environments (Körner and Larcher 1998, Körner 2003), which appears to have evolved convergent across a wide range of plant families (Wang et al. 2004). Its major advantage is the high moisture and nutrient storage capacity (Agakhanyantz and Lopatin 1978, Ruthsatz 1978). Patchy vegetation occurs because plants tend aggregate forming a mosaic of vegetation and bare ground.

Objectives

The general objective of this thesis is to evaluate the main effects of global change on mountainous vegetation, including changes in human land uses and climatic change. I address the effect of human intervention on vegetation, the effect of climate on vegetation and the combined effect of anthropogenic activities and climate change.

Specifically, we addressed the following specific objectives and hypothesis:

1. Evaluate plant species interactions as mechanism of vegetation succession of subalpine grasslands at the Central Pyrenees after land abandonment and its variation under differing climates (Chapter 1). The following hypothesis are considered:
    i. H1.1: The net interaction between the two main shrubs encroaching the subalpine grasslands, *E. horridum* and *B. sempervirens*, will vary with the vital stage of *B. sempervirens* (seedling, juveniles and adults).
    ii. H1.2: The interaction between *B. sempervirens* and *E. horridum* vary with climatic conditions associated to the altitude (High site *vs* Low site).
    iii. H1.3: *E. horridum* facilitation effect occurs through the amelioration of physical environment (low temperatures) rather than improving soil conditions (soil nutrient concentration).

2. Evaluate the possible management strategies (burning *vs* clearing) to control *E. horridum* expansion into the abandoned subalpine grasslands at the Central Pyrenees, and the role of the soil seed bank (Chapter 2).
    i. H2.1: Prescribed burning might favor *E. horridum* seedling establishment rather than clearing because fire favor seeds germination of some Mediterranean shrubs species.

ii. H2.2: *E. horridum* removal treatment, burning *vs* clearing, would differently affect the soil seed bank species richness, diversity and dynamics.

iii. H2.3: Burning might alter the soil conditions (nutrients concentration and texture) more than clearing.

- *Appendix 4 Chapter 2*. Monitor the vegetation recovery and evaluate the role of plant species interactions on vegetation recovery after a fire perturbation in an *E. horridum* community (a preliminary study).

iv. H2.4: Species abundance would increase during succession.

v. H2.5: Plant interactions, specially positive interactions, would play a determinant role in vegetation recovery

3. Improve our understanding of how climate might affect ecosystem dynamics through it direct effect on population process and it indirect effect on species interactions (Chapter 3).

   i. H3.1: Plant interactions are a fundamental mechanism in vegetation succession of subalpine grasslands.

   ii. H3.2: Plant interactions play a determinant role mediating the vegetation succession dynamics under a climate change.

   iii. H3.3: Global warming might accelerate *B. sempervirens* expansion, which might lead to a dominance of *B. sempervirens* over *E. horridum*. Under low temperatures, *B. sempervirens* expansion would be slowed-down and *B. sempervirens* and *E. horridum* might coexist.

4. Estimate the variation of developmental instability (DI) in a Mediterranean oak (*Q. faginea*) at the Central Pre-Pyrenees along an altitudinal gradient (xeric, mesic and cold sites) in two climatically contrasting years (wet *vs* dry conditions); and assess the relationship between biomass production (shoot length) and DI (Chapter 4).

   i. H4.1: DI can be an index of species adaptation, i.e. individuals that are adapted to environmental stress (living in the xeric site) might have low DI values under stressful conditions (dry year).

   ii. H4.2: Under stressful environmental conditions energy allocation might be invested in maintain correct development (low DI values) rather than in biomass production, i.e. there is trade-of between maintenance of correct development and growth.

5. Investigate the relationship between biotic interactions and plant community structure (plant spatial pattern and diversity) in Mediterranean alpine ecosystems, and to disentangle how biotic interactions and plant community structure change in response to environmental conditions (Chapter 5).
   i. H5.1: There is a positive relationship between positive interactions and community structure (plant spatial aggregation and plant diversity).
   ii. H5.2: There might be more positive interactions under high levels of environmental stress, i.e. at north slopes than at south slopes due to low temperatures, which might lead to high plant diversity under stressful conditions; and in acid soils than in basic, due to harsh soil condition.

The present thesis is organized in chapters that correspond to original research articles that are published or are going to be published in indexed journals. For that reason they are written in English and each one have the appropriate structure with an introduction, material and methods, results and discussion.

The main structure of the thesis is summarized in the following figure:

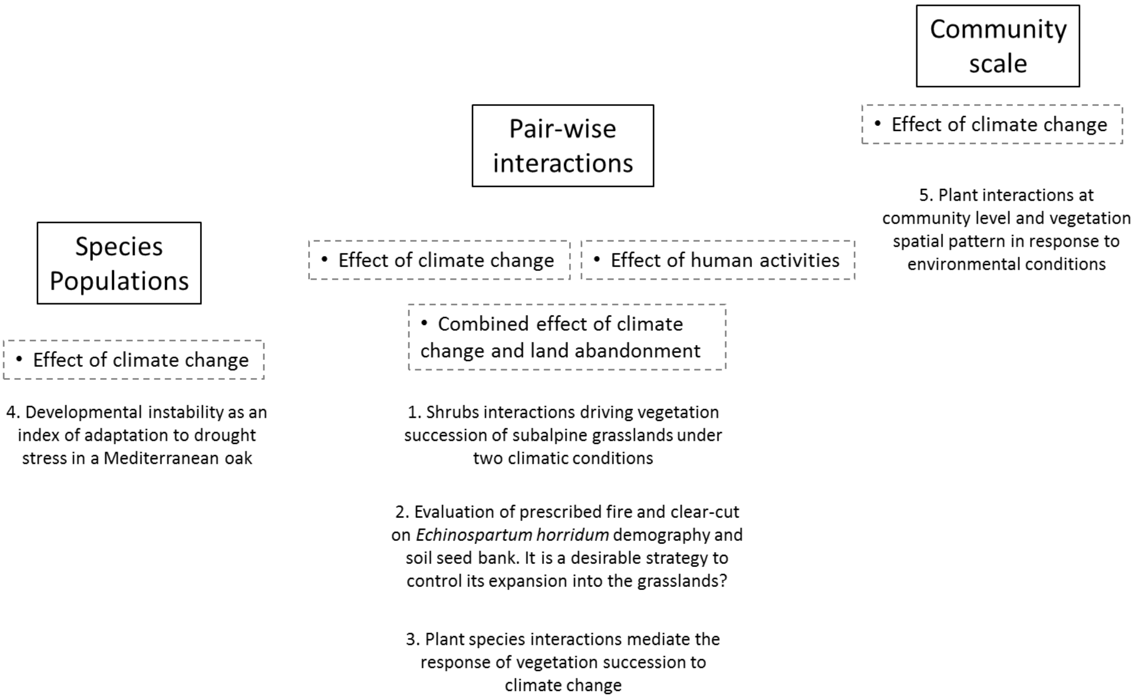


Figure 7. Scheme of the main chapters of the present thesis, the scale at which they operate and the drivers of ecosystems change that they address, climate change and human land use change.

- Outline of the thesis

Plant species do not live isolated in the ecosystem, every element of an ecosystem is contact and interacts with the others (Tylianakis et al. 2008). Therefore, I focus on plant species interactions as main mechanism that articulate the response of ecosystems to those drivers of change. I wonder how the direct effect of an ecosystem driver on a single species would affect other coexisting species, and therefore would have secondary effects on the whole community dynamics. In addition, the interaction between ecosystems drivers, in this case land abandonment and climate change, might modify vegetation dynamics. For that reason we monitored the response of two coexisting shrub species to a change in climatic conditions after land abandonment (Chapter 1). However, plant behavior in one or two years of study is not enough for predicting future community dynamics, especially when working with shrubs, therefore we used ecological modelling that allow to generate and test theories and hypotheses which are not possible with short term experiments (Park et al. 2015) (Chapter 3). We addressed as well possible strategies to face the consequences of these drivers of change, specifically on subalpine ecosystem. Grasslands conservation was assessed by means of investigating the possible strategies to control the expansion of the main colonizing subalpine shrub (Chapter 2). The effect of climate change was evaluated in a Mediterranean oak which is also involved in the natural vegetation processes after land abandonment at the Pyrenees (Chapter 4), and at a community scale in Sierra Nevada Mountain range (Chapter 5).

Chapter 1

Shrub interactions driving vegetation succession of subalpine grasslands under two climatic conditions

Paloma Nuche and Concepción L. Alados

Manuscript in revision in Journal of Plant Ecology.

Abstract

Introduction and Aims: The abandonment of agronomic practices in subalpine systems has led to shrub encroachment, which transformed grasslands into woodlands. The competitive and facilitative interactions among incoming shrub species might influence vegetation succession and might be affected by climate change. This study aimed to identify the nature of shrub species interactions between an early colonizer and a secondary succession shrub in the successional encroachment process and whether it differs between climates.

Methods: The interactions between the two main encroaching shrub species in the Central Pyrenees (Spain), *Buxus sempervirens* and *Echinospartum horridum*, were studied at the actual upper limit of altitudinal range of *B. sempervirens* where coexist with *E. horridum* (High site) and in a milder location at lower elevation (Low site). In the studied areas, *E. horridum* is the first shrub species that encroaches upon the abandoned grasslands and *B. sempervirens* enters the system after *E. horridum* has become established. A neighbor-removal experiment was used to assess the bidirectional interactions between *E. horridum* and *B. sempervirens,* and whether those interactions differed among the life stage of *B. sempervirens* (seedlings, juveniles, and adults), at the two study sites. Survival, annual crown growth and developmental instability (i.e., error in development caused by stress) (DI) were the performance indices used to quantify plant responses. Differences of mean air temperatures and soil nutrient amelioration were evaluated as possible mechanism of the facilitative effect of *E. horridum* on *B. sempervirens*.

Results and conclusions: E. horridum enhanced the survival of *B. sempervirens* seedlings under its crown, however, *B. sempervirens* adults outcompeted *E. horridum*. Therefore, the invasion of the grasslands by *E. horridum* facilitated the settlement and expansion of *B. sempervirens* and, hence, has promoted vegetation succession at the Pyrenees. In addition, climatic differences associated with elevation affected shrub interactions. Under low temperatures at the upper limit of altitudinal range of *B. sempervirens*, the presence of *E. horridum* enhanced the growth of *B. sempervirens* juveniles and at low elevation, under a milder climate, *B. sempervirens* exerted a high degree of competition toward *E. horridum*. An increase in temperatures associated to climate change would benefit *B. sempervirens* juveniles growth which might affect the successional process because it would increase their competitive ability when *B. sempervirens* juveniles become adults.

Key words: plant interactions; vegetation succession; climate change, subalpine ecosystems; developmental instability.

Introduction

The abandonment of agricultural practices in subalpine ecosystems is leading to shrub encroachment process that transforms grasslands into woodlands (McDonald et al. 2000, Gellrich et al. 2007, Gartzia et al. 2014). The interactions between the incoming shrubs species might influence the vegetation succession. Connell and Slatyer (1977) proposed three possible models to address autogenic succession. The first model included *facilitation* by 'early colonist' to the benefit of 'late succession species', which, once established, outcompete the former. The second and the third models included *no interaction* and *competition* by the 'early colonist' species to the 'late succession' ones, respectively. In addition, plant-plant interactions are a crucial component of the response of plant communities to climate change (Theurillat & Guisan 2001, Brooker 2006). However, studies in alpine ecosystems remain scarce (Anthelme et al., 2014), particularly in the context of succession (Barrio et al., 2013). To predict vegetation succession after land abandonment and identify the impacts of climate change, studies that assess species interactions under different climatic conditions are needed.

Positive interactions such as facilitation occur when one species enhances the survivorship, growth or fitness of another (Callaway 2007). The *Stress Gradient Hypothesis* (SGH) posits that positive interactions are common under high levels of environmental stress (Bertness and Callaway 1994; Callway and Walker 1997). The amelioration of the local microclimate by neighbors is one of the main direct mechanisms involved in positive plant-plant interactions (Callaway 2007). Low temperatures limit plant growth in alpine and subalpine ecosystems (Körner 2003) and cushion plants can act as nurse plants by creating a favorable the microenvironment under its crown (Cavieres et al., 2007). Global warming is expected to reduce temperature stress in alpine ecosystems (IPCC, 2013) and therefore reduce the positive effect of nurse plants as per the SGH. In a similar way, competitive interactions are expected to increase in alpine ecosystems following SGH. To date, however, evaluations of the SGH have been contradictory for various reasons, such as differences in species life-histories (Maestre 2009), variation on the performance variable used (Goldberg et al. 1999, Hastwell & Facelli 2003), and site-specific effects (Michalet et al., 2014).

The aim of our study was to investigate shrubs interaction between an 'early colonist' and a 'late succession' shrub, at various life stages of the ‘late succession’ species, as mechanism in vegetation succession of abandoned subalpine grasslands. We investigated the interaction between the two main shrubs species that have established in the semi-natural grasslands at the Central Pyrenees (Spain), *Echinospartum horridum* (Vahl) Rothm (an ‘early colonist’) and *Buxus sempervirens* L. (a ‘late succession colonist’), and whether climatic conditions might affect their interaction. Specifically, we aimed (1) assess the balance of interaction (competition *vs*. facilitation) between the two species at the life stages of *B. sempervirens* (seedling, juvenile and adult), (2) assess shrubs interactions at two locations which differ in environmental conditions: at the actual upper limit of altitudinal range of *B. sempervirens* (High site) and at a low elevation with a milder climate (Low site), and (3) evaluate the microclimate amelioration and soil nutrient amelioration as mechanism of interaction because *E. horridum* is a cushion-shaped leguminous N-fixing specie. To investigate the bidirectional interactions between those two shrubs we performed a reciprocal neighbor-removal experiment, at the two study sites, for *B. sempervirens* adults, juveniles and seedlings. We hypothesize that *E. horridum* promotes shrub encroachment because it enhances the survivorship of *B. sempervirens* seedlings, which, when they mature might outcompete *E. horridum*. We hypothesize as well that facilitation would predominate under stressful conditions for the facilitated seedlings and juveniles of *B. sempervirens* (at the High site) and that competition of *B. sempervirens* adults towards *E. horridum* would be more intense under less stressful conditions (at the Low site).

To evaluate plant response we used two common performance indicators, survival and growth (annual crown growth), and an integrative index of stress, Developmental Instability (DI). DI is an index of stress (Møller and Swadel 1997, Polak 2003, Beasley et al. 2013) because it is correlated with several abiotic stressors, including environmental factors such as drought and high temperature (Alados et al. 2001, Nuche et al 2014), as well as biotic stressors such as intra-specific competition (Komac and Alados 2012) and inter-specific competition and facilitation (Alados et al., 2006, Zvereva & Kozlov 2006). Under stress less energy is available for the correct functioning of organisms and the mechanisms intended to insure the proper development might be compromised, which leads to developmental errors (Freeman et al. 2003). In this study we used two indices of DI, fluctuating asymmetry (FA) and translational asymmetry (TA). Fluctuating asymmetry is the asymmetry of bilateral structures and translational asymmetry is the asymmetry in the allometric relationships among organisms' traits (Graham et al. 2010).

2. Materials and Methods

Study area and plant species

The study area included two sampling sites: at the upper limit of altitudinal range of *B. sempervirens* (High site at 1800 m) and at a milder location at lower elevation (Low site at 1400 m.a.s.l.) at the periphery of Ordesa and Monte Perdido National Park (PNOMP), in the Central Pyrenees, Spain (Table 1). The climate is Mediterranean mountainous and characterized by an equinoctial precipitation regime, cold winters and a short vegetative period. The average annual precipitation is 1758 mm (from the closet meteorological station "Refugio de Góriz"). Vegetation is a dense shrubland dominated by monospecific patches of *E. horridum* and scattered individuals of *B. sempervirens*. *Echinospartum horridum* is a thorny cushion chamaephyte endemic to the sub-Mediterranean territory of the Pyrenees. *Buxus sempervirens* is a broadleaf evergreen phanerophyte (up to 5 m high) widely distributed in the Mediterranean region and central Europe (Quézel and Médail 2003).

Field work and laboratory procedures

To quantify the effects of *E. horridum* on the life stages *B. sempervirens*, 36 units of coexisting adult *E. horridum* – adult *B. sempervirens*, 36 units of adult *E. horridum* – juvenile *B. sempervirens* and 36 units of adult *E. horridum* – seedling *B. sempervirens* were selected along two transects that were 10 m apart, at each study site (High and Low). Adult *E. horridum* were removed on half of the replicates (E. removal treatment, which had 9 replicates per vital stage on each transect; and Control, which had 9 replicates per vital stage on each transect, at each study site) (see Appendix B). *B. sempervirens* seedling were considered individuals smaller than 5 cm tall that were entirely below an *E. horridum* crown; juveniles were individuals between 20 and 90 cm tall growing inside a *E. horridum* but that had part of its crown out of it, and adults were individuals taller than 1m tall. None seedling showed evidence of grazing herbivores. To test the hypothesis of the competitive effect of *B. sempervirens* on *E. horridum*, 36 units of coexisting adult *B. sempervirens* - adult *E. horridum* and 36 units of coexisting juvenile *B. sempervirens* - adult *E. horridum* were selected along the former two transects, at each study site. On half of the units *B. sempervirens* adults were removed from the *E. horridum* neighborhood (B. removal treatment, which included 9 replicates per transect, and the Control, which had 9 replicates per transect, at each study site). The experiment was established in August 2010 and vegetation was surveyed at that time (before manipulations) and in August 2011. The survival and height of *B. sempervirens* seedlings, the crown size and DI of *B. sempervirens* juveniles and adults in the Control and in the E. removal treatment, and the crown size and DI of *E. horridum* adults in the Control and in the B. removal treatment were recorded. Mortality was only accounted when the seedling was found dead. Annual crown growth was quantified as the increase in crown size between the two years of study. Crown size was estimated based on the longest crown diameter and the diameter perpendicular to it. The crown height of *B. sempervirens* was also recorded. To quantify DI, we collected three annual shoots from each *B. sempervirens* juvenile and adult (in Control and E. removal treatment) and three annual spines from each *E. horridum* adult (in Control and B. removal treatment). An electronic caliper (resolution 0.01 mm) was used to measure the shoot length and the internode length from the base to the top of each shoot, to quantify translational asymmetry in *B. sempervirens*, and from the base to the thorny apex of opposite spines, which was used to calculate the fluctuating asymmetry in *E. horridum* (see Appendix C).

To test the hypothesis that *E. horridum* increases soil nutrient availability, soil samples were collected at the base of each *B. sempervirens* monitored: 9 replicates per vital stage and transect, at each treatment (Control and E. removal) and at each study site (High and Low). Soil samples were collected with a soil collector of 20cm in deep. The concentration (%) of the following nutrients were quantified in the soil samples: nitrogen (N), total carbon (C), organic carbon (Corg), organic matter (MO); pH and electrical conductivity (CE) were also quantified. After polishing soils samples, nitrogen and total carbon was quantified using an elemental analyzer, Variomax Elementar (DUMAS procedure); organic carbon was quantified with dichromate (Heanes 1984). Organic matter was quantified by incineration (at 550ºC). After sieve soil samples (2 mm mesh) texture was quantified (Malvern Mastersizer 2000).

Local environmental conditions

To assess the variation in the meteorological conditions within the elevation range, and to assess the crown effect of *E. horridum* within its local environment, we installed 4 air temperature and relative humidity sensors at the soil surface, under *E. horridum* crown (Control) and outside *E. horridum* crown (E. removal treatment) at each study site (a total of 8 sensors at each study site) (Table 1). Daily air temperature and relative humidity (± 0.5ºC) was recorded every 30 min from May 2011 to May 2013 using a Hobo U-23 pro v2 data logger (Onset Computer Co., Bourne, MA, USA). Photosinthetic Photon Flux (PPF, solar radiation from 400 to 700 nm) was measured in a clear sky day in August 2011 with a portable quantum meter (Apogee Instruments Inc., Logan, UT, USA) in Control and in E. removal treatment, in 15 *E. horridum* individuals as replicas at each study site.

Statistical analyses

Local environmental conditions

Temperature

To test for differences between local climate conditions under and outside *E. horridum* crown and between study sites, mean temperatures from data collected in the field were analyzed using linear mixed models (LMM). The growing season usually starts with snowmelt in April, and, even though growth can continue until the next winter, we focused on the period April - Jun as the most important period for plant growth. To test for differences in the local climate between study sites a linear mixed model (LMM) that had mean temperature (Apr-June) of the E. removal treatment as response variable. The factor Site, which accounted for differences between study sites (High *vs.* Low) were included as fixed factor. To test for differences in microclimate under and outside *E. horridum* crown, a linear mixed model with mean temperature (Apr-June) as response variable and Treatment as fixed factor (Control *vs.* E. removal) was performed at each study site, High and Low, separately. The factor Transect, which represented the replicates at Site level, and Year and Date because of temporal auto-correlation, were included as random factors in former models.

Solar radiation

To test for differences in solar radiation between study sites a general linear model (GLM) with the factor Site (High site *vs.* Low site) was performed with data of the E. removal treatment. The effect of the E. removal treatment in sun radiation was evaluated at each study site, High and Low, separately, with a GLM that included Treatment (Control *vs.* E. removal) as fixed factor.

Soil nutrients

To test for differences in soil nutrient content between study sites, a linear mixed model (LMM) was performed for each nutrient based on the data from the Control treatment. The factor Site was included in the model as fixed factor and Transect as random factor.

To test for differences in soil nutrient concentration between treatments (Control and E. removal) a linear mixed model was performed for each nutrient: nitrogen (%N), total carbon (%C), organic carbon (%C.org) and organic matter (%MO), pH and electrical conductivity (CE), for each vital stage (*B. sempervirens* juveniles and adults), at each study site (High and Low). The factor Treatment was included in the model as a fixed factor and Transect was included as random factor.

Seedling survival

To quantify the survival probability of *B. sempervirens* seedlings at each study site and in Control and E. removal treatment, a generalized linear mixed model (GLMM) with binomial distribution was used. The response variable 'seedling survival' had a value of 1 if the seedling survived from 2010 to 2011 and a value of 0 if the seedling was found dead in 2011. The factor Site, which accounted for variation between sites, and the factor Treatment, which accounted for the effect of the neighbor-removal experiment, were included as fixed factors. Transect, which represented the replicates at Site level, was included as random factor, and the variable 'seedling height' was included as a covariable. To test the effect of the variable 'seedling height' on the probability of seedling survival under different stressful conditions combination of the site and exposure, a generalized linear mixed model (binomial distribution) with seedling survival as response variable, seedling height as covariable and Transect as random factor was analyzed for each combination of Site and Treatment (High site - Control, High site - E. removal, Low site - Control and Low site - E. removal).

Annual Crown growth

Based on the assumption that *E. horridum* individuals are ellipsoidal, the crown size of *E. horridum* was estimated with the area of ellipse based on the longest diameter, D, and its perpendicular, d:

$$Area_E = \frac{D}{2} * \frac{d}{2} * \pi \qquad (1)$$

The crown size of *B. sempervirens* was calculated based on cylinder volume (Azmi et al., 1991, Robles et al., 2002) with the longest diameter, D, its perpendicular, d, and the height, h:

$$Volume_B = \pi * \left(\frac{(D+d)}{2}\right)^2 * h \qquad (2)$$

The annual crown growth of the two shrub species was calculated as follows:

$$annual\ crown\ growth = \frac{(Crown\ Size_{2011} - Crown\ Size_{2010})}{Crown\ Size_{2010}} \qquad (3)$$

Cylinder volume and ellipse area were used for calculating crown size for each species *B. sempervirens* and *E. horridum* respectively, due to species specific crown shape.

To test the effect of elevation on *B. sempervirens* (juveniles and adults) and on *E. horridum* performance a linear mixed model (LMM) that included annual crown growth as the response variable was performed for each study species. Site and Transect were included as fixed and random factors, respectively. To test the effect of *E. horridum* on *B. sempervirens* (juveniles and adults) performance and the effect of *B. sempervirens* adults on *E. horridum* performance, a linear mixed model that included annual crown growth as the response variable was performed for each study site (High and Low) and each study specie. Treatment and Transect were included as fixed and random factor, respectively. We expected that adult and juvenile *B. sempervirens* would differ their response to climatic conditions and in the degree of interaction with *E. horridum*; therefore, each life stage was evaluated individually.

Developmental Instability (DI)

Fluctuating Asymmetry (FA) was measured in *E. horridum* spines based on the random departure from bilateral symmetry (Palmer and Strobeck 1986). To assess the relationship between asymmetry and size we tested the correlation between absolute fluctuating asymmetry, $|L\text{-}R|$, and the sum of both spines, $(L+R)$, which was highly significant (r=0.18, p<0.0001); therefore, the raw data were transformed using logarithm, $|\ln L_i - \ln R_i|$ (Palmer and Strobeck 2003). To deal with half normal distribution, we applied the Box-Cox transformation (following Swaddle et al. 1994, Graham et al. 1998, and Freeman et al. 2004). We used *FA* as an index of developmental instability. High *FA* value indicates high DI.

$$FA = \left(\left|\ln L_i - \ln R_i\right| + 0.00005\right)^{0.33} \qquad (4)$$

To test the effect of altitude on *E. horridum* DI, we used data from *E. horridum* individuals that coexisted with *B. sempervirens* juveniles in a linear mixed model (LMM) that included FA as the response variable. Site was included as fixed factor and Transect, Individual and Shoot as random factors. To test the effect of *B. sempervirens* adults on *E. horridum* DI a linear mixed model that had FA as the response variable, Treatment as fixed factor and Transect and Individual as random factors was performed, at each study site.

Translational Asymmetry (TA) was estimated in *B. sempervirens* shoots based on the error in the following curve-fitting equation:

$$L(N) = kN^{a} e^{-bN} \qquad (5)$$

where *L* is the internode length, *N* is the internode order (measured from the bottom to the top, see Appendix A), *e* is the natural base, and *k*, *a*, and *b* are the fitted parameters (Alados et al. 1998, 2006, Freeman et al. 2004, Tan-Kirstanto et al. 2003). Curve-fitting accuracy was calculated after log-log linearization and posterior linear regression adjustments of each sample. (1 - R^2, the coefficient of determination) was used as a *TA* index, a measure of the degree of developmental instability. High *TA* value indicates high DI.

To test the effect of elevation on *B. sempervirens* DI, a linear mixed model (LMM) that had TA as the response variable, Site as fixed factor and Transect, Individual and Shoot as random factors was performed. To test the effect of *E. horridum* on *B. sempervirens* DI a linear mixed model that had TA as the response variable, Treatment as fixed factor and Transect and Individual as random factors was performed, at each study site. We expected that adult and juvenile *B. sempervirens* would differ in their response to elevation and in the degree of interaction with *E. horridum*; therefore, each life stage was evaluated individually. ‘Annual shoot length’ was included as covariable in every model to avoid a size effect.

Models were selected based on Akaike Information Criterion (AIC) (Zuur et al., 2009). The parameter estimations were made using the Restricted Maximum-Likelihood Estimation Method (REML) in the nmle package (Pinheiro et al, 2014) for the R 2.15.3 software (R Development Core Team), with the probability of statistical significance set to 0.05 (type I).

Results

Local environmental conditions

Temperature

Mean temperatures were lower at the High site than at the Low site during period months (Apr-Jun) (Table 1, Table 2). In that period (Apr-Jun) at both sites, mean air temperatures were lower outside *E. horridum* crowns (E. removal treatment) than they were under *E. horridum* crowns (Control) (Table 1, Table 2). The lowest temperatures occurred were at the High site in the E. removal treatment (Table 1).

Solar radiation

Levels of solar radiation were highest at the High site (Table 1, Table 2). In addition, the presence of *E. horridum* crowns diminish significantly the irradiance levels beneath them at both sites (Table 1, Table 2).

Soil nutrients

The soil nutrients concentrations did not differ significantly between study sites: Nitrogen ($F_{1,33}$=1.24, p=0.27), organic carbon ($F_{1,33}$=0.590, p=0.44), total carbon ($F_{1,33}$=0.079, p=0.77), C/N ($F_{1,33}$=0.0048, p=0.94), MO ($F_{1,33}$=0.12, p=0.72), pH ($F_{1,33}$=0.36, p=0.54), CE ($F_{1,33}$=0.13, p=0.71).

At the High site, soil nutrient concentrations, pH and electrical conductivity did not differ significantly between treatments (Control *vs.* E. removal); however, at the Low site, all nutrients concentrations were significantly lower in the E. removal treatment than they were in Control (Table 3, Fig. 3).

B. sempervirens response to *E. horridum* removal

B. sempervirens *Seedling Survival*

The survival of *B. sempervirens* seedlings was significantly higher under *E. horridum* crown (Control) than it was outside (in E. removal treatment) (z=8.03, p=0.02; Fig. 3). Seedling survival did not differ significantly between sites (z=0.27, p=0.52). Seedling height and survival were positively correlated (z=8.93, p=0.002). At the High site in E. removal treatment seedling height had a significant positive effect on seedling survival (z=2.033, p=0.04). In the E. removal treatment at the Low site, the effect was marginal (z=1.875, p=0.06). In the Controls at the High and Low sites seedling height and seedling survival there were not significantly correlated (z=0.573, p=0.56; z=0.970, p=0.33, respectively).

Annual Crown Growth of B. sempervirens *juveniles and adults*

Annual crown growth of juvenile *B. sempervirens* did not differ significantly between Sites ($F_{1,68}$=0.7737, p=0.47). At the High site juveniles' annual crown growth was significantly higher in Control than in the E. removal treatment, and a similar but non-significant trend was apparent at the Low site (Table 4; Fig. 4).

Annual crown growth of adult *B. sempervirens* did not differ significantly between sites ($F_{1,68}$=0.6925, p=0.49), and was not affected by the removal of *E. horridum* ($F_{1,33}$=2.109, p=0.15 at the High site, and $F_{1,33}$=0.4945, p=0.48 at the Low site).

Developmental Instability in B. sempervirens

Developmental instability of juvenile and adult of *B. sempervirens* did not significantly differ between the High and Low sites ($F_{1,135}$=3.34, p=0.21 and $F_{1,210}$=0.384, p=0.59, respectively) or between treatments (High site: $F_{1,105}$=0.632, p=0.43; Low site: $F_{1,105}$=0.0299, p=0.86 for adults; see for juveniles Table 4).

E. horridum *response to* B. sempervirens *removal*

E. horridum *Annual Crown Growth*

E. horridum had a non-significant tendency of having higher annual crown growth at the High site than at the Low site in individuals that were coexisting with *B. sempervirens* juveniles ($F_{1,32}$=3.44, p=0.07, Fig. 5a).

At the Low site, the growth rate of *E. horridum* was significantly higher in B. removal treatment than it was in the Control (Table 4, Fig. 5b), and a similar but non-significant trend was apparent at the High site.

Developmental Instability in E. horridum

Developmental instability of *E. horridum* that were coexisting with *B. sempervirens* juveniles did not differ between the High and the Low sites ($F_{1,178}$=6.15, p=0.13, Fig. 4a).

At High site developmental instability of *E. horridum* was marginally higher if they were coexisting with *B. sempervirens* adults in Control than if they were in the B. removal treatment (Table 4; Fig. 5c), and a similar but non-significant trend was apparent at the Low site.

Discussion

E. horridum enhanced the survival of *B. sempervirens* seedlings under its crown, and thus, it had a facilitative effect on the establishment of *B. sempervirens* seedlings. However, *B. sempervirens* that survive to maturity outcompeted *E. horridum*. The invasion of the grasslands by *E. horridum* facilitated the settlement and expansion of *B. sempervirens* and, hence, has promoted vegetation succession at the Pyrenees. In addition, climate differences associated with elevation affected facilitation and competitive interactions. In the low-temperature climate at the actual upper limit of the elevational range of *B. sempervirens, E. horridum* had a facilitative effect in increasing the growth of juvenile *B. sempervirens* beneath its crown. At the lower elevation, where climate was milder, adult *B. sempervirens* applied higher degree of competition towards *E. horridum*. An increase in temperatures associated with climate change would benefit the growth of juvenile *B. sempervirens*, which it might affect the successional process by increasing their competitive ability once those juveniles become adults.

The microclimate created by *E. horridum* was more important to the survival of *B. sempervirens* seedlings than was climate associated with the elevation. *B. sempervirens* survival was similar at both altitudes, therefore, seedling facilitation under warming temperatures fail to be reduced, as also reported by other authors (Cavieres and Sierra-Almeida, 2012). The microhabitat created by *E. horridum* crowns, where mean air temperatures and air humidity are higher, and levels of irradiance are lower than outside the crown, enhanced the survival of *B. sempervirens* seedlings. The cushion-like morphology of *E. horridum*, with short stature and compact form, acts as an efficient heat trap (Körner & De Moraes 1979) which also reduces loss of soil moisture (Cavieres et al. 2007). In addition, *B. sempervirens* is a shade-tolerant species that grows in the understory of dense forests (García-Plazaola 2008). That shade-tolerance trait probably makes the seedlings sensitive to high irradiance and allows them to develop under *E. horridum* crown. Despite its shade tolerance, *B. sempervirens* also occurs in areas that are exposed to high irradiance (García-Plazaola 2008), such as the tree line ecotone in our study site. Hormaetxe et al. (2005) demonstrated that in *B. sempervirens* leaves exposed to sun, pools of α-tocopherol, a photoprotective molecule, increased with leaf age. That might explain why seedling height increased the survival provability of *B. sempervirens* seedlings if they were not covered by *E. horridum* crown at the High site, which indicated that small seedlings are most vulnerable under the harsh conditions combination of high altitude and exposure.

The multiple effects that *E. horridum* exerts on its microenvironment prevented us to identifying the primary specific factor that dictates seedling survival; nevertheless, the net effect that *E. horridum* has on the survival of *B. sempervirens* seedling is manifest. The facilitation effect of *E. horridum* towards *B. sempervirens* seedlings is a mechanism in vegetation succession although its effects do not seem to vary with climatic conditions. In our study, however, there is still a 38% (on average between High and Low sites) of seedlings that did not have the protection of *E. horridum* survived, which suggests that the presence of *E. horridum* might accelerate the expansion rate of *B. sempervirens*, although *B. sempervirens* expansion might not be limited to stands in which *E. horridum* is not present.

Low temperatures negatively affected the growth of *B. sempervirens* juveniles at the High site, which was at the actual upper limit of altitudinal range of the species in the Lomar Valley, Central Pyrenees. The removal of *E. horridum* left juveniles exposed to lower temperatures than at the Low site during growing period (April-June), consequently the annual crown growth of juvenile *B. sempervirens* at the High site was reduced. At the highest elevation under low temperatures, *E. horridum* facilitated juvenile *B. sempervirens*, which can be explained by SGH. A decrease in air temperature diminishes rates of photosynthesis (Berry & Björkman, 1980, Sendall et al., 2015) and therefore C fixation and growth. An increase in rates of photosynthesis are not limited by water availability under *E. horridum* crown due to air humidity is higher than it is outside its crown. The milder climate at the Low site did not reduced juveniles growth after *E. horridum* was removed despite a reduction in soil nutrient concentrations. An increase of air temperatures is one of the main mechanisms by which nurse plants facilitate other plant species in alpine environments (Arroyo et al. 2003, Cavieres et al. 2007), which can be explained by the hypothesis that facilitation in alpine and subalpine ecosystems is principally originated by stresses that are not directly related to resource availability (Maestre et al., 2009, Anthelme et al. 2014).

In the Central Pyrenees, the developmental instability of *B. sempervirens* adults and juveniles did not vary significantly across the altitudinal gradient of study, which suggests that despite the lower temperatures of the High site *B. sempervirens* would be able to withstand high altitude environmental conditions. At the Pyrenees, the subalpine grasslands replaced the native forest to be used for agricultural activities during the last 500 years (Montserrat and Fillat 1990). Therefore, even though *B. sempervirens* juveniles have reduced growth when they are not facilitated by *E. horridum* the actual upper limit of *B. sempervirens* altitudinal range might not be its climatic limit.

The neighbor-removal experiment in our study showed that *B. sempervirens* outcompeted *E. horridum* at the Low site, where *E. horridum* had the lowest performance (reduced growth and high DI). *E. horridum* has narrow and v-shaped leaves, which is typical of plant species that live in cold environments (Körner 2003). The disadvantage of that morphotype is the poor light-harvesting potential caused by low leaf area (Körner & De Moraes 1979), which might be the reason why *E. horridum* was outcompeted by *B. sempervirens*, a taller shrub which creates a dense shade, especially at the Low site, where irradiance levels were lower. We do not know, however, the role that phenolic compounds of *B. sempervirens* leaves might play in this competitive interaction.

Developmental instability measurements did not show a significant effect in the neighbor removal treatment in *B. sempervirens* juveniles either in adults. This could be explained because the effect of *E. horridum* to *B. sempervirens* adults was not strong, as also observed in annual crown growth indicator. The DI effect observed in B. removal treatment in *E. horridum* probably would have been more apparent later, when the effect of the removal treatment would have persisted longer (Zvereva & Kozlov 2006). However, the large samples sizes needed for DI analyses prevented us from documenting DI in several consecutive years. The effect of the B. removal in *E. horridum* DI was more significant at the High site than it was at the Low site, whereas the effect on *E. horridum* annual crown growth was strongest at the Low site. This difference might be because the effect of the B. removal was obscured by the higher DI at the Low site.

Climate associated to elevation influenced the effect that *E. horridum* has had on soil nutrient availability. At the High site, the removal of *E. horridum* did not affect soil nutrient concentrations, however, at the Low site, the removal of *E. horridum* did significantly reduced the concentration of soil nutrients. The removal of *E. horridum* might have diminished the input that it could have provided to the soil through N fixation and litter deposition. At the Low site, where the temperatures were highest, nutrient cycling might have been accelerated (Bier et al., 2008) and, consequently, soil nutrient concentration reduced.

We conclude that *E. horridum* promotes shrub encroachment by favoring the survival of secondary successional species such as *B. sempervirens*. We would expect the competitive displacement (sensu Walter & Del Moral 2003) of *E. horridum* by *B. sempervirens* in the valley of the study area. In addition, rising temperatures associated to climate change can have a beneficial effect on *B. sempervirens* juveniles growth and increase the competitive ability of *B. sempervirens*, which would increase the negative effect to *E. horridum*, and might accelerate the rate of succession.

Acknowledgements

We gratefully acknowledge the financial support of the Spanish Economy and Competition Ministry (PN-MICINN) (CGL2008-00655/BOS and CGL2011-27259) and Spanish National Park organization (DIPA 125/2010 MMAMRM). We thank the AEMET for providing meteorological information, Bruce MacWhirter.

Table 1. Annual and April to June average of daily mean temperatures (ºC), annual and warm months (April to September) daily mean air relative humidity (%), and solar radiation in Control and in E. removal treatment at each study site (High and Low) in the Central Pyrenees, Spain.

	Air Temperature (ºC)			**Air Relative Humidity (%)**			**Solar radiation (μmol · m^{-2} · s^{-1})**	
	Annual mean	April – Jun mean		Annual mean	Apr-Sep mean			
		Control	E. removal		Control	E. removal	Control	E. removal
High site **(1800 m.a.s.l)**	7.38	11.86 ± 0.22	9.74 ± 0.11	71.32	76.88	71.32	8.4 ± 2.22	1746 ± 64.29
Low site **(1400 m.a.s.l.)**	8.43	13.27 ± 0.19	11.16 ± 0.14	75.61	79.87	75.61	2.2 ± 0.54	1238.8 ± 47.00

Table 2. *F* and *p* values of the linear mixed models for environmental variables: mean temperature (ºC) (Apr-Jun) and solar radiation (µE$m^{-2}s^{-1}$). Differences between sites were tested for the E. removal treatment, and differences between treatments (Control *vs*. E. removal) were tested at each study site, High and Low, in the Central Pyrenees, Spain.

Response variable	**Fixed factor**		*F value (d.f.)*	*p value*
Mean temperature	Site		41.14 (2, 387)	<0.0001
	Treatment (Control *vs*. E. removal)	High site	149.67 (1, 7279)	<0.0001
		Low site	740.21 (1, 6774)	<0.0001
Solar radiation	Site		19.79 (2, 32)	<0.0001
	Treatment (Control *vs*. E. removal)	High site	729.55 (1, 28)	<0.0001
		Low site	692 (1, 28)	<0.0001

Table 3. *F* and *p* values of the linear mixed models for studied soil nutrients: nitrogen, organic carbon, total carbon, C/N, organic matter, pH and electrical conductivity (CE), for *B. sempervirens* adults and juveniles, at each study site, High and Low, in the Central Pyrenees, Spain. Statistically significant differences are in bold.

Site	**Fixed factor**	**Response variable**	***B. sempervirens* Juveniles**		***B. sempervirens* Adults**	
			F value (d.f.)	*p value*	*F value (d.f.)*	*p value*
High	Treatment (Control vs. E. removal)	% N	0.313 (1,32)	0.579	1.249 (1,29)	0.272
		% C organic	0.365 (1,32)	0.549	1.027 (1,29)	0.319
		% C total	0.227 (1,31)	0.637	1.446 (1,29)	0.238
		C/N	0.046 (1,31)	0.830	2.073 (1,29)	0.160
		MO	0.374 (1,32)	0.544	---	---
		pH	0.532 (1,32)	0.470	0.212 (1,29)	0.650
		CE	0.464 (1,32)	0.500	2.865 (1,29)	0.106
Low	Treatment (Control vs. E. removal)	% N	4.464 (1,31)	**0.042**	18.815 (1,29)	**<0.001**
		% C organic	10.759 (1,31)	**0.002**	6.841 (1,31)	**0.013**
		% C total	7.228 (1,31)	**0.011**	16.690 (1,29)	**<0.001**
		C/N	9.174 (1,32)	**0.004**	6.121 (1,31)	**0.019**
		MO	4.973 (1,32)	**0.032**	---	**---**
		pH	1.860 (1,32)	0.182	3.256 (1,28)	0.081
		CE	0.889 (1,32)	0.353	0.879 (1,28)	0.356

Table 4. *F* and *p* values of the linear mixed models of species interaction indicators, annual crown growth and developmental instability (DI). Differences between treatments (Control vs. E. removal / B. removal) at each study site (High and Low) was evaluated, for *B. sempervirens* juveniles, and *E. horridum*. Statistically significant differences are in bold.

Response variable	**Fixed factor**	**Site**	***B. sempervirens* juveniles**		***E. horridum* (coexisting with *B. sempervirens* adults)**	
			F value (d.f.)	*p value*	*F value (d.f.)*	*p value*
Annual Crown Growth	Treatment (Control *vs.* Removal)	High	8.00 (1,33)	**0.0079**	1.515 (1,33)	0.2271
		Low	0.621 (1,33)	0.4362	6.481 (1,31)	**0.0161**
DI	Treatment (Control *vs.* Removal)	High	0.275 (1,61)	0.6038	3.886 (1,64)	**0.050**
		Low	1.488 (1,74)	0.2317	0.768 (1,67)	0.3839

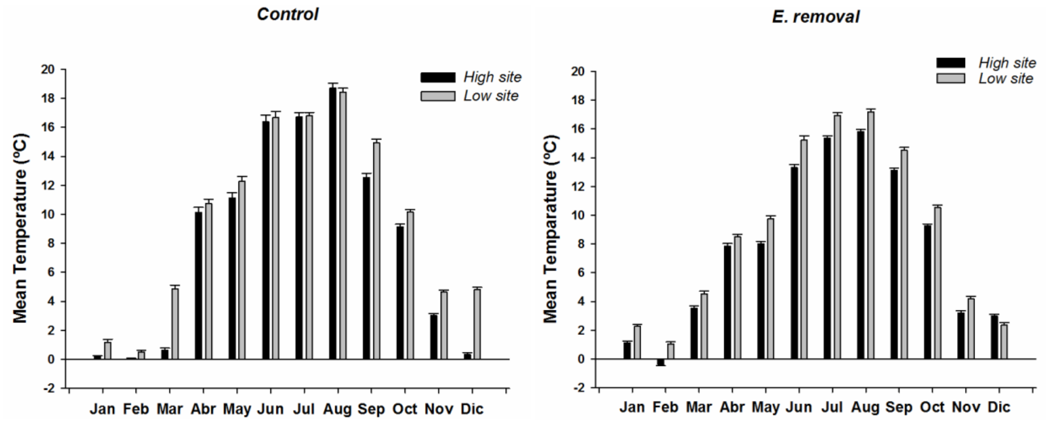


Figure 1. Monthly mean temperature (ºC) under *E. horridum* crown (Control) (a) and outside *E. horridum* crown (E. removal treatment) (b), at each study site (High and Low), within an elevation range in the Central Pyrenees, Spain.

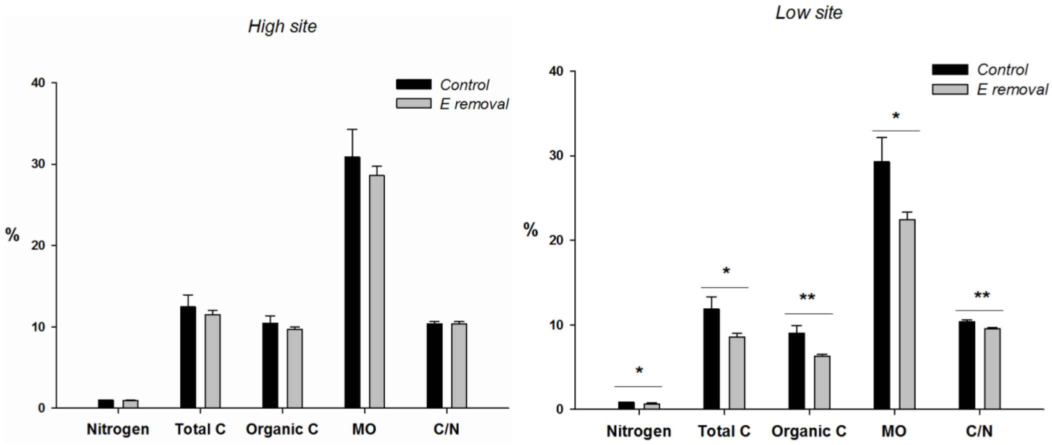


Figure 2. Concentration (%) of soil nutrients: nitrogen, total carbon (Total C), organic carbon (Organic C), organic matter (MO) and ration carbon/nitrogen (C/N), under *E. horridum* crown (Control) and where *E. horridum* had been removed (E. removal) form *B. sempervirens* juveniles neighborhood, at each study site, High (a) and Low (b), within an elevation range at the Central Pyrenees, Spain. *p<0.05, **p<0.01.

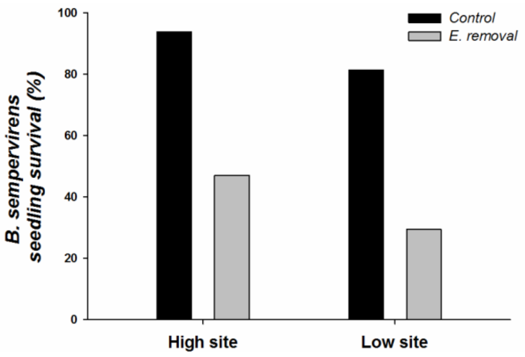


Figure 3. Seedlings survival (%) of *B. sempervirens* beneath the *E. horridum* crown (Control) and those grown where *E. horridum* have been removed (E. removal), at each study site (High and Low) within an elevation range in the Central Pyrenees, Spain.

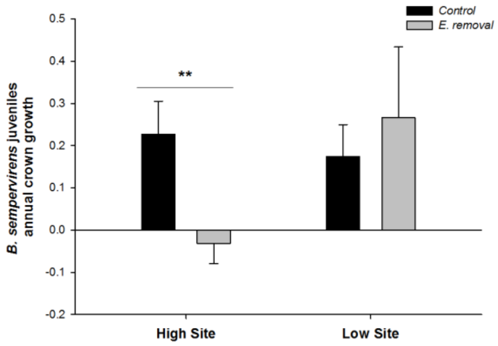


Figure 4. Annual crown growth of juvenile *B. sempervirens* under *E. horridum* crown (Control) and where *E. horridum* had been removed (E. removal), at each study site (High and Low) within an elevation range at the Central Pyrenees, Spain.

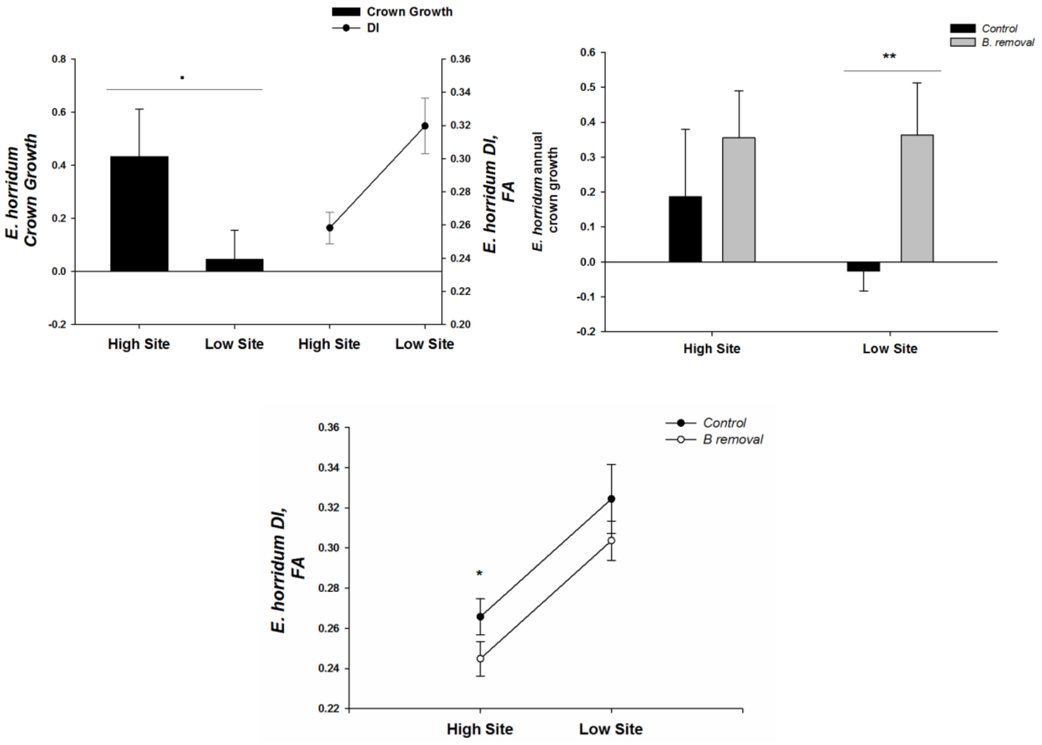


Figure 5. (a) Annual crown growth and developmental instability (DI) of *E. horridum* that coexisted with juveniles *B. sempervirens* (Control), (b) *E. horridum* annual crown growth when coexisting with adults *B. sempervirens* (Control) and when *B. sempervirens* adults had been removed (B. removal), and (c) *E. horridum* developmental instability (FA) in Control and B. removal treatment, at each study site (High and Low) within an elevation range at the Central Pyrenees, Spain.

Chapter 2

Effects of prescribed burning and clearcutting on *Echinospartum horridum* demography and soil seed bank. Are they appropriate for controlling the expansion of *E. horridum* into grasslands?

Paloma Nuche, Benjamin Komac, Maite Gartzia, Ramón Reiné and Concepción L. Alados

Abstract

Aims: The subalpine grasslands ecosystems have some of the highest biodiversity in Europe and are high-value natural resources. Those grasslands have been under threat because of the abandonment of traditional agro-pastoral activities and consequent invasion of woody species. At the Central Pyrenees (Spain) a variety of management strategies are carried out to stop the expansion of the highly encroaching shrub *Echinospartum horridum*, however, how these managements affect the recovery of subalpine grasslands is poorly understood.

Methods: This study evaluated controlled fires and mechanical removal of aerial vegetation as management procedures for controlling the expansion of *E. horridum* into subalpine grasslands at the Central Pyrenees, and the role of the soil seed bank. *E. horridum* demography (germination and survival) was monitored annually for three years; and the soil seed bank and the soil properties were monitored in two trials, in two *E. horridum* stands where the vegetation was previously removed by (1) fire (Burnt treatment), or by (2) mechanical removal (Clear-cut treatment); an undisturbed *E. horridum* stand was also monitored as the Control.

Results: The Burnt treatment favored germination and survival of *E. horridum* seedlings, Clear-cut treatment seems to be a better option because *E. horridum* seedlings fared worse (fewer germination and lower probability of survival) than did those in Burnt treatment. Both management treatments increased the seed density in the soil compared to Control, *E. horridum* removal favor that seeds that arrived by dispersion became established in the soil. The soil seed bank in the Burnt treatment had higher seed abundance, higher seed diversity and was more dynamic than was the Clear-cut treatment because burning entails a deeper soil perturbation. However, fire entails the loss of soil nutrients.

Conclusions: Both treatments favored the formation of a soil seed bank comprised herbs and grasses typical from subalpine grasslands; however, the soil seed bank would have limited capacity on its own to recover the former subalpine grassland because of the low species richness and the low seed abundance. We would recommend an integrated strategy that includes traditional shepherding, which favor seed dispersion.

Key words: subalpine grasslands, shrub encroachment, conservation management, soil seed bank.

1. Introduction

The abandonment of traditional agro-pastoral activities in the subalpine grasslands ecosystems has brought to the invasions of woody species, a process called shrub encroachment (McDonald et al. 2000, Sabatini et al. 2003, Lasanta-Martínez et al, 2005), which had led to loss of biodiversity and ecosystem services (Harrison et al. 2010, Ratajczak et al. 2012, Hooftman and Bullock 2012). Consequently, there has been a general interest in conservation these species-rich grasslands. In Europe, mountainous grasslands below the potential tree line were created by the removal of woody species and were maintained grace to the traditional practices such as livestock grazing and woody plant removal by fire or clearing (Montserrat and Fillat 1990, Mather and Fairbairn 2000, Savulescu and Mihai 2011, When et al. 2011). To revert woody encroachment similar traditional procedures should be used. The success of the management treatment would depend on the reproductive biology of the shrubs involved, e.g. clearcutting will not remove a resprouting shrub species completely nor will burning remove pyrophytes. In addition, the soil seed bank is vital to the reproductive success of a plant species and can be an important source for re-establishing aboveground vegetation (Leck 1989), whether it is desirable grasslands or undesirable shrubland. Therefore, the soil seed bank influences the restoration and persistence of grasslands.

The soil seed bank can contribute to the recovery of plant communities through seedling recruitment from germinated seeds that were produced by the local vegetation, from nearby locations, and even seeds from former plant communities that may be still present in the soil (Matus et al. 2003, Bossuyt and Honnay 2008). Shrub management treatments affect the soil seed bank in different ways: it can favor the germination of seeds already presents in the soil (Rivas et al. 2006), and it can favor the recharge of the soil seed bank given that the soil might be more accessible to seeds after the perturbation (Fernández et al. 2013). The removal of shrubs can favor the establishment of various species into the soil seed bank, which might be desirable grasses and herbs that are characteristic of the subalpine grasslands, could be undesirable ruderal species, which often appear in disturbed habitats (Grime 1974), or the shrub species that is being removed from the area (Potts et al. 2010). Abundance, diversity and temporal dynamics of the soil seed bank can be differently modified by burning or clearing. The removal of aerial vegetation through clearcutting can increase the amount of sunlight reach the soil surface, which can affect the soil seed bank. The effect of fire on the soil seed bank depends on the species germination cues (Leck 1989). Burning can affect abiotic factors involved in germination signaling, e.g. soil thermal and moisture regimes (Kenny 2000, Thomas et al. 2003); besides, the heat may destroy seeds or favor seeds germination depending on the species and on the temperature (de Luis 2005).

At the Central Pyrenees 24% of the subalpine grasslands were transformed to woody species under 2100 m.a.s.l. between 1980’s and 2000’s (Gartzia et al. 2014). The main shrub entering the calcareous grasslands is *Echinospartum horridum* (Vahl) Rothm. Two strategies to stop *E. horridum* expansion were considered based on traditional management practices: burning and clearing. Although information on different aspects of the environmental effects of burning and clearing has been reported in recent years (Fernández et al. 2015), the consequences of both management strategies need to be evaluated as optimal strategy to stop *E. horridum* expansion.

The main objective was to evaluate prescribed burning and mechanical removal of aerial vegetation as management procedures for controlling the expansion of *E. horridum* in subalpine grasslands in the Central Pyrenees (Spain), and their effect on the soil seed bank. Specifically we investigated the capacity of *E. horridum* to recover following a perturbation (fire or mechanical removal) and the effects of *E. horridum* removal on the soil seed bank and soil properties. *E. horridum* demography (annual germination and survival), the soil seed bank and the soil properties were monitored in two *E. horridum* stands where vegetation was previously removed by (1) fire (Burnt treatment) or (2) aerial vegetation mechanical removal (Clear-cut treatment). Two trials, in 2009 and in 2011, followed the changes in the soil seed bank and the soil properties after *E. horridum* had been removed. The seedling emergence method was used to identify and quantify species composition of the seed bank. We hypothesized that (1) burning will favor *E. horridum* germination because fire can break seed dormancy in some Mediterranean shrubs species (Rivas et al. 2006); and (2) that burning will have a greater effect on the soil seed bank and the soil properties than will clearcutting because the former causes the most perturbation.

2. Methods

2.1 The study area and plant species

The study area was near Torla village at the periphery area of Ordesa and Monte Perdido National Park, in the Central Pyrenees, Spain. The study area was a subalpine grassland at 1700 m.a.s.l. that had been encroached upon by *Echinospartum horridum* (Vahl) Rothm, a thorny cushion endemic to the Pyrenees which occurred as dense monospecific patches that harbored some herbs and graminoids. *E. horridum* flowers in mid-July and seeds maturation and explosive seed dehiscence occurs in early September, the seeds remain in the soil throughout autumn and winter and the peak germination occurs in mid-May. The climate is Mediterranean mountainous characterized by an equinoctial precipitation regime. The average annual precipitation is 1758 mm, and daily maximum and minimum average temperatures are 8.7º C and 1.5º C, respectively (from the closet meteorological station "Refugio de Góriz" at 2200 m.a.s.l.).

2.2 Field work and laboratory procedures

Management procedures

To monitor the effect of fire, in autumn 2008, 10 ha of *E. horridum* community were burnt by the EPRIF (Team for the Integral Prevention of Forest Fires, from the Ministry of Agriculture, Food and Environment) (Burnt treatment). At the same time, the aerial biomass of *E. horridum* was removed manually on a nearby 50 m^2 area (Clear-cut treatment). A stand of *E. horridum* was left untouched and served as the Control.

Soil properties

To test the hypothesis that management treatments might affect soil properties, six soil samples were collected in Burnt treatment, Clear-cut treatment and in Control in 2009 the year following the management treatment and in 2012 three years after the treatments. Particle size (% of sands, silts and clays), soil nutrients (% of nitrogen, carbon and organic matter), pH and electrical conductivity (EC) were quantified. Soil samples were collected using a 20-cm-deep soil collector. After polishing soils samples, nitrogen and total carbon was quantified using an elemental analyzer, Variomax Elementar (DUMAS procedure). Organic carbon content was quantified using dichromate (Heanes

1984). Organic matter was quantified by incineration (at 550ºC). Particle sizes were quantified after the soil samples had been sieved (2 mm mesh) (Malvern Mastersizer 2000).

E. horridum demography

To evaluate the *E. horridum* capacity for re-colonization after the application management treatments, the number of seedlings that germinated annually and their survival were monitored in 10 square plots (1 m x 1 m) in mid-May each year (2009, 2010, 2011) following management treatment until the year when seedlings were no longer discernible, in the Burnt, Clear-cut and Control areas.

Soil seed bank of E. horridum community

To study the soil seed bank of *E. horridum* community, 240 soil samples were collected in mid-June in two years, the first in the spring following the application management treatment in 2009, and the second in 2011. Based on a random stratified sampling design, four sampling units (Replica I) were assigned randomly within each treatment (Burnt and Clear-cut and Control), and 5 soil cores (Replica II) were collected at each sampling unit (20 sampling points per treatment). At each sampling point, soil cores (4-cm radius) were extracted from two depths: Deep I: 0-5 cm and Deep II: 5-10 cm. To quantify the soil seed bank, we used the Seedling Emergence Method (Ter Heerdt et al. 1996). Soil samples were washed over a 0.2-mm mesh sieve and the resulting concentrated samples were uniformly extended on pot previously filled with compost (Ter Heerdt et al. 1996). To avoid seed burial and germination within the compost, a thin nylon net (0.1mm mesh) was placed between samples and compost. Samples were kept in a germination chamber for four months under a controlled night (10 h at 15 ◦C) and day (14 h at 25 ◦C; 5–8 Klux, luminous flux area^{-1}) cycles. Emerged seedlings were identified and removed from the sample (Bekker et al. 1997). Samples were watered every three days and, during the last month, to induce germination, 1 g/l of gibberellic acid (GA3) was added to the irrigation water (Ikuma and Thimann 1960, Evans et al. 1996).

2.3 Statistical analysis

2.3.1 Soil properties

To test whether management treatments affected to the soil properties a General Linear Model (GLM) was performed for each nutrient (N, C, C/N and OM) (after arc-sin transformation of the data to fit a Gaussian distribution), pH and electrical conductivity in 2009 and 2012, individually. Soil texture was quantified in 2012, only, therefore, a model for each component: sand (particles between 1000-50 micron), silt (particles between 50-2 micron) and clays (particles <2 micron), based on the data from 2012. Treatment, which accounted for the differences between management treatments (Burnt treatment, Clear-cut and Control), was included as fixed factor. Tukey test were used to identify significant differences between factor levels.

2.3.2 E. horridum demography

To identify significant effects of the management treatment on *E. horridum* seedling emergence, a Generalized Linear Mixed Model (GLMM) that included number of germinated seedling as the response variable, Treatment as fixed factor, and the ten plots in the random factor was performed at each study year (2009, 2010 and 2011), individually. A Poisson distribution was used to model distribution of the residuals due to number of seedling emerged is a count.

To identify significant effects of the management treatments on *E. horridum* seedling probability of survival a GLMM that included proportion of seedling that survived from 2009 to 2010 as the response variable, Treatment as fixed factor and plot as random factor was performed. A binomial distribution was used given that the response variable is a proportion (success/fail). A similar model was performed to estimate the probability of survival of two year seedling, and the response variable was proportion of seedling that survived from 2009 to 2011.

2.3.3 Soil seed bank of E. horridum community

To test whether the management treatments affected total seed abundance, differences between Burnt, Clear-cut and Control were quantified at two depth (0-5 cm and 5-10 cm) in each year (2009 and 2011), individually. Zero inflated Poisson models (ZIP) (Zeileis et al., 2008, Zuur et al., 2009) were used given the high proportion of 0 values. Total number of soil seeds was the response variable and Treatment was a fixed factor. To test whether soil seed abundance varied significantly with time-since-fire/clearing, differences between years (2009 and 2011) were tested at each treatment, Burnt, Clear-cut and Control, for each depth (0-5 cm and 5-10 cm), individually. A Zero inflated Poisson model (ZIP) that included number of soil seeds as response variable and Year as fixed factor was performed. ZIP models were performed with the *pscl* package (Jackman 2015) in R 3.2.0 software.

To identify the type of soil seed bank that predominated in each management treatment (Burnt, Clear-cut and the Control), each plant species was classified as either transient (T), short term persistent (STP) and long term persistent (LTP), based on its relative abundance at two soil depths (following Thompson et al., 1997). T: the species had been present in the original vegetation before management treatment application but did not appeared in the seed bank or appeared only in the upper layer (0-5cm), STP: seeds are most abundant in the upper layer (0-5 cm) but they also occur in the deep layer (5-10 cm), LTP: seeds are most abundant in deep layer (5-10 cm). To test whether the occurrences of the types of seed bank (T, STP and LTP) varied significantly with time-since-fire/clearing, the differences between years (2009 and 2011) were analyzed at each management treatment (Control, Burnt and Clear-cut), individually. A GLMM was performed for each seed bank type (T, STP and LTP), that included seed bank type was the response variable, Year was the fixed factor, Replica I was the random factor, and Poisson distribution was used for the distribution of the residuals. Models were selected based on Akaike Information Criterion (AIC) (Zuur et al., 2009). The parameter estimations were made using the Restricted Maximum-Likelihood Estimation Method (REML) in the *nmle* package (Pinheiro et al. 2014), of R 3.2.0 software.

Shannon Diversity Index was calculated for each collected soil sample using the function *diversity* form the package *vegan* (Oksanen et al. 2015) in R 3.2.0. To test whether soil seed bank diversity was affected by management treatments, differences between Burnt, Clear-cut and Control were evaluated for each study year, 2009 and 2011, individually. A GLMM was performed that had Shannon Diversity Index as the response variable, Treatment as a fixed factor and Replica I as random factor. Pairwise comparison between treatments were made with Tukey test for mixed models in *multcomp* package (Hothorn et al. 2008) of R

3.2.0 software. To test whether soil seed bank diversity varies with time-since-fire/clearing, differences between years (2009 and 2011) were evaluated at each treatment, Burnt, Clear-cut and Control, individually. A GLMM was performed that had Shannon Index as response variable, Year as a fixed factor and Replica I as random factor. To quantify soil seed bank dynamics at each soil depth, the previous models were performed again but separately for each soil depth (0-5 cm *vs.* 5-10 cm), individually.

All the statistical analyses were performed in R 3.2.0 (R Development Core Team) software and the probability for statistical significance set to 0.05 (type I).

3. Results

3.1 The effect of fire on soil properties

In 2009, the year following prescribed burning and clearcutting, soil nutrient concentration in management treatments tending to be lower than they were in the Control site, however, three years after in 2012 the differences were significant (Table 1, Figure 1). The Burnt treatment had lower soil nutrients concentration than did the Control site and the Clear-cut treatment, and the concentrations in the latter two were similar (Figure 1).

In both years (2009 and 2012), the pH in the Clear-cut treatment was significantly lower than it was in the Control site, however the Burnt treatment did not differ significantly from the Control site (Table 1 and 2). In 2009 electrical conductivity of the soil was significantly lower in the Burnt treatment than in was in the Control site and Clear-cut treatment. In 2012, that difference was more pronounced and, in Clear-cut treatment, EC was significantly lower than it was in the Control site (Table 1 and 2).

Particle size was affected by fire: in Burnt treatment the proportion of the soils that was sand was significantly lower and the proportions of the soil that were clay and silt were significantly higher than they were in the Control site and in Clear-cut treatment which had similar proportions (Table 1, Figure 2).

3.2. E. horridum demography

In each year, the number of *E. horridum* germinated seedling was higher at the Burnt treatment than it was in the Control site (2009: z=17.32; 2010: z=13.73; 2011: z=10.50, p<0.0001 for each one), and also higher at the Clear-cut treatment than in Control site (2009: z=15.65; 2010: z=11.35; 2011: z=8.24, p<0.0001 for each one) (Figure 3a).

The survival of the first-year seedling *E. horridum* was significantly lower in the Clear-cut treatment than it was in the Control site (z=-3.49, p<0.001), but the probability of survival in the Brunt treatment was similar to those in the Control site (z=-1.24, p=0.22). The survival of the two-year-old seedlings was significantly higher in the Burnt treatment than it was in the Control site (z=2.03, p=0.042), but the probability of survival in Clear-cut treatment was similar to those in the Control site (z=0.514, p=0.61) (Figure 3b).

3.3 Effect of fire on E. horridum community soil seed bank

Soil seed bank composition

There was a total of 29 plant species forming the soil seed bank in *E. horridum* community and management treatments (Burnt and Clear-cut) and a total of 735 emerged seedlings (Figure 4 and Appendix 1). Species richness was highest in the Burnt treatment, with 23 species, and lower in the Clear-cut treatment, with only 16 species, and in the Control site, with 18 species (Figure 4, Appendix 1). At both management treatments species richness increased from 2009 to 2011, from 16 to 19 in the Burnt treatment and from 8 to 13 in the Clear-cut treatment. In the Control site, however, species richness (13 species) did not vary between years (Appendix 1). *Linum catharticum* (40.31%) and *Echium vulgare* was (13.75%) was the dominant species in the soil seed bank in the Burnt treatment (Figure 4). In the Clear-cut treatment the dominant species was *Paronychia kapela* (63.3%) and *Arenaria grandiflora* (25%) (Figure 4). In Control site, *L. catharticum* (32.45%) and *Euphorbia cyparisas* (13.24%) were the most abundant species (Figure 4).

Seed abundance

Seed density in the soil was higher in the Burnt treatment and in the Clear-cut treatment than in the Control site, being the highest in the Burnt treatment in 2011 (Table 3).

In the first 5 cm of the soil, the total number of seeds differed among treatments at each year (Figure 5a). In 2009, Clear-cut treatment had more seeds than did Control site (z=2.31, p=0.021), but an amount similar to that of the Burnt treatment (z=0.864, p=0.38); the Burnt treatment and the Control site had similar number of seeds (z=-1.59, p=0.11). In 2011, however, the Burnt treatment had more seeds than did the Clear-cut treatment (z=-2.76, p=0.006) or the Control site (z=7.25, p<0.0001); the Clear-cut treatment had more seeds than did the Control site (z=4.914, p<0.0001) (Figure 5a). Total seed number increased significantly from 2009 to 2011 at the two types of treatments (z=-2.218, p=0.02 and z=2.898, p=0.003, in the Burnt and in the Clear-cut, respectively), but not at the Control site (z=0.656, p=0.51).

In 5-10 cm below the soil surface, the total number of seeds differed significantly between treatments in each year (Figure 5b). In 2009, the Burnt treatment had fewer seeds than did the Control (z=-5.17, p<0.0001) or the Clear-cut treatment (F=4.14, p<0.0001), and the number of seeds in the Clear-cut treatment and in the Control were similar (z=-1.09, p=0.27). In 2011, however, the Burnt treatment and the Clear-cut treatment had more seeds than did the Control site (z=2.67, p=0.007; and z=2.89, p=0.004, respectively), and the Burnt and Clear-cut treatments had a similar number of seeds (z=0.40, p=0.68). Total seed number increased from 2009 to 2011 at each management treatment (z=6.57, p<0.0001 and z=2.75, p=0.006, Burnt and Clear-cut respectively), but not in Control site (z=-0.956, p=0.33).

Type of soil seed bank

At the Burnt treatment the number of T species decreased significantly (z=-2.63, p=0.008) and STP species significantly increased (z=6.42, p<0.0001) between 2009 and 2011. The number of LTP species did not differ significantly between years (z=-1.34, p=0.17) (Figure 6a).

Changes in the Clear-cut treatment were similar to those in the Burnt treatment; however, the differences between years were not statistically significant for T, STP or LTP (z=-1.79, p=0.07, z=1.19, p=0.23, and z=-0.505, p=0.61, respectively) (Figure 6b).

In the Control site the numbers of T and LTP species were similar between years (z=-0.448, p=0.65 and z=-1.346, p=0.17, respectively); however, the number of STP species increased significantly between 2009 and 2011 (z=2.98. p=0.002) (Figure 6c).

Soil seed bank diversity

In 2009, seed diversity did not differ significantly between the treatments and Control site, but, in 2011, soil seed bank diversity was significantly higher in the Burnt treatment than it was in the Clear-cut treatment and in the Control site, which had similar diversity (Table 1, Figure 7).

In the Burnt treatment seed bank diversity increased significantly between 2009 and 2011 (F=42.03, p<0.001); however, in the Clear-cut treatment and in the Control site diversity did not differ significantly between years (F=0.0094, p=0.93, and F=2.13, p=0.19, respectively) (Figure 7).

The same patterns in soil seed diversity were apparent at both soil depths (0-5 *vs.* 5-10 cm) (result did not shown).

4. Discussion

In the Central Pyrenees, Spain, prescribed burning favored the germination and survival of *E. horridum* seedlings; therefore, burning alone would be an ineffective management procedure for stopping the expansion of *E. horridum* into subalpine grasslands. Clearcutting appears to be a better option because *E. horridum* seedlings performed worse in the Clear-cut treatment, where the number of seeds that germinated and probability of survival were lower than they were in the Burnt treatment. In addition, prescribed burning altered soil properties substantially, but clearcutting had a modest effect on soil quality. The Burnt treatment had a more abundant, diverse and more dynamic soil seed bank than Clear-cut treatment, and harbored seeds of several species of well-preserved grasslands; however, the soil seed bank is still quite poor to enable self-recovery of the former grassland vegetation.

The high density of adult *E. horridum* in the undisturbed *E. horridum* stand (Control) might have inhibited seed germination and survival. In the Clear-cut treatment, the removal of aerial vegetation allowed sunlight to reach the soil, which promoted *E. horridum* seedlings germination. In addition to the increase in sunlight that reached the soil after vegetation removal, as in the Clear-cut treatment, the high *E. horridum* seedling establishment in the Burnt treatment might have been because burning helped to break *E. horridum* seeds dormancy. Fire can modify the seed germination response via physical and chemical signals involved in germination (de Luis et al. 2005, Rivas et al. 2006). Thermal shocks can scarify the coat of the seeds making it permeable (Keely 1987) and smoke might act as a chemical signal interacting with seed germination inhibitors (Tieu et al. 1999, Flematti et al. 2011, Tormo et al. 2014). In our study, the number of seedlings germinated annually decreased with time in both treatments, which might have been because the soil became more saturated and there is less space available. In addition, the soil seed bank of *E. horridum* might have been depleted; however, *E. horridum* seeds occurred in the first 5 cm of the soil and the year following the treatment application, which, by definition, would indicate that *E. horridum* formed transient seed banks, i.e. *E. horridum* seeds did not persist in the soil more than one year. Probably, some of the *E. horridum* seeds arrived by dispersal. In addition, it appears that *E. horridum* seeds have highly specific requirements and the standard conditions in the germination chamber might not have met those requirements. More research on the germination cues and seed rain of *E. horridum* seeds is needed. A study on another species from the same genre, *E. algibicium* seeds occurred in the deep soil layer (5-10 cm) (Aparicio and Guisande, 1997); however, soil seed persistence of a specie can differ among locations (Saatkamp et al. 2014) and in several species both persistent and transient soil seed bank have been documented (Thompson et al. 1997, Kleyer et al. 2008).

In our study, average seed density was similar to those in Mediterranean shrublands (Leck et al. 1989). As in our study, others have found that the density of soil seeds increased after burning and clearing (Fernández et al. 2013). After such big scale perturbations there is an empty niche to be colonized and therefore the soil seed bank can be recharged; however, the Clear-cut and Burnt treatments differed in the timing of seed recharge. In the short-term, Clear-cut treatment recharged the uppermost soil layer, but not the deep soil because the mechanical removal of aerial vegetation did not alter the soil structure. The deep soil layer was recharged later, after the seeds had had more time to become buried. After the Burnt treatment, even with the possibility of some seed mortality (Auld and Denham, 2006), fire might have promoted seeds germination because in the upper soil layer, ten of 14 species, and in the deep soil layer, four of six species were present in the aerial vegetation that established after the Burnt treatment (unpublished data). Burning favored soil seed germination on the short term, which was followed by a large soil seed bank recharge because burning altered soil structure. Both management treatments had long-term effects and the seeds can enter the soil several years after the perturbations. That pattern was more apparent in the upper soil layer because is the most accessible to seeds. During primary succession seed density and species richness of the soil seed bank increase as the new species invade and reach maturity (Walker and Del Moral 2003); however, as vegetation succession progress, the number of buried seeds decreases (Donelan and Thompson 1980) and usually the highest diversity occurs at intermediate levels of perturbation (Wilson 1990). In our study the high abundance and diversity observed three years after *E. horridum* removal might represent an intermediate snapshot during succession and it might decrease as succession proceeds.

The first seeds that reached the soil of the management treatments formed transient seed banks that subsequently became buried and formed persistent seed banks. That process was most acute in the Burnt treatment because burning causes a greater perturbation than clearcutting; however, species that formed a long-term persistent seed bank did not exhibit much variation, these species needs more time to arrive and to get buried in the soil (Bekker et al. 2000). Interestingly, the soil seed bank of an *E. horridum* mature community is also dynamic. Although the seed abundance and diversity remained constant, the type of soil seed bank varies among years, which might be because the fruit sets of the other species in the community might vary annually because of environmental (Hedhly 2011, Hayes et al. 2012) or genetic factors (Kelly et al. 2002).

In our study, the diversity of the soil seed bank was lower than the average diversity in grasslands vegetation (Monteiro et al. 2013). This is typical from soil seed banks that usually are highly specialized where few species are dominant

(Leck et al. 1989), which leads to a highly successful vegetation recovery after perturbation, because the dominant species are the first species in germinating, and creates a suitable habitat for the germination of the species that follow (Walker and Del Moral 2003). Burning increased seed bank diversity and species richness, but clearcutting did not, thus, the burned area was more prone to colonization by seeds than was clear-cut area. Is therefore prescribed burning a suitable procedure for recovering the subalpine grasslands? It would also depend on species composition of the soil seed bank. *Linum catharticum* the predominant species in the soil seed bank in the Burnt treatment and in the Control site, is a biennial herb that forms abundant soil seed banks because the seeds needs light to germinate (Milber 1994) (in Burnt treatment *L. catharticum* was abundant in 2011, but not in 2009, Appendix 1). The Burnt treatment favored *C. bonus-henricus*, *E. vulgare*, *G. pinetorum-pumilum*, *H. perforatum*, *P. kapela*, *P. saxifraga*, *R. mediterraneus*, *Taraxacum* sp. and *T. praecox* (present in Burnt treatment but absent in Control soil seed banks). Some of those species are typical of well-preserved natural grasslands, e.g. *T. praecox* (Fillat et al. 2008), but others, such as *E. vulgare*, which was the second species more abundant on the soil seed bank, are typical from disturbed areas. All of those were transient species when entered the system, and the seeds of five of nine species became buried and formed a persistent soil seed bank after two years. In addition, all of the species except *C. fontanum* were present in the aerial vegetation of Burnt treatment (unpublished data); therefore Burnt treatment can favor the germination of some species from the soil seed bank that can contribute to the recovery of the previous abandoned grasslands. In Clear-cut treatment, *P. kapela* and *A. grandiflora*, which are typical from well-preserved subalpine grasslands (Fillat et al. 2008), were predominant species in the soil seed bank. Clearcutting favored *E. vulgare*, *F. rubra*, *G. pinetorum-pumilum*, *P. kapela* and *P. vulgaris* (present in Clear-cut treatment but absent in Control soil seed banks). In 2009, four of those five species were transient species, and by 2011, *F. rubra*, *G. pinetorum-pumilum* and *P. vulgaris* had formed a long-term persistent seed bank. *E. vulgare* is ruderal specie that is typical from disturbed habitats; however *F. rubra*, *G. pinetorum-pumilum*, *P. kapela* and *P. vulgaris* are typical from well-preserved natural grasslands (Fillat et al. 2008). Thus, Clear-cut treatment, as well as Burnt treatment, can favor the establishment in the soil seed bank of some of the species that are typical of the subalpine grasslands; however, the low seed abundance and species richness would make difficult grassland restoration exclusively from the soil seed bank.

The collateral effects of *E. horridum* removal are important. Specifically, the proper management of soil resources is essential to vegetation restoration (Walker and Del Moral 2003). Burning has a significant long-term impact on the soil properties (Certini 2005). Soil nitrogen, carbon, organic matter and the size of soil particles decreased especially in the mid to long-term (three years after the Burnt treatment). Leaching, water erosion, infiltration and volatilization can reduce soil nutrients concentrations (Gimeno-García et al. 2000, Certini 2005) after perturbation. Natural changes in soil humidity and soil temperature dictate soil structure (Horn and Smucker 2005) and fire might alter soil structure by drastically modifying these two variables. In addition, partial organic matter combustion with fire might degrade soil particles (Mataix-Solera et al. 2011, Albalasmeh et al 2013). Others factors such as weather and soil characteristics can influence the extent of the effect of fire on the soil (Certini 2005); thus, the effect of burning on *E. horridum* stands might not be the same everywhere. The clear-cut treatment did not have a significant effect on soil structure and nutrient concentration because the mechanical removal of *E. horridum* did not alter the soil; however, the decomposition of *E. horridum* spines or roots can reduce soil pH (Paul et al. 2001). The effects of those soil modifications on grassland recovery are unknown and should be investigated (Basto et al. 2013).

We conclude that the low abundance and low species richness of the soil seed bank in the two management treatments and the rapid regeneration rate of *E. horridum* would not allow for the recovery of the subalpine grassland from the soil seed bank alone. Livestock grazing on *E. horridum* seedlings can help to minimize its establishment. In addition, an optimal protocol should include traditional shepherding between grasslands, which can promote phoretic dispersal of plant species (Fischer et al. 1996, Wagner et al. 2013) and increase the soil seed bank recharge in the soils of the management treatments. The peak in *E. horridum* seedling establishment takes place the year following the management treatment, therefore, *E. horridum* removal should better occur before annual seeds are released.

Acknowledgements

We gratefully acknowledge the financial support of the Spanish Ministry of Science and Innovation (BES-2009-017189), the Spanish Economy and Competition Ministry (PN-MICINN) (CGL2008-00655/BOS and CGL2011-27259) and Spanish National Park organization (DIPA 125/2010 MMAMRM). We thank the AEMET for providing meteorological information, Bruce MacWhirter

Year	**Fixed factor**	**Response variable**	***F value (d.f.)***	***p value***
2009	Treatment (Control , Burnt, Clear-cut)	% N	1.79 (2,15)	0.20
		% C total	1.44 (2,15)	0.27
		C/N	0.81 (2,15)	0.46
		OM	1.60 (2,15)	0.23
		pH	53.86 (2,15)	<0.0001
		EC	3.62 (2,15)	0.052
		Shannon	1.38 (2,57)	0.30
2012	Treatment (Control , Burnt, Clear-cut)	% N	11.59 (2,15)	<0.0001
		% C total	11.21 (2,15)	<0.001
		C/N	1.73 (2,15)	0.21
		OM	9.05 (2,15)	<0.0001
		pH	19.29 (2,15)	<0.0001
		EC	16.63 (2,15)	<0.0001
		Sand	7.09 (2,15)	0.0068
		Silt	5.24 (2,15)	0.019
		Clay	7.15 (2,15)	0.0066
		Shannon	6.88 (2,57)	0.015

Table 1. *F* and *p* values for each general linear model (GLM) of each soil nutrient investigated: nitrogen (%N), total carbon (C total), ratio Carbon/Nitrogen (C/N), organic matter (OM), physicochemical properties: pH and electrical conductivity (EC), and general linear mixed model (GLMM) for Shannon diversity index. Differences between treatments (Control, Burnt and Clear-cut) were investigated at each study year (2009 and 2012), separately.

	2009			**2012**		
	Control	*Clear-cut*	*Burnt*	*Control*	*Clear-cut*	*Burnt*
pH	7.19 ± 0.15	5.59 ± 0.16	7.42 ± 0.09	6.63 ± 0.13	5.70 ± 0.12	6.79 ± 0.15
EC	0.18 ± 0.03	0.13 ± 0.01	0.09 ± 0.02	0.15 ± 0.02	0.08 ± 0.006	0.08 ± 0.008

Table 2. Mean and standard error (se) of physicochemical soil properties of pH and electrical conductivity (EC), at each treatment (Control, Clear-cut and Burnt) and each study year (2009 and 2012), at a *E. horridum* community at the central Pyrenees, Spain.

Seed density (mean seed number/m^2 ± se)			
	Control	**Clear-cut**	**Burnt**
2009	2880.07 ± 921.82	4988.69 ± 1173.43	2725.78 ± 877.48
2011	4834.40 ± 684.33	8485.91 ± 2210.98	13371.73 ± 4614.52

Table 3. Density of seeds (mean seed number of seeds/m^2 ± se) on the soil seed bank at each management treatment (Clear-cut and Brunt) and in Control, at each year of study, 2009, the year following the management treatment application, and in 2011.

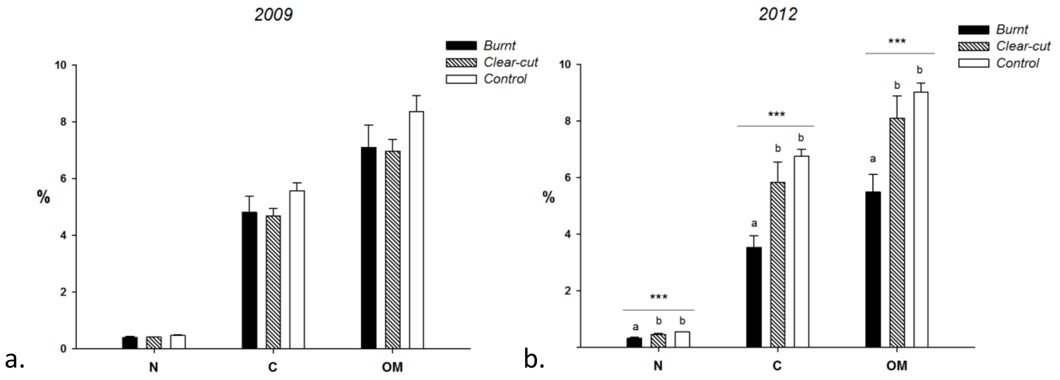


Figure 1. Soil nutrient concentrations: % nitrogen (N), % total carbon (C) and % organic matter (OM), in management treatments (Burnt, Clear-cut and Control), in an *E. horridum* community, at the Central Pyrenees, Spain, (a) the year after the application of the management treatment (2009), and (b) three years after the management treatment (2012). Different letters show differences between factor levels. *** p<0.001.

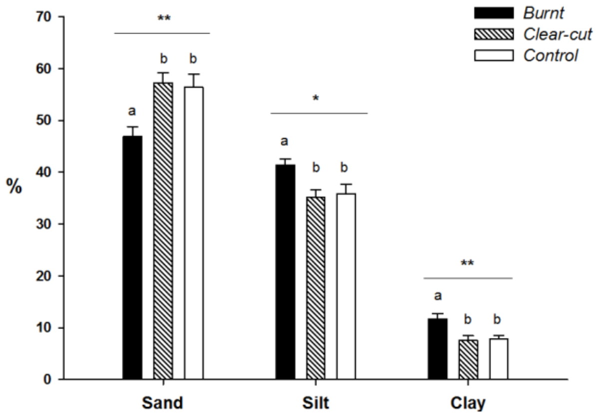


Figure 2. Proportions (%) of sands, silts and clays in the soils of management treatments (Burnt, Clear-cut and Control), in an *E. horridum* community, at the Central Pyrenees, Spain, in 2012, four years after management treatments. Different letters show differences between factor levels. *p<0.05, **p<0.01

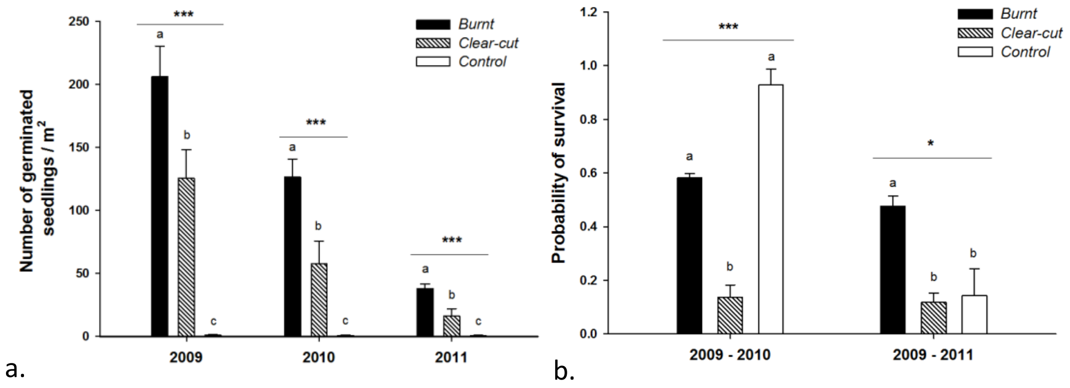


Figure 3. a) Number of *E. horridum* seedling germinated and b) survival probability of *E. horridum* seedlings in three years (2009, 2010 and 2011) after the application of management treatments (Brunt, Clear-cut, and Control) in 2008, in the Central Pyrenees, Spain. Different letters show differences between factor levels. *p<0.05, ***p<0.001

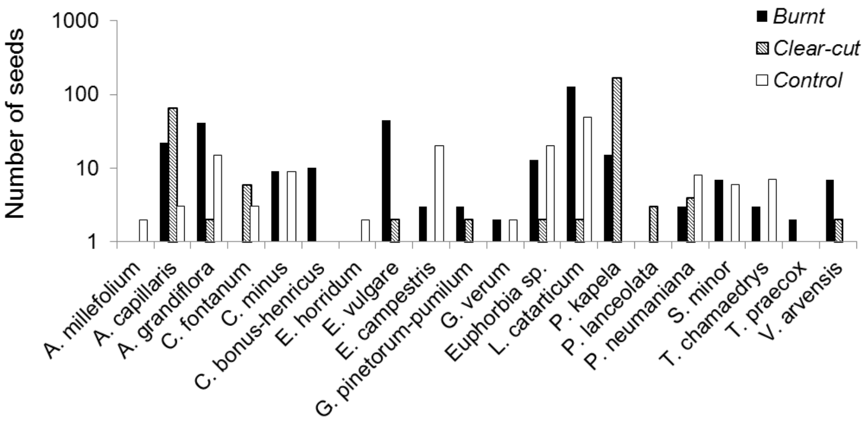
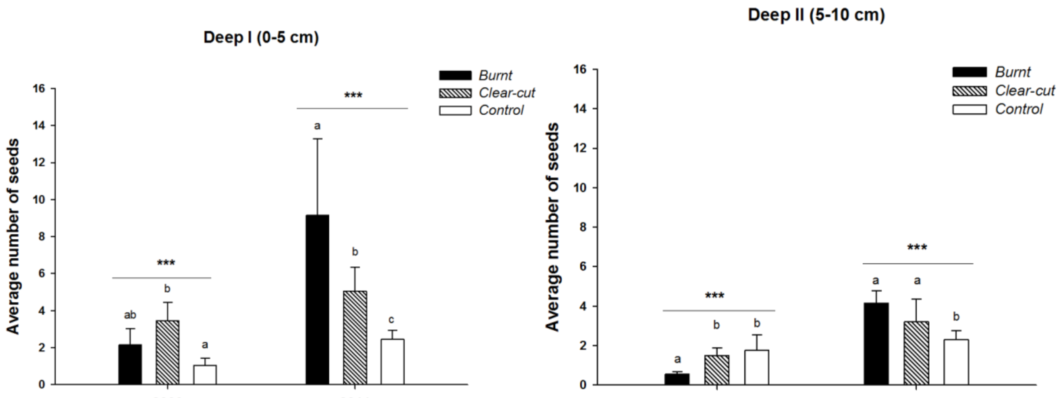


Figure 4. Number of seeds (logarithmic scale) of each species present in the soil seed bank at each treatment (Burnt, Clear-cut and Control). Species with very low abundance (<2seeds) were not represented.



a. b.

Figure 5. Total seed number of the soil seed bank in each management treatment (Burnt, Clear-cut and Control), in 2009 and in 2011, one and two years after management treatment application respectively, and at each soil depth (0-5 cm and 5-10 cm), in an *E. horridum* community in the Central Pyrenees, Spain. Different letters show differences between factor levels. *** p< 0.0001.

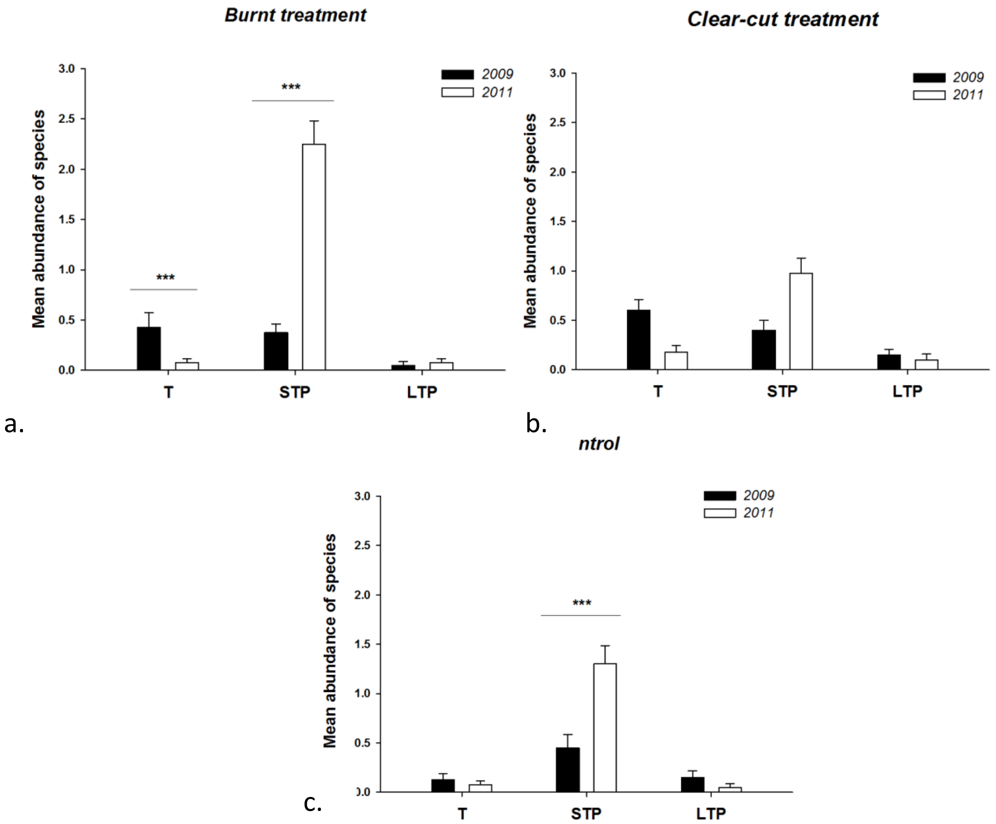


Figure 6. Mean abundance of species forming a type of seed bank (T: transient, STP: short term persistent, or LTP: long term persistent) at each management treatment, a) Burnt, b) Clear-cut and c) Control, and each study year, 2009 and 2011, one and two years after management treatment application respectively, at the Central Pyrenees, Spain. *** $p < 0.001$.

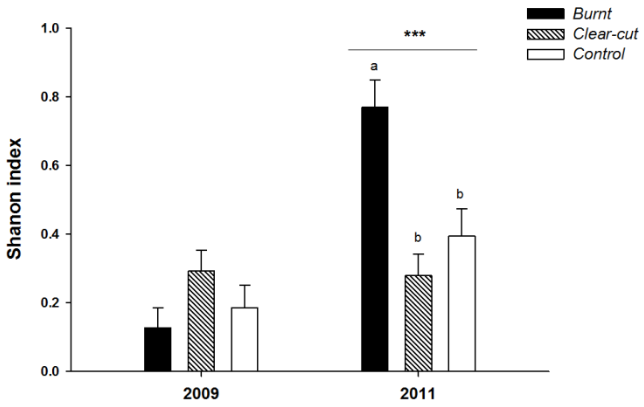


Figure 7. Shannon diversity index of the soil seed bank at each management treatment (Burnt, Clear-cut and Control), in two years (2009 and 2011), one and two years after management treatment application respectively, in an *E. horridum* community in the Central Pyrenees, Spain. Different letters means significant differences based on Tukey test. ***p< 0.0001.

Chapter 3

Plant species interactions mediate the response of vegetation succession to climate change

Paloma Nuche, Concepción L. Alados and Sonia Kéfi

Abstract

Woody encroachment and forest re-growth are secondary succession processes that are nowadays occurring worldwide mainly due to abandonment of traditional land uses. The interactions between the involved woody species determine the succession processes and speed. Given the ongoing climate change and its effect on the intensity of species interactions, an impact of climate change on the vegetation succession is expected. This study evaluated the impact of climate change on the vegetation succession through the modification of plant-plant interactions and recruitment, using a combination of modeling and field data from abandoned subalpine grasslands at the Pyrenees. We developed a cellular automata model to describe the vegetation dynamics of the ecosystem dominated by two shrub species: *Echinospartum horridum* (Vahl.) Rothm (an 'early colonist') and *Buxus sempervirens* L. (a 'late succession colonist'). Field data showed that inter-species interactions, as well as the establishment rate of *B. sempervirens* seedlings, vary with climatic conditions. We simulated the effect of a change in climatic conditions on the system dynamics by modifying the intensity of inter-specific interactions and seedling establishment as observed in field experiments. We focused on two possible climatic scenarios: a low temperature scenario, which would be the climatic conditions found at the upper limit of *B. sempervirens* distribution, and a high temperature scenario simulating global warming. Under low temperatures the vegetation succession rate would be slowed-down due to low *B. sempervirens* competitive ability and low *B. sempervirens* seedlings establishment; despite the high facilitation intensity towards *B. sempervirens* seedlings; and therefore both study species might coexist. Higher temperatures would speed-up the vegetation succession rate given the higher competitive ability of *B. sempervirens* and the higher *B. sempervirens* seedling establishment under high temperatures, despite the low value of seedling facilitation; therefore species coexistence is not possible and *B. sempervirens* would displace *E. horridum*. We conclude that plant interaction should be taken into account when evaluating the potential impact of climate change on ecosystems dynamics.

Key words: vegetation succession, plant interactions, climate change, cellular automata.

1. Introduction

Predicting how ongoing climate change will affect ecosystems is one of the most pressing challenges of ecology today. Predictions have typically focused on the direct effects of climate change on single species (Davis et al. 1998, Thuiller et al. 2005). Yet interactions between species can largely influence how climate change may impact ecosystems (Gilman et al. 2010). The interactions between the species present in a given community largely determine the community composition and its temporal dynamics (Connell and Slatyer 1977, Walker and Del Moral 2003). The prediction of the effect of changes in environmental conditions on ecological communities is however non-trivial because the species interactions themselves can vary with environmental conditions (Callaway and Walker 1997, Tylianakis et al. 2008).

Climatic conditions might affect plant communities in two ways: directly, by affecting key processes of population dynamics (e.g. recruitment; Slot et al. 2005, Coop and Givnish 2008, Batllori and Gutierrez 2008) and indirectly through the modification of plant species interactions (Callaway and Walker 1997, Tylianakis et al. 2008). For example, in a terrestrial ecosystem along an altitudinal gradient, two species whose altitudinal range partly overlaps can locally coexist and thereby interact. An increase in temperature could favor the establishment of the species growing at lower altitude (Castanha et al. 2013), and also increase its competitive ability (Choler et al. 2001), which could lead to a reduction of the other species abundance or even to an eventually local extinction.

At the upper limit of species altitudinal range at alpine and subalpine ecosystems low temperatures usually limit seed establishment (Coop and Givnish 2008, Batllori and Gutierrez 2008), and seedlings are frequently facilitated by adult plants because protect them from low temperatures and frost (Körner 2003, Cavieres et al. 2007). A direct effect of climate warming on recruitment and an indirect effect on seedling facilitation might affect community dynamics at high elevations.

The abandonment of traditional land uses is leading to secondary succession processes such as woody encroachment and forest re-growth in many areas around the globe (MacDonald et al. 2000, Rudel et al. 2000, Müller and

Zeller 2002). This is particularly true in many mountainous ecosystems which are transforming from grasslands to woodlands. An increase in mean air temperature of 2.2 – 5.2 Celsius degrees is predicted to occur in Mediterranean mountains in the 21st century (Nogués-Bravo et al. 2008). The combination of ongoing secondary succession and important climate change make those regions particularly interesting to decipher how climate change affects species, species interactions and therefore community dynamics and functioning.

This study will explore vegetation succession in abandoned subalpine grasslands under the two main drivers of environmental change land use and climate change (Sala 2000), using a combination modeling and data analysis from field experiments performed in Ordesa and Monte Perdido National Park, Central Pyrenees, Spain. Our general objective was to improve our fundamental understanding of how climate might affect ecosystem dynamics through effects on both species processes, recruitment and species interactions. More specifically we aimed to simulate the effect of a change in climatic conditions on the system dynamics by the modification of the intensity of shrubs interactions and seedling establishment.

Ecological modelling has been widely used to understand ecosystem dynamics and generate and test theories and hypotheses (Park et al. 2015), which couldn’t be possible by experiments on the short term. Here we developed a spatially explicit gird based model whose parameter values were obtained from field experiments. The model includes the two main shrubs entering the subalpine grasslands: *Echinospartum horridum* (Vahl) Rothm (an ‘early colonist’) and *Buxus sempervirens* L. (a ‘late succession colonist’), at two vital stages of *B. sempervirens*, seedlings and adults.

At the Central Pyrenees the mean annual temperature has raised over the period 1882 – 1970 of 0.83ºC (Bücher and Dessens 1991) and a marked increase in temperature, 2.8 - 4ºC, is predicted by the end of 21st century, where the southern slopes and highest mountainous areas are the most sensitive to a temperature increase (López-Moreno et al. 2008). The higher rate of woody encroachment of grasslands at the Central Pyrenees was at 1000 – 1600 meters a.s.l. (Gartzía et al., 2014), representing the actual *B. sempervirens* upper limit.

Therefore, current global warming might modify the rate of vegetation succession of abandoned grasslands at 1000-1600 m.a.s.l., and eventually might favour the upslope migration of *B. sempervirens*.

We based our model design on a field data experiment, which demonstrated that *E. horridum* facilitates the survival of seedlings of *B. sempervirens*, and once *B. sempervirens* became adult over-competes *E. horridum* (Nuche and Alados *in rev*). Previous field data showed that higher temperatures enhanced *B. sempervirens* seedling establishment and *B. sempervirens* competitive ability, and lower temperatures enhanced *E. horridum* facilitation towards *B. sempervirens* seedlings (Nuche and Alados *in rev*). Therefore we hypothesize that vegetation succession could be accelerated under high temperatures given that competitive ability of *B. sempervirens* adults towards *E. horridum* will increase and that *B. sempervirens* seedling establishment would also be enhanced, although the low facilitation towards *B. sempervirens* seedlings might counteract this tendency. Under low temperatures vegetation succession is expected to slow-down due to the low *B. sempervirens* seedling establishment and low competitive ability of *B. sempervirens*, however, it could be compensated with an increase in the facilitative effect of *E. horridum* towards *B. sempervirens* seedlings.

The main questions of the study are 1) are plant-plant interactions a fundamental mechanism in vegetation succession at the Pyrenees?, 2) do plant-plant interactions play a determinant role in mediating the response of plant communities to climate change?, 3) would global warming accelerate the vegetation succession rate?, 4) would low temperatures at the upper limit of altitudinal range slow-down vegetation succession rate? and, therefore 5) allow for both study shrubs coexistence rather than under high temperatures derived from current global warming at lower locations?

2. Methodology

2.1 Study system

The experimental study was conducted at the abandoned subalpine grasslands of Ordesa-Monte Perdido National Park, at the Lomar Valley, Central Pyrenees, Spain. The climate is Mediterranean mountainous. At the closet weather station ‘Refugio de Góriz’ (2.215 m a.s.l.) the average annual rainfall was 1728 mm between 1981 and 2006. The mean annual temperature for that period was ≈5 ºC, with daily temperatures between 25 ºC and -21 ºC. There are two dominant plant species, which we present hereafter: *Echinospartum horridum* (Vahl.) Rothm and *Buxus sempervirens* L.

Echinospartum horridum (Vahl.) Rothm, a calcicolous chamaephyte thorny cushion endemic of the Spanish Pyrenees and the southern France, is among the most highly invasive shrub that encroaches these grasslands. It reproduces both sexually, by seeds, and asexually. Asexual reproduction occurs through clonal propagation of decumbent branches, with root at nodes along the stems. New rooted stems can break off and become independent clonal fragments, which permits the plant to maintain and increase its cover, suppressing the establishment of other species and leading to the formation of monospecific patches. At the interior of these patches *E. horridum* have a lower growth rate and a higher crown death rate than plants at the edges of the patches (Komac, 2010). It spread preferentially from 1000 to 1700 meter a.s.l. although it can be found also from 390 to 2400 m a.s.l. (Marinas et al., 2004).

Buxus sempervirens L. is a broadleaf evergreen phanerophyte (up to 5 m high) widely distributed in the Mediterranean region and central Europe (Quézel and Médail 2003, Domenico et al. 2012). *B. sempervirens* have only sexual reproduction, although several secondary stems can develop from the stump. In the Iberian Peninsula it preferentially spread from 400 meter a.s.l. to 1700 m.a.s.l. (Tena 2009), although it can establish at very low altitude near the sea-level (Iparragirre et al. 1985) and up to 2000 m.a.s.l. at the Pyrenees (according to P. Chouard in Lenoble and Broyer, 1945).

The main mechanism in our study system is that *E. horridum* enhance the survival of *B. sempervirens* seedlings under its canopy, but as *B. sempervirens* seedlings grow, they over-compete the *E. horridum* nurse plant (Nuche and

Alados, *in rev*). In a previous experiment we found that the intensity of the interactions between those two species as well as the number of *B. sempervirens* seedlings recruited varied with temperature conditions.

2.2 General model structure

The model presented in this paper is a spatially-explicit grid-based model describing *E. horridum* - *B. sempervirens* dynamics based on our knowledge on demography, dispersion and species interactions from previous field data and field experiments (Nuche and Alados, *in rev*). We studied the interactions between *E. horridum* and *B. sempervirens* at two vital stages of *B. sempervirens*, seedling and adults.

The model is a two dimensional lattice of cells in which each cell has 8 nearest-neighbors and can be in one of the following five states: occupied by an *E. horridum* (S), occupied by a *B. sempervirens* seedling (Bs), occupied by a *B. sempervirens* adult (Ba), occupied by an *E. horridum* with a *B. sempervirens* seedling under its crown (BsS), or empty (E) (Figure 1).

The main processes occurring in the model are the following. *E. horridum* can spread locally by clonal expansion or by seed dispersal around the mother plant; seeds can germinate if they fall in an empty cell. *B. sempervirens* (adults) spread part of their seeds globally throughout the lattice and part of them locally in the nearest neighborhood. *B. sempervirens* seeds can establish on an empty cell or if the cell is occupied by an *E. horridum*. In that latter case, the cell becomes occupied by both *B. sempervirens* seedling – *E. horridum* (so-called BsS cells). BsS cells have the following possible transitions: 1. to become *B. sempervirens* seedling cell (Bs) if *E. horridum* dies; 2. to become *E. horridum* cell (S) if *B. sempervirens* seedling dies; and 3. to become *B. sempervirens* adult cell (Ba) if *B. sempervirens* seedling matures, in which case *E. horridum* would be displaced by the adult *B. sempervirens* (Figure 1). This is how vegetation succession is included in the model. In our model, *B. sempervirens* seed rain and germination are subsumed in establishment, as done in others models (Zeng 2006). *B. sempervirens* adults compete with the neighboring *E. horridum* cells (Nuche and Alados, *in rev*).

2.3 Transition between states

2.3.1 Colonization of empty cell

In the model, an empty cell can be colonized by *E. horridum* and *B. sempervirens* seedlings. The transition rate of an empty cell to a *E. horridum* (S), $W_{E,S}$ is denoted by:

$$W_{E,S} = \left((1 - q_{S|E}) * \ es_1 + vs_1\right) * \ q_{S|E} \qquad (1)$$

The first term in the equation is reproduction by seeds, where es_1 is seed establishment rate on an empty cell and, $(1 - q_{S|E})$ is the probability of a *E. horridum* cell to be at the edge of a patch, because *E. horridum* seeds can only colonize empty cells at the edge of a patch (Komac et al. 2012). The second term of the equation reflects vegetative reproduction, where vs_1 is the expansion rate of shrubs into neighboring empty cells. The two terms of the equation are multiplied by the probability that a neighboring cell is empty $q_{S|E}$ because shrub dispersal is local.

The transition rate of an empty cell to a *B. sempervirens* seedling (Bs), $W_{E,Bs}$ is denoted by:

$$W_{E,B_s} = \left(eb * \ q_{B_a|E} \ * \ (1 - \delta) + \ eb * \ \delta * \ p_{B_a}\right) * sb \qquad (2)$$

The first term of the equation reflects local reproduction, where eb is the seed establishment rate in a empty cell (seed production + recruitment), multiplied by the probability that an neighboring cell is empty, $q_{B_a|E}$, and the proportion of seeds dispersed locally, $(1 - \delta)$. The second term of the equation reflects the global dispersion, where the establishment rate of *B. sempervirens* seedlings through the lattice is multiplied by the global density of *B. sempervirens* adults, p_{B_a}, and the proportion of seeds globally dispersed, δ. sb is the early survival rate of the seedling within the first year when it is alone in the cell (i.e. without the nursing effect of an adults *E. horridum*).

2.3.2 Shrub encroachment

The establishment of *B. sempervirens* seedlings is enhanced if the cell is already occupied with an adult *E. horridum*:

$$W_{S,BsS} = \left(eb * q_{B_a|E} * (1-\delta) + eb * \delta * p_{B_a}\right) * (sb + fs) \qquad (3)$$

The transition is the same as the transition $W_{E,Bs}$ transition (equation 2), but including the facilitation term *fs* which reflects the enhanced survival of *B. sempervirens* seedlings because of the facilitation effect from *E. horridum*.

2.3.3 Mortality

After the death of an *E. horridum* and of a *B. sempervirens* (adult or seedling) the cells becomes empty. The mortality of *E. horridum* (S), $W_{S,E}$, is calculated as:

$$W_{S,E} = \left(d_s + c_1 * q_{S|S} + c_2 * q_{Ba|S}\right) \qquad (4)$$

where d_s is the basal mortality rate of adult plants; c_1 is additional mortality due to competition among neighboring *E. horridum* plants. Indeed, plants living in the interior of monospecific patches have a lower growth rate and higher crown death rate than plants at the edges of the patches (Komac et al. 2012); c_2 is the additional mortality due to intensity of competition between neighboring adults *B. sempervirens* and *E. horridum.*

The mortality of *B. sempervirens* adults (Ba) is a density-independent rate, d_{B_a}:

$$W_{B_a,E} = d_{B_a} \qquad (5)$$

The mortality of *B. sempervirens* seedlings (Bs) is a density-independent rate, d_{B_s}.

$$W_{B_s,E} = d_{B_s} \qquad (6)$$

2.3.4 Maturation of B. sempervirens

B. sempervirens seedlings mature at a constant rate and thereby become adults:

$$W_{B_s,B_a} = m \qquad (7)$$

where m is such that on average *B. sempervirens* seedlings become adult after 1/m years.

2.4 Estimation of parameter values

The parameter values of the model were estimated using data on *E. horridum* and *B. sempervirens* longevity, growth, demography, dispersal and seedling production. We also used data from field experiments to estimate the interaction intensity between the two shrub species for both *B. sempervirens* seedlings and adults. We used data from a previous study (Komac et al. 2012, Komac et al. 2013) and from two abandoned and shrub-encroached grasslands that differed in the time when they were abandoned in Ordesa and Monte Perdido National Park, Spain (Nuche and Alados, *in rev*, and additional data in Appendix). This allowed us to validate the model (Appendix 3).

E. horridum reproduce both sexually and clonally. The **clonal spreading rate of *E. horridum*** in empty sites, vs_1, was estimated by the difference in crown growth between two consecutive years (Table 1). The seedling **establishment rate of *E. horridum*** in empty sites, es_1, was calculated as the number of seedlings divided by the number of adults *E. horridum* per m^2 (Table 1). **Intra-specific competition of *E. horridum*, c_1**, was calculated as *E. horridum* mortality rate in the center of a patch minus *E. horridum* mortality rate at the edge of a patch (Table 1).

B. sempervirens reproduce only sexually. To estimate the **establishment rate of *B. sempervirens* seedlings, *eb***, the number of seedlings established per mother plant and per year were accounted during 7 consecutive years (from 2007 to 2014); ***eb*** was calculated as the average of seedlings established / 8 neighboring cells. The frequency distribution of seedlings dispersion (kernel) was recorded in 2007, where all seedlings in a circumference of 2 meters radius around a *B. sempervirens* target and their distance to the target were recorded. That allowed us to estimate the fraction of local *vs.* global dispersion of *B. sempervirens*. **Global dispersion**, $\boldsymbol{\delta}$, was calculated as the mean number of seedlings between 1 and 2 meters from the *B. sempervirens* mother plant (Table 1). Our estimation of mean maximum distance of *B. sempervirens* dispersion agrees with Debussche and Leppart 1992. **Survival rate of *B. sempervirens* seedlings** in the first year, $\boldsymbol{s_b}$, is the probability of survival outside *E. horridum* crown, and was the percentage of seedlings that survived without the protection of *E. horridum* in one year (Table 1). Mediterranean resprouter shrubs, such as *B. sempervirens*, reach **maturity, m,** and thus are able to produce seeds on average 9.1 years old (Pausas et al. 2004) (Table 1).

The **mortality rate of *E. horridum* ($\boldsymbol{d_S}$)** and ***B. sempervirens* adults ($\boldsymbol{d_{Ba}}$)**, was calculated as the inverse of their lifespan. The average age of an *E. horridum* adult plant at the edge of a patch was 35.6 yr (n=10 plants); therefore the mortality rate in the absence of competition was 1/35.6 ind yr^{-1}. Parameters of *E. horridum* shrub are deeper explained in Komac et al., 2013. Mortality rate of *B. sempervirens* was calculated as the inverse of the average age of the more abundant age class, 1/45 ind yr^{-1}. The **mortality rate of *B. sempervirens* seedlings**, $\boldsymbol{d_{Bs}}$, was estimated as the annual probability of survival during the first 10 years since after 10 years the plant is assumed to be an adult. In the field, the survival of *B. sempervirens* seedling was monitored annually from 2008 to 2012 (N=36). The average mortality rate was estimated as 0.62 which was the percentage of seedling that survived five years divided by five (Table 1).

In the field, the abundance of *B. sempervirens* seedlings ranging from 1 year old to 10 year old was quantified; the annual mortality rate was estimated as 1 minus the root square of the number of seedlings of 10 years old divided by the number of seedlings on 1 year old (Table 1).

The interaction between study shrubs have been evaluated using a previous neighbor removal experiment (Nuche and Alados. *in rev*). The **facilitation intensity**, *fs*, of *E. horridum* to *B. sempervirens* seedlings is calculated as the probability of survival under *E. horridum* minus the provability of survival outside *E. horridum* crown (Table 1). C**ompetition intensity of *B. sempervirens* adults to *E. horridum***, c_2, was calculated as the mortality rate of *E. horridum* when coexisting with *B. sempervirens* (Control) minus the mortality rate of *E. horridum* when *B. sempervirens* adults were removed from its neighborhood (B.

removal) (Table 1). *E. horridum* mortality in the neighbor removal experiment is calculated as the inverse of the lifespan; the lifespan is estimated from the relationship between crown size and age of *E. horridum* of a previous study (Komac et al. 2012). The higher *E. horridum* mortality rate in B. removal compared to *ds* could be due to the location of our study, which is at a lower altitude than the study of Komac et al. 2012.

2.5 Model Simulations

Simulations were run on 200*200 lattices with periodic boundary conditions and a stochastic asynchronous update (Ingerson and Buvel, 1984) using MATLAB 7.8. Each cell was assumed to be around 1 meter because averaged crown size (diameter) of *E. horridum* plants > 15 years and of *B. sempervirens* adults was ≈ 1 meter (Komac et al. 2012, Komac et al. 2013, Marinas, 2004, Nuche and Alados *in rev*). One time step was assumed to be a year. Random initial conditions were used for the simulations. Test with different initial conditions were performed and similar results were achieved. Each simulation was run until stationary state was reached. Model validation was performed (Appendix 3).

2.5.1 The role of plant interactions on vegetation succession

To investigate the role of plant interactions on vegetation succession several simulations were performed with different combination of values of the parameters that account for species interactions, *fs*, *E. horridum* facilitation towards *B. sempervirens* seedlings, and *c2*, *B. sempervirens* adults' competition towards *E. horridum*. The range of values for *fs* parameter was from 0, when the protection of *E. horridum* towards *B. sempervirens* seedlings does not lead to an increase in seedling survival (no facilitation), to 1, when the protection of *E. horridum* leads to 100% of *B. sempervirens* survival underneath its crown (facilitation is maximum). The range for *c2* was from 0, when *B. sempervirens* adult do not increase the probability of dying of *E. horridum*, to 1. The initial conditions were set to a very low abundance of *B. sempervirens* adults and *E. horridum*, i.e. 0.001 % each, no *B. sempervirens* seedlings, and the rest of cells were in state empty.

For each combination of parameters, logistic regressions were fit for *B. sempervirens* adults progression (Ba % cover) and the time step at which the inflexion point was achieved was recorded as indicator of vegetation succession rate. The carrying capacity (i.e. the maximum value of Ba cover reached at the end of the simulation) and the steepness of the curve (the exponential growth of the logistic curve) were also recorded as indicator of the *B. sempervirens* expansion.

2.5.2 The effect of temperature on vegetation succession

To investigate how temperature might affect vegetation succession, we used data of seven years of monitoring *B. sempervirens* establishment (Appendix 1); and from two sites which differed in temperature conditions (hereafter referred to as High Temp and Low Temp, respectively for high and low temperature conditions) (Nuche and Alados *in rev*). The variation of the intensity of interactions with temperature was estimated quantifying the intensity of interaction at each study site (High and Low sites, Nuche and Alados *in rev*). With previous information two temperature scenarios were simulated in the present study: a low temperature scenario and a high temperature scenario. The low temperature scenario represent the climatic conditions found by *B. sempervirens* at its actual upper limit of altitudinal range (High study site at 1600 m.a.s.l., Nuche and Alados *in rev*); and the high temperature scenario represent the current global warming in locations of *B. sempervirens* - *E. horridum* ecosystem at a mid-altitude (Low study site at 1200 m.a.s.l., Nuche and Alados *in rev*).

Intensity of *E. horridum* facilitation towards *B. sempervirens* juveniles showed an important variation with temperature, in the model was applied towards the *B. sempervirens* seedlings, *fs*. Under low temperatures the facilitation intensity of *E. horridum* towards *B. sempervirens* juveniles was 1.86 times higher than under high temperatures, calculated as the intensity of facilitation at the High site divided by the intensity of facilitation at the Low site. The intensity of facilitation at each site was estimated as the growth of *B. sempervirens* juveniles when *E. horridum* was removed from its neighborhood (E. removal treatment) minus the growth of *B. sempervirens* saplings when coexisting with *E. horridum* (in Control) divided by the growth of *B. sempervirens* juveniles in Control (Nuche and Alados *in rev*). Under high temperatures the intensity of competition of *B.*

sempervirens adults towards *E. horridum* was 2.3 times higher, calculated as the intensity of competition in Low site divided by the intensity of competition in High site. The intensity of competition at each site was estimated as the growth of *E. horridum* when *B. sempervirens* was removed from its neighborhood (B. removal treatment) minus the growth of *E. horridum* when coexisting with *B. sempervirens* (in Control) divided by the growth of *E. horridum* in Control (Nuche and Alados *in rev*).

High site has an annual mean air temperature of 7.38 ºC and Low site 8.43ºC (Nuche and Alados *in rev*). Therefore the variation in the intensity of plant interactions corresponds to a change in 1.05 ºC.

With these data, we could estimate the range of variation of the parameters (*eb*, *fs*, and *c2*) as previously explained for each of the two temperature conditions. We thereby modeled here climate indirectly by its known effects on the interspecific interactions and recruitment in the study system.

In the Low Temp scenario the competition ability of *B. sempervirens* towards *E. horridum*, *c2*, was set to a low value (c2=0.1) and the role of the intensity of facilitation (*fs*) and the variation in recruitment (*eb*) was evaluated. The range values for *eb* was from 0, to 0.8, because is a low number of *B. sempervirens* seedlings per m^2 quantified in the field during 7 years of monitoring (Appendix 2).

In the High Temp scenario *E. horridum* facilitation towards *B. sempervirens* seedlings, *fs*, was set to a low value (*fs*=0.2), and the role of the intensity of competition of *B. sempervirens* adults towards *E. horridum* and the variation in recruitment were investigated.

3. Results

3.1. The role of plant interactions on the system dynamics,

Independent of the precise combination of parameter values chosen and of the initial proportions of *B. sempervirens* adults (Ba) and *E. horridum* (S) in the lattice, the Ba cover increases in time during the simulations, while simultaneously the cover of S decreases until eventually disappears/goes extinct.

The invasion of Ba in the system accelerates when the competition intensity from Ba to S (i.e. the parameter, *c2*) and the facilitation from S to Bs (i.e. the parameter *fs*) increase. This means that for strong interspecies interactions, the number of iterations needed to reach the inflexion point of the Ba growth curve decreases (Figure 2e). In other terms, increasing the intensity of inter-species interactions, *c2* and *fs*, speeds-up vegetation succession, that is Ba invasion.

Simulations showed that an increase in *c2* would compensate a decrease of *fs* (Figure 2e, 6 *vs.* 7). Ba expansion is much accelerated with an increase in *fs* than with the same increase in *c2* (Figure 2e, 3 *vs.* 4 and 6 *vs.* 8). However, as competition changes with temperature (2.3 times) more than facilitation (1.86 times) an increase in temperature (e.g. 1.05ºC) would accelerate the Ba expansion (taking the rest of the parameters constant) (Figure 2e, 5 *vs.* 7).

3.2. Vegetation succession in the low vs. high temperature scenario,

We now investigate how the speed of the vegetation succession varies under two different temperature scenarios including both shrubs interactions and *B. sempervirens* establishment.

Consistently with Figure 2, in Low Temp scenario a low *c2* and *eb* values would lead to a decrease in Ba expansion rate despite high values of *fs*; and High Temp scenario, with high *c2* and *eb* values would speed-up Ba expansion rate despite the low *fs* value (Figure 3e¿III? *vs.* Figure 4d¿IV?).

At the Low Temp scenario, in the case where the Ba recruitment (*eb*) was low (inferior to 0.22), a minimum *fs* threshold was required to allow for Ba expansion, otherwise its population collapsed (Figure 3 a-d).

At the High Temp scenario, *B. sempervirens* expansion rate is accelerated until a *c2* threshold value beyond which the Ba expansion rate does not depend on competition anymore, and is also independent of the recruitment value, *eb* (Figure 4a and b). The carrying capacity did not vary with the competition parameter *c2* except at low values of eb.

3.3. Coexistence

Figure 5 shows that in the Low Temp scenario, the coexistence between *B. sempervirens* and *E. horridum* is possible under several combinations of parameter, *eb* and *fs*, values. However, in the High Temp scenario *B. sempervirens* colonizes up to 80% of the matrix and *E. horridum* almost disappear.

4. Discussion

The model built in this study successfully described the dynamics of the vegetation succession of subalpine grasslands at the Pyrenees, where the expansion of *B. sempervirens* leads to the progressive displacement of *E. horridum*. In addition, species interactions have been shown to be a fundamental mechanism on vegetation succession of abandoned subalpine grasslands at the Pyrenees. When species interactions were set to 0, the system dynamics were extremely low; this indicates that species interactions are a fundamental mechanism in species turnover. In the absence of a change in climatic conditions, the vegetation succession of grasslands takes place due to the abandoned agricultural activities at the 1200-1600 m. a.s.l., where the lack of grazing lets the seedlings of the woody species establish and develop (Rousset and Lepart 2002, Borchard et al. 2011).

Higher intensity (i.e. higher parameter values) of competition and facilitation gave rise to higher vegetation succession rate. Therefore, environmental conditions that lead to higher intensities of these plant-plant interactions are bound to speed-up vegetation succession (Walker and del Moral 2003, Dullinger *et al*. 2003, Batllori and Gutiérrez 2008). Do we expect to find in nature certain environmental conditions that would favor both facilitation and competition in our study system at the same time? A previous experiment showed that competition ability of *B. sempervirens* increased under higher temperatures and the intensity of *E. horridum* facilitation towards *B. sempervirens* seedling decreased (Nuche and Alados *in rev*). *B. sempervirens* population has two strategies to expand on *E.*

horridum stands, 1. being facilitated by *E. horridum* or 2. out-competing *E. horridum,* which could encompass a wide range of environmental conditions. The simulations showed that *B. sempervirens* expansion is much accelerated with an increase in facilitation than with the same increase in competition, which would mean that facilitation is a better strategy to *B. sempervirens* expansion, facilitation is more important in vegetation succession than competition. Facilitation would therefore mediate vegetation succession response to climate change more than competition. However, facilitation and competition did not vary with temperature to the same extent, competition have a greater variation than facilitation with the same change in temperature. Therefore competition would play a major role than facilitation in mediating the vegetation responses to climate change in our study system. For that reason vegetation succession is expected to be accelerated under higher temperatures and slowed-down under low temperatures.

In a scenario of global warming, higher temperatures are expected to increase the ability of adults' *B. sempervirens* to outcompete *E. horridum* and *B. sempervirens* seedling establishment, which would speed-up the vegetation succession at the Pyrenees even with very low seedling facilitation (low *fs* value). However, there is an intensity of competition from *B. sempervirens* adults towards *E. horridum* beyond which competition does not affect vegetation succession rate anymore, meaning that there is an upper limit in the increase of vegetation succession imposed by climate change. One possible explanation is that competitive effect is local and is applied only to the *E. horridum* individuals that are in the neighborhood of *B. sempervirens* adults. In addition, a high value of competition would lead in theory to a lower carrying capacity of *B. sempervirens* adults when the number of seedling establishment is not too high. This indicates that there is a trade-off between the local dispersion and the necessity of *B. sempervirens* seedlings to establish under an *E. horridum versus* the competitive effect that *B. sempervirens* adults apply towards the *E. horridum* of their neighborhood. When the competitive effect that *B. sempervirens* applied to *E. horridum* is high, the *B. sempervirens* seedlings do not establish so well because the facilitative effect is nullified. Even though *B. sempervirens* eventually displace *E. horridum*, they need *E. horridum* for the establishment of their seedlings. This could be a mechanism for both species coexistence. However, a high value of competitive ability and a low value of seedling establishment is not expected to occur at the same time because both increase with temperature, except on non-mast years. Masting is synchronous highly variable seed production among years by a plant population (Kelly 1994). Whether mast seeding results from weather conditions or is an evolved reproductive strategy is still under debate (Kelly et al. 2002). Lázaro et al. 2006 showed that another species of the same genre, *B. balearica*, had a masting behavior in island populations (in Balearic Islands) rather than in the mainland, partially related to rainfall events. However, in our study we did not find important inter-annual variability on recruitment (Appendix 2). Therefore, masting behavior might not be observable at recruitment phase or *B. sempervirens* might not behave as masting specie in our study sites. A detailed study on *B. sempervirens* reproductive biology is required, especially on the relationship of its masting behavior and climate.

At the upper-limit of altitudinal range, *B. sempervirens* would be exposed to low temperatures which limit seedling establishment and adults' competitive ability. Despite the fact that an increase in the intensity of facilitation of *E. horridum* towards *B. sempervirens* seedlings compensate for a decrease in the number of seedling established, vegetation succession rate is slowed-down due to the lower competition ability of adult *B. sempervirens*.

Low summer temperatures limit seed establishment by diminishing adults flower and fruit production (Hedhly 2011), *B. sempervirens* flower bud formation and fruit set takes place in July and August (Milla et al. 2012). *B. sempervirens* seedling establishment lower than 0.11 seedling m^{-2}, 1 seedling in 9 m^{2}, would disrupt the expansion of *B. sempervirens*. *B. sempervirens* is therefore expected to be able to migrate upslope until low temperatures limit its seeds production under a value of 9 seedlings per m^{2}. Infer a temperature value for this isotherm would be very simplistic because the relationship between fruit production and temperature might not be linear and might also depend on other environmental or genetic parameters (Lázaro et al. 2006). If seedling establishment is between 1 seedlings 5-9 m^{-2} the intensity of facilitation would determine the expansion or the collapse of *B. sempervirens* population. Therefore, under extremely low temperatures the presence of *E. horridum* is needed for the establishment of *B. sempervirens*.

In the low temperature scenario *B. sempervirens* and *E. horridum* coexist under certain combination of parameter values (mainly low *eb* values, see Figure 5). In nature, the community dominated by *B. sempervirens* and *E. horridum* have been described as a mature and stable community, named *Berberidion p.p.* (Tena 2009). It would be adaptive for *B. sempervirens* to not exert a very high competitive displacement towards *E. horridum* given that is needed for *B. sempervirens* seedling survival, especially under harsh climatic conditions under low temperatures. Contrary, in the high temperature scenario, *B. sempervirens* dominates and *E. horridum* abundance is lower than 10% due to the high values of competition and establishment of *B. sempervirens*.

If current global mean air temperature increases as predicted by climate change, the low temperatures at the actual upper limit of *B. sempervirens* altitudinal range will not limit *B. sempervirens* recruitment and competition ability towards *E. horridum* anymore. A *B. sempervirens* upslope migration could therefore been expected (Dullinger et al. 2003).

Camarero et al. 2004 demonstrated in *Pinus uncinata* at its upper altitudinal limit at the Central Pyrenees that an increase of spring-summer temperatures is positively related with seedling establishment but negatively related with advance in tree line, resulting in an increase of tree density rather than an upward migration of the tree line position. They explained these results because higher temperatures could suppose drought stress in Mediterranean mountains. *B. sempervirens* is a species of the sub Mediterranean territory, locations with a Mediterranean climate but not very dry either hot, *B. sempervirens* prefers the climate of mid mountain with certain humidity (Lenoble and Broyer, 1945). Therefore, it could be possible that the lack of water would limit the upslope migration of *B. sempervirens* under climate change. However, the presence of *E. horridum* might counteract the lack of water resources given that humidity is higher underneath its crown (Nuche et al *in rev*, Cavieres et al 2007). We unknown if there might be other abiotic factors that varies with altitude, such as sun radiation or poor soil conditions, which might limit *B. sempervirens* upslope migration.

Although several studies have suggested the importance of plant interactions in mediating ecosystem responses to climate change (Walther 2010, Van der Putten 2012), the majority of studies on the upper limit of altitudinal distribution of woody species have focused on the effect of climate on one single species (Camarero et al. 2004, Danby et al. 2007) and have therefore disregarded the importance of species interactions.

Here we showed how shrub-shrub interactions are a fundamental process to understand the dynamics of this ecotone. Taking into account plant-plant interactions on the upper limit of altitudinal range of shrubs, which might be highly sensitive to a change in climatic conditions and where several species usually coexist, makes taking into account species interactions fundamental.

It is noteworthy that we here only considered the dominant species of the system. However, other species could come into the system continuing the vegetation succession, such as *Pinus sylvestris*, which would alter the balance of interaction between *B. sempervirens* and *E. horridum*. Besides, we did not take into account the variation in *E. horridum* performance with climatic conditions. Komac et al. (2013) showed that *E. horridum* vegetative growth was also favored by higher temperatures, but this study was conducted on a higher and therefore colder location than the experiments of the present study where performed.

We conclude that plant interactions are a key mechanism in vegetation succession, and that a change in climatic conditions can modify the vegetation succession process by the modification of these plant interactions. Therefore plant interaction should be taken into account when evaluating the potential impact of climate change on ecosystems dynamics.

Acknowledgements

We gratefully acknowledge the financial support of the Spanish Ministry of Science and Innovation (BES-2009-017189), the Spanish Economy and Competition Ministry (PN-MICINN) (CGL2008-00655/BOS and CGL2011-27259) and Spanish National Park organization (DIPA 125/2010 MMAMRM). We thank the AEMET for providing meteorological information, Bruce MacWhirter.

Transition type	**Symbol**	**Interpretation**	**Estimation from field data**	**Value**	**Source**
Mortality	d_s	Mortality rate of *E. horridum*	1/(life span)	1/35.6=0.028	Komac et al. *in rev* Komac et al., 2013
	d_{Ba}	Mortality rate of *B. sempervirens* adults	1/(life span)	1/45 = 0.02	Unpublished data
	d_{Bs}	Mortality rate of *B. sempervirens* seedlings	Provability of survival the first 5 years / number of years	0.6/5=0.155≈0.2	Appendix 3
Vegetative reproduction	vs_1	Vegetative reproduction rate of *E. horridum* on empty sites	(D2-D1)/D1, with D1 and D2 the crown diameters of *E. horridum* in two consecutive years; calculated in high slope	0.109	Komac et al., 2013
Seed establishment	es_1	Establishment rate of *E. horridum* seedlings on empty sites	seedlings/number of adult *E. horridum* on high slopes m^{-1}	0.38	Komac et al. 2013
	eb	Establishment rate of *B. sempervirens* seedlings on empty sites	Average number of seedlings in the first meter near a *B. sempervirens* adult / m^2	1.32	Unpublished data
	s_B	Early survival of *B. sempervirens* seedlings without facilitation of *E. horridum*	Probability of survival in the first year	0.2	Nuche et al. *in rev*
Global dispersion	δ	Dispersion of *B. sempervirens* seedlings throughout the lattice	Num seedlings farther than 1 meter to the mother plant per mother plant	0.48/8 = 0.06	Nuche et al. *in rev*
Species Interaction	c_1	*E. horridum* intra-specific competition	*E. horridum* mortality rate in the center of a patch-*E. horridum* mortality rate at the edge of a patch	0.0015	Komac et al., 2013
	c_2	Competition intensity of *B. sempervirens* adults on *E. horridum*	(*E. horridum* mortality rate in Control - *E. horridum* mortality rate in B. removal)	0.105-0.085=0.20	Nuche et al. *in rev*
	fs	Early survival of *B. sempervirens* seedlings when facilitated by *E. horridum*	%*B. sempervirens* seedling survival under *E. horridum* - %*B. sempervirens* seedling survival outside *E. horridum*	0.8 - 0.2 = 0.6	Nuche et al. *in rev*
B. sempervirens Maturity	m	Maturation rate of *B. sempervirens* seedling	Time to achieve sexual maturity	10	Pausas et al. 2004

Table 1. Model parameters, their meaning and values.

	Low Temperature scenario	High Temperature scenario
eb	[0 : 0.8]	[0.7 : 3]
fs	[0 : 0.8]	0.2
c2	0.1	[0 : 1]

Table 2. Parameter values and range of parameter values under the two temperature scenarios.

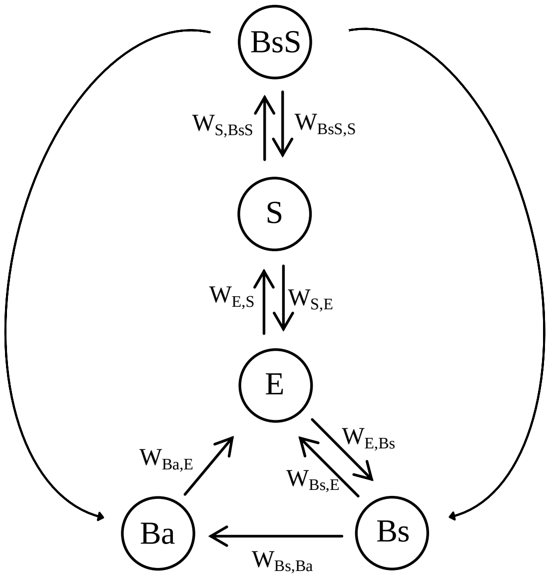


Figure 1. Conceptual model showing the possible states of de model: cell occupied by *E. horridum* (S), *B. sempervirens* seedlings (Bs), by both *E. horridum* and *B. seedling* (BsS), by *B. sempervirens* adults (Ba), or empty (E). $W_{\sigma\sigma'}$, are the rates of transition between the states. Their mathematical expressions are given in the text.

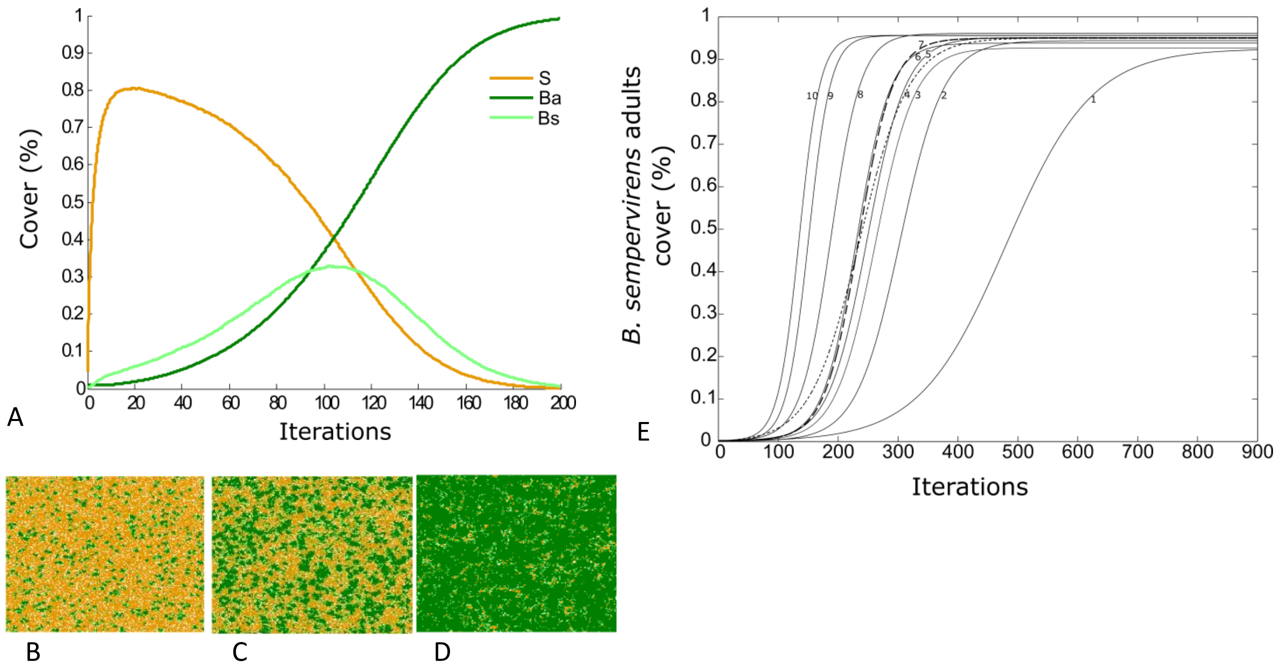


Figure 2. a) Cover (%, number of occupied cells) of each state: S, *E. horridum*, Ba, *B. sempervirens* adults, and Bs, *B. sempervirens* seedlings, through model progression. Three snapshots of the lattice through model progression B) in 60 iteration, C) in 100 iteration, D) in 140 iteration. E) Logistic regression of adults *B. sempervirens* through model progression under different combination of parameter values, **1**: *c2*=0, *fs*=0; **2**: *c2*=0.14, *fs*=0.14; **3**: *c2*=1, *fs*=0; **4**: *c2*=0, *fs*=1; **5**: *c2*=0.1, *fs*=0.28; **6**: *c2*=0.85, *fs*=0.14; **7**: *c2*=0.28, *fs*=0.28; **8**: *c2*=0.14, *fs*=0.85; **9**: *c2*=0.85, *fs*=0.85, **10**: *c2*=1, *fs*=1. All other parameter values are set to values indicated in the methods (Table 1).

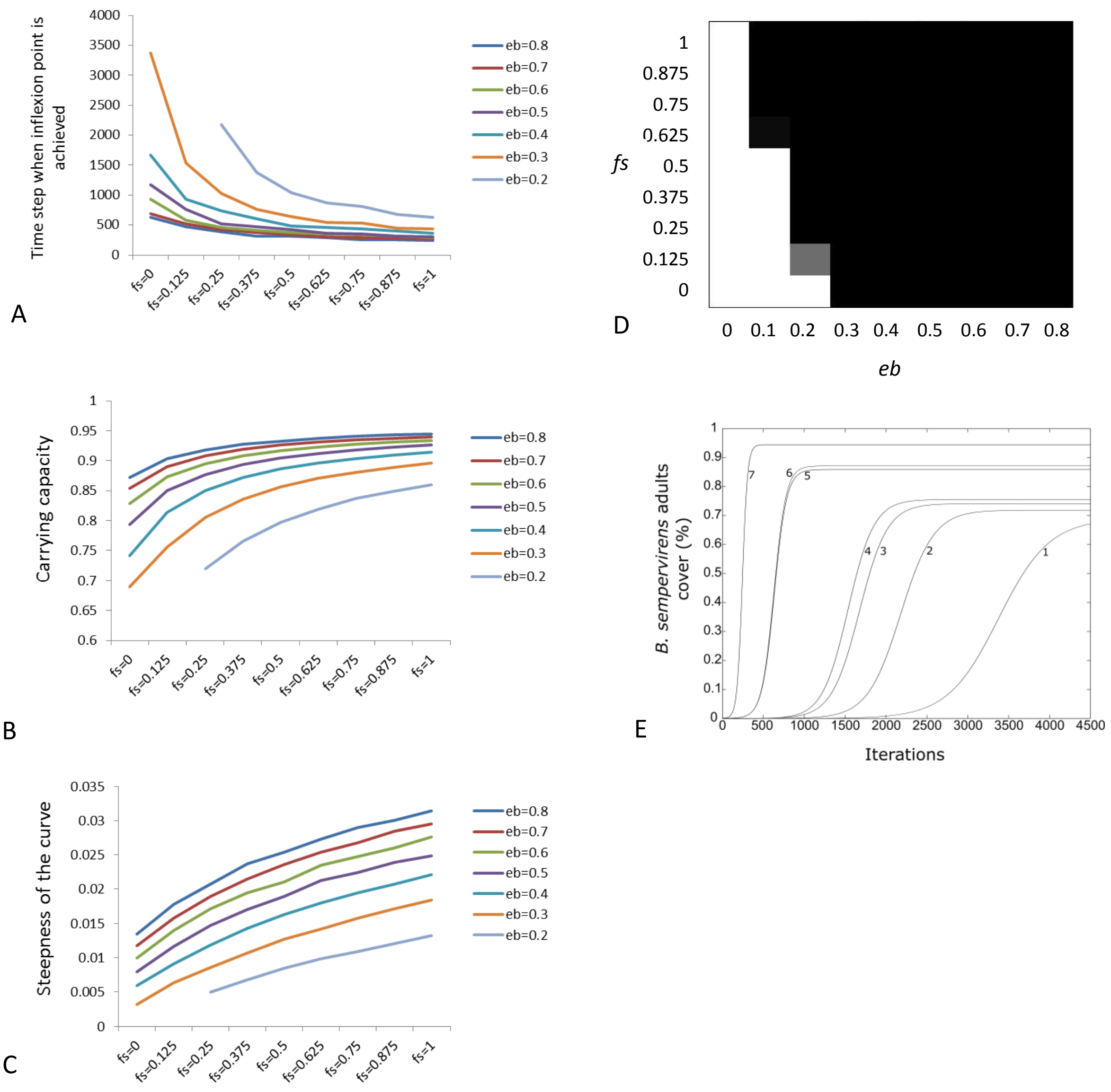


Figure 3. Low temperature scenario (*c2*=0.1). A) Time step when inflexion point is achieved in the logistic fit of *B. sempervirens* (Ba) progression. B) Carrying capacity and C) steepness of the curve, for several simulations with different combination of parameter values, *eb*, *B. sempervirens* seedling establishment and *fs*, *E. horridum* facilitation towards *B. sempervirens* seedlings. D) State diagram, black, there is Ba final cover, white, there is not Ba at the end of the simulation. E) Logistic regression of adults *B. sempervirens* through model progression, when 1: *fs*=0 and *eb*=0.3, 2: *fs*=0.25 and *eb*=0.2, 3: *fs*=0 and *eb*=0.4, 4: *fs*=0.125 and *eb*=0.3, 5: *fs*=1 and *eb*=0.2, 6: *fs*=0 and *eb*=0.8, 7: *fs*=1 and *eb*=0.8. All other parameter values are set to values indicated in the methods (Table 1).

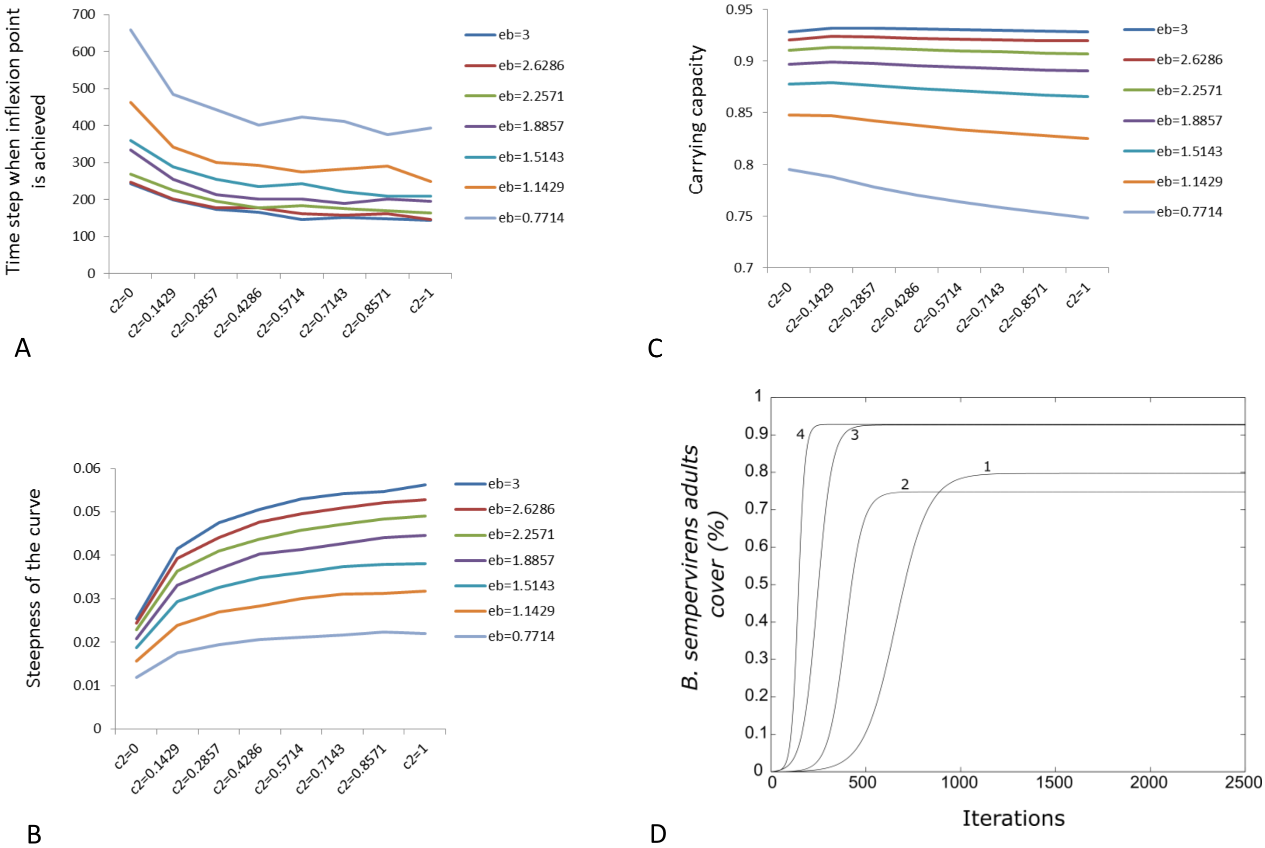


Figure 4. High temperature scenario (*fs*=0.2). A) Time step when the inflexion point is achieved, B) the carrying capacity and C) the steepness of the curve of the logistic regression of *B. sempervirens* adults cover through the simulation at each combination of parameter values, *c2*: competition effect of *B. sempervirens* adults on *E. horridum* and *eb*, number of *B. sempervirens* seedling established. D) Logistic regression of adults *B. sempervirens* through model progression, when I: *c2*=0 and *eb*=0.7; II: *c2*=1 and *eb*=0.7; III: *c2*=0 and *eb*=3, IV: *c2*=1 and *eb*=3. All other parameter values are set to values indicated in the methods (Table 1).

Chapter 4

Developmental instability as an index of adaptation to drought stress in a Mediterranean Oak

Paloma Nuche, Benjamin Komac, Jesús Julio Camarero and Concepción L. Alados

Chapter 5

Plant interactions at community level and vegetation spatial pattern in response to environmental conditions in alpine Mediterranean ecosystem

Concepción L. Alados, Paloma Nuche, Hugo Saiz , Juan Escós , Teresa Navarro and Yolanda Pueyo

Abstract

The change in environmental conditions influences not only species abundance, but also the interaction among plant species. It has important implications on ecosystem performance and dynamic, high relevant in the perspective of actual climate change. In particular, the importance of positive interactions preventing the loss of diversity is very important in harsh environments, as the alpine Mediterranean ecosystems, which are under the pressure of cold temperatures and the water shortage in determinant periods of the year. This make particularly relevant to investigate the relationship between environmental conditions and plant community interactions and its influence on plant community structure (species diversity and vegetation spatial patterns). We compared sites that differed in soil properties and climatic conditions to disentangle how biotic interactions and plant community structure change in response to environmental conditions. We compared North and South oriented slopes in two environments (Acid vs Basic soils). According with theory we observed higher number of positive interactions and higher vegetation aggregation in the most stressful colder habitats. This in turn resulted in higher species diversity. A clear positive relationship was observed between the positive interaction balance and the vegetation aggregation patterns as predicted. Under the expected global warming scenario we predict the breakage of plant-plant association that can be assessed by the reduction of vegetation aggregation patterns and the loss of species diversity. These effects are more evident in the poor soil nutrients acid soils than in the richer basic soils.

Keywords**:** *alpine, diversity, facilitation, spatial patterns, Sierra Nevada*

1. Introduction

In recent decades, climate warming and increased frequency of extreme events (Stocker et al. 2013), have led to an increasing interest to the study of the effects of global change on vegetation composition and species diversity (Guisan et al. 1995, Chapin III et al. 2000, Theurillat and Guisan 2001). This is particularly important for arctic and alpine ecosystems, where recent studies have reported important changes in plant composition, biomass and diversity (Klein et al. 2004, Walker et al. 2006, Elmendorf et al. 2012). Nevertheless, the intensity and direction of change can differ among species and regions (Clavel et al. 2011, Elmendorf et al. 2012, Jiménez-Alfaro et al. 2014, Rosbakh et al. 2014). While at high elevations plant species richness has increased in recent decades due to climate warming (Stöckli et al. 2011, Venn et al. 2012, Wipf et al. 2013), at low elevations in subalpine belts this effect is less evident and can even revert (Vittoz et al. 2009, Rosbakh et al. 2014). One of the mechanisms involved on this change in arctic and alpine communities referred to the reduction of specialist species and homogenization of species distribution (Jurasinski and Kreyling 2007, Clavel et al. 2011, Ross et al. 2012), as more generalist species are able to invade upper zones. This would result in the loss of not only species diversity but also functional diversity which could ultimately drive to changes in the processes operating in the whole ecosystem (Tilman et al. 1997).

The change in the organisms interactions in response to climate change has received less attention despite some authors have hypothesized that species interactions can dramatically alter species responses to climate change (Tylianakis et al. 2008, Gilman et al. 2010, Adler et al. 2012) influencing the abundance of competitors and the intensity and direction of competition, of relevant influence on the response of plant community dynamic to climate change (Farrer et al. 2014). The importance of positive interactions preventing the loss of diversity have been reported in several studies (Hacker and Gaines 1997, Michalet et al. 2006, Le Bagousse-Pinguet et al. 2014) particularly in harsh environments (Cavieres et al. 2014).

Stress gradient hypothesis predicts an increasing frequency, intensity and importance of positive interactions with increasing levels of abiotic stress and disturbances (Bertness and Callaway 1994, Brooker and Callaghan 1998) although under extreme stress conditions positive interactions may decline (Maestre et al. 2005, Michalet et al. 2006, Smit et al. 2007). Further studies have suggested that the life history of the interacting species and the nature of the stress can change the sign of this relationship (Michalet et al. 2006, Maestre et al. 2009, Malkinson and Tielbörger 2010). Thus, this relation is not always linear along an environmental stress gradient, and some authors proposed a modification of SGH predicting the increase of the importance of positive plant-plant interactions only until moderate level of stress (Holmgren and Scheffer 2010). In addition, it has been argued that the discrepancy between studies can be based on either unrelated stress factors acting at the same time, or species-specific interactions (Soliveres et al. 2015). In any case, most of the studies on plant-plant interactions concentrate on interactions between selected pairs of species rather than interactions at the whole community level (see (Soliveres et al. 2015) for review), but see (Saiz and Alados 2012), despite we need to know the response of the community to environmental pressures to forecast scenarios of global change and to determine the importance of positive and negative plant interaction on the diversity and structure of plant communities (Hacker and Gaines 1997).

Vegetation spatial patterns are related with the direction and strength of plant interactions, which lead to processes of niche differentiation and /or construction, and ultimately enhance the coexistence of more species than would be expected from a random distribution (Kikvidze et al. 2005). Few studies have addressed explicitly the relative importance of plant-plant interactions on spatial patterns, but see (Kikvidze et al. 2005, Mitchell et al. 2009, Maestre et al. 2010). Theoretical investigations revealed that vegetation spatial patterns emerge in response to self-organizing processes (Rietkerk et al. 2004) where plant facilitation is one of their leading mechanisms (Kéfi et al. 2007b), while in field studies this relationship is assumed.

The aim of this work was (1) to investigate the relationship between biotic interactions and plant community structure (plant spatial pattern and diversity) in Mediterranean alpine ecosystems, and (2) to disentangle how biotic interactions and plant community structure change in response to environmental conditions.

We conducted an observational study in a dry, high mountain system in southeast Spain and compared North and South oriented slopes in two environments (Acid vs Basic soils). We measured T^a and soil properties in each site to assess the environmental conditions of each study site. We used north and south slopes as a proxy of climate warming, as higher temperatures are reached in south slopes in comparisons with north slopes (Zhou et al. 2013). Although many studies have used altitudinal gradient as a surrogated of climate change (Körner 2007), north-south orientations provide important climatic variation that have been also used to simulate long term effect of contrasting environmental conditions (Escos et al. 2000, Alados et al. 2001, Sternberg and Shoshany 2001, Pueyo et al. 2007). The effect of soil pH on species richness is a well-known phenomenon (Ellenberg 1988). In Mediterranean ecosystems, acid soils are less diverse than basic soils (Ojeda et al. 1996, Ozkan et al. 2009) probably due to the low soil nutrients observed in acid soils (Ozkan et al. 2006),which may influence the resilience of the plant community to climate change (i.e. more diverse communities have been presented as being more resilient to environmental changes (Walker 1995, Elmqvist et al. 2003)).

We hypothesize that (H1) it exists a positive relationship between the predominance of positive interactions and vegetation spatial aggregation. (H2) Positive interactions are a mechanism to maintain high diversity in plant communities under stress. Thus, in stressing conditions by low temperatures (north slopes), positive biotic interactions (and thus, plant spatial aggregation) will predominate, maintaining high diversity in the plant community. On the contrary, under more benign temperatures (south slopes) negative biotic interactions will predominate. The effect of stress by low temperatures on biotic interactions and plant community structure will be evaluated under two contrasting environments, acidic and basic soils. We expect that (H3) nutrient-poor acidic soils will present a stressful environment by itself, with smaller species pool than nutrient-rich basic soils, and reinforcing the effect of low temperatures on plant community.

2. **Material and Methods**

2.1 Study Area

The study was conducted in the Sierra Nevada National Park, south-eastern Spain. The study area consisted of three locations, and within each location two sites with different exposition (north-south) were selected (location A north 37º06’N, 2º 58’W, 2270 m a.s.l. altitude; location A south 37º 05’N, 2º 58’W, 2275 m a.s.l.; location B north 37º06’N, 3º 24’W, 2279 m a.s.l.; location B south 37º06’N, 3º24’W, 2299 m a.s.l.; location C north 37º07N, 3º25W, 2180 m a.s.l., and location C south 37º07N, 3º25W, 2172 m a.s.l.). The distance between paired North-South sites in each location was c. 0.5-1 km. Average mean Tª in the closet meteorological station (37º 09’ N, 3º 15’ W) between 2009 and 2014 (both included) is 7.74 ºC, and total annual precipitation is 538.09 mm (www.magrama.gob.es/es/red-parques-nacionales/red-seguimiento/datos-seguimiento.aspx).

Soil substrate is siliceous in location A and B and it is basic material in location C. Basic outcrop at high elevation is scarce as a natural output of mountain formation genesis, location C is the only one around 2100 m available in Sierra Nevada mountain range.

Vegetation is a dwarfland matorral dominated by cushion plants as

Erinacea anthyllis Link, *Genista versicolor* Boiss, *Juniperus communis* L. and *Astragalus nevadensis* Boiss, and herbaceous as *Cerastium boissieri* Gren., *Festuca indigesta* Boiss and *Hieracium castellanum* Boiss. & Reut.

2.2 Data collection and analysis

To monitor the environmental conditions four air temperature probes were installed at each site (two probes at each slope) at 1m of height. Daily air temperature was recorded every hour from Jun 2012 to July 2014 using a Hobo U-23 pro v2 data logger (Onset Computer Co., Bourne, MA, USA). We calculated mean, maximum and minimum daily temperatures for each month separately. We compared average maximum daily temperatures as well as April (starting of vegetation growth peak after the winter break) maximum daily temperatures among the study sites.

We collected 36 soil samples from the upper 15 cm of soil in July 2012 close to the studied transects (6 samples per site). Soil samples were dried in the laboratory and passed through a 2 mm sieve. The soil pH, organic matter content (OM), total carbon (% Total C) and nitrogen (% N), and the C/N relationship were quantified for each sample. After polishing soil samples, total carbon and nitrogen was quantified using an elemental analyzer, Variomas Elementar (DUMAS procedure); organic carbon was quantified with dichromate (Heanes 1984). Organic matter was quantified by multiply % organic Carbon by the factor Van Bemmelen 1.724 (Heanes 1984), which assume that OM has 58 % of organic C.

We sampled 24 transects of 500 m each, eight per each location (four at north slope site and four at south slope site) at the peak of the vegetation growing season, in July 2011. We used the point-intercept method (every 20 cm) (Goodall

1952) to record the presence of individual plants along the 500-m transects. Species diversity was measured by Shannon index, H' (Shannon 1948), $H'=\sum_{i=1}^{N} p_i \ln \frac{1}{p_i}$, where, p_i is the probability of the frequency of the i-th species, and N is the number of species. In our case p_i represent the probability of the occurrence of the species in the 500-m transect.

We employed Detrended Fluctuation Analysis (DFA) (Peng et al. 1992) to characterize the vegetation spatial pattern from presence/absence data of 500 m transects. A number of studies have applied DFA to spatial and temporal sequences (for reviews see Rutherford et al. 2004, Asher et al. 2009). The analytical protocol is described in Peng et al. (1992, 1995), and its application to vegetation spatial data in Alados et al (1999). The following description follows that from the above studies.

In order to analyze transect data via DFA, we first extracted 2050 points length vegetation sequences at 20 cm intervals. We transformed these data into a binary sequence [$z(i)$], in which the species or vegetation under examination was denoted 1 and its absence (lag) was denoted -1. We generated a random walk from these binary sequences by integrating the sequence series [$y(s)$] such that $y(s) = \sum_{i=1}^{\mathrm{N}=1} \mathrm{z}(i)$. The entire sequence was divided into non-overlapping boxes of length n, and a least squares regression line was then fit to the data within each box [$y_n(s)$]. If $\hat{y}_n(s)$ is the regression estimate for $y_n(s)$ at each box size n, then the average fluctuation [$F(n)$] of $y_n(s)$ about $\hat{y}_n(s)$ at each box size follows the modified root mean-square equation $F(n) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_n(s) - \hat{y}_s(s))^2}$

We repeated this procedure for all time scales (box sizes), which were the nearest integers to 2^2, $2^{2.5}$, 2^3 …2^9. The resultant relationship fit the power-law

$$F(n) \sim n^{\propto}$$

where α=0.5 represents a non-correlated, random sequence (white noise), and $\alpha \neq 0.5$ represents a long-range correlation of the plant distribution sequence, which is said to have ‘memory’, i.e. what happens in one step depends on what appears in previous consecutive steps across a long sequence. The parameter is equivalent to the Hurst exponent (Hausdorff et al. 1997), although it is a superior method for quantifying long range autocorrelations (Hu et al. 2001). Theoretically, it is unaffected by the magnitude of fluctuations but is affected by the sequential ordering of the fluctuations.

With the same 500-m transects, we estimated the number of positive and negative associations among the matrix of co-occurring species for each transect by comparing the probability of observed and expected pair of species at the upper and lower limit of the 95% confidence intervals of the Poisson distribution (Saiz and Alados 2012). We considered that two species were co-occurring when they appeared at the same sampling point along the transect. We assumed that recurrent local co-occurrences reflect plant interactions and not only plants that share habitat requirements (Saiz and Alados 2012, Soliveres and Maestre 2014). We computed the number of positive, K+ and negative, K- associations between pairs of species on each transect. We assumed that two species that appeared together more often than expected benefit from the association, while when they segregate more than expected, interference can be assumed (Tilman and Kareiva 1997, Tirado and Pugnaire 2005). We computed the interaction balance (K+ -K-)/(K+ + K-) since it is a symmetrical estimate around zero widely used in ecology literature (Armas et al. 2004). The mechanisms and consequences of the overall net competition balance of interactions is better understood if we decompose their competition and facilitation components (Callaway and Walker 1997, Michalet et al. 2014). We then calculated the number of positive (K+) and negative (K-) associations divided by the number of species (S) in the transect to control the influence of species richness in association frequency.

We performed General Lineal Model (GLM) comparing North vs South slopes and Site (Acid vs Basic soil sites) for each soil and vegetation variables. Soil variables included, soil nutrients (nitrogen (%N), total carbon (%C total), Carbon/Nitrogen ratio (C/N), organic matter (MO)) and pH. Vegetation variables included vegetation structure (% bare soil, species richness, and vegetation spatial patterns (Alpha-DFA)), plant-plant interactions (standardized number of positive (K+/S), and negative (K-/S) associations, and ratio of positive to negative associations ((K+ - K-)/(K+ + K-)). Linear regression was used to test relations between pair of variables. Variables used as percentages were converted to its angular transformation. All the statistical analyses were performed with R (R Core Team 2013).

3. **Results**

3.1 Effect of environmental conditions (slope and soil type) on air temperature and soil nutrient content

The results of average maximum daily T^a (Table 1), as well as April maximum daily T^a is higher in South oriented slope than in North oriented slope at the Acid and Basic sites. Soil analyses revealed that pH vary significantly between Acid and Basic sites and between slopes, being higher in North than in South slopes (Table 1).

Percentage of Organic Matter, percentage of total C, percentage of N, and C/N ratio varied significantly with soil acidity and orientation. Means ± se values presented in Table 1 back transformed showed that basic soils are richer in soil nutrients than acid soils and South slopes soils are richer than North slopes. The C/N ratio only differed significantly between Acid and Basic soils (Table 1), but not difference was observed between North and South slopes.

3.2 Relationship between biotic interaction and plant community structure (plant spatial pattern and diversity)

We observed the ratio of positive to negative associations was positively related with vegetation spatial patterns, Alpha-DFA, although only marginally significant ($F_{1,\ 22} = 3.64$, $P = 0.06$) (Fig. 1). Separated analyses for positive and negative associations revealed a significant effect of the number of positive associations on Alpha-DFA ($F_{1,\ 22} = 5.09$, $P < 0.05$); rather than between number of negative associations and Alpha-DFA ($F_{1,\ 22} = 0.07$, n.s.) (Fig. 1).

The relation between species richness and the ratio of positive to negative associations was positive and statistically significant ($F_{1,\ 22} = 19.03$, $P < 0.001$, $R^2 = 0.46$), as also was the relation between Shannon index and the ratio of positive to negative associations ($F_{1,\ 22} = 5.95$, $P < 0.05$, $R^2 = 0.21$) (Fig. 2).

Species richness and Shannon index increased significantly with Alpha-DFA ($F_{1,\ 22} = 4.34$, $P <0.05$, $R^2 = 0.35$; $F_{1,\ 22} = 9.74$, $P <0.01$, $R^2 = 0.31$; for species richness and Shannon index respectively) (Fig. 3). We also observed a positive relationship between percent of bare soil and Alpha-DFA ($F_{1,\ 22} = 16.87$, $P < 0.001$, $R^2 =0.43$), (Fig. 3).

3.2 Effect of environmental conditions on biotic interactions and plant community structure (plant spatial patterns and diversity)

In general, the Basic site presented more number of positive associations (1.72± 0.20) than Acid site (1.34± 0.14) (Table 2), and the North sites presented more number of positive associations (1.66 ±0.18) than South sites (1.27±0.15), although at a significant level of $P = 0.1$ (Table 2). A significant interaction between orientation and soil type was present for the ratio of positive to negative associations as well as for negative interactions (Table 2). Separated analyses for Acid and Basic soil showed that the ratio of positive to negative associations was significantly larger in Basic soils in North oriented slopes in comparison with South slopes, Fig 4, ($F_{1,\ 6} = 14.20$, $P < 0.001$). We did not observe any significant differences between North and South slopes in Acid soils ($F_{1,\ 14}$ = 0.03, n.s.). Plant-plant positive association showed the same trends than plant interaction balance but it was only marginally significant (Table 2, Fig. 4). Negative plant associations was significantly larger in Basic and South slope in comparison with North slope ($F_{1,\ 6}$ = 31.52 $P < 0.001$), however was larger in Acid and North slope than in South slope ($F_{1,\ 14}$ = 4.88, $P < 0.05$).

Vegetation spatial aggregation (Alpha-DFA) was higher in Basic rather than in Acid soils, and in North rather than South slopes (Table 2, Fig. 5).

Basic soil showed larger diversity (Shannon index) than Acid soils, although only a marginal difference was observed for species richness. Shannon diversity index also increased in North slopes in comparison with South slopes. Not significant interaction effects between soil and orientation was observed in any case.

4. Discussion

Comparisons among the mechanisms of vegetation pattern formation of alpine habitat dominated by cushion plants under contrasting conditions of insolation and T^a (North vs South slopes) and soil nutrients conditions (Acid vs Basic soils) revealed the importance of plant interaction in pattern formation. Acid soils showed lower concentrations of OM, C and N than Basic soils. Nitrogen is considered the most limiting to alpine plant productivity since it depends on biological activity that in turn depends on soil temperature (Bliss 1971) and its concentration is positively correlated with soil organic matter (OM) (Körner 2003). We also observed in this study a lower concentration in soil OM as well as C and N when obtained from North oriented slopes, where also averaged temperatures were lower. Comparisons of the rate of litter decomposition and associated microbial parameters along an altitudinal gradient in the Alps revealed higher rate of decomposition at higher temperatures (Schinner 1982). C/N concentrations observed in this study, between 9 and 14, was similar to the observed in grasslands in Bavarian Alps at 2000 m (Körner 2003). C/N ratio present lower values in Basic and more productive soils in this study in concordance with the lower values observed in the most productive sites reported in other studies (Rehder 1970). Other studies also revealed poor soil nutrients in Acid soils in Mediterranean ecosystems (Ozkan et al. 2006).

Vegetation spatial patterns measured by Alpha-DFA increased in the colder North, with lower mean maximum Tº in April (the starting of the growing season) than in the warmer South slopes. According with the SGH we should expect higher number of positive interactions in the most stressful habitats (Bertness and Callaway 1994), and consequently we should expect higher Alpha-DFA in North slopes, as we did. Conversely, we observed that the ratio of positive to negative associations was higher in North than in South slopes, whereas negative plant-plant association was higher in Basic and South slopes, where competition for water is more important. Thus, under harshest environments, facilitation ameliorate the range of conditions of facilitated plants (Bruno et al. 2003), enlarging the realized niche of species (Hacker and Gaines 1997), resulting in a high ecosystem performance, higher diversity and richness (Cavieres and Badano 2009). The North-South difference in the ratio of positive to negative interactions was larger in Basic than Acid soils, supporting the hypothesis that facilitation can be more important under relatively more benign conditions than in the most stressful environments (Holmgren and Scheffer 2010, Soliveres et al. 2011).

Other studies also observed that colder temperatures of north slopes showed more positive interactions and higher aggregated distribution (Kikvidze et al. 2005) supporting the hypothesis that abiotic stress as temperature are drivers of species interactions (Callaway et al. 2002, Michalet et al. 2014) and that interactions in turn drive spatial patterns (Haase 2001). In alpine environments, greater facilitation is observed at higher elevations (Callaway et al. 2002, Cavieres et al. 2002, Michalet et al. 2014) where cushion plants promote nutrient availability (Yang et al. 2010, Anthelme et al. 2012). For example, in Sierra Nevada the cushion Chamaephyte *Arenaria tetraqueta* under higher stress at the high end of the elevation gradient increased facilitation effect but not at the low end (Schöb et al. 2013).

South oriented slopes, present higher insolation and desiccation than north slopes (Isard 1986) resulting in lower water balance under similar conditions of precipitation and run-off. In particular in Mediterranean regions, drought periods induce soil desiccation and reduce nutrients availability for plants (Mooney et al. 1965, Reverter et al. 2010), enhanced in south slopes where temperature are higher. In agreement with these, we observed lower plant diversity in South slopes in comparison with North slopes. Previous studies have also reported that as degradation increases, spatial vegetation patterns and species distributions became randomly distributed (Alados et al. 2003, Alados et al. 2004) plant community association patterns become simplified (Saiz and Alados 2014), and vegetation composition becomes increasingly similar over time (Ross et al. 2012).

Vegetation patchiness emerge in response to self-organizing processes (Rietkerk and van de Koppel 2008) being plant-plant interactions one of its most important drivers, as facilitative interactions between neighbors can lead to patch nucleation and coalescence (Yarranton and Morrison 1974, Kéfi et al. 2007a, Cutler et al. 2008). Consistent with the theory, we observed a positive relationship between the ratio of positive to negative interactions with the vegetation aggregation as predicted. We also observed that species richness increases in sites with aggregated spatial distribution (Kikvidze et al. 2005, Alados et al. 2006, Pueyo et al. 2013), in spite of the increase of bare soil, confirming the hypothesis that vegetation patchiness is a better indicator of ecosystem degradation than percent of bare soil (Kéfi et al. 2007a).

We expect that under global warming the dwarfland vegetation of Mediterranean mountains lead to reduction of species diversity, and the loss of self-organizing vegetation processes as plant-plant facilitation that enhance ecosystem performance, being this effects more pronounced in the most frequent acid substrate than in the basic soils.

Table 1. Mean and standard error and GLM (General Lineal Model) comparing North vs South slopes and Site (Acid vs Basic soil sites) for climate (average maximum daily temperature as well as April maximum daily T^{a}), soil nutrients (nitrogen (%N), total carbon (%C total), Carbon/Nitrogen ratio (C/N), organic matter (MO)), and physicochemical properties (pH), at the Sierra Nevada National Park, Spain * P < 0.5, ** P < 0.01, *** P < 0.001

		Acid N	Acid S	Basi N	Basi S
Climate					
	Mean max T^{a} April	13.54±6.55	18.40±8.94	12.39±8.55	14.92±7.92
	Mean max annual T^{a}	14.65±9.69	19.26±10.15	16.77±10.90	17.68±9.97
Soil					
	pH	5.54±0.03	4.97±0.03	7.61±0.12	7.19±0.31
	OM (%)	5.38±0.51	6.14±0.33	5.62±1.46	9.05±1.49
	C total (%)	3.09±0.34	3.86±0.25	3.61±0.94	6.41±0.90
	N (%)	0.26±0.03	0.31 ±0.02	0.39±0.08	0.57±0.09
	C/N	12.27±0.83	11.52±0.50	8.25±0.63	9.17±0.52

GLM

Response variable	Slope F	Site F	Interaction F	df
Climate				
Mean max T^{a} April	20.94***	0.64	0.90	1,296
Mean max annual T^{a}	104.58***	16.66***	81.40***	1,4471
Soil				
pH	27.39**	409.21***	0.45	1, 32
OM (%)	10.65 **	7.60**	4.75*	1, 32
C total (%)	17.62 **	16.44**	5.29*	1, 32
N (%)	11.32 **	40.02**	2.43	1, 32
C/N	0.03	26.31**	1.80	1, 32

Table 2. GLM (General Lineal Model) comparing North vs South slopes and Site (Acid vs Basic soil sites) for plant-plant interactions (standardized number of positive (K+/S), and negative (K-/S) interactions, and ratio of positive to negative interactions ($(K^+ - K^-)/(K^+ + K^-)$)), vegetation structure (Alpha-DFA, percentage of bare soil), species richness (Shannon index), at the Sierra Nevada National Park, Spain * P < 0.5, ** P < 0.01, *** P < 0.001

Response variable	df	Slope F	Site F	Interaction F
Interaction				
K^+/S	1, 20	2.91	2.39	0.30
K^-/S	1, 20	0.67	6.03*	27.22**
$(K^+ - K^-)/(K^+ + K^-)$	1, 20	3.12	0.10	4.77*
Vegetation				
Alpha DFA	1, 20	6.78 *	34.67 **	1.15
Bare soil	1, 20	0.80	15.24 **	0.02
Richness	1, 20	5.27*	3.36	0.27
Shannon	1, 20	9.16 **	11.90 **	0.65

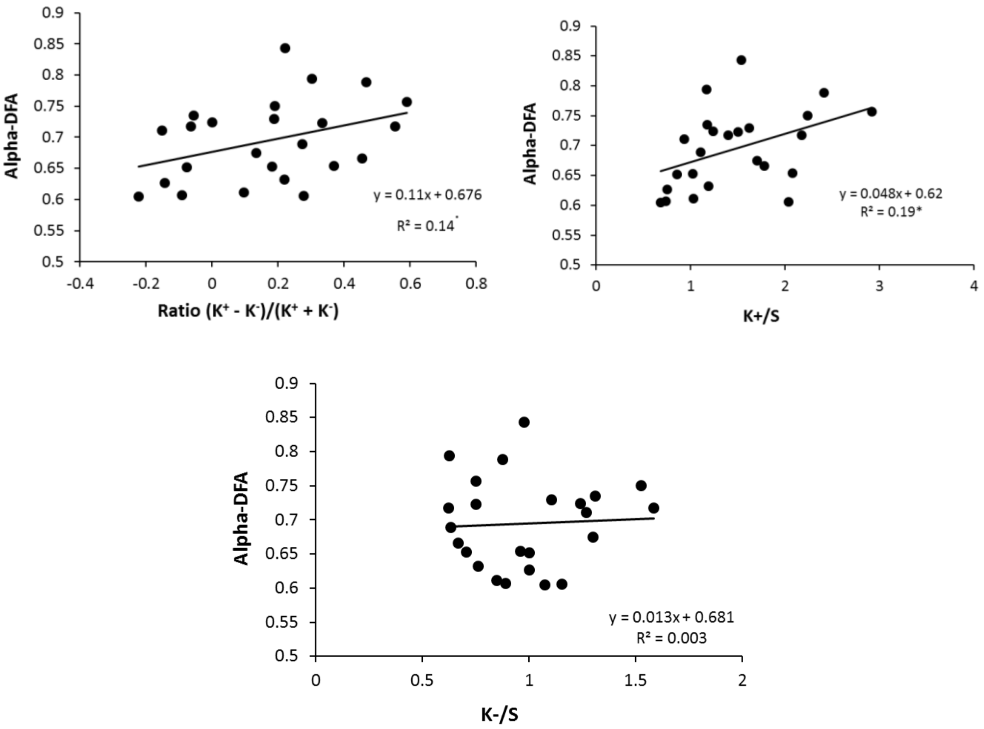


Figure 1. Relationship between vegetation spatial aggregation (Alpha-DFA) and the abundance of species interactions, positive (K+/S), negative, (K-/S) and the ratio of positive to negative interactions.

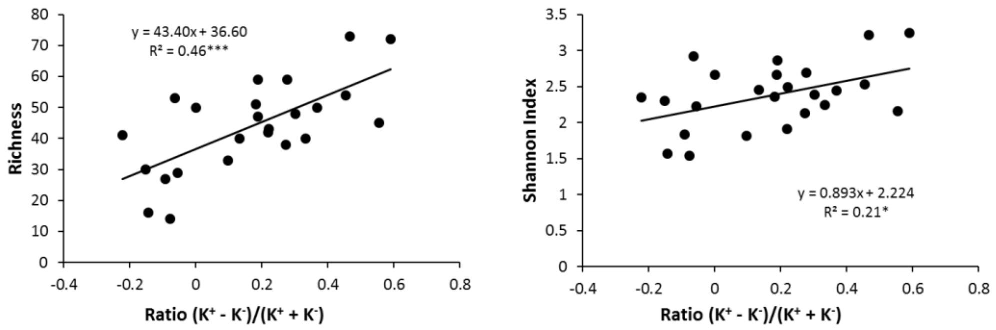


Figure 2. Relationship between species richness and diversity (Shannon index) and the ratio of positive to negative species interactions

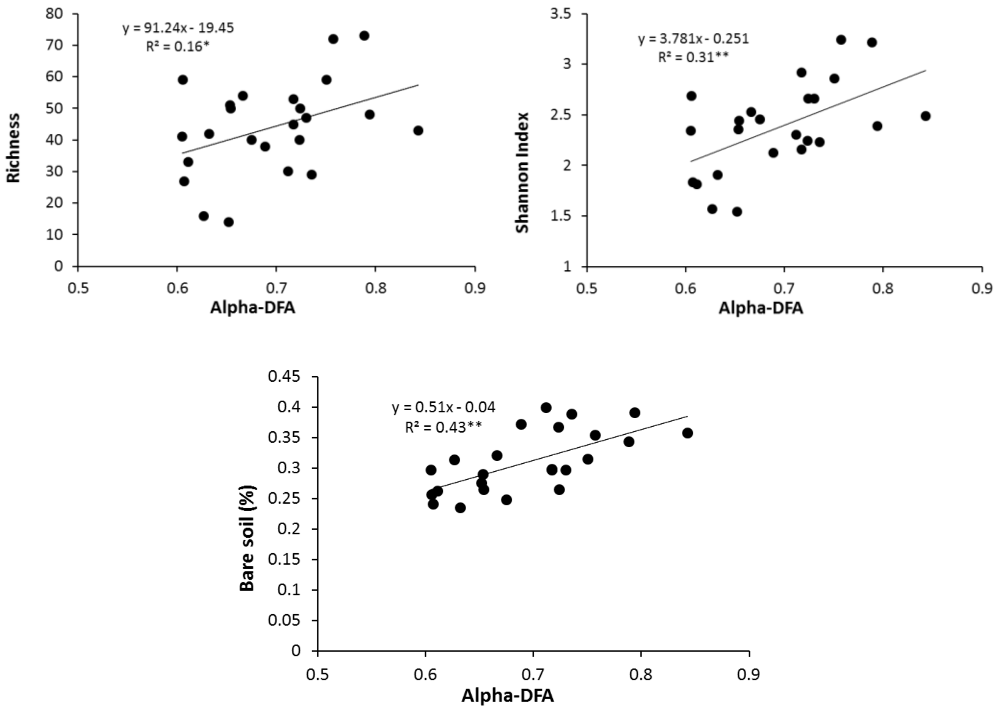


Figure 3. Relationship between species richness, plant diversity (Shannon index) and percentage of bare soil with vegetation spatial aggregation (Alpha-DFA).

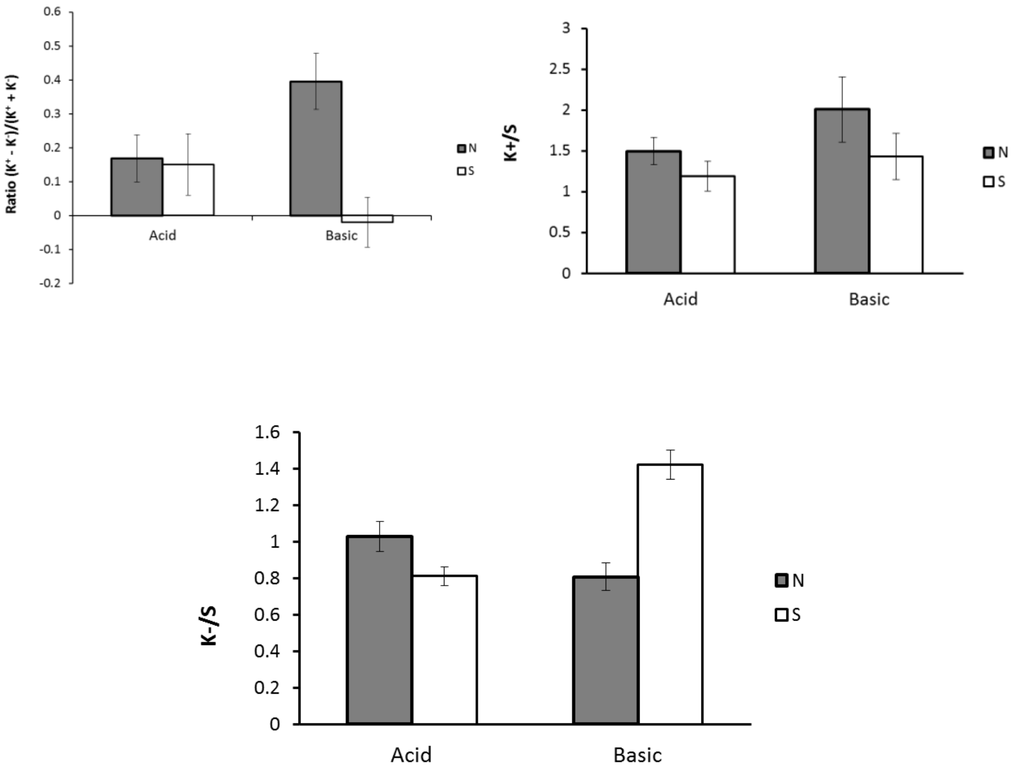


Figure 4. Mean and standard error (se) of the ratio of positive to negative plant interactions, only positive and only negative plant interactions, in acid *versus* basic soils and in North *versus* South slopes, at the study sites at Sierra Nevada National Park (Spain).

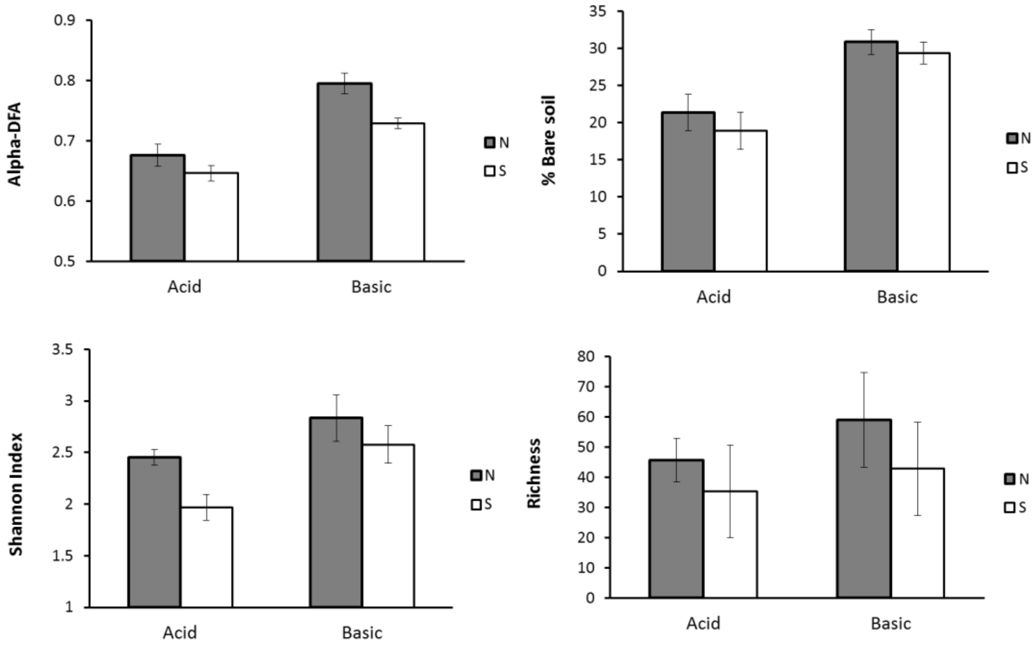


Figure 5. Mean and standard error (se) of the Alpha-DFA, percentage of bare soil, Shannon index, and species Richness, in acid *versus* basic soils and in North *versus* South slopes, at the study sites at Sierra Nevada National Park (Spain).

Discussion

At the Central Pyrenees, subalpine grasslands are abandoned and consequently woody encroachment occurs (Figure 1). The first shrubs breaking the grasslands structure is *E. horridum* and the second shrub species that enter the ecosystem is *B. sempervirens* (Figure 1). Interactions between *E. horridum* and *B. sempervirens* are crucial for the vegetation succession, were *E. horridum* facilitates *B. sempervirens* seedlings and juveniles, but once they growth old *B. sempervirens* adults outcompete *E. horridum* (Chapter 1, Figure 1). In addition, the intensity of interaction between *E. horridum* and *B. sempervirens* juveniles and adults vary with climate conditions (Chapter 1, Figure 1), therefore climate change can have an effect on vegetation succession after land abandonment by modifying interaction between shrubs. Under cold conditions, the expansion rate of *B. sempervirens* will be slow mainly due to its low competitive ability and performance under low temperatures; therefore the two shrubs species can coexist. Under warmer temperatures, *B. sempervirens* expansion would be fast due to its high competitive ability and performance under warm temperatures; thus *B. sempervirens* dominate over *E. horridum*, which could eventually disappear (Chapter 3, Figure 1). Management treatments (burning and clearcutting) were applied to control *E. horridum* expansion and to revert the shrubland to a grassland ecosystem. After such perturbations, the role of the soil seed bank in vegetation recovery is determinant, the species germination rates and seedling survival are crucial to the establishment of the next plant community (Chapter 2, Figure 1). If *E. horridum* is removed only once, the *E. horridum* community will establish again with a dominance of *E. horridum* (80% of cover) after six years (Appendix 4 Chapter 2, Figure 1).

Plant interactions were a mechanism for alpine plant community structure in Sierra Nevada (Chapter 5). Positive interactions promoted vegetation spatial aggregation, which supported high plant diversity, especially under stressful low temperatures (Chapter 5). Therefore, current climate change might affect alpine plant community structure, i.e. diversity and spatial patterns, by the modification of plant species interactions (Figure 2). The Mediterranean oak showed an adaptive strategy to face climatic stress (Chapter 4); however, the adaptive capacity of organisms to climatic stress would depend on the pace of climate change.

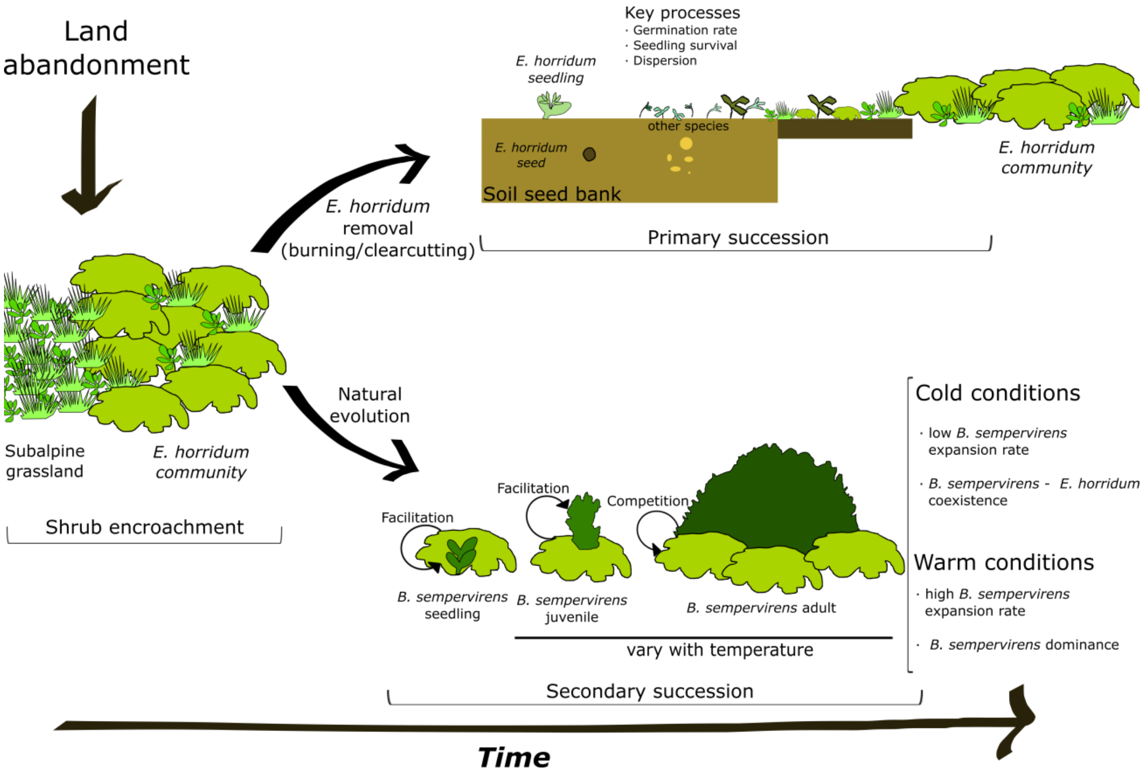


Figure 1. Diagram showing the main processes studied at the Central Pyrenees.

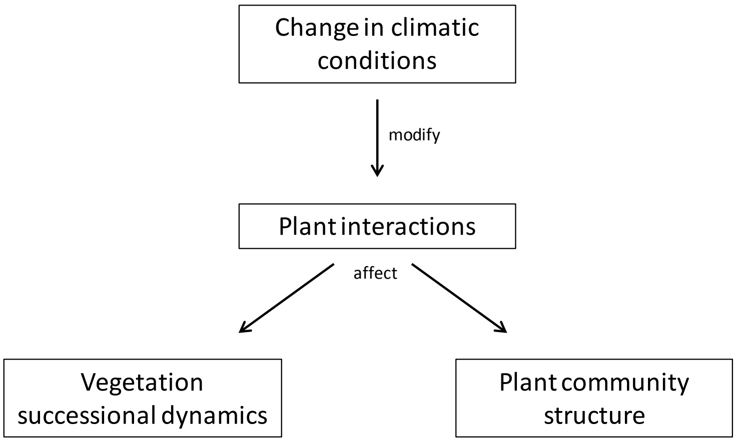


Figure 2. Diagram showing main studied processes. A change in climatic conditions can modify the intensity or predominance of plant interactions, which would in turn affect the ecosystem processes where they take part.

- Species interactions and vegetation succession

Shrubs interactions have shown to be a fundamental mechanism on vegetation succession at the Central Pyrenees after land abandonment, where *E. horridum* enhanced the survival of *B. sempervirens* seedlings under its crown, but when they grow older, *B. sempervirens* adults outcompeted *E. horridum* (H1.1) (Chapter 1). Interspecific facilitation has been broadly evidenced in alpine communities, where stress and physical disturbance are strong limiting factors for plant survival and growth (Choler et al. 2001, Callaway et al. 2002, Cavieres et al. 2006). Associations between seedlings of one species and sheltering adults of another species have been broadly referred as the ‘‘nurse plant syndrome’’ (Turner et al. 1966, Franco and Nobel 1989, Acuña-Rodriguez et al. 2006). Many studies suggest that the positive effects of benefactors are strong when beneficiaries are young and small, and when the beneficiaries grow older and larger, competitive interactions might dominate (Archer et al. 1988, Walker and Vitousek 1991, Chapin et al. 1994, Le Roux et al. 2013, St. Clair et al. 213) (see Figure 3 and 5 in the General Introduction). Both types of interactions, facilitation and competition, favored *B. sempervirens* expansion on *E. horridum* stand, therefore plant interactions have a determinant role on the vegetation succession of subalpine grasslands. Connell and Slatyer (1977) described the facilitation by the 'early colonist' to the benefit of the 'late successional species', which, once established, outcompete the former, as a model describing autogenic succession (only mediated by internal vegetation processes, sensu Tansely 1935). In addition, climatic conditions associated to the altitude affected competitive and facilitative interactions (H1.2). *B. sempervirens* juveniles were facilitated by *E. horridum* under the low temperatures at the High site and *B. sempervirens* adults applied a higher competitive effect toward *E. horridum* at the Low site where the climate was milder. The effect that *E. horridum* might have on soil nutrient concentration due to it is a N-fixing leguminous species with high litter release was not the mechanism of facilitation, which supports the hypothesis that in alpine ecosystems facilitation is related to physical stress and not to resource availability (H1.3). In this context, evaluating the effect of a variation in temperature conditions on shrubs interactions and the secondary effects that can have on vegetation succession can help to understand how climate change might be coupled with human impact on ecosystems. The cellular automata model built in Chapter 3 describe the main dynamics of this ecosystem, which is vegetation succession, i.e. *B. sempervirens* expansion in *E. horridum* stands. The model allowed evaluating the effect of a change in climatic conditions on vegetation succession based on field data in two ways: directly, by affecting key processes of population dynamics (e.g. recruitment) and indirectly through the modification of plant species interactions. Plant interactions have been shown to be a fundamental mechanism on vegetation succession because when the parameters that account for plant interactions were set to 0 the succession was extremely low (H3.1). In addition, simulations showed that climate change can have an effect on vegetation succession through the modification of plant species interactions (H3.2). Specifically, under low temperatures *B. sempervirens* expansion rate would be slowed down due to low *B. sempervirens* competitive ability and low *B. sempervirens* seedling establishment, despite the high facilitation intensity of *E. horridum* towards *B. sempervirens* seedlings (H3.3). These low values of establishment and competition under low temperatures would favor the coexistence of both study shrubs because *B. sempervirens* would not be able to displace *E. horridum* very much. The low temperatures would be found by *B. sempervirens* at its upper limit of altitudinal range where it coexist with *E. horridum* (Figure 3b). On the other hand, high temperatures derived from current global warming would speed-up vegetation succession rate given the high competitive ability of *B. sempervirens* and the high *B. sempervirens* seedling establishment under warm temperatures, despite the low seedling facilitation intensity (H3.3). Under high temperatures *E. horridum* would be faster displaced by *B. sempervirens* than under low temperatures, thus both shrubs coexistence might not be possible. Nevertheless, other plant species might come into this shrubland altering the balance of interaction between *E. horridum* and *B. sempervirens*, e.g. *Pinus sylvestris*. In Ordesa valley at the Central Pyrenees Dolezal et al. (2004) showed how *B. sempervirens* differently affected the seedling establishment and spatial structure of tree species (*Fagus sylvatica* and *Abies alba*), therefore *B. sempervirens* can also favor vegetation succession of abandoned grasslands and determine the future canopy composition.

We quantified shrub interactions *intensity* (sensu Welden and Slauson (1986), Brooker et al. 2005) as a reduction of the growth of species A as a consequence of the presence of species B (and *vice versa*), being A and B *E. horridum* and *B. sempervirens* respectively. We then evaluated the relative *importance* of competition and facilitation on ecosystem dynamics (rather than on species sensu Welden and Slauson 1986, Brooker et al. 2005) with cellular automata model. Simulations showed that facilitation have a greater effect on vegetation succession than competition because a variation in facilitation parameter caused a bigger variation in *B. sempervirens* expansion rate than the same change in competition parameter. However, facilitation and competition do not vary to the same extent with the same change in temperature. Competition varied more than facilitation with a change in temperature (Chapter 1), therefore, under a change in climatic conditions, competition will have higher impact on vegetation succession than facilitation (Chapter 3) (see Figure 5 in the General Introduction).

Warming temperatures might climb the upper limit of altitudinal range for *B. sempervirens*; however other environmental factors that varies with altitude could counterbalance the positive effect of temperature on *B. sempervirens*. Several studies showed already an upslope migration of plant species (Peñuelas and Boada 2003, Lenoir et al. 2009, Jump et al. 2012, Pauli et al. 1996), but other studies did not (Camarero and Gutierrez 2004). In Mediterranean mountains the lack of water associated to the increased temperatures in Mediterranean mountains can limit seedling establishment (Pauli et al 2012). However, the presence of *E. horridum* might counteract the lack of water resources given that *E. horridum* retains soil humidity (Chapter 1 and Cavieres et al. 2007).

Several studies showed that the outcome of plant-plant interactions change with vital stage (Chapin et al. 1994, Pugnaire et al. 1996a). In our study the reciprocal neighbor removal allows to conclude that the interactions effectively vary with plant vital stage because *B. sempervirens* seedlings are facilitated by *E. horridum* and *B. sempervirens* adults outcompete *E. horridum*. Most of the studies focus on the single effect of one species to another and reciprocal interactions are rarely investigated (Bronstein 2009). In our study, the assessment of the reciprocal interaction is required to reproduce ecosystem dynamics, i.e.: succession, the ultimate effect on ecosystem dynamics that the facilitative effect of *E. horridum* towards *B. sempervirens* seedlings would have when seedlings become adults. Three types of facilitation can be considered regarding the effect that the facilitated species have on the facilitator: 1. antagonistic (+/-), the facilitator species receives a negative interaction back from the facilitated, 2. neutral (+/0), when there is no effect, named commensalism, and 3. positive (+/+), if the facilitated exert a positive effect to the facilitator, named mutualism (Callaway 2007). *E. horridum – B. sempervirens* interaction is a relationship of antagonistic facilitation. *E. horridum* facilitation cost is to be displaced by *B. sempervirens*, and the extent of *E. horridum* displacement would depend on climatic conditions. The climatic conditions that favor *B. sempervirens* fitness (growth and reproduction), i.e. warmer temperatures, would lead to a greater displacement of *E. horridum* and an eventually local extinction, as shown by our cellular automata model (Chapter 3). Interestingly, although *B. sempervirens* outcompete *E.*

horridum, it is required to *B. sempervirens* expansion and when the competitive effect of *B. sempervirens* adults is very high *B. sempervirens* colonization decline.

Several studies accounted for reciprocal species interactions in alpine ecosystems also showing negative effects of the facilitated species to their facilitators nurse plants (Michalet et al. 2011, Craston et al. 2012, Schöb et al. 2014a). Those studies evaluated the evolutionary potential of facilitation, but less studies focus on the importance of the costs of facilitation as successional driver.

Facilitative interactions range from more specific to generalist (Bronstein 2009). Benefits may only be conferred by individuals that exhibit certain morphological traits, architectures or sizes. Some of these traits have been well documented in nurse plants; however, the traits of the facilitated plants have received less attention (Bronstein 2009). The possibility of a species to receive the facilitative effect of a nurse plant might depend on some specific trait. For instance the shade tolerance trait of *B. sempervirens* allows them to establish underneath *E. horridum*. Although it has been described a negative effect of the nurse plants to the underneath facilitative plants due to light interception (Franco and Noble 1989), the net effect was facilitative in *B. sempervirens* seedlings. Other strictly heliophilous species might not be capable of establishing underneath a nurse plant, e.g. the seedlings of dominant heliophilous shrubs are prevented from establishing, the germination of *E. horridum* seeds was very low in a mature *E. horridum* community (Chapter 2). Plant functional traits of the nurse as well as the facilitative plant have been shown to be fundamental in plant-plant interactions (Soliveres et al. 2014) and determinant in vegetation succession (Noble and Stayler 1980).

- Management solutions to consequences of land abandonment at the Pyrenees

Once *E. horridum* enter the abandoned subalpine grasslands secondary succession takes place (Chapter 1 and 3, Figure 1). A quarter of the subalpine grasslands has already disappeared (Gartzía et al. 2014). Therefore, is of high importance to improve our knowledge about minimizing *E. horridum* expansion to conserve the high value subalpine grasslands.

Prescribed burning and clearcutting were used to remove *E. horridum* individuals that formed high dense monospecific patches and assess the vegetation recovery and the *E. horridum* regeneration after the perturbation (Chapter 2, Figure 1). *E. horridum* regenerated better after prescribed burning than after clearcutting, with higher germination rates and higher seedling survival probability (H2.1) (Chapter 2). Thus, clearcutting appeared to be a better management treatment than burning. Both management treatments, burning and clearcutting, increased seed density of the soil seed bank compared to an intact *E. horridum* community; thus *E. horridum* removal favored the establishment in the soil of seeds coming by dispersion (H2.2). In addition, prescribed burning had a more diverse and more dynamics soil seed bank than clearcutting because burning caused more soil perturbation (H2.2). Both treatments, burning and clearcutting, favored the formation of a soil seed bank composed by herbs and grasses typical from subalpine grasslands; however, the soil seed bank would have little utility on its own to recover the subalpine grasslands given the low species richness and the low seed abundance. Burning had negative effects in the soil which includes diminution of the nutrients content and soil particle size (H2.3), which could lead to the loss of soil resources and therefore hindering subalpine grasslands restoration. Although burning favored the soil seed bank more than clearcutting, *E. horridum* regenerated better in burning than in clearcutting, and burning also compromise soil resources. In addition, vegetation recovery after prescribed burning (Appendix **3** of Chapter 2) showed how *E. horridum* cover progressively increased (H2.4) reaching 80% in 5 years. The removal of *E. horridum* once would not be enough to recover the subalpine grassland. Komac et al. (2013) showed that to control *E. horridum* expansion, removal treatments should be periodic. Prescribed burning and clearcutting were evaluated as management procedures to remove *E. horridum* and to allow subalpine grassland recovering because those were the traditional human practices used in the past to remove shrubs at those grasslands. Nowadays, *E. horridum* forms big monospecific patches that might occupy a whole hillslope, whereas in the past isolated individuals spread in the grassland stand, which probably were more easily removed and entailed smaller soil perturbation. In addition, the precise traditional procedure is not well known either; for instance, the better moment to remove *E. horridum* along the year, autumn *vs.* spring, is also unknown. We observed that *E. horridum* reach maturity and therefore is capable of producing flowers at the age of four years (personal observation and Montserrat and Montserrat 1984). Therefore, four years after *E. horridum* removal, its established seedlings from the seed bank, would have become adults and would overrun the soil again. To recover subalpine grassland herbs and grasses typical of those Pyrenean subalpine grasslands have to establish precisely in the period when *E. horridum* is removed before it establish again. Here is when the soil seed bank plays a determinant role. Many authors agree that restoration based only on the soil seed bank is possible at locations that were not degraded more than five years ago because of seed senescence (Bossuyt and Honnay 2008). An integrated strategy that includes traditional shepherding to favor seed dispersion by animals would favor the establishment of those grasses and herbs typical of subalpine grasslands (Wagner et al. 2013).

• Plant performance indicators of abiotic and biotic stress: DI *vs.* growth

Several indices of species performance were used to assess the species responses to climatic conditions and biotic interactions (Chapter 1 and 4).

Common indices such as plant size and growth (annual crown growth, annual shoot length, branch biomass), were applied together with a holistic index, developmental instability (DI).

The Mediterranean oak *Quercus faginea* (Chapter 4) showed an adaptive response to climatic stress (H4.1) by means of reducing its investment in biomass production towards the maintenance of a correct development (H4.2). The individuals at the xeric site showed a lower DI value after a drought year than the individuals at the mesic and cold sites, and they were also smaller. Deviations from perfect symmetry in a population that is on average symmetric may be the outcome of energy dissipation and diversion (Parsons 2005). Stress diverts energy from production to maintenance (Odum 1985). *Q. faginea* generated an adaptive response to water shortage and hence it might be able to adapt to rising temperatures and decreased precipitation predicted for the Mediterranean region (Nogués-Bravo et al. 2008) if the pace of environmental change is slow enough (Kozlowski 2002).

Although non-significant, growth and DI varied with climatic conditions associated to altitude in *E. horridum* and *B. sempervirens* juveniles and adults (Appendix **4** Chapter 1). *B. sempervirens* had a non-significant tendency of having higher DI upwards and *E. horridum* downwards. *B. sempervirens* altitudinal range distribution is lower in altitude than altitudinal range of *E. horridum* in the study valley (Figure 3), being the High study site *B. sempervirens* upper limit of altitudinal range and the Low study site was close to *E. horridum* lower limit of altitude in the study valley (Figure 3). In mountainous ecosystems altitudinal distribution of plant species is arranged at least in part by climatic conditions associated to altitude (Körner 2003). However, at their limit of altitudinal climate optimum, plants might not be able to adapt due to physiological and morphological constraints and might present some degree of developmental error and limited growth.

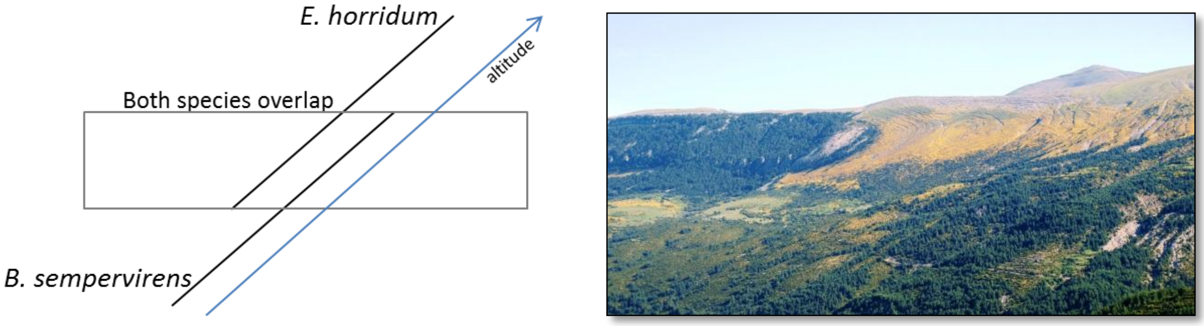


Figure 3. Schematic distribution of the two study species *E. horridum* (yellow) and *B. sempervirens* (light green) in the Lomar Valley, near Fanlo village at the periphery area of the Ordesa and Monte Perdido National Park, at the Central Pyrenees, Spain.

Trade-off theory predicts that a change in one trait might be associated to a detrimental change in another (Stearns 1989), negative correlations between organisms traits might occur, especially under stressful conditions or low productive environments (Biere 1995). The trade-off found in *Q. faginea* between the maintenance of correct development (low DI) and biomass production (low growth) under high environmental stress conditions could be a problem when both, DI and growth, are used as indices of species performance to evaluate species interactions. Neighbor removal could suppose an increment or a decrease on environmental stress given that the mechanism by which plant species interact with neighbors mainly occurs through the modification of the local environment, e.g. competing for a limiting soil resource or increasing soil fertility (Callaway 2007). The performance indicator used to evaluate the response of the plant to the neighbor removal is therefore decisive given that traits might not respond to stress in the same way. Discrepancies on plant interactions theory have arisen derived from the performance variable used (Goldberg et al. 1999, Hastwell and Facelli 2003). If, for instance, survival is used instead of growth as species interactions index, the effect of the neighbor removal might not be detected given that growth is a more sensitive measure. Trade-offs between organism traits that appear only under high levels of environmental stress might lead to confusion when evaluating plant-plant interactions along environmental gradients. In my opinion it has not been paid much attention of the implications of the physiological trade-offs on the plant interaction theory, especially, on how those trade-offs might vary along environmental gradients when attempting to evaluate *stress gradient hypothesis*. Holmgren et al. (2012) demonstrated how the trade-off between being adapted to light or shade makes drought more harmful at intermediate levels of light conditions, and therefore, facilitation is more important at intermediate levels of stress along a stress gradient. Leaf size and shoot length are frequently used as indices of plant vigor (James et al. 1994, Yasur and Rani 2013, Weijer et al. 2013) because they are easy to measure. However, growth not always represent better condition, for instance, animal tumor and plant galls have an enhanced grow resulting from a process of higher stress (Tracy et al. 1995). DI could be a suitable index of species interactions which might inform, together with growth, the global strategy of the plant.

DI was an index of species interactions (Chapter 1), specifically competition, in *E. horridum* because DI was higher when coexisting with *B. sempervirens* adults than when they were removed from its neighborhood. Other studies applied fluctuating asymmetry as index of intraspecific competition (Rettig et al. 1997, Komac and Alados 2012), but to our knowledge DI was never used as index of interspecific interactions. Komac and Alados (2012) also found a trade-off between developmental stability (DS) and biomass production in *E. horridum*. However, in our study, there is no apparent trade-off between biomass production and DS in *E. horridum*, i.e. growth and DI have similar pattern along in the two study sites (High *vs* Low) and in the neighbor removal treatment (Control *vs* B. removal), being at the Low site and in Control when coexisting with *B. sempervirens* adults where *E. horridum* had lower annual growth and high DI, which indicates that this situation is more stressful for it.

• Plant interactions and Vegetation spatial pattern

Positive interactions were a mechanism of vegetation spatial aggregation (H5.1) in Sierra Nevada (Chapter 5), confirming therefore the theoretical predictions (Rietkerk et al. 2004, Kéfi et al. 2007b). Plant diversity and species richness were also favored by positive interactions (H5.1) (Cavieres et al. 2002, Le Bagousse-Pinguet et al. 2014, Michalet et al. 2015a), suggesting that facilitative interactions increase the realized niche of species (Michalet et al. 2006). North slopes with a lower soil nutrients concentration (OM, C and N) and averaged lower temperatures, also had higher ratio of positive to negative associations, higher plant diversity and higher plant spatial aggregation than South slopes (H6.2). The dominance of positive interactions under low temperatures at alpine systems has been widely documented (Callaway et al. 2002, He et al. 2013). Alpine cushions usually act as nurse plant systems (Cranston et al. 2012, Reid et al. 2010) where their facilitative effects are commonly manifest as a higher density and diversity of species within the compact cushion canopy compared with open areas (Cavieres et al. 2013). Kikvidze et al. 2005 showed that under warm climates, such as at South slopes at our study sites, the vegetation spatial aggregation is lower, with no dominance of positive or negative interactions (competition might be counterbalanced with facilitation) and therefore the species diversity is also lower. Rising temperatures derived from current climate change might favor that north slopes climate resembles south slopes climate leading therefore to a lower rich and diverse alpine plant community.

• Evaluation of Stress Gradient Hypothesis (SGH) (H7.1)

The *fathers* of SGH considered 'environmental stress' at an ecosystem level based on Grime (1979) concept of stress, i.e. they quantified environmental harshness based on the ecosystem primary production (Callaway and Walker 1997). Ecosystems with low primary production, such as alpine ecosystems or deserts, were considered stressful environments. However, other authors (Körner 2004) argued that stress is suffered by individual organisms and therefore 'stress' is not a quality of an ecosystem and therefore cannot be estimated at ecosystem level, precisely because organism became adapted to the environmental stress were they evolved and developed. If we take the first view, SGH is confirmed in our study because facilitation is found at high altitude in a high stressful climate and competition is found at low altitude in a less stressful climate. We found more intense facilitation under high environmental stress for the facilitated species, *B. sempervirens* juveniles, and more intense competition under lower level of stress for *B. sempervirens* (adults); which meet the SGH. However, if we take the second view, individual stress level view, SGH would work for *B. sempervirens*, i.e. *B. sempervirens* is facilitated under higher levels of environmental stress for *B. sempervirens* (at the High site), but not for *E. horridum*. At the Low site, which is a high level of environmental stress for *E. horridum,* it was not facilitated, it was indeed outcompeted. Some authors have proposed a refinement of the SGH which take into account the life history of interacting species (Maestre 2009, Malkinson and Tielbörger 2010, Soliveres et al. 2011). In alpine ecosystems the general conceptual model is that stressful conditions rises with altitude (Körner 2003), however plant species have evolved for being adapted to their environment, therefore such climatic conditions might not be stressful for inhabiting organisms. Environmental stress at ecosystem level (Grime 1979) should be used to assess responses at an ecosystem level rather at an individual level (Körner 2004), for that reason, evaluating the effects of climate change at a community scale might shed light in the complex interactions between ecosystems and stress gradients.

Our study at plant community scale at the Sierra Nevada Mountain Range showed that positive interactions were more abundant at the North slopes than at the South slopes in basic soils. Low temperatures of the north slopes favored the abundance of positive plant interactions in the alpine plant community. Acid soils have lower nutrient content than basic soils, in addition, low soil pH limit the plants adsorption of some micronutrients, which is an important cause of stress (Harter 1983). Positive plant interactions were less abundant on acid soils than on basic soils despite acid soils are highly stressful for plant. There are two types of stresses that might affect the outcome of plant-plant interactions, resource or non-resource related stress. Temperature act as a non-resource related stress and positive interactions tend to be more easily detected than when the stress is related to a resource, such as water shortage or low nutrient availability. When is a resource related stress, facilitative interactions are more difficult to be detected given that might be counterbalanced with competitive interactions for the limiting resources. Therefore, at the community scale, there were more positive interactions on the "medium-stress" level in basic soils at North slopes than in the highly stressful acid soils. This result supports the hypothesis that facilitation would be dominant under intermediate stress levels (Homlgren and Scheffer 2010, Holmgren et al. 2012). Facilitation might promote diversity at intermediate environmental severity levels by expanding the realized niche of stress-intolerant competitive species into harsh physical conditions (Hacker and Gaines 1997, Michalet et al. 2006). However, if environmental conditions become extremely severe, i.e. acid soils in our study sites, the positive effects of the benefactors might decrease (Kitzberger et al. 2000) and plant diversity is therefore reduced.

• What to do next? Short and long-term perspectives

In order to assess the impacts of drivers of environmental change, such as climate change and change in land uses, on ecosystem, deeper research is needed. In the present thesis we evaluated the effect of environmental conditions associated to the altitude as a surrogate of a change in climatic conditions. The use of altitudinal gradients on ecology is quite widespread because a climatic variation in space substitutes a climatic variation in time. It has been successful and useful in many studies (Callaway et al. 2002, Serrano-Ortiz et al. 2015), as also in the present work. Altitudinal gradients have however some limitations given that several environmental variables vary with altitude, which make difficult to decipher the effect of each one on the object of study. The main abiotic variable that varies with altitude is temperature, however, other variables, such as wind, sun radiation, relative soil or air humidity, might have important effects too.

Field experiments that try to isolate each variable, for instance, the use of open-top chambers, or irrigation water exclusion, might be helpful. In addition, long-term field experiments with more than two years of monitoring would be desirable given that the study species are shrubs or trees with slow growth. Unfortunately, PhD grants and research projects are relatively short to make possible the monitoring of the vegetation for a long time. For that reason, north *vs.* south slopes were also used to assess the effect that a change in climatic conditions might have on a plant community on the long-term.

In addition, future research directions bring me to the deepening on the *E. horridum – B. sempervirens* studied system. An important factor to consider when evaluating the effect of climatic conditions in alpine and subalpine ecosystems is the role of the snow pack. Snow pack can isolate underneath vegetation and maintain winter temperatures around 0 degrees therefore protecting plant from freezing. Snow might also have indirect effects on shrubs interactions by the melting water, especially in a Mediterranean mountain with frequent droughts. Thus, investigating the effect that snow layer might have on the interaction between the studied shrubs, *E. horridum* and *B. sempervirens* can shed light on effects of climate change on secondary vegetation succession after land abandonment at the Pyrenees. Another effect that it has not been investigated in detail is the effect of land abandonment on soil erosion, which is indeed quite controversy (Caviezel et al. 2014). The effect that *E. horridum* might have on soil dynamics (formation/erosion) after it has invaded the abandoned subalpine grasslands should be investigated. *E. horridum* increase soil nutrients concentrations (Chapter 1 and Palacio, S personal communication), especially nitrogen and carbon, because is an N-fixing leguminous that indeed produces a great amount on organic matter which might improve soil quality favoring therefore following successional species; however its role on soil formation and/or erosion is relatively unknown. Caviezel et al. (2014) explains how plants with thick roots such as shrubs and trees might favor the loss of the fine soil material that is more easily retained by the fines roots of herbs and grasses. In addition, the assessment of the effects of *E. horridum* removal (prescribed burning and clearcutting) on soil erosion is of vital importance to vegetation recovery. We have seen in Chapter 2 that *E. horridum* removal only once is not enough to recover the subalpine grassland, and periodic treatments (Komac et al. 2013) together with livestock grazing would be necessary. The assessment of the effect of periodic burnings on soil quality is determinant given that the negative effects of burning on soil might be multiplicative.

On the other hand, plants not only interact through the aboveground biomass but also through roots and microbe interactions in the belowground. To assess the belowground processes that might take part in shrubs interactions, a better understanding of the differential effect of *E. horridum* on soil nutrient concentration under different temperatures (High site *vs.* Low site in Chapter 1) would be necessary. This pattern probably respond to low temperatures slow-down decomposition rates.

The role of plant interactions has shown to be central on successional dynamics and plant community structure. Evaluating the role of plant interactions in primary succession after a perturbation, such as prescribed burning or clearcutting (Chapter 2), can help to understand this process. In addition, the recent developed methodology of species networks (Saiz 2014) can shed light in understanding successional processes.

Finally, it would be very useful to predict the time necessary to *B. sempervirens* expansion into *E. horridum* stands. In the cellular automata model which describes *E. horridum - B. sempervirens* dynamics (Chapter 3) we focused on the effect of climate on *B. sempervirens* expansion, highlighting the role of plant interactions. Isolating the effect of single parameters on the dynamics allow to understand its role on the ecosystem, but if we would like to make precise predictions of the rate of vegetation succession more detailed information is needed. Incorporate information about the effect that climate can have on *E. horridum* in the model would allow to evaluate the combined response of both study shrubs to climatic conditions. However, very complete models makes them also very complex models, limitations in ecological models usually arise for the needs of simplification in order to be able understand model results.

Main Conclusions

1. Plant interactions are a mechanism for vegetation succession of abandoned subalpine grasslands (Chapter 1 and 3) and a mechanism for vegetation pattern formation and plant diversity at alpine vegetation (Chapter 5).

2. Climatic conditions associated to the altitude (High *vs* Low) and to the slope orientation (North *vs.* South) affected the plant interactions intensity (Chapter 1) and abundance (Chapter 5), respectively. Therefore, species interactions must be taken into account when evaluating the potential impact of climate change on ecosystems.

3. At the Central Pyrenees, *Echinospartum horridum* and *Buxus sempervirens* encroaches abandoned subalpine grasslands leading to secondary vegetation succession. *E. horridum* ("early colonist") - *B. sempervirens* ("late colonist") interaction vary with vital stage of the *B. sempervirens*, i.e. *B. sempervirens* seedling are facilitated by *E. horridum*, but once they become adults, outcompete *E. horridum*. This is the mechanism for vegetation secondary succession of abandoned subalpine grasslands at the Central Pyrenees (Chapter 1). The cellular automata model built in Chapter 3 that describes the dynamics of the system, i.e. *B. sempervirens* expansion in *E. horridum* stand, showed that when there are not interactions between shrubs, the dynamics is extremely low, indicating that shrubs interactions are fundamental in vegetation succession.

4. Climatic conditions associated to the altitude affected the intensity of *E. horridum - B. sempervirens* interaction: the competitive effect of *B. sempervirens* adults towards *E. horridum* is more intense at lower elevation (i.e. under warmer temperatures), and the facilitative effect of *E. horridum* towards *B. sempervirens* juveniles is more intense at higher elevation (i.e. under colder temperatures) (Chapter 1).

5. A change in climatic conditions can have an effect on secondary succession of subalpine grasslands by the modification of shrubs interactions and by its direct effects on *B. sempervirens* recruitment (Chapter 3). *B. sempervirens* expansion would be accelerated under warming conditions derived from current climate change which would favor the displacement of *E. horridum*. Low temperatures, found at the upper limit of *B. sempervirens* altitudinal range, would slow down vegetation succession rate and therefore *B. sempervirens* and *E. horridum* coexistence would be possible (Chapter 3).

6. *E. horridum* removal only once will not allow to recovery the previous subalpine grassland because burning favored *E. horridum* seedlings germination and survival (Chapter 2). Burning favored *E. horridum* establishment, while also favored a more diverse and species rich soil seed bank, than clearing (Chapter 2). However, the low species abundance at the soil seed bank would not make possible the recovery of previous grasslands on its own. A frequency of *E. horridum* removal is then required and we would recommend an integrated strategy that includes traditional sheepherding to favor soil seed bank recharge.

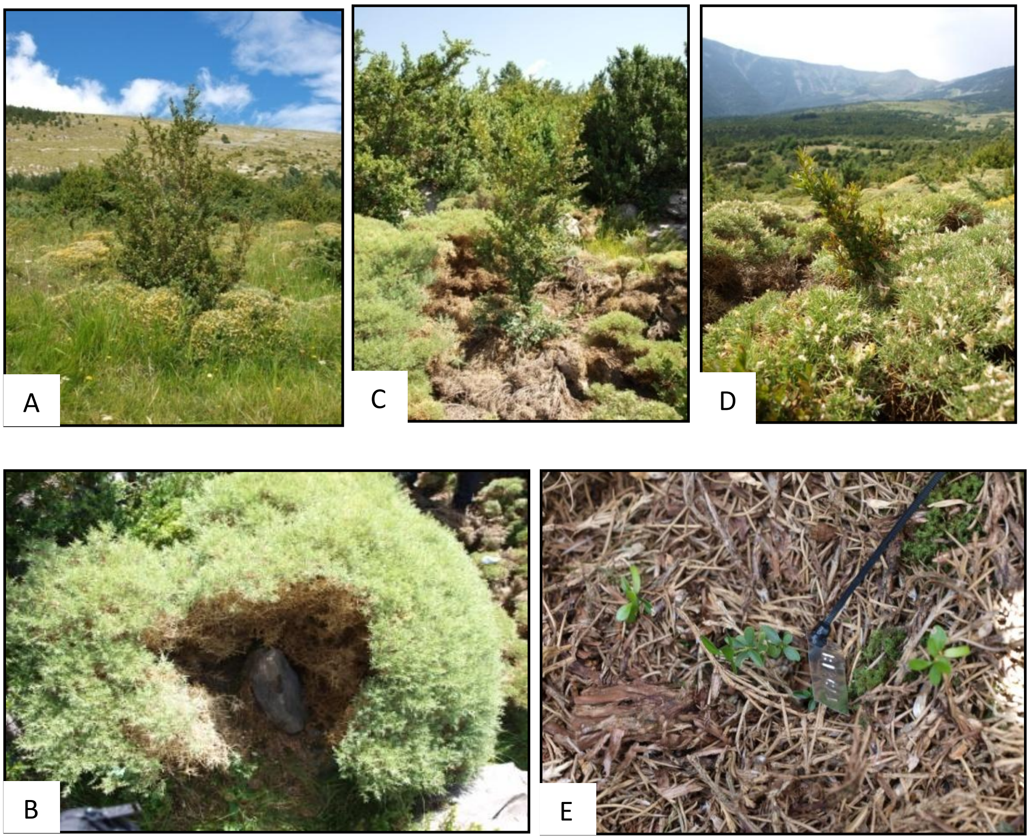
7. Developmental instability can be used as an indicator of environmental stress, and it can indicate a degree of adaptiveness of the species to the specific environmental conditions (Chapter 4). In addition, developmental instability can be an index of pair species interactions (Chapter 1).

8. Positive plant interactions favored plant spatial aggregation and plant diversity in Sierra Nevada, Spain (Chapter 5), especially under stressful conditions by low temperatures. Environmental stressful conditions derived from a related-resource stress, i.e. acid soils with lower nutrient content than basic soils, have less abundance of positive plant interactions than more nutrient rich but cold site on basic soil-north slopes.

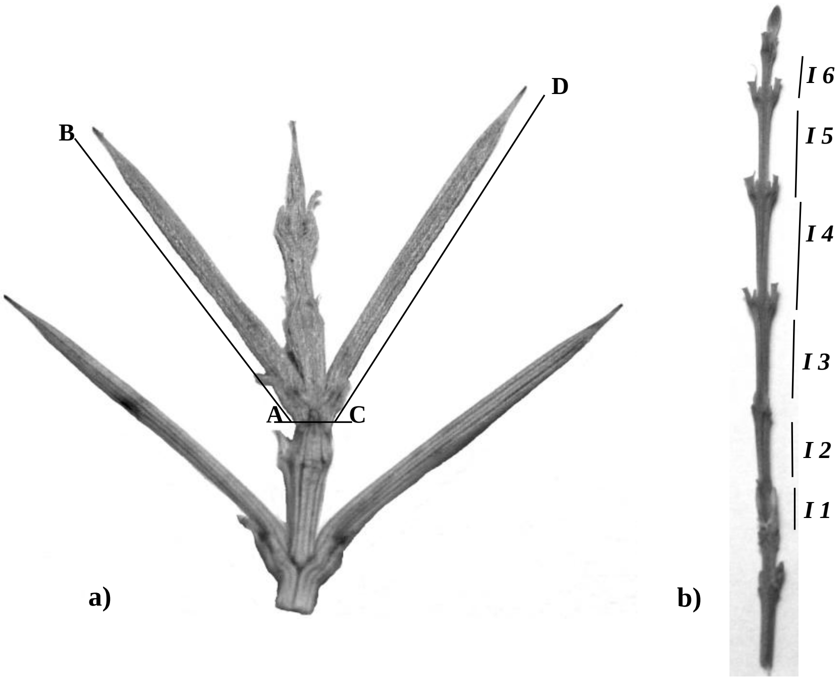
Appendices

Appendix of the Chapter 1

Appendix 1. Study site at the 'Lomar Valley' in the periphery area of Ordesa and Monte Perdido National Park (PNOM), at the Pyrenees, Spain. Light green are *E. horridum* and dark green are *B. sempervirens*.



Appendix 2. The study species and neighbor-removal experiment at the study sites. a) Coexisting adult *B. sempervirens* and *E. horridum*; b) *E. horridum* where coexisting *B. sempervirens* had been removed; c) Adult *B. sempervirens* where coexisting *E. horridum* had been removed; d) Juvenile *B. sempervirens* emerging from *E. horridum* crown; e) *B. sempervirens* seedling in which surrounding *E. horridum* had been removed.



Appendix 3. a) Spines of *E. horridum* and b) annual shoot of *B. sempervirens*. Spines were measured from the base to the apex, A-B for left side and C-D for right side to calculate fluctuating asymmetry. The lengths of all internodes on the shoots were measured (I1, I2, I3, etc) to calculate translational asymmetry.

	B. sempervirens adults		*B. sempervirens* juveniles	
	DI ($1-R^2$)	Annual Growth	DI ($1-R^2$)	Annual Growth
High site (1200 m.a.s.l)	0.1284 ± 0.0087	0.0365 ± 0.03	0.1338 ± 0.0128	(see Figure 4 Chapter 2)
Low site (1200 m.a.s.l)	0.1111 ± 0.0108	0.1336 ± 0.04	0.0894 ± 0.0104	

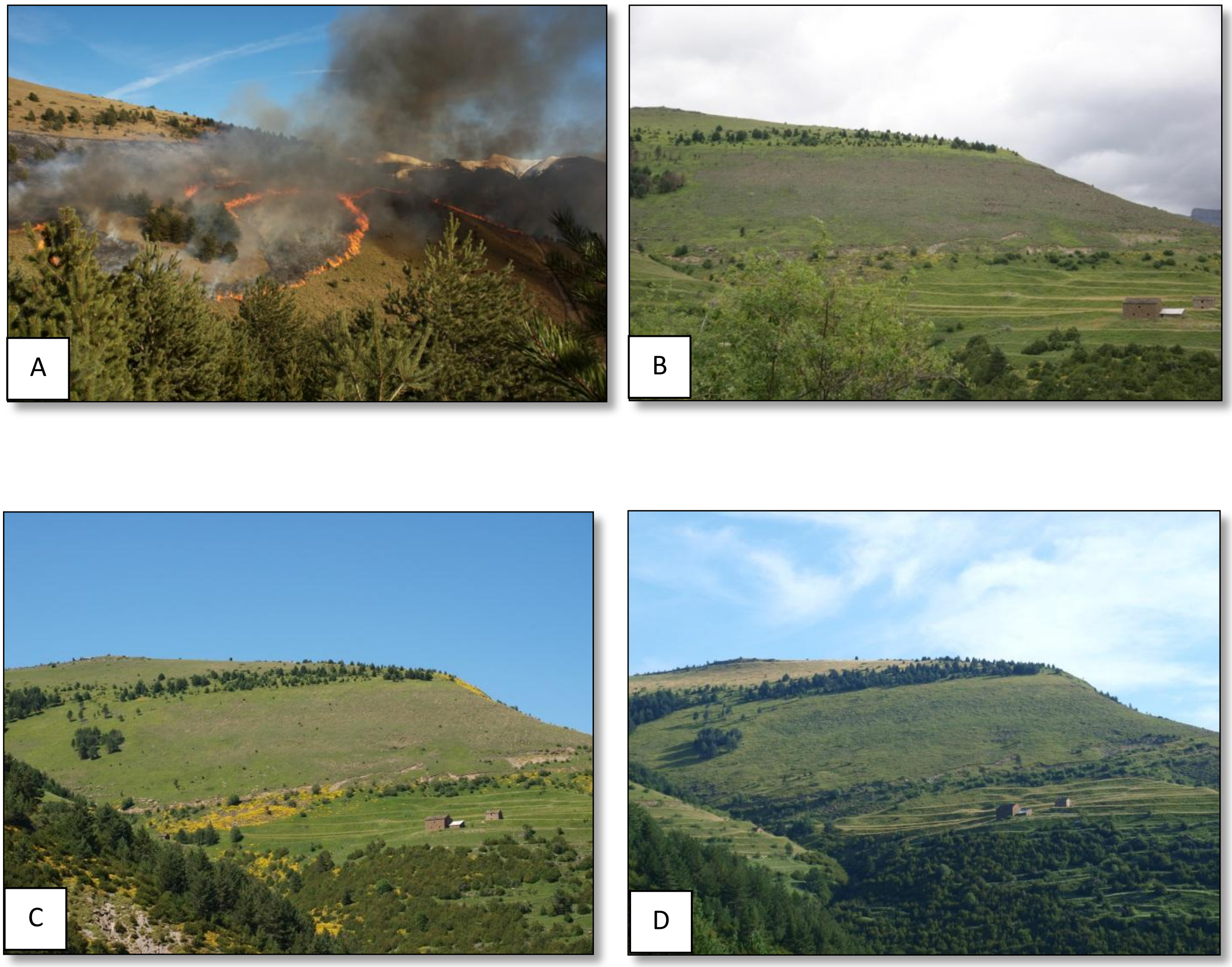
Appendix 4. Average annual growth and DI (±SE) in Control plots for *B. sempervirens* adults and juveniles at each study site, High and Low sites, at the Central Pyrenees (Spain).

Appendix of the Chapter 2

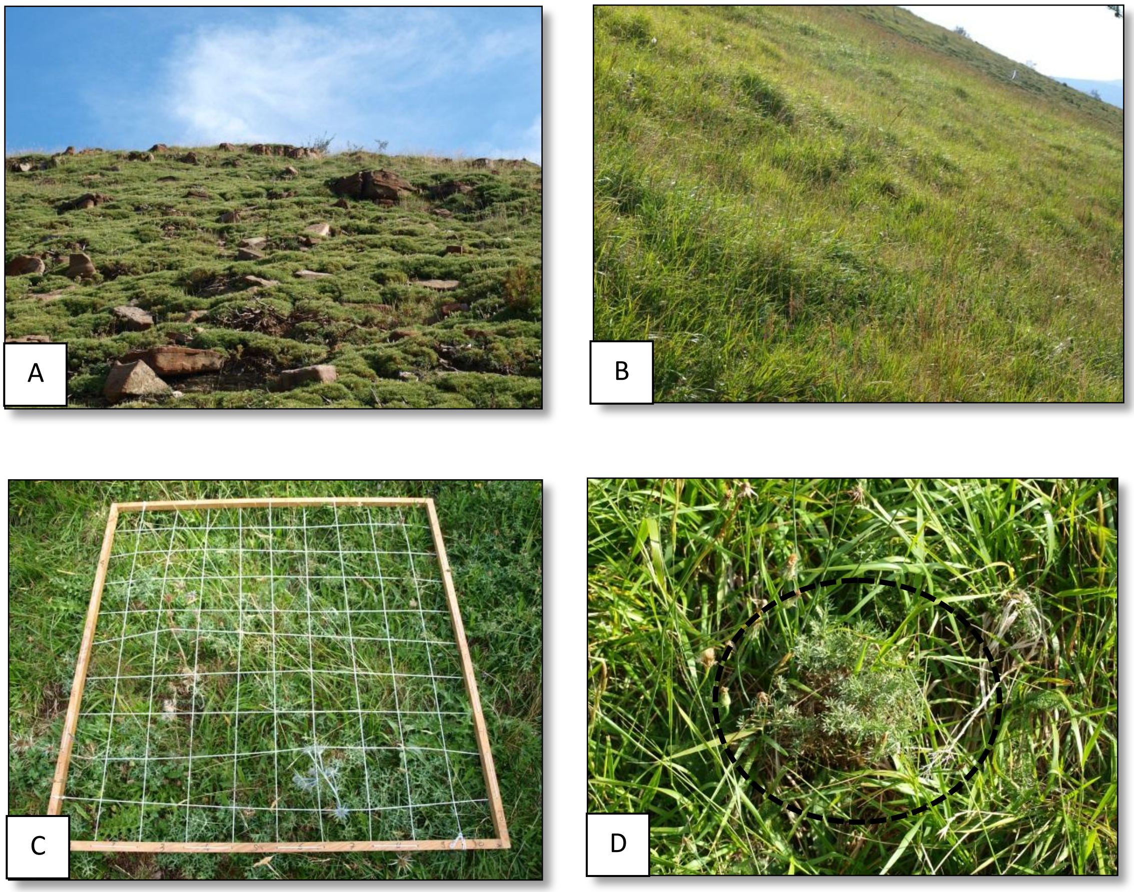
Appendix 1. Distribution of the soil seeds of the species found at each management treatment (Burnt, Clear-cut and Control), study year (2009 and 2011) and depth (I, 0-5 cm, and II 5-10 cm). T: transient, STP: short term persistent, LTP: long term persistent. Type of soil seed bank in Thomson et al. 1997 and Kleyer et al. 2008 data bases, and in Navarra et al. 2011.

	Control				**Burnt**				**Clear-cut**				**Total**	**Thomson et al. 1997 and Kleyer et al. 2008**
	2009		**2011**		**2009**		**2011**		**2009**		**2011**			
		Type		**Type**		**Type**		**Type**		**Type**		**Type**		
Achillea millefolium	2	LTP	0	T	0	T	0	T	1	T	0	T	3	Transient and persistent
Agrostis capillaris	0	T	3	STP	3	STP	16	STP	44	STP	22	STP	91	Transient and persistent
Arenaria grandiflora	6	STP	9	STP	6	STP	35	STP	1	STP	1	T	58	Other Arenaria sp. with persistent soil seed bank
Campanula rotundifolia	0	T	1	STP	0	T	0	T	0	T	0	T	1	Transient and persistent
Cerastium fontanum	1	LTP	2	LTP	0	T	0	T	0	T	6	STP	9	Transient and persistent
Chaenorhinum minus	1	LTP	8	STP	2	T	7	STP	0	T	1	LTP	19	Transient and persistent
Chenopodium bonus-henricus	0	T	0	T	0	T	10	STP	0	T	0	T	10	Transient
Echinospartum horridum	2	T	0	T	1	T	0	T	0	T	0	T	3	---
Echium vulgare	0	T	0	T	8	T	36	STP	0	T	2	T	46	Transient
Erucastrum nasturtiifolium	1	T	0	T	1	LTP	0	T	0	T	0	T	2	*E. gallicum* transient and persistent
Eryngium bourgatii	7	STP	13	STP	1	T	2	STP	0	T	0	T	21	*E. campestre* transient soil seed bank
Euphorbia sp.	5	STP	15	STP	3	STP	10	STP	0	T	2	STP	45	Persistent
Festuca rubra	0	T	0	T	0	T	0	T	0	T	1	LTP	1	Transient and persistent
Galium pinetorum-pumilum	0	T	0	T	0	T	3	STP	1	T	1	LTP	5	*G. pumilum* transient and persistent
Galium verum	2	STP	0	T	1	T	1	LTP	0	T	0	T	4	Transient and persistent
Hypericum perforatum	0	T	0	T	1	T	0	T	0	T	0	T	1	Transient and persistent

	Control				**Burnt**				**Clear-cut**				**Total**	**Thomson et al. 1997 and Kleyer et al. 2008**
	2009		**2011**		**2009**		**2011**		**2009**		**2011**			
		Type		**Type**		**Type**		**Type**		**Type**		**Type**		
Linum catharticum	22	STP	27	STP	5	STP	124	STP	2	LTP	0	STP	180	Transient and persistent
Paronychia kapela	0	T	0	T	14	T	1	LTP	45	STP	123	STP	183	Navarra et al. 2011
Pimpinella saxifraga	0	T	0	T	0	T	1	T	0	T	0	T	1	Transient and persistent
Plantago lanceolata	0	T	1	T	0	T	1	LTP	0	T	3	T	5	Transient and persistent
Potentilla neumaniana	5	LTP	3	STP	2	STP	1	T	3	T	1	LTP	15	Transient and persistent
Prunella vulgaris	0	T	0	T	0	T	0	T	0	T	1	LTP	1	Transient and persistent
Rhinanthus mediterraneus	0	T	0	T	0	T	1	T	0	T	0	T	1	Other Rhinanthus sp. with transient and persistent soil seed banks
Sanguisorba minor	0	T	6	STP	1	T	6	STP	0	T	1	T	14	Transient and persistent
Taraxacum sp.	0	T	0	T	1	T	0	T	0	T	0	T	1	Transient and persistent
Teucrium chamaedrys	1	T	6	STP	0	T	3	STP	0	T	0	T	10	Transient and persistent
Thymus praecox	0	T	0	T	0	T	2	STP	0	T	0	T	2	Transient and persistent
Umbelliferae sp.	1	T	0	T	0	T	0	T	0	T	0	T	1	Transient and persistent
Veronica arvensis	0	T	1	LTP	1	LTP	6	STP	2	STP	0	T	10	Transient and persistent
Total	56		95		54		266		99		165			
Total	151				320				264				735	



Appendix 2. The study area at the periphery of Ordesa and Monte Perdido National Park, near Torla, Central Pyrenees (Spain) and the progression following the Brunt treatment. A) Burning in November 2008 (by Komac, B). B) After Burn treatment in 2009 (by Komac, B), C) in 2012 (by Nuche, P.), and D) in 2015 (by Nuche, P.). Note that the burnt treatment is progressively greened.



Appendix 3. The study sites, Burnt and Clear-cut treatments in August 2015. A) At the Burnt treatment, with a cover of 90% of *E. horridum*, B) a detail of the temperature and humidity sensor, C) Clear-cut mainly dominated by grasses (mostly *Brachypodium pinnatum*), D) plot in Clear-cut treatment, note de different species composition, in the plot there is a dominance of species ruderal such as *Eryngium vulgare*, *Carlina acaulis* and *Ononis spinosa*. E) and F) *E. horridum* individuals in Clear-cut treatments, note the small size and low compact crown surrounded by grasses. All the pictures were taken by Nuche, P.

Appendix 4.

Objective: In order to evaluate if prescribed burning can be a desirable strategy to remove the highly invasive shrub *Echinospartum horridum* Valh and recover the subalpine grasslands, the vegetation recovery of *E. horridum* burnt stands were monitored annually in Torla (the same study site of Chapter 2) in the Central Pyrenees, Spain.

Methods: The prescribed burning was performed in autumn 2008 in an *E. horridum* community. Vegetation recovery was monitored annually until *E. horridum* recolonized again the area, in 2014, hence six years of monitoring were conducted. Monitoring was performed every year in mid-July when vegetation was fully developed.

Three transects were sampled each year (2008-2014). The point-intercept method was used, each transect had 250 m length and every 10 centimeters every species was recorded (Godall 1952).

Several indices were calculated as indicators of ecosystem evolution after burning. Species richness (number of species), diversity (Shannon and Evenness), percentage of bare soil, *E. horridum* cover, plant positive and negative interactions, the ratio between positive to negative plant interactions.

Results: The percentage of bare ground was high the year following prescribed burning and it progressively diminishes, meanwhile, *E. horridum* cover increased (Figure 1). Species richness and Shannon index showed to be the highest at intermediate years (Figure 2). Species richness experiment a slightly increase the last two years, which might show how the mature *E. horridum* stand after the fire perturbation is also rich in species.

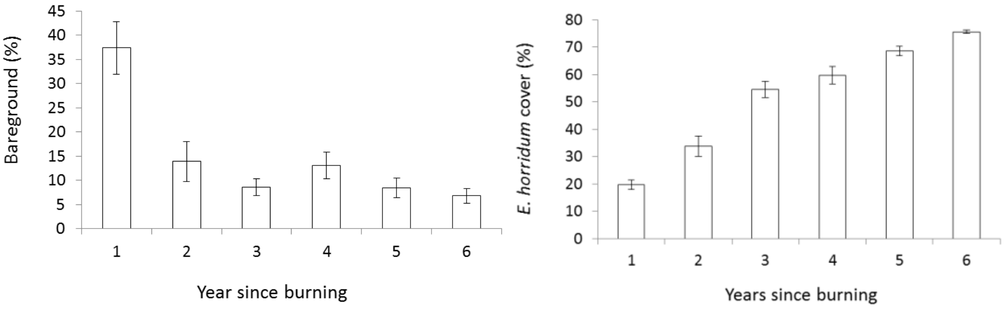


Figure 1. A) Bare ground percentage and B) cover of *E. horridum* (%) each year after prescribed burning at Torla, in the Central Pyrenees (Spain).

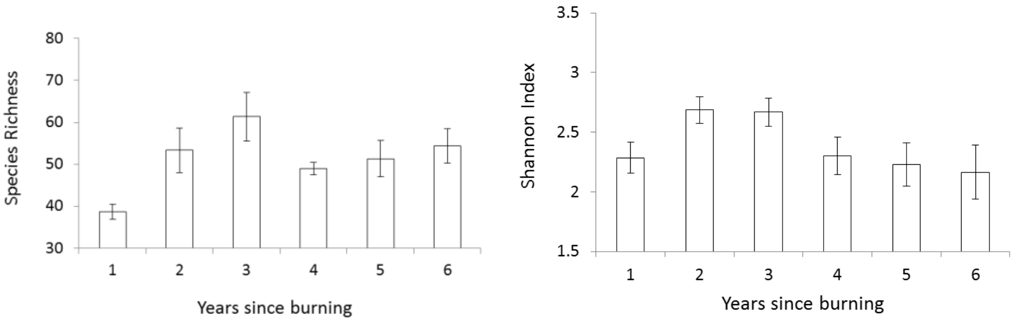
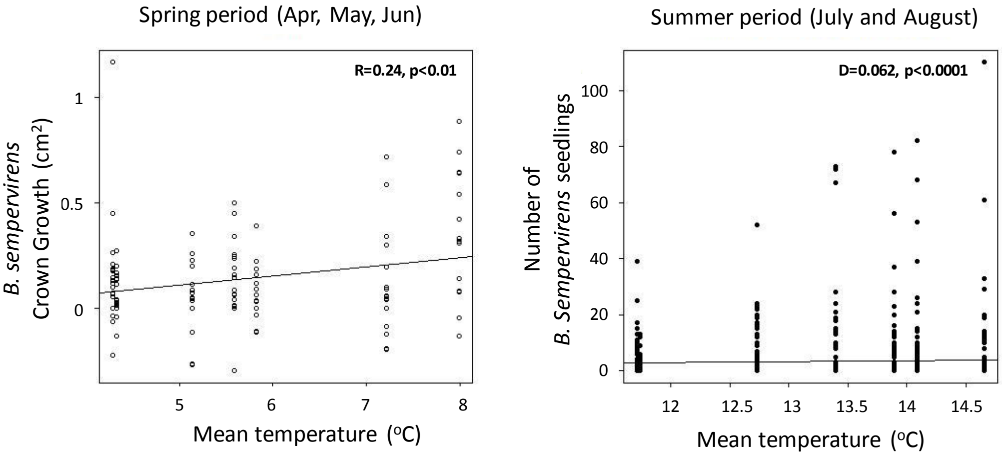
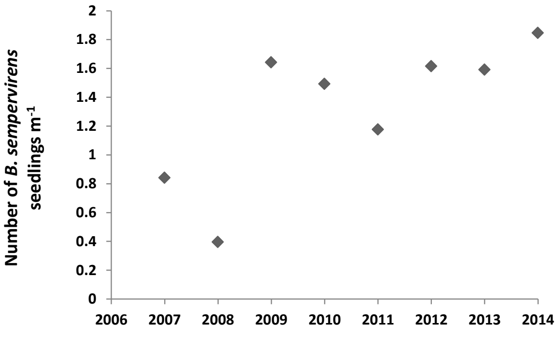


Figure 2. A) Species richness and B) Shannon index in each year after prescribed burning at Torla, in the Central Pyrenees (Spain).

Appendix of the Chapter 3



Appendix 1. *B. sempervirens* adults annual crown growth (cm^2) in relation to mean temperature of the spring period (April-June) and the number of *B. sempervirens* established in relation to mean temperature of the summer period (July and August). R^2 is the coefficient of determination of a linear model for annual crown growth, and D is the deviance of a generalized mixed model for number of seedling (Poisson family distribution).



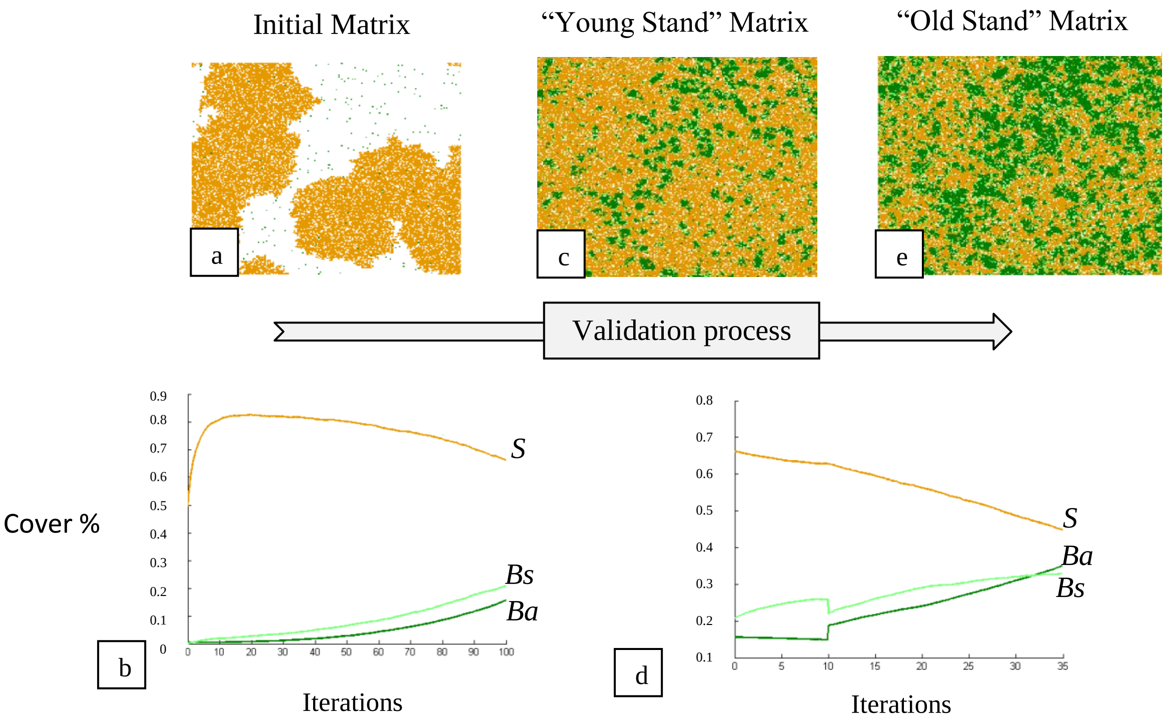
Appendix 2. Average number of *B. sempervirens* seedling per m^2 established yearly at the study sites, in the Central Pyrenees, Spain.

Appendix 3. **Methods: Model validation**

Model validation was made with field data. Two grasslands locations which differ in the time when they were abandoned and thus when the shrub colonization started, called for the purposes of the study "Old Stand", for the stand which was abandoned firstly, and "Young Stand" for the stand which was abandoned afterwards, were used for model validation. They differ in 35 years approximately (calculated by average *B. sempervirens* age in each stand from dendrochronology analyses, unpublished data). At present, we can observe two moments through the evolution of the system by comparing both sites, which allow validating the model. By setting the starting conditions of the model to the "Young Stand" conditions and running the model for 35 time steps (1 time step =1 year) we would expect to observe in the model the same trend found in "Old Stand". To start the model validation a matrix with self-organized *E. horridum* obtained from a former cellular automata model (Komac et al. 2013) and a very few cover of *B. sempervirens* adults (0.01%) (Appendix 3.2 a) was run during the number of iterations needed to achieve the cover of the "Young Stand" (Appendix 3.2 b), 0.23% for *B. sempervirens* adults and 0.66% for *E. horridum* (Appendix 3.1 and Appendix 3.2 c). This "Young Stand Matrix" was run for 35 iterations simulating the vegetation succession process (Appendix 3.2 d). The tendency of the evolution of the species in the model reflects the observed trend in the field and at the end of the simulation (Appendix 3.2 e) the abundance of *B. sempervirens* and *E. horridum* was similar to their abundances in the field in the "Old Stand" (Appendix 3.1).

	Field Cover (%)		**Field Cover (%) Re-scaled up**		**Model Cover (%)**	
	"Young Stand"	**"Old Stand"**	**"Young Stand"**	**"Old Stand"**	**Initial Conditions**	**Final conditions**
B. sempervirens	0.148	0.314	0.24	0.57	0.23	0.49
E. horridum	0.449	0.229	0.74	0.42	0.66	0.44
Empty	0.008	0.002	0.01	0.01	0.11	0.06
Total	0.606	0.545	1	1	1	1

Appendix 3.1. % cover of study species, *E. horridum*, *B. sempervirens*, and Empty sites, in the field, at "Young Stand" and "Old Stand".



Appendix 3.2. Cellular automata model with 200 * 200 cells: a) Initial matrix, validation process starting conditions. b) Coverage (% of cells occupied by each state) *E. horridum*, *B. sempervirens* seedling and adults, at each iteration in the progression from the matrix (a) to the matrix (c). c) Matrix with 23% coverage of *B. sempervirens* after 1000 iterations. d) Coverage (% of cells occupied by each state) *E. horridum*, *B. sempervirens* seedlings and adults at each iteration in the progression from the matrix (c) to the matrix and (e). e) Final matrix. Dark green cells represent *B. sempervirens* adult (Ba), light green cells represent *B. sempervirens* seedling (Bs), orange cells represent *E. horridum* (S), and white cells represent empty space capable of being colonized (E).

Affiliation of the co-authors

Benjamin Komac	Centre d’Estudis de la Neu i la Muntanya d’Andorra (CENMA – IEA). Andorra.
Hugo Saiz Bustamante	Departamento de Conservación de la Biodiversidad y Restauración de Ecosistemas. Instituto Pirenaico de Ecología (IPE-CSIC). Zaragoza, España.
Jesús Julio Camarero	Departamento de Conservación de la Biodiversidad y Restauración de Ecosistemas. Instituto Pirenaico de Ecología (IPE-CSIC). Zaragoza, España.
Juan Escós	Escuela Politécnica Superior de Zaragoza Universidad de Zaragoza Huesca, España.

Maite Gartzía	Departamento de Conservación de la Biodiversidad y Restauración de Ecosistemas. Instituto Pirenaico de Ecología (IPE-CSIC) Zaragoza, España.
Ramón Reiné Viñales	Escuela Politécnica Superior de Zaragoza Universidad de Zaragoza. Huesca, España.
Teresa Navarro	Departamento de Biología Vegetal Facultad de Ciencias Universidad de Málaga. Málaga. España.
Yolanda Pueyo Estaún	Departamento de Conservación de la Biodiversidad y Restauración de Ecosistemas. Instituto Pirenaico de Ecología (IPE-CSIC). Zaragoza, España.

Agradecimientos

En primer lugar quiero agradecer a mi tutora de tesis, Inma Alados, por darme la oportunidad de realizar esta tesis. Durante estos años he aprendido muchas cosas tanto en el plano académico como en el personal, y eso se lo debo en parte a ella, y también al resto del equipo. Quiero agradecer a Yolanda Pueyo, a Maite Gartzía, Hugo Saiz, a Ángel de Frutos, a Yacine Kouba, a Ben Komac, a Guille Bueno por todo lo que me han enseñado en el curro de campo con algunos y en el despacho o incluso en bares con otros. Agradezco la compañía de todas las personas con las que he compartido momentos en el IPE, empezando por mis compañeros de despacho Edu, Ceci y Fabián; y por los juepinchos con los demás, Javi, Ana F., Iker, Sam, María Gallega, Josu, Antonio I., Alberto Barcos, Kike, Jesús, R., Natalia, y a los de los viejos tiempos Diego, Leti, Elena cita, Montse cita, *& company*.

También aprovecho para dedicar un cachito de esta tesis a los que me acogieron en las dos estancias que he hecho al extranjero, Ray Callaway y Sonia Kéfi, las dos muy gratificantes.

No podía olvidarme de la familia Palazio, me han acogido estupendamente en todas las campañas de campo en Nerín.

No me olvido de mis tricantinos del alma ... Mery, Soni, Ainho, Jelen, Albita, Pablulu, Pablete, Ral, Kiko, Jony, Richy especialmente,

Especial agradecimiento a mis padres por haberme apoyado en mis ilusiones aunque pareciera un camino sin mucho futuro, y a mi hermanita por ponerse siempre de mi parte.

Especial gratitud a Jesús por su constante apoyo.

References

1. Adler, P. B., H. J. Dalgleish, and S. P. Ellner. 2012. Forecasting plant community impacts of climate variability and change: when do competitive interactions matter? Journal of Ecology, 100: 478-487.
2. Agakhanyantz, O.E. and Lopatin, I.K. 1978. Main characteristics of ecosystems of Pamirs, USSR. Artic and Alpine Research, 10: 397-407.
3. Agrawal, A.A., Ackerly, D.D., Adler, F., Arnold, A.E., Cáceres, C, F Doak, D.F., Post, E., Hudson, J.P., Maron, J., Mooney, K.A, Power, M., Schemske, D., Stachowicz, J., Strauss, S., Turner, M.G. and Werner, E. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment, 5: 145-152.
4. Alados, C.L., ElAich, A., Papanastasis, V.P., Ozbek, H., Navarro, T., Freitas, H., Vrahnakis, M., Larrosi, D. and Cabezudo, B. 2004. Change in plant spatial patterns and diversity along the successional gradient of Mediterranean grazing ecosystems. Ecological Modelling, 180: 523-535.
5. Alados, C.L., Giner, M. and Pueyo, Y. 2006. An assessment of the differential sensitivity of four summer-deciduous chamaephytes to grazing and plant interactions using translational asymmetry. Ecological Indicators, 6: 554–566. doi:10.1016/j.ecolind.2005.08.019
6. Alados, C.L., Gotor, P., Ballester, P., Navas, D., Escós, J.M., Navarro, T. and Cabezudo, B. 2006. Association between competition and facilitation processes and vegetation spatial patterns in alpha steppes. Biological Journal of the Linnean Society, 87:103-113.
7. Alados, C.L., Navarro, T., Cabezudo, B., Emlen, J.M. and Freeman, C. 1998. Developmental instability in gynodioecious *Teucrium lusitanicum*. Evolutionary Ecology, 12: 21–34.
8. Alados, C.L., Navarro, T., Escós, J., Cabezudo, B. and Emlen, J.M. 2001. Translational and fluctuating asymmetry as tools to detect stress in stress-adapted and –non-adapted plants. International Journal of Plant Sciences, 162: 607-616.
9. Alados, C.L., Pueyo, Y., Giner, M.L., Navarro, T., Escós, J., Barroso, F., Cabezudo, B. and Emlen, J.M. 2003. Quantitative characterization of the regressive ecological succession by fractal analysis of plant spatial patterns. Ecological Modelling, 163: 1-17.
10. Alados, C.L. and Weber, D.N. 1999. Lead effects on the predictability of reproductive behavior in fathead minnows (*Pimephales promelas*): A mathematical model. Environmental Toxicology and Chemistry, 18: 2392-2399.
11. Albalasmeh, A.A., Berli, M., Shafer, D.S., Ghezzehei, T.A. 2013. Degradation of moist soil aggregates by rapid temperature rise under low intensity fire. Plant soil. 362: 335-344. DOI 10.1007/s11104-012-1408-z
12. Álvarez-Martínez, J.M., Suárez-Seoane, S., Stoorvogel, J.J., de Luis Calabuig, E., 2014. Influence of land use and climate on recent forest expansion: a case study in the Eurosiberian-Mediterranean limit of north-west Spain. Journal of Ecology, 102: 905–919. doi:10.1111/1365-2745.12257

13. Anthelme, F., Buendia, B., Mazoyer, C. and Dangles, O. 2012. Unexpected mechanisms sustain the stress gradient hypothesis in a tropical alpine environment. Journal of Vegetation Science, 23: 62-72.
14. Anthelme, F., Cavieres, L.A. and Dangles, O. 2014. Facilitation among plants in alpine environments in the face of climate change. Frontiers in Plant Science, 5: 1-15.
15. Aparicio, A., Albaladejo, R.G. and Ceballos, G.L. 2002. Genetic differentiation in silicicolous Echinospartum (Leguminosae) indicated by allozyme variability. Plant Systematics and Evolution, 230: 189–201.
16. Aparicio, A. and Guisande, R. 1997. Replenishment of the endangered *Echinospartum algibicum* (Genisteae, Fabaceae) from the soil seed bank. Biological conservation, 81: 267-273.
17. Archer, S. 1989. Have southern Texas savannas been converted to woodlands in recent history? American naturalist, 4: 545–561.
18. Armas, C., Ordiales, R. and Pugnaire, F. I. 2004. Measuring plant interactions: a new comparative index. Ecology, 85: 2682-2686.
19. Arroyo, M.T.K., Cavieres, L.A., Peñaloza, A. and Arroyo-Kalin, M.A. 2003. Positive associations between the cushion *Azorella monantha* (Apiaceae) and alpine plant species in the Chilean Patagonian Andes. Plant Ecology, 169: 121-129.
20. Asher, L., Collins, L.M., Ortiz-Pelaez, A., Drewe, J.A., Nicol, C.J. and Pfeiffer, D.U. 2009. Recent advances in the analysis of behavioural organization and interpretation as indicators of animal welfare. Journal of the Royal Society Interface, 6: 1103-1119.
21. AUCT. PL. Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. 2009. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid.
22. Auld, T.D. and Denham, A.J. 2006. How much seed remains in the soil after a fire? Plant Ecology, 187:15-24. DOI 10.1007/s11258-006-9129-0
23. Azmi, M., Razali, W., Mohd, W. and Fauzidah, A. 1991. Characteristics and volume–weight relationship of four Malaysiam bamboos. Journal Tropical of Forest Science, 4: 87–93.
24. Bakkenes, M., Alkemade, J.R.M., Ihle, F., Leemans, R. and Latour, J.B. 2002. Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. Global Change Biology, 8: 390–407.
25. Barbero, M., Bonin, G., Loisel, R., and Quézel, P. 1990. Changes and disturbances of forest ecosystems caused by human activities in the western part of the Mediterranean basin. Vegetation, 87: 151-173.
26. Barrio, I.C., Bueno, C.G., Nagy, L., Palacio, S., Grau, O., Munilla, I., García, M.B., Garcia-Cervigón, A.I., Gartzia, M., Gazol, A., Lara-Romero, C., Anadon-Rosell, A., Ninot, J.M., Chocarro, C., Alados, C.L., Fillat, F. and Zamora, R. 2013. Alpine Ecology in the Iberian Peninsula: What Do We Know, and What Do We Need to Learn? Mountain Research and Development, 33: 437–442. doi:10.1659/MRD-JOURNAL-D-13-00052.1
27. Basto, S., Drca-Fornell, C., Thompson, K. and Rees, M. 2013. Effect of pH buffer solutions on seed germination of *Hyperichum pulchrum*, *Campanula rotundifolia* and *Scabiosa clumbaria*. Seed Science and Technology, 41: 298-302

28. Batllori, E., Gutiérrez, E. 2008. Regional tree line dynamics in response to global change in the Pyrenees. Journal of Ecology, 96: 1275–1288. doi:10.1111/j.1365-2745.2008.01429.x
29. Beasley, de A.E., Bonisoli-Alquati, A. and Mousseau, T.A. 2013. The use of FA as a measure of environmentally induced developmental instability: A meta-analysis. Ecological Indicators, 30: 218-226.
30. Bekker, R.M., Verweij, G.L., Smith, R.E.N., Reiné, R., Bakker, J.P., and Schneider, S. 1997. Soil seed banks in European grasslands: Does land use affect regeneration perspectives? Journal of Applied Ecology, 34: 1293–1310.
31. Bekker, R.M., Verweij, G.L., Bakker, J.P. and Fresco, L.F. 2000. Soil seed bank dynamics in hayfield succession. Journal of Ecology, 88: 594-607.
32. Benito Alonso, J.L. 2010. La vegetación del Parque Nacional de Ordesa y Monte Perdido (Pirineo Aragonés). Monografía de Botánica Ibérica nº 6. Segunda edición.
33. Bertness, M.D. and Callaway, R.M. 1994. Positive interactions in communities. Trends in Ecology and Evolution, 9: 191-193.
34. Bertness, M.D. and Ewanchuk, P. 2002. Latitudinal and climate-driven variation in the strength and nature of biological interactions in New England salt marshes. Oecologia, 132: 392–401. doi:10.1007/s00442-002-0972-y
35. Bertness, M.D. and Shumway, S.W. 1993. Competition and facilitation in marsh plants. American Naturalist, 4: 718-724.
36. Biere, A. 1995. Genotypic and Plastic Variation in Plant Size: Effects on Fecundity and Allocation Patterns in Lychnis Flos-Cuculi Along a Gradient of Natural Soil Fertility. The Journal of Ecology, 83: 629-642. doi:10.2307/2261631
37. Bliss, L.C. 1971. Arctic and alpine plant life cycles. Annual Review of Ecology and Systematics, 2: 405-438.
38. Borchard, F., Berguer, H.J., Bunzel-Drüke, M. and Fartman, T. 2011. Diversity of plant-animal interactions: possibilities for a new plant defense indicator value? Ecological Indicators, 11: 1311-1318.
39. Bossuyt, B. and Honnay, O. 2008. Can the seed bank be used for ecological restoration? An overview of the seed bank characteristic in European communities. Journal of vegetation science, 19: 875-884. doi: 10.3170/2008-8-18462.
40. Brandt, J.S., Haynes, M.A., Kuemmerle, T., Waller, D.M. and Radeloff, V.C. 2013. Regime shift on the roof of the world: Alpine meadows converting to shrublands in the southern Himalayas. Biological Conservation, 158: 116–127.
    doi:10.1016/j.biocon.2012.07.026
41. Bravo, D.N., Araújo, M.B., Lasanta and T., Moreno, J.I.L. 2008. Climate Change in Mediterranean Mountains during the 21st Century. AMBIO: A Journal of the Human Environment, 37: 280–285.
    doi:10.1579/0044-7447(2008)37[280:CCIMMD]2.0.CO;2
42. Brooker, R.W. 2006. Plant-plant interactions and environmental change. New Phytologist, 171: 271-284.
43. Brooker, R.W. and Callaghan, T.V. 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. Oikos, 81: 196-207.
44. Brooker, R.W., Callaway, R.M., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Michalet, R., Pugnaire, F.I., Valiente-Banuet, A. and Whitham, T.G. 2009.

Don't Diss Integration: A Comment on Ricklefs's Disintegrating Communities. The American Naturalist, 174: 919–927. doi:10.1086/648058

45. Brooker, R., Kikvidze, Z., Pugnaire, F.I., Callaway, R.M., Choler, P., Lortie, C.J. and Michalet, R. 2005. The importance of importance. Oikos, 109: 63–70.
46. Bruno, J.F., Stachowicz, J.J. and Bertness, M.D. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution, 18: 119–125.
47. Bücher, A. and Dessens, J. 1991. Secular trend of surface temperature at an elevated observatory in the Pyrenees. American Meteorological Society, 4: 859-868.
48. Bullock, S.H. 1991. Herbivory and the demography of the chaparral shrub *Ceanothus greggii* (Rhamnaceae). Madroño, 38, 63-72.
49. Callaway, R.M. 1997. Positive interactions in plant communities and the individualistic-continuum concept. Oecologia, 112: 143–149.
50. Callaway, R.M. 2007. Positive interactions and interdependence in plant communities. Dordrecht, the Netherlands: Springer.
51. Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C. J., Michalet, R., Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E. T., Armas, C., Kikodze, D. and Cook, B.J. 2002. Positive interactions among alpine plants increase with stress. Nature, 417: 844-848.
52. Callaway, R.M., Walker, L.R. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology, 78: 1958–1965.
53. Camarero, J.J. and Gutiérrez, E. 2004. Pace and pattern of recent treeline dynamics: response of ecotones to climatic variability in the Spanish Pyrenees. Climatic Change, 63: 181-200.
54. Camarero, J.J., Gutiérrez, E. and Fortín, M.J. 2000. Spatial pattern of subalpine forest-grassland ecotones in the Spanish Central Pyrenees. Forest Ecology and Management, 134: 1-16.
55. Camarero, J.J., Palacio, S. and Montserrat-Martí, G. 2013. Contrasting seasonal overlaps between primary and secondary growth are linked to wood anatomy in Mediterranean sub-shrubs. Plant Biology, 15: 798–807. doi:10.1111/j.1438-8677.2012.00702.x
56. Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S. and Naeem, S. 2012. Biodiversity loss and its impact on humanity. Nature, 486: 59–67. doi:10.1038/nature11148
57. Castanha, C., Torn, M.S., Germino, M.J., Weible, B. and Kueppers, L.M. 2013. Conifer seedling recruitment across a gradient from forest to alpine tundra: effects of species, provenance and sites. Plant Ecology and diversity, 6: 307-318.
58. Cavieres, L., Arroyo, M.T.K., Peñaloza, A., Molina-Montenegro, M. and Torres, C.. 2002. Nurse effect of *Bolax gummifera* cushion plants in the alpine vegetation of the Chilean Patagonian Andes. Journal of Vegetation Science, 13: 547. doi:10.1658/1100-9233(2002)013[0547:NEOBGC]2.0.CO;2
59. Cavieres, L.A. and Badano, E.I. 2009. Do facilitative interactions increase species richness at the entire community level? Journal of Ecology, 97: 1181-1191.

60. Cavieres, L.A., Badano, E.I., Sierra-Almeida, A. and Molina-Montenegro, M.A. 2007. Microclimatic Modifications of Cushion Plants and Their Consequences for Seedling Survival of Native and Non-native Herbaceous Species in the High Andes of Central Chile. Arctic, Antarctic, and Alpine Research, 39: 229–236. doi:10.1657/1523-0430(2007)39[229:MMOCPA]2.0.CO;2
61. Cavieres, L.A., Brooker, R.W., Butterfield, B.J., Cook, B.J., Kikvidze, Z., Lortie, C.J., Michalet, R., Pugnaire, F. I., Schöb, C., Xiao, S., Anthelme, F., Björk, R.G., Dickinson, K.J.M., Cranston, B.H., Gavilán, R., Gutiérrez-Girón, A., Kanka, R., Maalouf, J.P., Mark, A.F., Noroozi, J., Parajuli, R., Phoenix, G.K., Reid, A.M.. Ridenour, W.M., Rixen, C., Wipf, S., Zhao, L., Escudero, A., Zaitchik, B.F., Lingua, E., Aschehoug, E.T. and Callaway, R.M. 2014. Facilitative plant interactions and climate simultaneously drive alpine plant diversity. Ecology Letters, 17: 193-202.
62. Cavieres, L.A. and Sierra-Almeida, A. 2012. Facilitative interactions do not wane with warming at high elevations in the Andes. Oecologia, 170: 575-584.
63. Caviezel, C., Hunziker, M., Schaffner, M. and Kuhn, N.J. 2014. Soil-vegetation interaction on slopes with bush encroachment in the Central Alps - adapting slope stability measurements to shifting process domains. Earth surface processes and landforms, 39: 509-521.
64. Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia, 143: 1-10.
65. Chapin III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C. and Díaz, S. 2000. Consequences of changing biodiversity. Nature, 405: 234-242.
66. Choler, P., Michalet, R. and Callaway, R.M. 2001. Facilitation and competition on gradients in alpine plant communities. Ecology, 82: 3295–3308.
67. Clavel, J., Julliard, R. and Devictor, V. 2011. Worldwide decline of specialist species: toward a global functional homogenization? Frontiers in Ecology and the Environment, 9: 222-228.
68. Collins, S.L. and Calabrese, L.B. 2012. Effects of fire, grazing and topographic variation on vegetation structure in tallgrass prairie. Journal of Vegetation Science, 23: 563–575. doi:10.1111/j.1654-1103.2011.01369.x
69. Colwell, R.K., Dunn, R.R. and Harris, N.C. 2012. Coextinction and Persistence of Dependent Species in a Changing World. Annual Review of Ecology, Evolution, and Systematics, 43: 183–203. doi:10.1146/annurev-ecolsys-110411-160304
70. Connell, J.H. 1983. On the Prevalence and Relative Importance of Interspecific Competition: Evidence from Field. The American Naturalist, 5: 661-696 .
71. Connell, J.H. and Slatyer, R.O. 1977. Mechanisms of Succession in Natural Communities and Their Role in Community Stability and Organization. The American Naturalist, 111: 1119-1144.
72. Coop, J.D. and Givnihs, T. J. 2008. Constraints on Tree Seedling Establishment in Montane Grasslands of the Valles Caldera, New Mexico. Ecology, 89: 1101-1111.
73. Cranston, B.H., Callaway, R.M., Monks, A. and Dickinson, K.J.M. 2012. Gender and abiotic stress affect community-scale intensity of facilitation and

its costs: *Gender, stress and facilitation*. Journal of Ecology, 100: 915–922. doi:10.1111/j.1365-2745.2012.01981.x

74. Cutler, N.A., Belyea, L.R. and Dugmore, A. J. 2008. The spatiotemporal dynamics of a primary succession. Journal of Ecology, 96: 231-246.
75. Danby, R.K. and Hik, D.S. 2007. Variability, contingency and rapid change in recent subartic alpine tree line dynamics. Journal of Ecology, 95: 352-363.
76. Davis, A.J., Jenkinson, L.S., Lawton, J.H., Shorroks, B. and Woods, S. 1998. Making mistakes when predicting shifts in species ranges in response to global warming. Nature, 391: 786-783.
77. De Candolle, A.P. 1855.Géographie Botanique Raisonnée.
78. De Luis, M, Raventós, J. and González-Hidalgo, J.C. Factors controlling seedling germination after fire in Mediterranean gorse shrubland. Implication for fire prescription. Journal of environmental management, 76:159-166.
79. Debussche, M. and Lepart, J. 1992. Establishment of woody plants in Mediterranean old fields: opportunities in space and time. Landscape Ecology, 6: 133-145.
80. Del Río, M., Schütze, G. and Pretzsch, H. 2014. Temporal variation of competition and facilitation in mixed species forests in Central Europe. Plant Biology, 16: 166–176. doi:10.1111/plb.12029Díaz, S., Tilman, D. and Fargione, J., et al. 2005. Millennium Ecosystem Assessment. Chapter 11: Biodiversity Regulation of Ecosystem Services.
81. D'Odorico, P., Fuentes, J.D., Pockman, W.T., Collins, S.L., He, Y., Medeiros, J.S., DeWekker, S. and Litvak, M.E. 2010. Positive feedback between microclimate and shrub encroachment in the northern Chihuahuan desert. Ecosphere, 1: 1-11. doi:10.1890/ES10-00073.1
82. Domenico, F.D., Lucchese, F. and Magri, D. 2012. Buxus in Europe: Late Quaternary dynamics and modern vulnerability. Perspectives in Plant Ecology, Evolution, Systematics, 14: 354-362.
83. Dominguez, P., Jacobson, A.G. and Jefferies, R.P.S. 2002. Paired gill slits in a fossil with a calcite skeleton. Nature, 417: 841–844. doi:10.1038/nature00805
84. Dullinger, S., Dirnböck, T. and Grabherr, G. 2003. Patterns of shrub invasion into high mountain grasslands of the northern calcareous Alps. Artic, Antarctic and Alpine research, 35:434-441.
85. Donelan, M. and Thompson, K. 1980. Distribution of viable seeds along a successional series. Biological conservation, 17: 297-311.
86. Ehrlich, P.R. and Raven, P.H. 1964. Butterflies and Plants: A Study in Coevolution. Evolution, 18: 586. doi:10.2307/2406212
87. Ellison, A.M., Bank, M.S., Clinton, B.D., Colburnm, E.A., Elliott, K., Ford, C.R., Foster, D.R., Kloeppel, B.D., Knoepp, J.D., Lovett, G.M., Mohan, J., Orwig, D.A., Rodenhouse, N.L, Sobczak, W.V, Stinson, K.A, Stone, J.K, Swan, C.M, Thompson, J., Von Holle, B, and Webster, J.R. 2005. Loss of foundation species: consequences for the structure and dynamics of forested. Frontiers in Ecology and the Environment, 3: 479–486.
88. Ellenberg, H. 1988. Vegetation ecology of Central Europe. Cambridge
89. Elmendorf, S.C., Henry, G.H.R., Hollister, R.D., Björk, R.G., Bjorkman, A.D., Callaghan, T.V., Collier, L.S., Cooper, E.J., Cornelissen, J.H.C., Day, T.A., Fosaa, A.M. and Gould, W.A. 2012. Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. Ecology Letters, 15: 164-175.

90. Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B. and Norberg, J. 2003. Response diversity, ecosystem change, and resilience. Frontier in Ecology and Environment, 1: 488–494.
91. Eriksson, O., Cousins, S.A.O. and Bruun, H.H. 2002. Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. Journal of Vegetation Science, 13: 743–748. doi:10.1111/j.1654-1103.2002.tb02102.x
92. Escós, J., Alados, C.L., Pugnaire, F.I., Puigdefabregas, J. and Emlen, J. 2000. Stress resistance strategy in an arid land shrub: interactions between developmental instability and fractal dimension. Journal of Arid Environments, 45: 325-336.
93. Evan, W. 2004. Why should we constrain stress and limitation? Why conceptual terms deserve broad definitions. Journal of Vegetation Science, 15: 569. doi:10.1658/1100-9233(2004)015[0569:WSWCSA]2.0.CO;2
94. Evans, A.S., Mitchell, J. and Cabin, R.J. 1996. Morphological side effects of using Gibberellic acid to induce germination: Consequences for the study of seed dormancy. American Journal of Botany, 83: 543–549.
95. Farrer, E.C., Ashton, I.W., Knape, J. and Suding K.N. 2014. Separating direct and indirect effects of global change: a population dynamic modeling approach using readily available field data. Global Change Biology, 20: 1238-1250.
96. Fensham, R.J., Fairfax, R.J. and Archer, S.R. 2005. Rainfall, land use and woody vegetation cover change in semi-arid Australian savanna. Journal of Ecology, 93: 596–606. doi:10.1111/j.1365-2745.2005.00998.x
97. Fernández, C., Vega, J.A. and Fonturbel, T. 2013. Effects of fuel reduction treatments on a gorse shrubland soil seed bank in the north of Spain: Comparing mastication and prescribed burning. Ecological engineering, 57: 79-87.
98. Fernández, C., Vega J.A. and Fonturbel, T. 2015. Does shrub recovery differ after prescribed burning, clearing and mastication in a Spanish heathland? Plant ecology, 216: 429-437.
99. Fillat, F., García-González, R., Gómez, D. and Reiné, R. 2008. Pastos del Pirineo. Consejo Superior de Investigaciones Científicas.
100. Fischer, S.F., Poshcold, P. and Beinlich, B. 1996. Experimental studies on the dispersal of plants and animals on sheep in calcareous grasslands. Journal of Applied Ecology, 33: 1206-1222.
101. Flematti, G.R., Merritt, D.J., Piggott, M.J., Trengove, R.D., Smith, S.M., Dixon, K.W., Ghisalberti, E.L. 2011. Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination. Nature communications, 2: 1-6. DOI: 10.1038/ncomms1356
102. Foley, J.A. 2005. Global Consequences of Land Use. Science, 309: 570–574. doi:10.1126/science.1111772
103. Freckleton, R.P. and Watkinson, A.R. 1997. Measuring plant neighbor effects. Functional Ecology, 11: 532-536.
104. Freeman, D., Brown, M.L., Dobson, M., Jordan, Y., Kizy, A., Micallef, C., Hancock, L.C., Graham, J.H. and Emlen, J.M. 2003. Developmental instability: measures of resistance and resilience using pumpkin *(Cucurbita pepo* L.). Biological Journal of the Linnean Society, 78: 27–41.
105. Freeman, D.C., Brown, M.L., Duda, J.J., Graham, J.H., Emlen, J.M., Krzysik, A.J., Balbach, H., Kovacic, D.A. and Zak, J.C. 2004. Developmental

instability in Rhus copallinum L.: multiple stressors, years, and responses. International Journal of Plant Sciences, 165: 53–63.

106. García-Plazaola, J.I., Esteban, R., Hormaetxe, K. and Becerril, J.M. 2008. Seasonal reversibility of acclimation to irradiance in leaves of common box (*Buxus sempervirens* L.) in a deciduous forest. Flora - Morphology, Distribution, Functional Ecology of Plants, 203: 254–260. doi:10.1016/j.flora.2007.03.007
107. García-Plazaola, J.I., Hernández, A., and Becerril, J.M. 2000. Photoprotective responses to winter stress in evergreen Mediterranean ecosystems. Plant Biology, 2: 530–535.
108. Gartzia, M., Alados, C.L. and Perez-Cabello, F. 2014. Assessment of the effects of biophysical and anthropogenic factors on woody plant encroachment in dense and sparse mountain grasslands based on remote sensing data. Progress in Physical Geography, 38: 201–217. doi:10.1177/0309133314524429
109. Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology, 94: 295–304. doi:10.1111/j.1365-2745.2006.01098.x
110. Gellrich, M., Baur, P., Koch, B. and Zimmermann, N.E. 2007. Agricultural land abandonment and natural forest re-growth in the Swiss mountains: A spatially explicit economic analysis. Agriculture, Ecosystems and Environment, 118: 93-108.
111. Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W. and Holt, R.D. 2010. A framework for community interactions under climate change. Trends in Ecology and Evolution, 5: 325-331. doi:10.1016/j.tree.2010.03.002
112. Giorgi, F. and Lionello, P. 2008. Climate change projections for the Mediterranean region. Global and Planetary Change, 63: 90–104. doi:10.1016/j.gloplacha.2007.09.005
113. Gimeno-García, E., Andreu, V. and Rubio, J.L. 2000. Changes in organic matter, nitrogen, phosforus, and cations, in soil as a result of fire and water erosion in a Mediterranean climate. European Journal of Soil Science, 51: 201-210.
114. Goldberg D.E., Rajaniemi, T., Gurevitch, J. and Stewart-Oaten, A. 1999. Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. Ecology, 4: 1118-1131.
115. Goldewijk, K.K. 2001. Estimating global land use change over the past 300 years: The HYDE Database. Global Biogeochemical Cycles, 15: 417–433. doi:10.1029/1999GB001232
116. Goodall, D.W. 1952. Some considerations in the use of point quadrats for the analysis of vegetation. Australian Journal of Sciences Research Bulletin, 5: 1-41.
117. Grace, J.B. 1991. A Clarification of the Debate Between Grime and Tilman. Functional Ecology, 5: 583-587. doi:10.2307/2389475
118. Grace, J.B. 1993. The effects of habitat productivity on competition intensity. Tree, 8: 229-230.
119. Graham, J.H., Emlen, J.M., Freeman, D.C., Leamy, L.J. and Kieser, J.A. 1998. Directional asymmetry and the measurement of developmental instability. Biological Journal of the Linnean Society, 64: 1–16.
120. Graham, J.H., Raz, S., Hel-Or, H. and Nevo, E. 2010. Fluctuating asymmetry: methods, theory and applications. Symmetry, 2: 466–540.

121. Graham, B.F. 1960. Transfer of Dye Through Natural Root Grafts of *Pinus strobus* L. Ecology, 41: 56-64. doi:10.2307/1931939
122. Grime, J.P. 1973. Competitive exclusion in herbaceous vegetation. Nature, 242: 344-345
123. Grime, J.P. 1974. Vegetation classification by reference to strategies. Nature, 250: 26-31
124. Grime, J.P. 1979. Plant strategies and vegetation processes. John Wiley, New York, New York, USA.
125. Grime, J.P. 1989. The stress debate: symptom of impeding synthesis? Biological Journal of Linnean Society, 37: 3-17.
126. Guisan, A., Theurillat, J.P. and Spilchiger, R. 1995. Effects of climate change on alpine plant diversity and distribution: the modelling and monitoring perspectives. Pages 129-135 *in* A. Guisan, J. I. Holten, R. Spilchiger, and L. Tessier, editors. Potential ecological impacts of climate change in the Alps and Fennoscandian mountains. Conservatoire et Jardin Botaniques Genève.
127. Gunderson, L.H. 2000. Ecological resilience - in theory and application. Annual review of Ecology, Evolution and Systematics, 31: 425-439.
128. Haase, P. 2001. Can isotropy vs. anisotropy in the spatial association of plant species reveal physical vs. biotic facilitation? Journal of Vegetation Science, 12: 127-136.
129. Hacker, S.D. and Gaines, S.D. 1997. Some Implications of Direct Positive Interactions for Community Species Diversity. Ecology, 78: 1990. doi:10.2307/2265939
130. Hastwell, G.T. and Facelli, J.M. 2003. Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. Journal of Ecology, 6: 941-950.
131. Harrison, P.A, Vandewalle, M., Sykes, M.T., Berry, P.M., Bugter, R., de Bello, F., Feld, C. K., Grandin, U., Harrington, R. Haslett, J.H., Jongman, R.H.G, Luck, G.W., Martins da Silva, P., Moora, M., Settele, J., Sousa, J.P. and Zobel, M. 2010. Identifying and prioritizing services in European terrestrial and freshwater ecosystems. Biodiversity and conservation, 19: 2791-2821.
132. Harter, R.D. 1983. Effect of soil pH on adsorption of lead, cooper, zinc and nickel. Soil Science Society of America Journal, 47: 47-51.
133. Hausdorff, J.M., Mitchell, S.L., Firtion, R., Peng, C.K., Cudkowicz, M.E., Wei, J.Y. and Goldberger, A.L. 1997. Altered fractal dynamics of gait: reduced stride-interval correlations with aging and huntingtons disease. Journal of Applied Physiology, 82: 262-269.
134. Hayes F., Williamson, J. and Mills, G. 2012. Ozone pollution affects flower numbers and timing in a simulated BAP priority calcareous grasslands community. Environmental Pollution, 163: 40-47. doi:10.1016/j.envpol.2011.12.032
135. He, Q., Bertness, M.D. and Altieri, A.H. 2013. Global shifts towards positive species interactions with increasing environmental stress. Ecology Letters, 16: 695–706. doi:10.1111/ele.12080
136. Heanes, D.L. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Communications in soil science and plant analysis, 15: 1191-1213.
137. Hedhly, A. 2001. Sensitivity of flowering plant gametophytes to temperature fluctuations. Environmental and experimental botany, 74: 9-16.

doi:10.1016/j.envexpbot.2011.03.016

138. Hódar, J.A. 2002. Leaf fluctuating asymmetry of Holm oak in response to drought under contrasting climatic conditions. Journal of Arid Environments, 52: 233–243. doi:10.1006/jare.2002.0989
139. Holmgren, M., Gómez-Aparicio, L., Quero, J.L. and Valladares, F. 2012. Non-linear effects of drought under shade: reconciling physiological and ecological models in plant communities. Oecologia, 169: 293–305. doi:10.1007/s00442-011-2196-5
140. Holmgren, M., Scheffer, M., 2010. Strong facilitation in mild environments: the stress gradient hypothesis revisited: Strong facilitation in mild environments. Journal of Ecology, 98: 1269–1275. doi:10.1111/j.1365-2745.2010.01709.x
141. Hooftman, D.A.P and Bullock, J.M. 2012. Mapping to inform conservation: A case study of changes in semi-natural habitats and their connectivity over 70 years. Biological conservation, 145: 30-38. DOI: 10.1016/j.biocon.2011.09.015
142. Horn, R. and Smucker, A.J. M. 2005. Structure formation and its consequences for gas and water transport in unsaturated arable and forest soils. Soil and Tillage Research, 82: 5-14.
143. Hormaetxe, K., Esteban, R., Becerril, J.M. &and García-Plazaola, J.I. 2005. Dynamics of the alpha-tocopherol pool as affected by external (environmental) and internal (leaf age) factors in *Buxus sempervirens* leaves. Physiologia Plantarum, 125: 333–344. doi:10.1111/j.1399-3054.2005.00568.x
144. Hothorn, T., Bretz, F. and Westfall, P. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal, 50: 346-363.
145. Hoùerou, H.N. 1981. Impact of man and his animals on Mediterranean vegetation. In: di Castri F.D., Goodall W. and Specht R.L. Eds: Mediterranean-type shrublands. Elsevier, Amsterdam, pp. 479-522.
146. Hough, R.A. 1995. Developmental Instability as a biomonitor of environmental stress. In Biomonitors and Biomarkers as Indicators of Environmental Change: A Handbook.
147. Hu, K., Ivanov, P.C., Chen, Z., Carpena, P. and Stanley, H.E. 2001. Effect of trends on Detrended Fluctuation Analysis. Physical Review E, 64: 1-19.
148. Humboldt, A. and Bonpland, A. 1805. Essai sur le Geographie des Plantes.
149. Ikuma, H. and Thimann, K.V. 1960. Action of Gibberelic acid on lettuce seed germination. Plant Physiology, 35: 557–566.
150. Ingerson, T.E., Buvel, R.L., 1984. Structure in asynchronous cellular automata. Physica D: Non-linear phenomena, 10: 59-68.
151. Iparragirre Aseginolaza, C. et. al. 1985. Araba, Bizkaia eta Gipuzkoako landare katalogoa. Catálogo florístico de Álava, Vizcaya y Guipúzcoa, Gobierno Vasco. Departamento de Política Territorial y Transportes. Vitoria-Gasteiz.
152. IPCC. 2013. Summary for policy makers ”in Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. Cambridge: Cambridge University Press, 3–29.

153. Isard, S.A. 1986. Factors Influencing Soil Moisture and Plant Community Distribution on Niwot Ridge, Front Range, Colorado, U.S.A. Arctic and Alpine Research, 18: 83-96.
154. Jackman, S. 2015. pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University. Department of Political Science, Stanford University. Stanford, California. R package version 1.4.9. URL http://pscl.stanford.edu/
155. Jackson, R.B. and Caldwell, M.M., 1993. Geostatistical Patterns of Soil Heterogeneity Around Individual Perennial Plants. The Journal of Ecology, 81: 683. doi:10.2307/2261666
156. Jaksic, F.M. and Fuentes, E.R. 1980. Why are Native Herbs in the Chilean Matorral More Abundant Beneath Bushes: Microclimate or Grazing? The Journal of Ecology, 68:665. doi:10.2307/2259427
157. James, J.C., Grace, J. and Hoad, S.P. 1994. Growth and Photosynthesis of *Pinus sylvestris* at its Altitudinal Limit in Scotland. The Journal of Ecology, 82: 297. doi:10.2307/2261297
158. Jiménez-Alfaro, B., Marcenó, C., Bueno, A., Gavilán, R. and Obeso, J.R. 2014. Biogeographic deconstruction of alpine plant communities along altitudinal and topographic gradients. Journal of Vegetation Science, 25: 160-171.
159. Jofre, R. and Lambal, S. 1988. Soil water improvement by trees in the rangelands of Southern Spain. Acta Oecologia, 9: 405-422.
160. Jump, A.S., Huang, T.J. and Chou, C.H. 2012. Rapid altitudinal migration of mountain plants in Taiwan and its implications for high altitude biodiversity. Ecography, 35: 204–210. doi:10.1111/j.1600-0587.2011.06984.x
161. Jump, A.S. and Peñuelas, J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters, 8: 1010–1020. doi:10.1111/j.1461-0248.2005.00796.x
162. Jurasinski, G. and Kreyling, J. 2007. Upward shift of alpine plants increases floristic similarity of mountain summits. Journal of Vegetation Science, 18: 711-718.
163. Keely, J.E. 1987. Role of Fire in Seed Germination of Woody Taxa in California Chaparral. Ecology, 68: 434-433.
164. Kéfi, S., Rietkerk, M., van Baalen, M. and Loreau, M. 2007. Local facilitation, bistability and transitions in arid ecosystems. Theoretical Population Biology, 71: 367–379. doi:10.1016/j.tpb.2006.09.003
165. Kéfi, S., Rietkerk, M., Alados, C.L., Pueyo, Y., Papanastasis, V. P., ElAich, A. and De Ruiter, P.C. 2007a. Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. Nature, 449: 213-217.
166. Kelly, D. 1994. The evolutionary ecology of mast seeding. Biological Journal of the Linnean Society, 9: 465-470.
167. Kelly, D. and Sork, V.L. 2002. Mast seeding in perennial plants: why, how, where? Annual review of Ecology and systematics, 33: 427-447.
168. Kikvidze, Z., Pugnaire, F.I., Brooker, R.W., Choler, P., Lortie, C.J., Michalet, R. and Callaway R.M. 2005. Linking patterns and processes in alpine plant communities: a global study Ecology, 86: 1395-1400.
169. Klein, J.A., Harte, J. and Zhao, X.Q. 2004. Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau. Ecology Letters 7:1170-1179.

170. Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P, Thompson, K., Sonnenschein, M., Poschlod, P., Van Groenendael, J.M., Klimeš, L., Klimešová, J., Klotz, S., Rusch, G.M., Hermy, M., Adriaens, D., Boedeltje, G., Bossuyt, B., Dannemann, A., Endels, P., Götzenberger, L., Hodgson, J.G., Jackel, A-K., Kühn, I., Kunzmann, D., Ozinga, W.A., Römermann, C., Stadler, M., Schlegelmilch, J., Steendam, H.J., Tackenberg, O., Wilmann, B., Cornelissen, J.H.C., Eriksson, O., Garnier, E., Peco, B. 2008. The LEDA Trait base: A database of life-history traits of Northwest European flora. Journal of Ecology, 96: 1266-1274.
171. Kim, D.G. and Kirschbaum, M.U.F. 2015. The effect of land-use change on the net exchange rates of greenhouse gases: A compilation of estimates. Agriculture, Ecosystems and Environment, 208: 114–126. doi:10.1016/j.agee.2015.04.026
172. Kitzberger, T., Steinaker, D.F. and Veblen, T.T. 2000. Effects of Climatic Variability on Facilitation of Tree Establishment in Northern Patagonia. Ecology, 81: 1914. doi:10.2307/177281
173. Knapp, A.K and Seasted, T.R. 1986. Detritus accumulation limits productivity of Tallgrass prarie. BioScience, 36: 662-668.
174. Komac, B. 2010. Effets des modifications de l’utilisation des terres sur la conservation des pâturages subalpins du Parc National de Ordesa Mont-Perdu, Laboratoire d’Ecologie Alpine. Université de Savoie, Chambéry, France.
175. Komac, B. and Alados, C.L. 2012. Fluctuating asymmetry and Echinospartum horridum fitness components. Ecological Indicators, 18: 252–258.
doi:10.1016/j.ecolind.2011.11.028
176. Komac, B., Kefi, S., Nuche, P., Escós, J. and Alados, C.L. 2013. Modeling shrub encroachment in subalpine grasslands under different environmental and management scenarios. Journal of Environmental Management, 121: 160–169. doi:10.1016/j.jenvman.2013.01.038
177. Körner, C. 2003. Alpine plant life: Functional plant ecology of high mountains. 2nd edition. Springer, Berlin.
178. Körner, C. 2004. Limitation and stress – always or never ? Journal of Vegetation Science, 14: 141. doi:10.1658/1100-9233(2003)014[0141:LASAON]2.0.CO;2
179. Körner, C. 2007. The use of ‘altitude’ in ecological research. Trends in Ecology and Evolution, 22: 569–574.
180. Körner, C. and De Moraes, J.A.P.V. 1979. Water potential and diffusion resistance in alpine cushion plants on clears summerdays. Oecologia Plantarum, 14, 109-120.
181. Körner, C. and Larcher, W. 1988. Plant Life in cold climates. Society for experimental biology, 42: 25-57
182. Körner, C. and Oshawa, S, et al. 2005. Millennium Ecosystem Assessment. Chapter 24: Mountain systems.
183. Kouba, Y. and Alados, C.L. 2014. La expansión del quejigo (Q*uercus faginea*) en el Prepirineo Aragonés durante la segunda mitad del siglo XX. Pirineos 169, 2-7. doi:10.3989/Pirineos.2014.169002
184. Kouba, Y., Alados, C.L. and Bueno, C.G. 2011. Effects of abiotic and anthropogenic factors on the spatial distribution of *Quercus faginea* in the

Spanish Central Pyrenees. Plant Ecology, 212: 999–1007. doi:10.1007/s11258-010-9880-0

185. Kouba, Y., Camarero, J.J. and Alados, C.L. 2012. Roles of land-use and climate change on the establishment and regeneration dynamics of Mediterranean semi-deciduous oak forests. Forest Ecology and Management, 274: 143–150. doi:10.1016/j.foreco.2012.02.033
186. Kozlowski, T.T. and Pallardy, S.G. 2002. Acclimation and Adaptive Responses of Woody Plants to Environmental Stresses. The Botanical Review, 68: 270–334. doi:10.1663/0006-8101(2002)068[0270:AAAROW]2.0.CO;2
187. Lang, J.M and Benbow, M.E. 2013. Species interactions and Competition. Nautre Education Knowledge, 4: 1-10
188. Lasanta-Martínez, T. 1989. Evolución reciente de la agricultura de montaña. Geoforma Edición, Logroño.
189. Lasanta-Martínez, T. and Vicente-Serrano, S. 2007. Cambios en la cubierta vegetal en el pirineo aragonés en los últimos 50 años. Pirineos, 162: 125-154.
190. Lasanta-Martínez, T., Vicente-Serrano, S.M., Cuadrat-Prats, J.M. 2005. Mountain Mediterranean landscape evolution caused by the abandonment of traditional primary activities: a study of the Spanish Central Pyrenees. Applied Geography, 25: 47–65. doi:10.1016/j.apgeog.2004.11.001
191. Lazić, M.M., Carretero, M.A., Crnobrnja-Isailović, J. and Kaliontzopoulou, A. 2015. Effects of Environmental Disturbance on Phenotypic Variation: An Integrated Assessment of Canalization, Developmental Stability, Modularity, and Allometry in Lizard Head Shape. The American Naturalist, 185: 44–58. doi:10.1086/679011
192. Le Bagousse-Pinguet, Y., Maalouf, J.-P., Touzard, B. and Michalet, R. 2014. Importance, but not intensity of plant interactions relates to species diversity under the interplay of stress and disturbance. Oikos, 123: 777–785. doi:10.1111/oik.00961
193. Leck, M.A, Parker, V.T. and Simpson, R.L. 1989. Ecology of soil seed banks. Academic Press, INC. San Diego, Califronia.
194. Lenoble, F., Broyer, C. 1945. Sur la distribution du *Buxus sempervirens* L. en France. Bulletin de la Société Botanique de France, 92: 118–131. doi:10.1080/00378941.1945.10834422
195. Lenoir, J., Gégout, J., Pierrat, J., Bontemps J. and Dhôte, J. 2009. Differences between tree species seedling and adult altitudinal distribution in mountain forest during the recent warm period (1986-2006). Ecography, 32: 765-777.
196. Livinsgtone, R.B. Influence of Birds, Stones and Soils on the Establishent of Pasture Juniper, *Juniperus communis*, and Red Cedar, *J. virginiana* in New England pastures. Ecology, 53: 1141-1147.
197. Loidi, J and Herrera, M. 1990. The *Quercus pubescens* and *Quercus faginea* forest in the Basque country (Spain): distribution and topology in relation to climatic factors. Vegetatio, 90: 81-92.
198. López-Moreno, J.I., Goyette, S. and Bnistom, M. 2008. Climate change prediction over complex areas: spatial variability of uncertainties and predictions over the Pyrenees from a set of regional climate models. International Journal of Climatology, 28: 1535-1550. DOI: 10.1002/joc.1645
199. Lortie, C.J., Brooker, R. W., Kikvidze, Z. and Callaway, R.M. 2004. The value of stress and limitation in an imperfect world: A reply to Körner. Journal of Vegetation Science, 15: 577-580

200. Löve, D. 1970. Subartic and subalpine: Where and What? Artic and Alpine Research, 2: 63-73.
201. MacDonald, D., Crabtree, J., Wiesinger, G., Dax, T., Stamou, N., Fleury, P., Gutierrez Lazpita, J. and Gibon, A. 2000. Agricultural abandonment in mountain areas of Europe: Environmental consequences and policy response. Journal of Environmental Management, 59: 47–69. doi:10.1006/jema.1999.0335
202. Maestre F.T., Bautista, S. and Cortina, J. 2003. Positive, negative and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. Ecology, 82: 3186-3197.
203. Maestre, F.T., Bowker, M.A., Escolar, C., Puche, M.D., Soliveres, Maltez-Mouro, S., Garcãa-Palacios, S.P., Castillo-Monroy, A.P., Martãnez, I. and Escudero, A.N. 2010. Do biotic interactions modulate ecosystem functioning along stress gradients? Insights from semi-arid plant and biological soil crust communities. Philosophical Transactions of the Royal Society, B: Biological Sciences, 365: 2057-2070.
204. Maestre, F.T., Callaway, R.M., Valladares, F., Lortie, C.J. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology, 97: 199–205. doi:10.1111/j.1365-2745.2008.01476.x
205. Maestre, F.T. and Cortina, J. 2004. Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. Proceedings of the Royal Society of London, Series B, 271: 331-333.
206. Maestre, F.T., Valladares, F., Reynolds, J.F. 2005. Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments: Facilitation and stress in arid environments. Journal of Ecology, 93: 748–757. doi:10.1111/j.1365-2745.2005.01017.x
207. Malkinson, D. and Tielbörger, K. 2010. What does the stress-gradient hypothesis predict? Resolving the discrepancies. Oikos, 119: 1546–1552. doi:10.1111/j.1600-0706.2010.18375.x
208. Maltez-Mouro, S, García, L.V., Freitas, H. 2009. Influence of forest structure and environmental variables on recruit survival and performance of two Mediterranean tree species (*Quercus faginea* Lam. and *Q. suber* L.). European Journal of Forest Research, 128: 27-36.
209. Marcott, S.A., Shakun, J.D., Clark, P.U. and Mix A.C. 2013. A reconstruction of regional and global temperature for the past 11,300 years. Science, 339: 1198-1201.
210. Margaris, N.S., Koutsidou, E. and Gioura, C.H. 1996. Changes in traditional Mediterranean land-use systems. En: Brandt, J. and Thomas, J.B. (Eds.), Mediterranean desertification and land use. Wiley and Sons: 29-42, Chichester.
211. Marinas, A., García-González, R., Aldezabal, A., Palacio, S., and Gómez-García, D. 2004. Interés ecológico y pastoral del erizón (*Echinospartum horridum* (Vahl) Rothm.). In: García-Criado, B., García Ciudad, A., Vazquez de Aldana, B.R., Zabalgogeazcoa, I. (Eds.), Pastos y ganadería extensiva. SEEP-IRNASA, Salamanca, pp. 117e122.
212. Martel, J., Lempa, K. and Haukioja, E. 1999. Effects of stress and rapid growth on fluctuating asymmetry and insect damage in birch leaves. Oikos, 86: 208-216.

213. Mataix-Solera, J., Cerdà, A., Arcenegui, V. and Zavala, L.M. 2011. Fire effects on soil aggregation: A review. Earth Science Reviews, 109: 44-60.
214. Mather, A.S. and Fairbairn, J. 2000. From floods to reforestation: the forest transition in Switzerland. Environment and History, 6: 399–421.
215. Matus, G., Tóthmérész, B. and Papp, M. 2003. Restoration prospects of abandoned species-richsandy grasslands in Hungary. Applied Vegetation Science, 6: 169-178.
216. Mayers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.B.A. and Kent, J. 2000. Biodiversity hotspot for conservation priorities. Nature, 403: 853-858.
217. Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I., Valiente-Banuet. A. and Callaway, R.M. 2006. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? Ecology Letters, 9: 767-773.
218. Michalet, R., Chen, S., An, L., Wang, X., Wang, Y., Guo, P., Ding, C. and Xiao, S. 2015. Communities: are they groups of hidden interactions? Journal of Vegetation Science, 26: 207–218. doi:10.1111/jvs.12226
219. Michalet, R., Schöb, C., Lortie, J.C., Brooker, R.W. and Callaway, R.M. 2014. Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. Functional Ecology, 20: 75-86.
220. Michalet, R., Xiao, S., Touzard, B., Smith, D.S., Cavieres, L.A., Callaway, R.M., Whitham, T.G. 2011. Phenotypic variation in nurse traits and community feedbacks define an alpine community: Alpine community genetics. Ecology Letters, 14: 433–443. doi:10.1111/j.1461-0248.2011.01605.x
221. Milber, P. 1994. Germination ecology of the grassland biennial *Linum catharticum*. Acta Botanica Neerlandica, 43: 261-269.
222. Milla, R., Castro-Díez P. and Montserrat-Martí, G. 2010. Phenology of Mediterranean woody plants from NE Spain: Synchrony, seasonality, and relations among phenophases. Flora, 205: 190-199. doi:10.1016/j.flora.2009.01.006
223. Mitchell, M.G., Cahill, J.F.J. and Hik, D.S. 2009. Plant interactions are unimportant in a subarctic-alpine plant community. Ecology, 90: 2360-2367.
224. Molina-Montenegro, M.A., Oses, R., Torres-Díaz, C., Atala, C., Núñez, M.A. and Armas, C. 2015. Fungal endophytes associated with roots of nurse cushion species have positive effects on native and invasive beneficiary plants in an alpine ecosystem. Perspectives in Plant Ecology, Evolution and Systematics, 17: 218–226. doi:10.1016/j.ppees.2015.02.003
225. Møller, A.P. and Swaddle, J.P. 1997. Asymmetry, Developmental Stability and Evolution. Oxford University Press.
226. Monteiro, A.T., Fava, F., Gonçalves, J., Huete, A., Gusmeroli, F., Parolo, G., Spano, D. and Bocchi, S. 2013. Landscape context determinants to plant diversity in the permanent meadows of Southern European Alps. Biodiversity and Conservation, 22: 937-958. DOI 10.1007/s10531-013-0460-1
64. Montserrat, P. and Fillat, F. 1990. The systems of management grasslands in Spain. In A. Breynmeyer (Ed.). Management grasslands. Elsevier, Amsterdam. Pp 37-70.

227. Montserrat, P.J.M. and Montserrat G. 1984. Estudio de las comunidades de *Echinospartum horridum* en el pirineo español. Acta Biológica Montana, 5:249-257
228. Montserrat-Martí, G., Camarero, J.J., Palacio, S., Pérez-Rontomé, C., Milla, R., Albuixech, J. and Maestro, M. 2009. Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees, 23: 787–799. doi:10.1007/s00468-009-0320-5
229. Mooney, H.A., Hillier, R.D. and Billings, W.D. 1965. Transpiration rates of alpine plants in Sierra Nevada of California. American Midland Naturalist, 74: 374-386.
230. Müller, D. and Zeller, M. 2002. Land use dynamics in the central highlands of Vietnam: a spatial model combining village survey data with satellite imagery interpretation. Agricultural economics, 27: 333-354.
231. Navarra, J.J., Kohfeldt, N., Menges, E.S. and Quintana-Ascencio, P. F. 2011. **S**eed **B**ank **C**hanges with **T**ime-since-**f**ire in **F**lorida **R**osemary **S**crub. Fire ecology, 7: 17-31.
232. Newman, E.I. and Ritz, K. 1986. Evidences on the pathways of phosphorus transfer between vesicular-arbuscular mycorrhizal plants. New phytologist, 104: 77-87.
233. Noble, I.R. and Stayler, R.O. 1980. The use of vital attributes to predict successional changes in plant communities subject to recruitment disturbances. Vegetatio, 43: 5-21.
234. Nogués-Bravo, D., Araújo, M.B., Lasanta, T. and López-Moreno, J.I. 2008. Climate change in Mediterranean Mountains during the 21^{st} Century. AMBIO, 37: 280-285.
235. Nuche, P., Komac, B., Camarero, J.J. and Alados, C.L. 2014. Developmental instability as an index of adaptation to drought stress in a Mediterranean oak. Ecological Indicators, 40: 68–75. doi:10.1016/j.ecolind.2013.12.023
236. Odum, E.P. 1985. Trends expected in Stressed Ecosystems. BioScience, 35: 419-422.
237. Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R. B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H. and Wagner, H. 2015. vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan
238. Olano, J.M., Almería, I., Eugenio, M. and von Arx, G. 2013. Under pressure: how a Mediterranean high-mountain forb coordinates growth and hydraulic xylem anatomy in response to temperature and water constraints. Functional Ecology, 27: 1295–1303. doi:10.1111/1365-2435.12144
239. Ojeda, F., T. Marañón, and J. Arroyo. 1996. Patterns of ecological, chorological and taxonomic diversity at both sides of the Strait of Gibraltar. Journal of Vegetation Science, 7: 63-72.
240. Ozkan, K., Senol, H., Basayigit, L., Gulsoy, S. and Mert, A. 2006. Buldan bati daglik bolgesinde sacli mesenin (*Quercus cerris* L. var. cerris) yayilisini sinirlandiran faktorler Pages 589 - 594 Restricting factors of distribution of Turkey oak (*Quercus cerris* L. var. cerris) in Buldan mountainous area, Buldan Symposium, Ankara.
241. Ozkan , K., H. Senol, S. Gulsoy, A. Mert, H. Suel, and Y. Eser. 2009. Vegetation-environment relationships in Mediterranean mountain forests on

limeless bedrocks of southern Anatolia, Turkey. Journal of Environmental Engineering and Landscape Management, 17: 154–163.

242. Palacio, S. 2006. Fenomorfología y variaciones estacionales de nitrógeno y carbohidratos en seis especies de caméfitos leñosos mediterráneos. Una aproximación funcional. Tesis Doctoral. Departamento de Biología Vegetal. Universidad de Barceleona.
243. Palmer, A.P. 1994. Fluctuating asymmetry: A primer. Pp 335-364. In: Developmental Instability: Its Origins and Evolutionary Implications. (T. Markow, Ed.). Kluwer, Dordrecht.
244. Palmer, A.P. and Strobeck, C. 1986. Fluctuating asymmetry: Measurement, Analysis, Patterns. Annual Review of Ecology and Systematics, 17: 391-421.
245. Palmer, A.P. and Strobeck, C. 2003. Fluctuating Asymmetry Analyses Revisited. In M. Polak (Ed.), Developmental instability: Causes and consequences, Pp 14–34. New York: Oxford University Press.
246. Park, Y.S., Baehr, C., Larocque, G.R., Sánchez-Pérez, J.M. and Sauvage, S. 2015. Ecological Modelling for Ecosystem Sustainability. Ecological Modelling, 306: 1–5. doi:10.1016/j.ecolmodel.2015.04.008
247. Parsons, P.A. 2005. Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. Biological Reviews, 80: 589. doi:10.1017/S1464793105006822
248. Paul, K.I., Black, A. S. and Conyers, M.K. 2001. Influence of fallow, wheat and subterranean clover on pH within an initially mixed surface soil in the field. Biology and fertility of soils, 33: 41-52.
249. Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Benito Alonso, J.L., Coldea, G., Dick, J., Erschbamer, B., Calzado, R.F., Ghosn, D., Holten, J.I., Kanka, R., Kazakis, G., Kollár, J., Larsson, P., Moiseev, P., Moiseev, D., Molau, U., Mesa, J.M., Nagy, L., Pelino, G., Puşcaş, M., Rossi, G., Stanisci, A., Syverhuset, A.O., Theurillat, J.P, Tomaselli, M., Unterluggauer, P., Villar, L., Vittoz, P., Grabherr1, G. 2012. Recent plant diversity changes on Europe's Mountain Summits. Science, 336: 353-355.
250. Pauli, H., Gottfierd, M. and Grabherr, G. 1996. Effects of climate change on mountain ecosystems - upward shifting of alpine plants. World review resource, 8: 382-390.
251. Pausas, J.G., Bradstock, R.A., Keith, D.A., Keeley, J.E. and the GCTE (Global Change of Terrestrial Ecosystems). 2004. Plant functional traits in relation to fire in crown-fire ecosystems. Ecology, 85: 1085-1100.
252. Peng, C.K., Buldyrev, S.V., Goldberger, A.L., Havlin, S., Sciortino, F., Simons, M. and Stanley, H.E. 1992. Long-range correlations in nucleotide sequences. Nature, 356: 168-170.
253. Peng, C.K., Havlin, S., Stanley, H.E. and Goldberger A.L. 1995. Quantification of Scaling Exponents and Crossover Phenomena in Nonstationary Heartbeat Time-Series. Chaos, 5: 82-87.
254. Pennings, S.C., Selig, E.R., Houser, L.T. and Bertness, M.D. 2003. Geographic variation in positive and negative interactions among salt marsh plants. Ecology, 84: 1527–1538.
255. Peñuelas, J. and Boada, M. 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). Global Change Biology, 9: 131–140.
256. Pinheiro J, Bates D, DebRoy S, Sarkar D & R Core Team. 2014. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version, 3: 1-117, http://CRAN.R-project.org/package=nlme.

257. Polak, M. 2003. Developmental instability: Causes and consequences. New York: Oxford University Press.
258. Post, E. 2013. Ecology of Climate Change: The Importance of Biotic Interactions: The Importance of Biotic Interactions. Princeton University Press.
259. Potts, J.B., Marino, E., Stephens, S.L. 2010. Chaparral shrub recovery after fuel reduction: a comparison of prescribed fire and mastication techniques. Plant Ecology, 210: 303 – 315. DOI 10.1007/s11258-010-9758-1
260. Price, J.N. and Morgan, J.W. 2008. Woody plant encroachment reduces species richness of herb-rich woodlands in southern Australia. Austral Ecology, 33: 278–289. doi:10.1111/j.1442-9993.2007.01815.x
261. Pueyo, Y., Alados, C.L., Maestro, M. and Komac, B. 2007. Gypsophile vegetation patterns under a range of soil properties induced by topographical position. Plant Ecology, 189: 301-311.
262. Pueyo, Y., Moret-Fernandez, D., Saiz, H., Bueno, C.G. and Alados. C.L. 2013. Relationships Between Plant Spatial Patterns, Water Infiltration Capacity, and Plant Community Composition in Semi-arid Mediterranean Ecosystems Along Stress Gradients. Ecosystems, 16: 452-466.
263. Quézel, P. and Barbero, M. 1990. Les foréts méditerranéennes problemes poses par leur signification historique, ecologique et leur conservation. Acta Botanica Malacitana, 15: 145–178.
264. Quézel, P. and Medial, F. 2003. Écologie et biogéographie des forêts du bassin méditerranéen. Collection environnement. Elsevier.
265. R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http:// www.R-project.org/. Accessed Sept 2011.
266. Ratajczak, Z., Nippert, J.B. and Collins, S. 2012. Woody encroachment decreases diversity across North American grasslands and savannas. Ecology, 93: 697-703.
267. Rathcke, B. 1983. Competition and facilitation among plants for pollination. In Real L (ed) Pollination Biology. Academic, New York, 305-329.
268. Rehder, H. 1970. Zur Ökologie insbesondere Stickstoffversorgung subalpiner und alpiner Pflanzengesellschaften im Naturschutzgebiet Schachen (Wettersteingebirge). Dissertationes Botanicae 6. Cramer, Stuttgart, Germany.
269. Reid, A.M., Lamarque, L.J. and Lortie, C.J. 2010. A systematic review of the recent ecological literature on cushion plants: champions of plant facilitation. Web Ecology, 10: 44–49.
270. Rettig, J.E., Fuller, R.C., Corbett, A.L. and Getty, T. 1997. Fluctuating asymmetry indicates levels of competition in an even-aged polar clone. Oikos, 80: 123-127.
271. Reverter, B.R., Sánchez-Cañete, E.P., Resco, V., Serrano-Ortiz, P., Oyonarte, C. and Kowalski, A.S. 2010. Analyzing the major drivers of NEE in a Mediterranean alpine shrubland. Biogeosciences, 8: 2601-2611.
272. Rey Benayas, J.M., Navarro, J., Espigares, T., Nicolau, J.M. and Zavala, M.A. 2005. Effects of artificial shading and weed mowing in reforestation of Mediterranean abandoned cropland with contrasting Quercus species. Forest Ecology and Management, 212: 302–314. doi:10.1016/j.foreco.2005.03.032

273. Ricklefs, R.E., 2008. Disintegration of the Ecological Community: American Society of Naturalists Sewall Wright Award Winner Address. The American Naturalist, 172: 741–750. doi:10.1086/593002

107. Rietkerk, M., Dekker, S.C., de Ruiter, P.C. and van de Koopel, J. 2004. Self-orgnized patchiness and catastrophic shifts in ecosystems. Science, 305:1926-1929.

108. Rietkerk, M. and Van De Koppel, J. 2008. Regular pattern formation in real ecosystems. Ttrends in Ecology and Evolution, 23: 169-175.

274. Rivas, M., Reyes, O. and Casal, M. 2006. Influence of heat and smoke on the germination of six leguminous shrubby species. International Journal of Wildland Fire, 15: 73-80.

275. Robles, A.B., Allegretti, L.I. and Passera, C.B. 2002. *Coronilla juncea* is both a nutritive fodder shrub and useful in the rehabilitation of abandoned Mediterranean marginal farmland. Journal of Arid Environments, 50: 381-392.

276. Rosbakh, S., Bernhardt-Römermann, M. and Poschlod, P. 2014. Elevation matters: contrasting effects of climate change on the vegetation development at different elevations in the Bavarian Alps. Alp Botany, 124: 143-154.

277. Ross, L.C., Woodin, S.J., Hester, A.J., Thompson, D.B.A. and Birks, H.J.B. 2012. Biotic homogenization of upland vegetation: patterns and drivers at multiple spatial scales over five decades. Journal of Vegetation Science, 23: 755-770.

278. Rousset, O. and Lepart, J. 2002. Neighborhood effects on the risk of an unpalatable grass being grazed. Plant Ecology, 165: 197-206.

279. Rudel, T.K., Perez-Lugo, M. and Zichal, H. 2000. When Fields Revert to Forest: Development and Spontaneous Reforestation in Post- War Puerto Rico. The Professional Geographer, 52: 386-397.

280. Rutherford, K.M.D., Haskell, M.J., Glasbey, C., Jones, R.B. and Lawrence, A.B. 2004. Fractal analysis of animal behaviour as an indicator of animal welfare. Pages S99-S103.

281. Ruthsatz, B. Las plantas en cojín de los semi-desiertos andinos del Noreste Argentino. Darwiniana, 21: 491-539.

282. Saatkamp, A., Poschol, P. and Venable, D.L. 2014. The functional role of soil seed banks in natural communities. Chap 11, in Fenner, M. 2014. Seeds: The ecology of regeneration in plant communities. 3erd edition. CABI Publishing.

283. Sabatini S, Albertosi A, Argenti G, Bianchetto E, Staglianò N. 2003. Improvement of pastures encroached by shrubs through animal grazing in an Italian alpine environment. In: Proceedings of the 12th Symposium of the European Grassland Federation, “Optimal forage systems for animal production and the environment” (Kirilov A, Todorov N, Katerov I eds). Pleven, Bulgaria, pp. 74-76.

284. Saiz, H. 2014. Redes de asociación planta-planta: organizacion de las especies y de la comunidad vegetal, y adaptación del sistema a factores de estrés en ecosistemas semi-áridos. Universidad de Zaragoza.

285. Saiz, H. and Alados, C.L. 2012. Changes in semi-arid plant species associations along a livestock grazing gradient. Plos One, 7: 1-9.

286. Saiz, H. and Alados, C.L. 2014. Effect of livestock grazing in the partitions of a semiarid plant-plant spatial signed network. Acta Oecologica, 59: 18-25.

287. Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirxo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans,

R., Lodge, D.M., Moony, H.A., Oesterheld, M., Poff, N.L. Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H. 2000. Global biodiversity scenarios for the year 2100. Science, 827: 1770-1774.

288. Sammul, M., Kull, K., Oksanen, L. and Veromann, P. 2000. Competition intensity and its importance: results of field experiments with Anthoxanthum odoratum. Oecologia, 125: 18–25.
289. Sankaran, M., Ratnam, J. and Hanan, N. 2008. Woody cover in African savannas: the role of resources, fire and herbivory. Global Ecology and Biogeography, 17: 236–245. doi:10.1111/j.1466-8238.2007.00360.x
290. Savulescu, I. and Mihai, B. 2011. Mapping forest landscape change in Iezer Mountains, Romanian Carpathians. A GIS approach based on cartographic heritage, forestry data and remote sensing imagery. Journal of Maps, 2011: 429-446.
291. Schinner, F. 1982. Soil microbial activities and litter decomposition related to altitude. Plant and Soil, 65: 87-94.
292. Schöb, C., Armas, C., Guler, M., Prieto, I. and Pugnaire, F.I. 2013. Variability in functional traits mediates plant interactions along stress gradients. Journal of Ecology, 101**:** 753-762.
293. Schöb, C., Michalet, R., Cavieres, L.A., Pugnaire, F.I., Brooker, R.W., Butterfield, B.J., Cook, B.J., Kikvidze, Z., Lortie, C.J., Xiao, S., Al Hayek, P., Anthelme, F., Cranston, B.H., García, M.-C., Le Bagousse-Pinguet, Y., Reid, A.M., le Roux, P.C., Lingua, E., Nyakatya, M.J., Touzard, B., Zhao, L., Callaway, R.M. 2014a. A global analysis of bidirectional interactions in alpine plant communities shows facilitators experiencing strong reciprocal fitness costs. New Phytologist, 202: 95–105. doi:10.1111/nph.12641
294. Schöb, C., Prieto, I., Armas, C. and Pugnaire, F.I. 2014b. Consequences of facilitation: one plant’s benefit is another plant’s cost. Functional Ecology, 28: 500–508. doi:10.1111/1365-2435.12185
295. Schwind, P.J. 1976. Environmental impacts of land use change. Journal of Environmental systems, 6: 125-145.
296. Shakun, J.D., Clark, P.U., He, F., Marcott, S.A., Mix, A.C., Liu, Z., Otto-Bliesner, B., Schmittner, A., Bard, E. 2012. Global warming preceded by increasing carbon dioxide concentrations during the last deglaciation. Nature, 484: 49–54. doi:10.1038/nature10915
297. Shannon, C.E. 1948. A mathematical theory of communication. Bell System Technical Journal, 27: 379-423.
298. Slot, M, Wirth, C., Schumacher, J., Mohren, G. M. J., Shibistova, O., Lloyd, J. and Ensminger, I. 2004. Regeneration patterns in boreal Scots pine glades linked to clod-induced photoinhibition. Tree physiology, 25: 1139-1150.
299. Smit, C., Vandenberghe, C., Ouden, J.D. and Müller-Schärer, H. 2007. Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. Oecologia, 152: 265-273.
300. Soliveres, S., Eldridge, D.J., Maestre, F.T., Bowker, M.A., Tighe, M. and Escudero, A. 2011. Microhabitat amelioration and reduced competition among understory plants as drivers of facilitation across environmental gradients: Towards a unifying framework. Perspectives in Plant Ecology, Evolution and Systematics, 13: 247–258. doi:10.1016/j.ppees.2011.06.001
301. Soliveres, S., Maestre, F.T., Bowker, M.A., Torices, R., Quero, J.L., García-Gómez, M., Cabrera, O., Cea, A.P., Coaguila, D., Eldridge, D.J., Espinosa, C.I., Hemmings, F., Monerris, J.J., Tighe, M., Delgado-Baquerizo, M.,

Escolar, C., García-Palacios, P., Gozalo, B., Ochoa, V., Blones, J., Derak, M., Ghiloufi, W., Gutiérrez, J.R., Hernández, R.M. and Noumi, Z. 2014. Functional traits determine plant co-occurrence more than environment or evolutionary relatedness in global drylands. Perspectives in Plant Ecology, Evolution and Systematics, 16: 164–173. doi:10.1016/j.ppees.2014.05.001

302. Soliveres, S., Schöb, C. and Maestre, F.T. 2015. Moving forward on facilitation research: response to changing environments and effects on the diversity, functioning and evolution of plant communities. Biological Reviews, 90: 297-313.
303. Soliveres, S. and Maestre. F.T. 2014. Plant–plant interactions, environmental gradients and plant diversity: A global synthesis of community-level studies. Perspectives in Plant Ecology, Evolution and Systematics, 16: 154-163.
304. Stearns, S.C. 1989. Trade-Offs in Life-History Evolution. Functional Ecology, 3: 259-268. doi:10.2307/2389364
305. Sternberg, M. and M. Shoshany. 2001. Influence of slope aspect on Mediterranean woody formations: Comparison of semiarid and an arid site in Israel. Ecological Research, 16: 335-345.
306. Stocker, T., Qin, D., Plattner, G., Tignor, M., Allen, S., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley P. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ Press, Cambridge, UK and New York, NY, USA.
307. Stöckli, V., Wipf, S., Nilsson, C. and Rixen C. 2011. Using historical plant surveys to track biodiversity on mountain summits. Plant Ecology and Diversity, 4: 415-425.
308. Swaddle, J.P., Witter, M.S. and Cuthell I.C. 1994. The analysis of fluctuating asymmetry. Animal Behavior, 48: 986-989.
309. Tan-Kirstanto, A., Hoffman, A., Woods, R., Batterham, P., Cobbett, C. & Sinclair, C. 2003.Translational asymmetry as a sensitive indicator of cadmium stress in plants: a laboratory test with wild-type and mutant Arabidopsis thaliana. New Phytologist, 159: 471–477.
310. Tansley, A.G. 1935. The Use and Abuse of Vegetational Concepts and Terms. Ecolgoy, 16: 284-307.
311. Tena, D. 2009. 5110. Formaciones estables xerotermófilas de Buxus sempervirens en pendientes rocosas (Berberidion P.P.). in: V.V.A.A. Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. Madrid: Ministerio de Medio Ambiente, y Medio Rural y Marino. Primera Edición. ISBN: 978-84-491-0911-9. pp 67.
312. Ter Heerdt, G.N.J., Verweij, G.L., Beller, R.M., and Bakker, J.P. 1996. An improved method for seed bank analysis: Seedling emergence after removing the soil by sieving. Functional Ecology, 10: 144–155.
313. Theurillat, J.P and Guisan, A. 2001. Potential impact of climate change on vegetation in the European alps: a review. Climatic change, 50: 77-109.
314. Thomas, P.B., Morris, E.C. and Auld, T.D. 2003. Interactive effects of heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region. Austral Ecology, 28: 674-683.
315. Thompson, K., Bakker, J. and Bekker, R. 1997. The soil seed banks of North West Europe: methodology, density and longevity. Cambridge University Press.

316. Thorpe, A.S., Aschehoug, E.T., Atwater, D.Z. and Callaway, R.M. 2011. Interactions among plants and evolution: Plant interactions and evolution. Journal of Ecology, 99: 729–740. doi:10.1111/j.1365-2745.2011.01802.x
317. Thuiller, W., Lavorel, S., Araujo, M.B., Sykes, M.T. and Prentice, I.C. 2005. Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences, 102: 8245–8250. doi:10.1073/pnas.0409902102
318. Tielbörger, K. and Kadmon, R. 2000. Temporal environmental variation tips the balance between facilitation and interference in desert plants. Ecology, 81: 1544–1553.
319. Tieu, A., Dixon, K.A., Sivasithamparam, K., Plummer, J.A. and Sieler, I.M. 1999. Germination of four species of native to Western Australia plants using plant-derived smoke. Australian Journal of Botany, 47: 207-219.Tilianakis, J.M., Didham, R.K., Bascompte, J. and Wardle, D.A. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters, 11: 1351-1363.
320. Tilman, D. and Kareiva, P.M. 1997. Spatial Ecology : The Role of Space in Population Dynamic and Interspecific Interactions. Princeton University Press, NJ, USA.
321. Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. and Siemann, E. 1997. The Influence of Functional Diversity and Composition on Ecosystem Processes. Science, 277: 1300-1302.
322. Tirado, R., Pugnaire, F.I. 2005. Community structure and positive interactions in constraining environments. Oikos, 111: 437–444.
323. Tonsor, S.J., Elnaccash, T.W. and Scheiner, S.M. 2013. Developmental instability is genetically correlated with phenotypic plasticity, constraining heritability, and fitness: plasticity reduces heritability. Evolution, 67: 2923-2935.
    doi:10.1111/evo.12175
324. Tormo, J., Moreira, B. and Pausas, J.G. 2014. Field evidence of smoke-stimulated seedling emergence and establishment in Mediterranean Basin flora. Journal of vegetation science, 25: 771-777.
325. Travis, J.M.J., Brooker, R.W., Clark, E.J. and Dytham, C. 2006. The distribution of positive and negative species interactions across environmental gradients on a dual-lattice model. Journal of Theoretical Biology, 241: 896–902.
    doi:10.1016/j.jtbi.2006.01.025
326. Tylianakis, J.M., Didham, R.K., Bascompte, J. and Wardle, D.A. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters, 11: 1351–1363. doi:10.1111/j.1461-0248.2008.01250.x
327. Van Auken, O.W. 2000. Shrub invasions of North American semiarid grasslands. Annual Review of Ecology and Systematics, 31: 197–215.
328. Van der Putten, W.H. 2012. Climate change, aboveground-belowground interactions, and species range shifts. Annual Review of Ecology, Evolution and Systematics, 43: 365-383.
329. Venn, S., Pickering, C. and Green, K. 2012. Short-term variation in species richness across an altitudinal gradient of alpine summits. Biodiversity and Conservation, 21: 3157-3186.

330. Vicente-Serrano, S., Lasanta, T. and Cuadrat, J.M. 2000. Transformaciones en el paisaje del pirineo como consecuencia del abandono de las actividades económicas tradicionales. Pirineos, 155: 111-133.
331. Villar, L. and Benito, J.L. 2004. Los bosques del Parque Nacional de Ordesa y Monte Perdido (Pirineo central español): cartografía, valor ecológico y conservación. Naturalia Maroccana, 2: 155–162.
332. Villar, L.P., Montserrat, P., García, M.B. 1990. Sobre algunas plantas de distribución latepirenaica, su corología e interés biogeográfico. Fontqueria, 28: 33–38.
333. Villar, L., Romo, A.M. and Perdigo, M.T. 1993. The beechwoods of the central prepyrenees (Spain). A preliminary survey for conservation. Biological conservation, 66: 85–93.
334. Vittoz, P., Randin, C., Dutoit, A., Bonnet, F. and Hegg, O. 2009. Low impact of climate change on subalpine grasslands in the Swiss Northern Alps. Global Change Biology, 15: 209-220.
335. Walker, B. 1995. Conserving Biological Diversity through Ecosystem Resilience. Conservation Biology, 9: 747-752.
336. Walker, L.W. and Del Moral, R. 2003. Primary Succession and Ecosystem Rehabilitation. Cambridge.
337. Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H.R., Ahlquist, L.E., Alatalo, J.M., Bret-Harte, M.S., Calef, M.P., Callaghan, T.V., Carroll, A.B., Epstein, H.E., Jónsdóttir, I.S., Klein, J.A., Magnússon, B., Molau, U., Oberbauer, S.F., Rewa, S.P., Robinson, C.H., Shaver, G.R., Suding, K.N., Thompson,C.C., Tolvanen, A., Totland, Ø., Turner, P.L., Tweedie, C.E., Webber, P.J. and Wookey, P.A. 2006. Plant Community Responses to Experimental Warming across the Tundra Biome. Proceedings of the National Academy of Sciences of the United States of America, 103: 1342-1346.
338. Walker, L.R. and Chapin III, F.S. 1987. Interactions among processes controlling successional change. Oikos, 51: 131-135.
339. Wagner, H.H., Rico, Y., Lehnert, H. and Boehmer, H.J. 2013. Process-based long-term evaluation of an ecological network of calcareous grasslands connected by sheep herding. Ecography, 36: 374–382. doi:10.1111/j.1600-0587.2012.07463.x
340. WallisDeVries, M.F., Poschlod, P. and Willems, J.H. 2002. Challenges for the conservation of calcareous grasslands in northwestern Europe: integrating the requirements of flora and fauna. Biological Conservation, 104: 265–273.
341. Walther, G.R. 2010. Community and ecosystems responses to recent climate change. Philosophical transactions of the Royal Society of Science, 365: 2019-2024. doi: 10.1098/rstb.2010.0021
342. Weijers, S., Auliaherliaty, L., van Logtestijn, R. and Rozema, J. 2013. Effects of manipulated precipitation and shading on Cassiope tetragona growth and carbon isotope discrimination: a high arctic field study. Arctic, Antarctic, and Alpine Research, 45: 132–142.
343. Welden, C.W. and Slauson, W.L. 1986. The intensity of competition versus its importance: an overlooked distinction and some implications. The quarterly review of biology, 61: 23-44.
344. When, S., Pedersen, B. and Hanssen, S.K. 2011. A comparison of influence of cattle, goat, sheep and reindeer on vegetation changes in mountain cultural landscapes in Norway. Landscape and urban Planning, 102: 177-187.

345. Wigelt, A. and Jolliffe, P. 2003. Indices of plant competition, 91: 707-720.
346. Wilson, J.B. 1990. Mechanisms of species coexistence: twelve explanations for Hutchinson's 'paradox of the plankton': evidence from New Zealand plant communities. New Zealand Journal of Ecology, 13: 17-42.
347. Wilson, M.V. 2007. Measuring the components of competition along productivity gradients. Journal of Ecology, 95: 301–308. doi:10.1111/j.1365-2745.2007.01215.x
348. Wipf, S., Stöckli, V., Herz, K. and Rixen, C. 2013. The oldest monitoring site of the Alps revisited: accelerated increase in plant species richness on Piz Linard summit since 1835. Plant Ecology and Diversity, 6: 447-455.
349. Wisz, M.S., Pottier, J., Kissling, W.D., Pellissier, L., Lenoir, J., Damgaard, C.F., Dormann, C.F., Forchhammer, M.C., Grytnes, J.A., Guisan, A., Heikkinen, R.K., Høye, T.T., Kühn, I., Luoto, M., Maiorano, L., Nilsson, M.C., Normand, S., Öckinger, E., Schmidt, N.M., Termansen, M., Timmermann, A., Wardle, D.A., Aastrup, P. and Svenning, J.C. 2013. The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. Biological Reviews, 88: 15–30. doi:10.1111/j.1469-185X.2012.00235.x
350. Yan, H., Liu, J., Huang, H.Q., Tao, B., Cao, M. 2009. Assessing the consequence of land use change on agricultural productivity in China. Global and Planetary Change, 67: 13–19. doi:10.1016/j.gloplacha.2008.12.012
351. Yang, Y., Y. Niu, L. A. Cavieres, and H. Sun. 2010. Positive associations between the cushion plant Arenaria polytrichoides (Caryophyllaceae) and other alpine plant species increase with altitude in the Sino-Himalayas. Journal of Vegetation Science, 21: 1048-1057.
352. Yarranton, G.A. and Morrison, R.G. 1974. Spatial dynamics of primary succession: nucleation. Journal of Ecology, 62: 417-428.
353. Yasur, J. and Rani, P.U. 2013. Environmental effects of nanosilver: impact on castor seed germination, seedling growth, and plant physiology. Environmental Science and Pollution Research, 20: 8636–8648. doi:10.1007/s11356-013-1798-3
354. Young, T.P., Petersen, D.A. and Clary, J.J. 2005. The ecology of restoration: historical links, emerging issues and unexplored realms: Ecology of restoration. Ecology Letters, 8: 662–673. doi:10.1111/j.1461-0248.2005.00764.x
355. Zeileis, A., Kleiber, C. and Jackman, S. 2008. Regression Models for Count Data in R. Journal of Statistical Software 27(8). URL http://www.jstatsoft.org/v27/i08/.
356. Zhou, X., Istanbulluoglu, E. and Vivoni, E.R. 2013. Modeling the ecohydrological role of aspect-controlled radiation on tree-grass-shrub coexistence in a semiarid climate. Water Resources Research, 49: 2872-2895.
357. Zhou, Z., Zhang, X., Gan, Z., 2015. Changes in soil organic carbon and nitrogen after 26 years of farmland management on the Loess Plateau of China. Journal of Arid Land, 7: 806–813. doi:10.1007/s40333-015-0051-y
358. Zomeni, M., Tzanopoulos, J., Pantis, J.D. 2008. Historical analysis of landscape change using remote sensing techniques: An explanatory tool for agricultural transformation in Greek rural areas. Landscape and Urban Planning, 86: 38–46. doi:10.1016/j.landurbplan.2007.12.006
359. Zuur, A., Ieno, E.I., Walker, N.J., Saveliev A.A. and Smith, G.M. 2009. Mixed Models and Extensions in Ecology with R. Springer.

360. Zvereva, E.L. and Kozlov, M.V. 2006. Facilitation of bilberry by mountain birch in habitat severely disturbed by pollution: Importance of sheltering. Environmental and experimental botany, 60: 170-176.