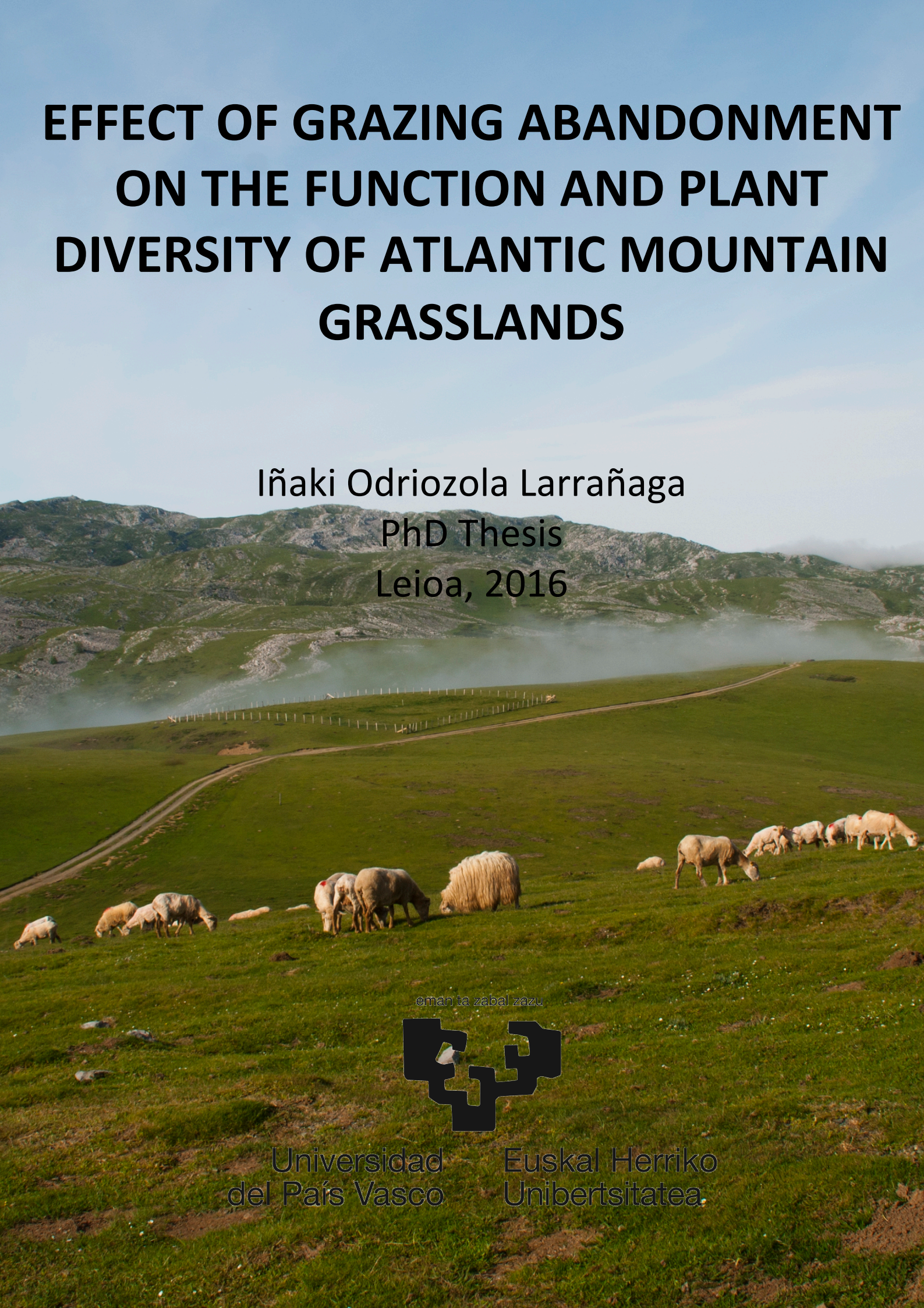
EFFECT OF GRAZING ABANDONMENT ON THE FUNCTION AND PLANT DIVERSITY OF ATLANTIC MOUNTAIN GRASSLANDS

Iñaki Odriozola Larrañaga

PhD Thesis

Leioa, 2016



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Leioa, May 2016

Supervised by Arantza Aldezabal Roteta
and Gonzalo García-Baquero Moneo

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SUMMARY

This doctoral thesis explored the consequences of the abandonment of extensive grazing by mixed livestock (sheep, cattle, and horses) in the highly productive Atlantic grasslands of the Aralar Natural Park, Basque Country (northern Iberian Peninsula). All large herbivores were experimentally excluded from four field plots by using 50 × 50 m fences. A grazing plot was delineated next to each exclusion plot where herbivores grazed continuously during the growing season (May to November).

Grazing generally enhances nutrient cycling and mineralisation in productive grasslands. Soil physical properties and forage properties were measured in the field plots to understand how grazing affects soil processes. All measurements were carried out after 8–10 years of exclusion. Grazing affected nutrient cycling by modifying forage quality, soil thermal regimes, and water content. It prevented litter accumulation, which provided less insulation to the soil and enhanced mean summer temperatures and variability. Higher temperatures combined with higher forage quality and, generally, without water stress, enhanced nutrient cycling and mineralisation in grazed areas.

The effect of grazing on community physiognomy and biodiversity was then studied. Disturbance by large herbivores maintains high levels of plant diversity in productive grasslands; possibly because grazing provides an equalising mechanism that suppresses the competitive exclusion of weak plant species by dominant species. Since species competition occurs at fine spatial scales, a fine-scale, spatially explicit survey design was used. After 10 years of the cessation of herbivory, i.e. after long-term suppression of the strong equalising mechanism previously present, competitive species created large intraspecific patches and out-competed weaker species, thereby reducing species diversity.

Competitive exclusion is assumed to be strong in productive grasslands because niche stabilisation is expected to be weak (i.e. they are rich in resources). However, in one of the field plots, pH showed high heterogeneity, which exerted moderately strong stabilisation in the plant community. Using two field plots with contrasting

plant niche stabilisation, the buffering effect of niche stabilisation on competitive exclusion was studied. The applied statistical techniques combined phylogenetically structured plant traits and spatially structured soil descriptors through species abundance. Under weak niche stabilisation, grasses with superior competitive ability (i.e. tall canopy with lateral vegetative spread) outcompeted dicots in all branches in the phylogenetic tree, resulting in strong reduction of species and phylogenetic diversity. However, under moderately strong niche stabilisation, competitive exclusion by superior species was counter-balanced by niche stabilisation, and resulted in a less important loss of species and phylogenetic diversity.

To conclude, given that 10 years of grazer exclusion retarded nutrient cycling and mineralisation and resulted in loss of spatial heterogeneity of floristic composition and plant diversity in the field plots, traditional grazing by mixed livestock (sheep, cattle, and horses) was a key ecological factor for maintaining soil function and plant diversity in Atlantic grasslands.

CHAPTER 1. General Introduction

1.1. History of grazing

During the past 66 million years (i.e. the Cenozoic era) of the world, the continental plates reached their present configuration and, consequently, the uplift of the Alpine-Himalayan belt (Alpine orogeny) occurred between 35–25 million years ago (Ma) (Condie & Sloan 1998). North of the Iberian Peninsula, the Alpine orogeny resulted in the uplift of the Cantabrian Range, Pyrenees, and Basque Mountains. The Aralar Massif, where the fieldwork in the temperate grassland system of the present study was performed, is located in the Basque Mountains. The historical changes in the configuration of the continents resulted in changes in the ocean circulation, which reduced the atmospheric carbon dioxide concentration and global temperature (Pagani et al. 2011). Coupled with these environmental changes, the evolution and diversification of grasses (and many other angiosperms) occurred, and grassland ecosystems expanded globally under seasonally dry conditions (Tallis 1991).

THE ORIGINS OF THE GRAZER-ANGIOSPERM INTERACTION

Although molecular evidence suggests that flowering plants (angiosperms) may have originated between 183–147 Ma (Bell et al. 2010), the first known macrofossil evidence for angiosperms has been dated from c.140 Ma (early Cretaceous) (Friis et al. 2011). Concomitantly, the first fossil evidence for the consumption of angiosperms by a relatively large grazer (an ankylosaur) is also ancient (Cretaceous) (Molnar & Clifford 2000), suggesting that herbivores have possibly been consuming angiosperms for 140 million years. During this long period, both angiosperms and herbivores have adapted to herbivory, e.g. one hypothesis proposes that mammals and grasses (Poaceae) have been co-evolving from their origin in the Cretaceous-Paleogene (i.e. Mesozoic-Cenozoic) transition (Janis 1993), 70–60 Ma. The adaptations in the dentition and skeletal structure found in fossil hoofed mammals support this hypothesis (Stebbins 1981; Strömberg 2011). Additionally, there is palaeocological evidence that the ancestral ruminant was a small, forest-dwelling species that adapted to consumption of a diet rich in intracellular carbohydrates. With the expansion of grasslands in the late Miocene, mixed feeders and grazing species radiated, and ruminants evolved

adaptations to increase their efficiency in digesting fibre (Pérez-Barbería et al. 2004).

THE ORIGINS OF TEMPERATE GRASSLANDS

From the Miocene (about 20 Ma) both floral and faunal evidence indicate that open habitat grasslands developed in western Eurasia (Strömberg 2005). It is uncertain how close these Eurasian open habitat grasslands were to modern temperate grasslands, but evidence suggests that "true" temperate grasslands did not appear until c. 2 Ma (Pleistocene) (Janis 1993; Strömberg 2011).

The extension (relative cover) and evolution of open habitats during the Holocene (a period that approximately encompasses the last 12000 years) in Europe have motivated extensive debates among scientists (Hejcman et al. 2013). Although Europe was possibly densely forested during the Holocene climatic optimum (i.e. the Atlantic period), these forests did not constitute closed canopy covers throughout all Europe, but open woodlands with scattered islands of small steppe-like (open) areas in the lowlands (Hejcman et al. 2013). Evidence from central Europe suggests that open habitats remained relatively stable, ranging from 12 to 30% of the European area (Kuneš et al. 2015). Therefore, before the introduction of extensive agriculture, the landscape of Europe was possibly patchy, alternating both types of habitats (Hejcman et al. 2013), with large herbivores such as wild horses (*Equus* sp.), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), aurochs (*Bos primigenius*), and European bison (*Bison bonasus*) keeping the forests open through grazing and browsing (Vera 2000).

SEMI-NATURAL GRASSLANDS

Major changes started globally when early farmers domesticated sheep, goats, cattle, onagers, and donkeys in the Middle East, dromedaries in the Arabian Peninsula, horses in southern Russia, camels and yacs in Central Asia, lamas in South America, water buffalo and zebu in South Asia, gaur, gayal, and banteng in Southeast Asia, and reindeer in northern Scandinavia and northern Siberia (Prins & Gordon 2008). People moved from these centres of origin across the landscapes,

taking with them their domesticated grazers and browsers, and spreading farming across the Old World (Prins & Gordon 2008).

The spread of farmers from Levant across Europe resulted in the spread of agriculture (firstly in around 7000 BP and then again in around 4600 BP): people cleared primeval forests and grew arable crops, and indigenous large herbivores were gradually replaced by livestock (Bakker & Londo 1998). Much of the open landscape was new, but most plant species that formed emerging grasslands and heathlands were already present in open forests, forest clearings, fringes along streams, fens and bogs, and in larger open areas along the coast (Bakker & Londo 1998). Hence this semi-natural landscape constituted the original flora and fauna, but the original biological communities were deeply transformed by human activities (Bakker & Londo 1998).

DECLINE OF THE EUROPEAN SEMI-NATURAL LANDSCAPE

By the mid-19th century, agricultural mechanisation and the massive introduction of industrial fertilisers created the current intensively cultivated landscape in Europe. Flora and fauna were heavily influenced by humans, many species (such as weeds) were nearly eradicated by pesticides, and non-indigenous species were introduced (Westhoff 1983). Nevertheless, the modern cultivated landscape still contains semi-natural remnants, particularly in southern Europe. In 1996, low-intensity farming systems constituted 82% of the agricultural area in Spain, 61% in Greece, 60% in Portugal, 35% in Ireland, 31% in Italy, 25% in France, 23% in Hungary, 14% in Poland, and 11% in the United Kingdom (Bignal & McCracken 1996).

Extensive livestock systems are a good example of low-intensity farming systems. These grazing systems are characterised by the continuous sustainable use of large areas of grasslands, heaths, and woodlands; virtually all the remaining high nature conservation value grasslands across Europe are associated with low-intensity livestock systems (Bignal & McCracken 1996). However, permanent grasslands that mainly sustain extensive grazing systems have declined (Pe'er et al. 2014), and further progressive reductions in extensively-managed livestock are expected

in Atlantic agro-ecosystems (Rounsevell et al. 2006), especially in mountain areas affected by the removal of European Union subsidies for marginal grazing land (Strijker 2005).

EXTENSIVE GRAZING IN THE ATLANTIC BASQUE COUNTRY

The Aralar Natural Park, where the experiments of the present study were conducted, is located in the Basque Country, North Iberian Peninsula. Since managed livestock (sheep, horses, and feral cattle) have been grazing in the mountain areas of the Basque Country since the Neolithic period, livestock grazing has a long tradition in this region of southwestern Europe (Caro Baroja 1971). A characteristic of the Neolithic period in this region is the presence of ancient funeral buildings called dolmens ("trikuharri" in Basque); dolmens were collective burials of shepherds from the surrounding areas and their usage is documented since 5300 BP (Moraza 2010). At present, traditional livestock grazing is still common in areas with abundant dolmens in the mountain areas of the Basque Country, whereas, at lower altitudes, cultivated grasslands and intensive cattle production dominate the landscape (Caro Baroja 1971).

The historical importance of livestock grazing as an economic activity in the Basque Country has contributed to the Basque language. For example, as in other languages, the words wealth and livestock are related in Basque: wealthy is "aberats" in Basque, and livestock "abere": those who possess much livestock are considered wealthy. Due to the abrupt geography conferred to the Atlantic Basque Country by the Basque Mountains, ruminant livestock farming, and particularly sheep production, is still active. Nevertheless, the current influence of the primary sector on the Basque economy is minor, with less than 1% Gross Domestic Product (EUSTAT 2011). Although not quantitatively important, livestock grazing and, in particular, sheep farming, has other societal and qualitative values: sheep grazing conforms to a local agricultural system displaying strong links with territorial identity (Batalla 2015). A good example is the legally regulated production of "Idiazabal Cheese", which is obtained from the milk of the local "Latxa" breed (Batalla 2015); approximately 59% of the cheese is produced in farms directly by the shepherd-farmers themselves (EUSTAT 2013). Other activities of high

traditional value associated with sheep farming are the long-distance seasonal movements called transhumances. One of these annually connects Erronkari in the Pyrenees and Bardenas of Navarre; others connected several Pyrenean zones with the Landes in France. All these transhumances appear to have their origins in the Middle Ages (Caro Baroja 1971).

However, the most extended traditional livestock farming in the Atlantic Basque Country is seasonal grazing based in short-distance transhumances that connects farms located at relatively low altitudes (occupied in winter) with local massifs (extensively used in summer). These transhumances occur throughout the Atlantic Basque Country, in Lower Navarre, in valleys west to Erronkari (Aezkoa, Zaraitzu…), and, at the massifs and ranges of Gorbea, Aizkorri, Entzia, Andia, Aralar, and Urbasa (Caro Baroja 1971). Short-distance transhumances to the Aralar massif have been regulated legally and managed by the local communities (so-called Enirio-Aralar Communal land), since at least 600 years ago (Moraza 2010).

As it occurs throughout Europe (Pe'er et al. 2014), the use of mountain grasslands by domestic livestock is declining slowly in most of the Pyrenean-Basque-Cantabrian grassland systems (Ruiz et al. 2009). Additionally, significant management changes –shorter duration in the mountains, lower stocking rate, and less shepherd control –have been detected in the extensive livestock systems of the Basque Mountains where, historically, shepherded livestock grazed from May to November (Aldezabal et al. 2015). The high average age of the shepherds (45–50 years old) and overall trend of rural population movement, abandoning farming activities, suggests this decreasing trend will be accentuated in the future (Ruiz et al. 2009).

1.2. Ecology of grazing: the keystone function of large herbivores

As discussed above, the pre-agricultural landscape in Europe is considered to have been patchy, with the presence of open areas (Hejcman et al. 2013), and wild herbivores maintaining the open area by grazing and browsing (Vera 2000). Now

that most wild large herbivores are extinct in Europe, extensively managed grazing has become crucial for the conservation of European grasslands (Sutherland 2002). In fact, there is sufficient evidence that herbivores play key functions in both natural and semi-natural ecosystems. Grazing directly affects the structure and composition of plant communities, and is fundamental for the survival of many other species, especially those from open habitats. Additionally, grazing also exerts direct impacts through defoliation, trampling, and faeces and urine deposition, which may modify ecosystem processes such as productivity, turnover, and the distribution of nutrients over time.

GRAZING AND ECOSYSTEM FUNCTIONING

Large vertebrate herbivores contribute to the productivity and functioning of ecosystems through their effect on microbial activity, nutrient cycling, and soil nutrient availability (Bardgett & Wardle 2003). An important mechanism is herbivore-induced change in the quality and quantity of resources that are returned to the soil as dung, urine, and plant litter (McNaughton et al. 1997; Bardgett & Wardle 2003). In productive grasslands, herbivores enhance nitrogen (N) mineralisation through dung and urine deposition, and promoting fast growing species and high quality, palatable (low carbon (C)/N ratio) regrowth. In contrast, in unproductive grasslands, herbivores promote slow growing, defended, unpalatable (high C/N ratio) plant species, thereby decreasing litter quality and N mineralisation (Bardgett & Wardle 2003).

The classical view of herbivore-induced changes in resource quality and quantity predicts the consequences for nutrient mineralisation in many cases; however, inconsistent results have been reported (Schrama, Veen, et al. 2013). To overcome these limitations, Schrama et al. (2013) proposed an extended theoretical framework, where herbivores affect N cycling through changes in resource quality and quantity, and through changes in soil physical conditions. Indeed, grazing strongly affects soil physical conditions such as temperature and water content (Greenwood & McKenzie 2001; Gass & Binkley 2011). Trampling by herbivores compacts the soil organic layer, reducing the insulation of soil and modulating the soil thermal regime (Aalto et al. 2013); soil temperature, in turn, influences

microorganism activity, the decomposition rate of organic matter, and nutrient cycling (Butenschoen et al. 2011; Karhu et al. 2014). The effects of soil temperature and water availability on soil microbial activity are interdependent (Poll et al. 2013), and grazing also affects soil water content: defoliation and trampling reduce plant and soil organic layer, enhancing water evaporation; however, defoliation also reduces transpiration by decreasing solar radiation intercepted by the leaves (Bremer et al. 2001). Additionally, trampling by grazers affects soil water content by modifying soil water infiltration rates (Schrama, Heijning, et al. 2013). Although the complexity of the pathways by which grazing may affect soil processes and nutrient cycling, studies integrating changes in resource quality and soil physical properties are limited (Schrama, Veen, et al. 2013).

GRAZING AND BIODIVERSITY

Since Tansley & Adamson (1925) published the results of the first known grazer exclusion experiment, the effect of grazing on grassland diversity has been debated among ecologists. Due to the contrasting effects of herbivory reported in differing grassland systems (e.g. Hyder et al. 1966; McNaughton 1983; Sala et al. 1986), Milchunas et al. (1988) proposed a generalised grazing model based on the intermediate disturbance hypothesis (Grime 1973; Connell 1978) that explained many of the apparently inconsistent responses. According to the model (Milchunas et al. 1988), the effect of grazing on diversity depends on aboveground productivity, evolutionary history of grazing, and grazing pressure. For example, in productive grasslands with a long evolutionary history of grazing (such as Atlantic grasslands) the relationship between plant diversity and grazing pressure is expected to be hump-shaped, with the highest diversity at moderate grazing pressure. Additionally, in such conditions, grazing is expected to strongly affect diversity. Divergent selection for competition for light and resistance to grazing would have resulted in a species pool composed of both tall and short species and quick competitive exclusion of short species for light would be expected with a hypothetical cessation of grazing (Milchunas et al. 1988). Simultaneously, Westoby et al. (1989) developed their state-and-transition model, which assumes that diversity in grassland systems may have multiple equilibrium states separated by

thresholds. If, after a change in grazing regime (or other abiotic factor), a shift in diversity exceeds a threshold, the change may be irreversible. Even if the previous grazing intensity is restored, diversity may reach an equilibrium state different from the original. The strength of the Milchunas et al. (1988) generalised model is that it attempts to develop universal forecasting, and the strength of the Westoby et al. (1989) state-and-transition model is its versatility and applicability to diverse field situations. Therefore, Cingolani et al. (2005) modified and combined the above models, thus producing a more applicable theoretical framework. Cingolani et al. (2005) argued that grazing would possibly result in irreversible changes in grasslands with a short history of grazing, as vegetation is less adapted to the impact of herbivory. Hence, Cingolani et al. (2005) retained the original forecasting of the generalised model for grasslands with a long history of grazing, but modified the application to systems with a short grazing history (Fig. 1.1). Lastly, Oesterheld & Semmartin (2011) highlighted the existing operational

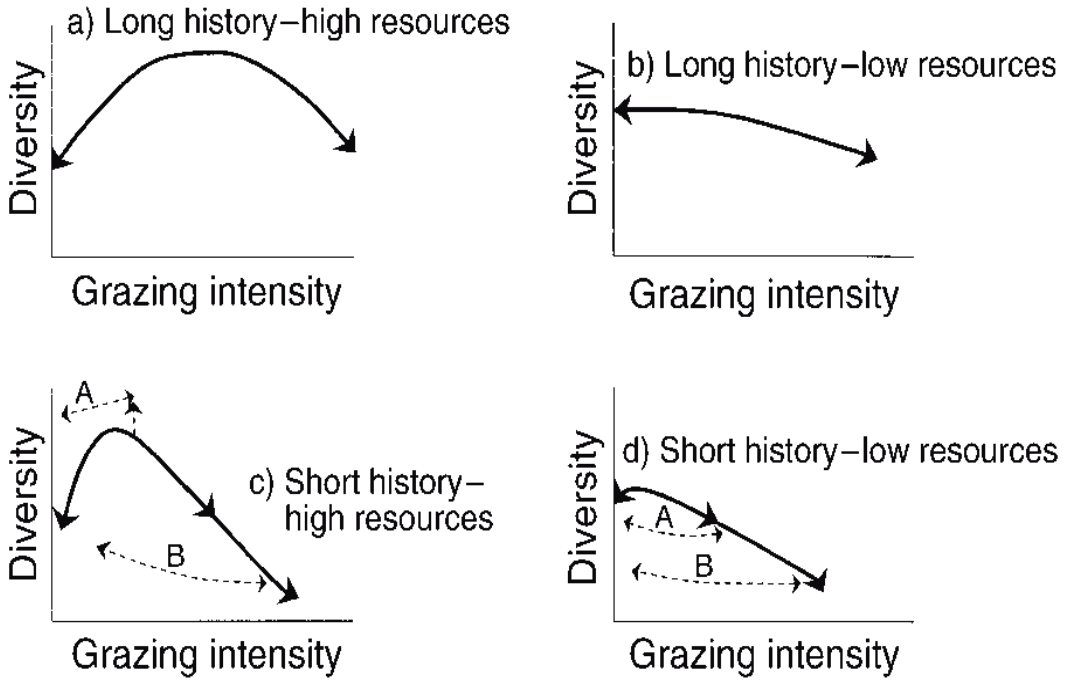

Fig. 1.1. Figure taken from Cingolani et al. (2005). Plant diversity response to grazing intensity in scenarios with contrasting productivity and grazing evolutionary history. Solid lines represent the original model (Milchunas et al. 1988) equilibrium curves, and dashed lines (A and B in panels c and d) represent additional equilibrium curves postulated by Cingolani et al. (2005). In productive grasslands with a long history, as the ones studied in this dissertation, a hump-shaped relationship is predicted, with the highest diversity at intermediate grazing intensity (Case a).

problems to determine the evolutionary history of grassland systems and proposed potential mechanisms that could operate across the axis of primary productivity. The predictions of the generalised model (Milchunas et al. 1988), as modified by Cingolani et al. (2005), remain unchanged. The model is still the standard reference for any work on the effect of grazing on species composition.

MODERN THEORY OF SPECIES CO-EXISTENCE

Recent theoretical and methodological developments in ecology could contribute to a more thorough understanding of the effects of herbivory on biodiversity. The last decade has seen substantial statistical advances in multivariate multiscale spatial analyses (Dray et al. 2012). Given that natural or semi-natural species assemblages are inherently multivariate, and that assembly processes, such as competition, occur in space, these methodological advances are invaluable in the context of community ecology. Additionally, the relatively recent emergence of the modern coexistence theory (Chesson 2000; Adler et al. 2007) has provided a new framework to understand species coexistence and, therefore, the maintenance of biodiversity. In this dissertation, the effects of herbivory on grassland plant community are framed in that context.

Most ecological work implicitly assumes that species differ in their niches. Indeed, it has been shown that species may differ, for example, in the use of multiple limiting resources (Tilman 1982). Chesson (2000) proposed a new paradigm where species coexistence in communities is determined by species niche differences, and their relative competitive ability differences. In this framework (Chesson 2000; Adler et al. 2007), there are two mechanisms that promote species coexistence: stabilizing processes, such as niches that make species to limit themselves more than they limit others; and equalising processes, such as grazing, that equalise the competitive ability differences of species. Niches cause intraspecific competition to be stronger than interspecific competition: when a species increases in abundance, the pressure of competition that it exerts upon itself also increases; consequently, its per capita growth rate is reduced relative to other species. As a consequence, competitive exclusion is limited –this phenomenon is termed as negative frequency dependence (Chesson 2000; Adler et

al. 2007). In contrast, relative competitive ability differences enhance competitive exclusion of inferior competitors by superior competitors (Mayfield & Levine 2010), and equalising processes limit competitive exclusion by reducing species competitive ability differences (Chesson 2000). In summary, it is the balance between niche-based stabilising processes (i.e. species niche differences) and competitive ability differences that determines coexistence (Chesson 2000; Adler et al. 2007; Mayfield & Levine 2010) (Fig. 1.2).

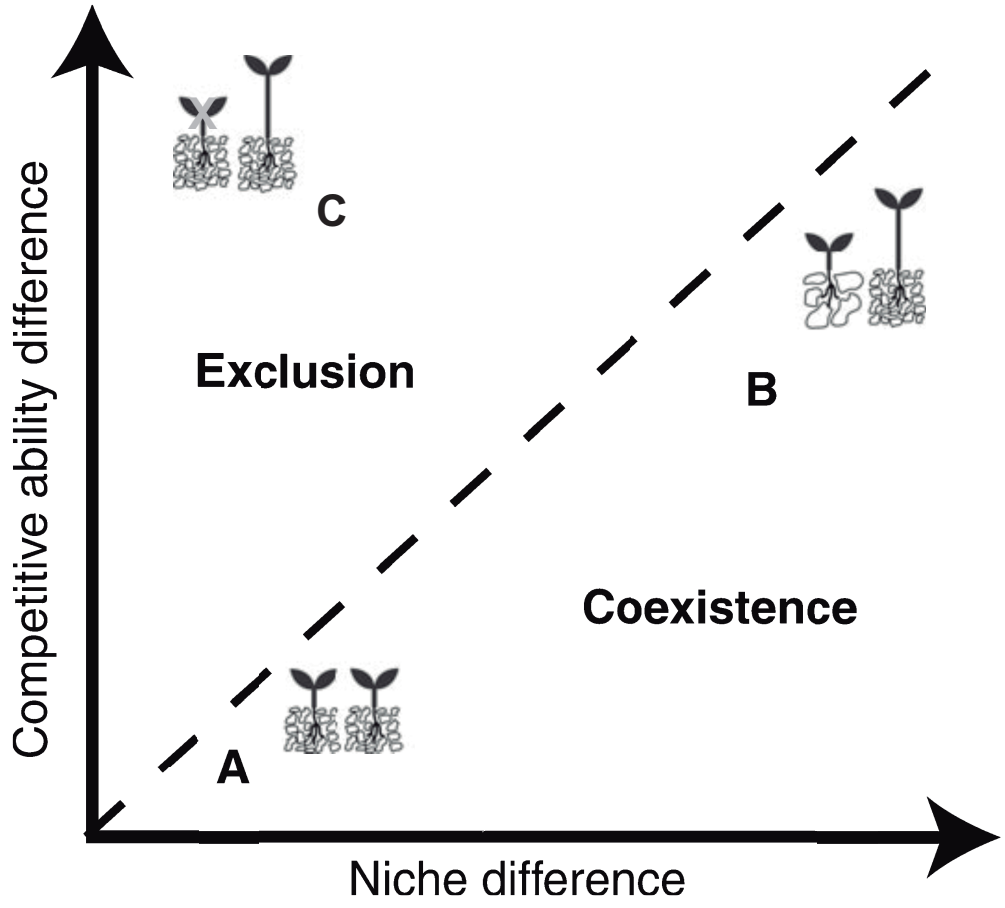

Fig. 1.2. Modified from Mayfield & Levine (2010). Coexistence occurs when stabilisation by niche differences overcomes competitive exclusion by competitive ability differences. Coexistence occurs in Case A, because weak stabilisation by niches exceeds subtle competitive ability differences. Coexistence occurs in case B, because strong niche stabilisation exceeds large competitive ability differences. Competitive exclusion occurs in Case C, because strong competitors out-compete weak competitors in absence of niche stabilisation.

GRAZING AS AN EQUALISING DISTURBANCE

In grasslands, non-selective shoot herbivory (which is mainly performed by ruminant and non-ruminant grazers) is a strong equalising mechanism (Wilson 2011) because it reduces species height and lateral spread differences (Deléglise et al. 2011) and consequently, the ability of species to compete for light and space. As mentioned, species may differ in their use of multiple limiting resources (Tilman 1982), which may be considered as niche dimensions exerting strong stabilisation (Harpole & Tilman 2007). Infertile and unproductive grasslands possibly have multiple limiting resources and niche processes could stabilise the growth of plant species. In contrast, in productive grasslands, niche stabilisation mechanisms are expected to be weak (Harpole & Tilman 2007) and competitive species are expected to exclude weaker species by out-competing them for light (Hautier et al. 2009). Therefore, in highly productive grasslands, similar to the ones here studied, an equalising mechanism, such as disturbance by non-selective shoot herbivory, is expected to play a greater role in species coexistence (Wilson 2011) than stabilisation by niches.

1.3. General objectives and dissertation structure

The general aim of this doctoral thesis was to provide an enhanced understanding of the role of traditional mixed grazing systems by sheep, horses, and cattle in maintaining soil function and plant diversity of Atlantic mountain grassland systems. Specifically, the main objectives were as follows:

1. The study aimed to elucidate the complex relationships between grazer-induced changes in the physical properties of soil and resource quality that drive changes in soil function and nutrient cycling. This objective has been discussed in Chapter 2 (*Livestock grazing modifies the effect of environmental factors on soil temperature and water content in a temperate grassland*).

2. Another objective was to understand the mechanisms dominant species use to outcompete weaker competitors in the absence of disturbance by herbivores. This objective has been discussed in Chapter 3 (*Grazing exclusion unleashes competitive plant responses in Atlantic mountain grasslands*).

3. The study also assessed the role of competitive ability and niche differences in the outcomes of competition in Atlantic grasslands. This objective has been discussed in Chapter 4 (*Patterns of species relatedness created by competitive exclusion depend on niche stabilisation: evidence from Atlantic grasslands*)

Finally, the General Discussion (Chapter 5) and General Conclusions (Chapter 6) are presented at the end of this dissertation.

1.4. Study area

The grazer exclusion experiment was conducted in the Aralar Natural Park, Basque Country (northern Iberian Peninsula). Four field sites on relatively flat terrain were selected: Oidui (42° 59' 16.6'' N, 2° 5' 59.4'' W; 876 m.a.s.l), Alotza (43° 0' 10.6'' N, 2° 5' 22'' W; 1223 m.a.s.l), Uzkuiti (43° 0' 50'' N, 2° 4' 3'' W: 1300 m.a.s.l), and Igaratza (42° 59' 9.25'' N, 2° 2' 9.7'' W: 1247 m.a.s.l) (Fig. 1.3). To simulate grazing cessation, permanent fenced plots (50 × 50 m each) were installed in May 2005. Next to each exclusion plot (*E* level), we delineated a grazed plot (*G* level), where sheep, cattle, and horses grazed continuously during the vegetative period (from May until November) (Fig. 1.4).

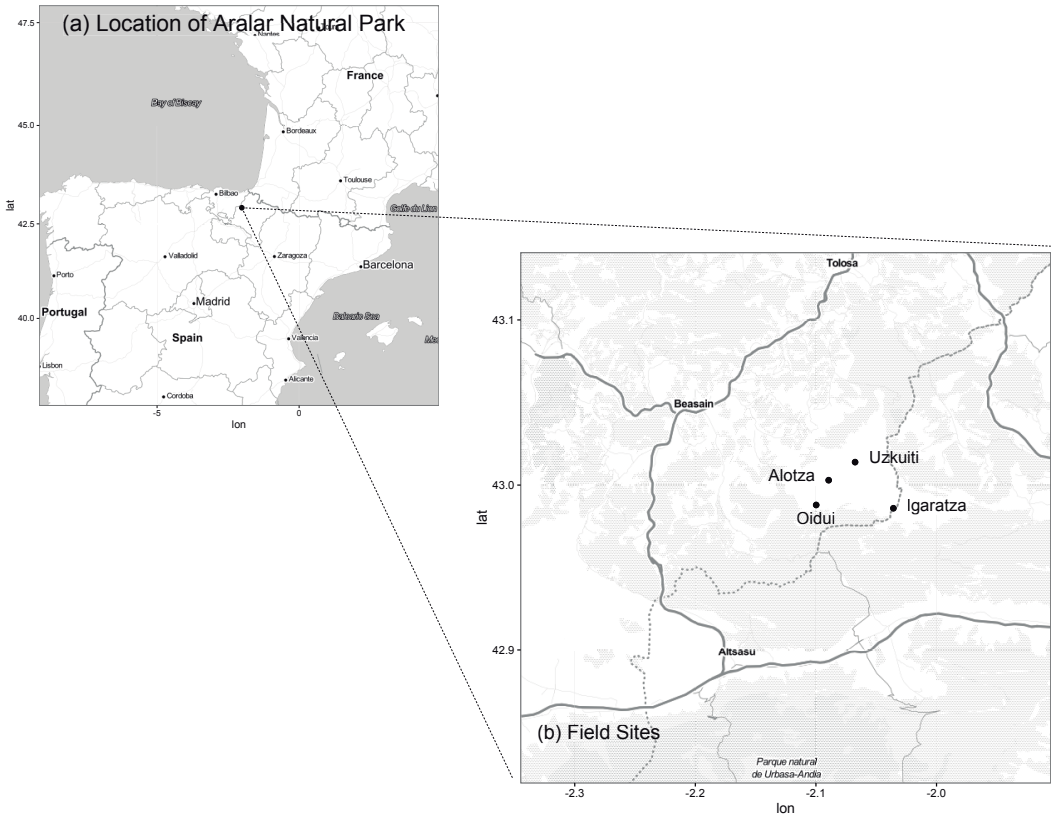

Fig. 1.3. Map of the study area: a) The Aralar Natural Park, Basque Country (northern Iberian Peninsula); b) Four field sites in the park were selected to conduct the grazer exclusion experiment.

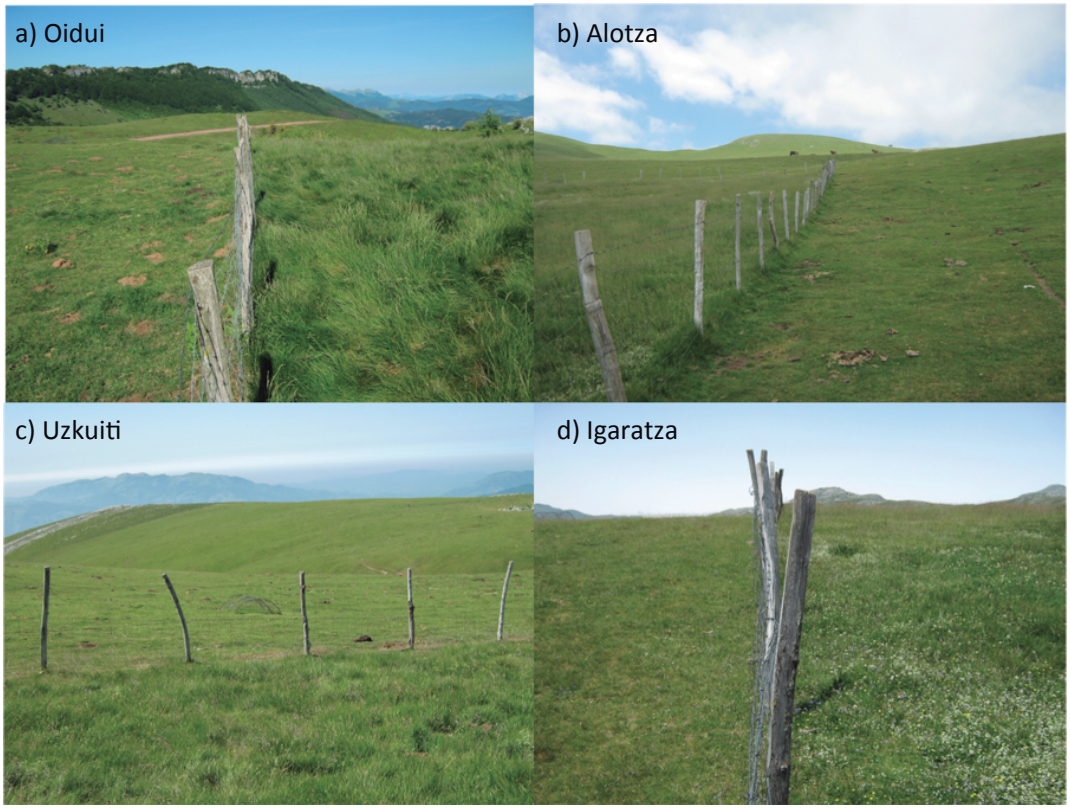

Fig. 1.4. Field sites in Aralar Natural Park: a) Oidui, b) Alotza, c) Uzkuiti and d) Igaratza.

Aralar Natural Park is a protected area including 11000 ha. The area has an oceanic climate, with a mean annual temperature of 12.4°C and annual precipitation of >1400 mm. The area traditionally used for seasonal (from May until November) livestock (beef cattle, dairy sheep, and horses) grazing occupies about 2077 ha (18.9% of the park area). In contrast to most Atlantic mountain grasslands, in the Aralar Natural Park grazing intensity has remained near constant for the last decade (Fig. 1.5), and is therefore suitable for grazer exclusion experiments. The primary vegetation type is a highly productive (mean aboveground net primary productivity of 3.37 t dry mass ha^{-1} $year^{-1}$ with standard error of 0.88) native grassland on a calcareous substrate (Gibbons and Moreno 2002), which contains mainly perennial species (Loidi 1982) and corresponds to the priority habitat "Species-rich *Nardus* grasslands" (code 6230) of the Habitat Directive (92/43/EEC, European Commission 2013).

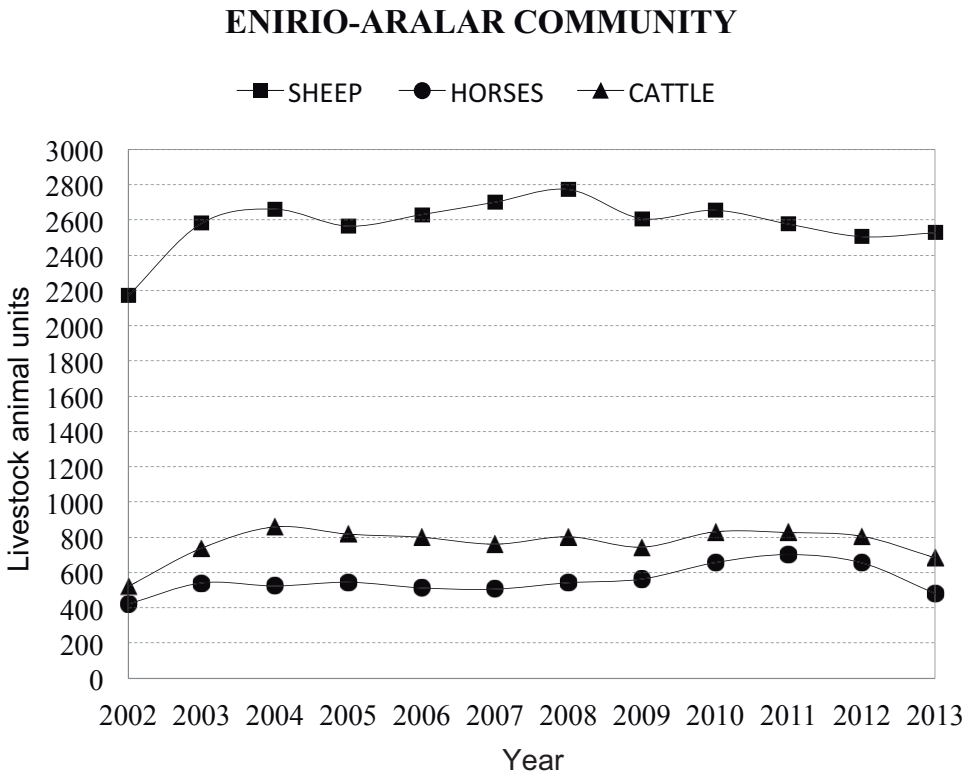

Fig. 1.5. Evolution of sheep, cattle, and horses abundance in Aralar Natural Park during the last 14 years.

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CHAPTER 2. Livestock grazing modifies the effect of environmental factors on soil temperature and water content in a temperate grassland

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Abstract

Grazing by large herbivores modulates the soil temperature and water content as well as the quality of resources returned to the soil. Therefore, in order to predict the effects of grazing on complex interacting soil processes and plant production, an integrated approach is needed. We hypothesized that grazing accelerates nutrient cycling by increasing (i) soil temperature and fluctuations, (ii) water-holding capacity, and (iii) forage quality. To test this biological hypothesis, we conducted a field experiment simulating grazing abandonment conditions in semi-natural mountain grassland plots. Our results show that grazing accelerates soil processes through all three hypothesized mechanisms. Since grazing maintains a thin organic layer that provides less insulation to the soil, higher mean temperatures and large daily temperature fluctuations were recorded in grazed areas (less insulated); these daily fluctuations were as large as seasonal variation in the ungrazed plots. The response of the soil water content to grazing was complex. Although overall exclusion reduced the soil water content, particularly in coarse-textured soils, this trend was reversed during long periods of high solar radiation (i.e. high evaporation). Forage quality was reduced in all plots when grazers were excluded. Experiments attempting to realistically simulate grazing may benefit from these findings, particularly in very productive grasslands where the thickness of the organic layer increases rapidly under grazing exclusion.

Key-words: soil thermal regime; seasonality; soil moisture; organic layer; forage quality.

2.1. Introduction

Grazing by large herbivores drives the main soil processes, such as nutrient cycling and microbial activity, as well as primary production (Bardgett and Wardle, 2003; Guitan and Bardgett, 2000; McNaughton et al., 1997a), through defoliation, trampling, and faeces-urine deposition. This occurs through the modulation of the soil's physical characteristics (temperature and water content) (Gass and Binkley,

2011; Greenwood and McKenzie, 2001) and the quality of the resources returned to the soil (Bardgett and Wardle, 2003; McNaughton et al., 1997b). For these reasons, in order to predict the effects of grazing on soil processes and plant production an integrated approach is needed (Butenschoen et al., 2011; Schrama, Veen, et al., 2013a). The high rates of nutrient cycling measured under grazing conditions in temperate grasslands (Bardgett et al., 1998; Frank and Evans, 1997; McNaughton et al., 1997a; Semmartin et al., 2004) are probably a consequence of the combined effects of the abovementioned mechanisms; however, comprehensive studies are few (Schrama et al., 2013b).

Grazing (the consumption of plant biomass by livestock) modifies soil temperature over time, which, in turn, influences microorganism activity, the decomposition rate of organic matter, and nutrient cycling (Butenschoen et al., 2011). This occurs because the thickness of the soil organic layer affects the soil temperature (Aalto et al., 2013), with thicker organic layers providing more insulation. Under conditions of moderate to high stocking rates, more aboveground plant biomass is removed, and the incorporation of plant residues into the soil is reduced (Bilotta et al., 2007). Thus, dry matter accumulation is prevented and a thinner layer of plant litter is formed, which provides less insulation to the soil. Therefore, under grazing conditions, soil temperature is expected to be more variable and more dependent on environmental temperature than under non-grazing conditions (Gan et al., 2012; Zhao et al., 2011).

The effects of soil temperature on microbial biomass and activity depend on soil water availability (Poll et al., 2013). The consumption of plant biomass also affects the soil water content, with the counteracting effects of increased evaporation and reduced transpiration. Less insulated soils receive more solar radiation, which raises the temperature and increases evaporation; however, defoliation decreases the solar radiation intercepted by leaves and consequently decreases transpiration (Bremer et al., 2001). In addition, infiltration also affects the soil water content. In general, the way in which grazing as a whole affects the circulation, the retention capacity, and the availability of soil water may depend on interactions with the soil's physical and chemical properties (Greenwood and McKenzie, 2001), and

environmental conditions. For example, compaction due to trampling is greater in fine-textured wet soils than in coarse-textured dry soils (Schrama et al., 2013a). Therefore, grazing is expected to increase the soil water-holding capacity, particularly in coarse-textured soils.

Soil temperature and moisture enhance microbial activity and biomass, if available substrates are not limiting (Frey et al., 2008). Grazing, too, may affect plant litter quality (Bardgett et al., 1998; Wardle et al., 2004). In temperate, highly productive grasslands (as those studied here), grazing results in an increase in species with a high nitrogen content and a low lignin content (Milchunas & Lauenroth, 1993; Semmartin, Aguiar, Distel, Moretto, & Ghersa, 2004). Generally, low carbon to nitrogen ratios promote nutrient release, thus accelerating nutrient cycling, whereas high ratios induce microbial immobilization (Semmartin et al., 2004). These effects are highly relevant to ecosystem functioning, because the amount of soil nitrogen derived from plant litter in grazed grasslands can be two to eight times greater than nitrogen derived from urine and faeces (Chaneton et al., 1996; Holland et al., 1992). Therefore, grazing is likely to promote plants with higher nitrogen to carbon ratios, consequently increasing forage quality.

Given that grazing accelerates nutrient cycling in temperate and productive grasslands (Bardgett et al., 1998; Frank and Evans, 1997; McNaughton et al., 1997a; Semmartin et al., 2004), we hypothesized that the mechanism involved in livestock grazing is a combination of increasing soil temperature and fluctuations, increasing the water-holding capacity, and increasing forage quality. To test this biological hypothesis, we conducted an '*in situ*' experiment simulating grazing abandonment conditions on a historically grazed semi-natural mountain grassland, and assessed the effects of grazing on the thickness of the organic layer, the soil thermal regime, the soil water content, and forage quality.

2.2. Materials and Methods

STUDY AREA

Experimental manipulations were carried out in the Aralar Natural Park

(42°59′48′′N, 2°06′51′′W), an 11,000-ha protected area located in the Basque Country (Northern Spain). The vegetation in the Park is comprised of a mosaic of gorse-heather shrublands and grasslands, which support livestock (mainly 18,000 dairy sheep of the Latxa breed) that are managed in an extensive grazing system. The area has oceanic climatic conditions, with a mean annual temperature of 12.4°C and an annual precipitation of more than 1400 mm. Despite the marked seasonality of the weather conditions at the site, drought periods are uncommon, as long periods without precipitation are unusual (Table 2.1). The area traditionally used by livestock (beef cattle, dairy sheep, and horses) occupies 2077 ha of the Park (18.9%), and its usage varies seasonally from May to November. Vegetation is mainly represented by native grasslands that are included in the Habitat Directive (European Commission, 2003). The most relevant vegetation type for livestock maintenance is *Jasiono-Danthonietum* grassland (code 6230, subtype a), primarily comprising *Festuca rubra* s.l., *Agrostis capillaris*, *Galium saxatile*, *Trifolium repens*, *Luzula campestris*, and *Cerastium fontanum*.

Table 2.1. Mean temperature, precipitation, and solar radiation values for the study years and seasons. Data were collected from the weather station of San Miguel of Aralar (Gobierno de Navarra, 2013), located near the study area.

Year	Season	Temperature (°C)	Precipitation (l/m^2)	Solar radiation (W/m^2)
2011-12	Summer	13.4	85.4	191.1[b]
2011-12	Winter	-0.6	205.4	73.52
2012-13	Summer	14.5	86.3	206.2
2012-13	Winter	0.8	759.8[a]	60.8

Annotations: [a] 2012–13 winter was very rainy, hence the high precipitation recorded. [b] 2011–12 summer was very cloudy, the lowest solar radiation in last 11 years was recorded.

EXPERIMENTAL DESIGN

In order to simulate grazing cessation, four permanently fenced plots (50 × 50 m each) were erected in May 2005 at four experimental sites: Oidui (*Oid*), Igaratza (*Iga*), Alotza (*Alo*), and Uzkuiti (*Uzk*). Around each excluded plot (*E* level) we delineated a grazed plot (*G* level) where sheep, cattle, and horses were allowed to graze continuously during the vegetative period (from May to October or November). All four sites were located on flat terrain, and differed only slightly in

pH and elevation (except *Oid*), but more noticeably in soil texture and grazing intensity (Table 2.2). The thickness of the organic layer (defined as soil A horizon plus fresh litter), the soil temperature, the soil water content (as a measure of available moisture in $m^3\ m^{-3}$), and forage quality were surveyed between 2010 and 2013 (after five to eight years of exclusion). There is a strong seasonal pattern in the Park (Table 2.1), so the effect of season was included in the analyses. January and February were used to represent winter, and July and August represented summer. The organic layer was measured in 2012; for this, we took 20 measurements in each experimental unit by digging a hole in the soil with a spade and directly measuring the organic layer using a metallic ruler. Soil temperature and water content were measured at 15 cm soil depth and at 2-hour intervals from April 2011 to March 2013, using Em50 data loggers connected to an ECH2O sensor system (Decagon Devices Inc., Pullman, WA, USA). Soil temperature data were only collected at two of the experimental sites (*Oid* and *Iga*). Forage sampling was carried out in July 2010, at the point of peak standing biomass. For this, three subsamples (1 m^2 each) were randomly collected to represent each experimental unit. The following variables were measured in % DM^{-1}: phosphorus (P), measured by a colorimetric determination technique; crude protein (CP), i.e. nitrogen content, measured by the Kjeldahl method and multiplied by 6.25; neutral detergent fibre (NDF), measured by the Weende method; and enzymatic digestibility (NDFcel), the enzymatic solubility of NDF in cellulase (Riveros and Argamenteria, 1987). The first three relate to forage quality, and the fourth is a direct measure of digestibility.

Table 2.2. Elevation, pH, grazing intensity, and texture measured at the four study sites.

Site	Elevation (m.a.s.l.)	pH	Grazing intensity				Texture (%)		
			L.U. (ha day^{-1})	Sheep (%)	Cattle (%)	Horse (%)	Sand	Silt	Clay
Oidui	876	4.95	2.4	46	21	33	34	36	30
Igaratza	1,247	4.75	2.3	63	0	37	34	34	32
Alotza	1,224	5.05	4.5	54	12	34	20	42	38
Uzkuiti	1,300	5.05	4	40	26	34	17	44	39

Annotations: L.U. = Grazing Livestock Unit. A cattle of over 2 years is taken as 1.0 L.U. All other grazing stocks are given equivalents as follows: sheep (0.122), horses (1.2).

DATA ANALYSIS

To assess the differences in soil mean temperature and soil daily temperature variation between the *E* and *G* plots, four time series were decomposed into trend, regular, and irregular (random) components. We used an additive decomposition model for the time series (Montgomery et al. 1990; Makridakis et al. 1997). Each time series corresponded to a combination of site (*Oid* and *Iga*) and year (2011 and 2012), and they comprise the grazing period, which also represents the vegetative period. Prior to decomposition, graphical inspection confirmed that all the series could be considered as additive combinations of seasonal, trend, and irregular components (Gardner, 1985). This model can be expressed as: $X_t = m_t + s_t + W_t$, $t = 1, 2, 3,\ldots$ where m_t is the trend component, s_t is the seasonal (regular) component of period T that satisfies $s_t = s_{t+T}$ for all t and $s_1 + \ldots + s_T = 0$, and W_t is the irregular (random) component. Since we used daily temperatures, the seasonal component represented the daily oscillation in temperature. Consequently, a period of $T = 12$ (one record every two hours) and a centred moving average of order 61 proved to be experimentally adequate. Models were fitted using the *stl* function in the stat package R (R Core Team, 2012). The assumption that the irregular component has a mean of zero and constant variance, i.e. $E(W_t) = 0$ and $V(W_t) = \sigma^2$, was evaluated graphically.

To test for the effect of abandonment on soil water content, winter and summer soil water contents were defined using the above criteria and used as response variables. The blocking factor *site* (four levels: *Oid*, *Iga*, *Alo,* and *Uzk*), and the fixed factors *exclusion* (two levels: *E* and *G*) and *year* (two levels: *2011–12* and *2012–13*) were used as explanatory variables. The experiment had a randomized complete block design (RCB) (Casella, 2008). Since the measures were repeated in two different years, i.e. the experiment was replicated twice, the blocks (*sites*) were crossed with replications (*years*). Therefore, assuming that the interactions involving *exclusion* were zero to obtain a valid test of exclusion, this dataset could be modelled as described in Table 3.11 and equation 3.28 in Casella (2008). To test for the effect of abandonment on the thickness of the organic layer, the blocking factor *site* and the fixed factor *exclusion* were used as explanatory variables. In this

analysis, the measures were not repeated twice, so the model (a classical RCB) was more straightforward (Casella, 2008; Table 3.2, equation 3.1). Linear models were fitted using the *lm* function in the stats package in R (R Core Team 2012); the model residuals met the assumptions of homogeneity of variance and normality of error (Grafen and Hails, 2002).

To explore the effect of grazing abandonment on forage quality, experimental units were ordinated by measured forage properties (Q mode). We used the unconstrained non-metric multidimensional scaling (nMDS; Legendre and Legendre, 2012) method, based on Euclidean distances. To down-weight the effects of the most influential variables, and also to standardize by site, data were Hellinger-transformed before the distance matrix was constructed (Legendre and Gallagher, 2001). The nMDS was performed using the *metaMDS* function in the vegan package in R (Oksanen et al., 2012), which also requires the permute package (Simpson, 2012).

2.3. Results

Substantial differences in soil temperature variation were observed between treatment (*exclusion*) levels (Figs. 2.1 and 2.2). Soil temperatures (in 2011 and2012) in the *E* plots were approximately 23 times more stable in *Oid,* and about 7 times more stable in *Iga,* than in the *G* plots. For the *G* plots, the range of the regular (diurnal) temperature component was approximately 1.8°C, and the range of the irregular temperature component was approximately 1.3°C. In striking contrast, there was hardly any temperature variation in the *Oid E* plot, and for the *Iga E* plot the regular variation was approximately 0.4°C and the irregular variation amounted to a mere 0.1°C (Figs. 2.1 and 2.2; regular and irregular components of the decomposition). Average temperatures were higher in the *G* plots than in the *E* plots for almost all of the study. In *Oid*, the temperature was approximately 5°C higher in the *G* plot than in the *E* plot in both study years, whereas in *Iga,* the temperature was approximately 2°C higher in the *G* plot (Figs. 2.1 and 2.2; trend component of the decomposition).

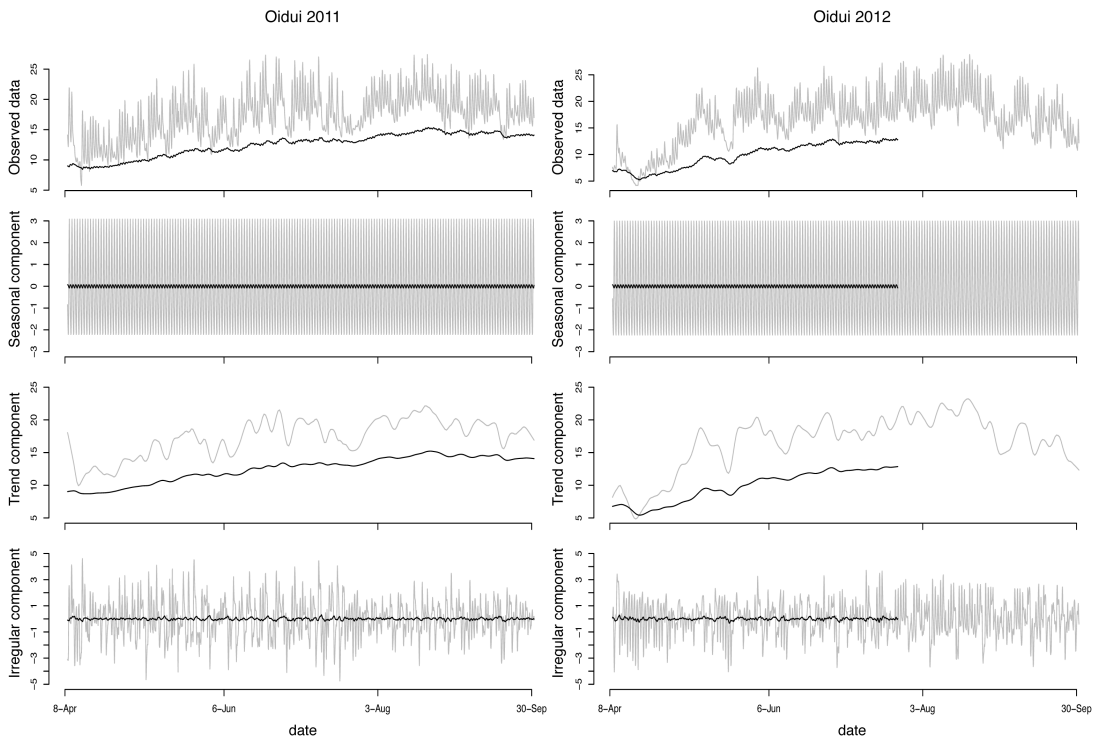

Fig. 2.1. Decomposition of daily temperature in Oidui, in 2011 (*left*) and 2012 (*right*). Original time series (*top*), regular variation or diurnality (*second*), trend component (*third*), and irregular variation (*bottom*) in Grazed (*G*) plots (*grey* line) and Excluded (*E*) plots (*black* line). Note that the irregular and regular temperature variations in Oidui *E* are almost zero. The second year *E* time series is shorter because the logger malfunctioned.

Overall, exclusion reduced the soil water content, although this assertion needs further explanation. In the summer, *E* significantly reduced the soil water content from 0.285 cm^3/cm^3 to 0.195 cm^3/cm^3, with a standard error for the difference of 0.015, which represents a 30% change. However, in the winter, the observed change was from 3.373 cm^3/cm^3 to 3.323 cm^3/cm^3, with a standard error of 0.006, which represents a mere 1.5% change. Moreover, the soil water content was 0.11 cm^3/cm^3 lower in the second summer than in the first one, with a standard error of 0.015 (Table 2.3). However, the summer model must be interpreted carefully. To interpret the *exclusion* effect confidently, this RCB statistical model requires that the interactions involving *exclusion* were non-significant, and the *site* × *exclusion* interaction was marginal; otherwise, the *F*-test numerator might be inflated and there is a risk of falsely declaring significance (Table 2.3). From the data, it seems that in the second summer, which was also the driest one, *exclusion* interacted with

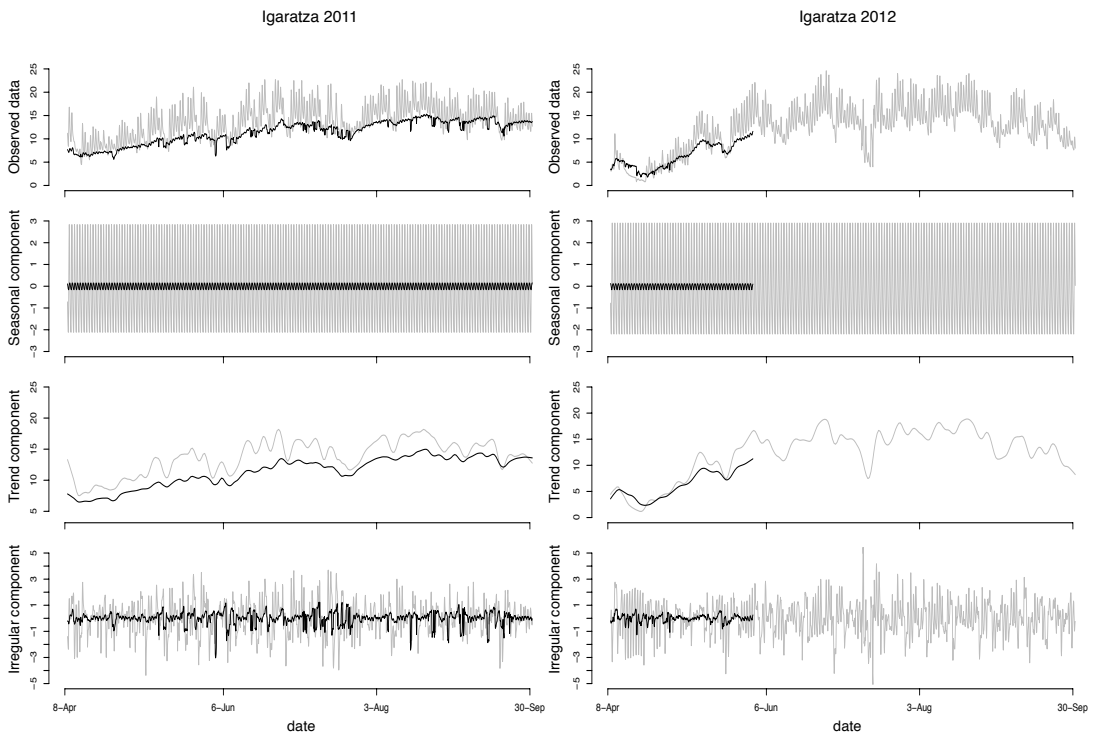

Fig. 2.2. Decomposition of daily temperature in Igaratza, in 2011 (*left*) and 2012 (*right*). Original time series (*top*), regular variation or diurnality (*second*), trend component (*third*), and irregular variation (*bottom*) in Grazed (*G*) plots (*grey* line) and Excluded (*E*) plots (*black* line). The second year *E* time series is shorter because the logger malfunctioned.

soil *texture.* The overall trend of more water in the *G* plots was reversed in *Alo* (mean water content: $G = 0.14$, $E = 0.164$) and *Uzk* (mean water content: $G = 0.272$, $E = 0.356$), which were the two clayey-silty sites (Table 2.2). This interaction may be the cause of the marginal *p*-value for the *site × exclusion* interaction.

The mean thickness of the organic layer was 7 cm under *E* and 2.75 cm under *G.* Therefore, exclusion significantly increased the organic layer thickness by 4.3 cm, with a standard error of 0.624 (Table 2.4).

Table 2.3. ANOVA table for the winter and summer soil water content.

Summer soil water content						Winter soil water content				
	df	SS	MS	*F*	*p*	df	SS	MS	*F*	*p*
year	1	0.046	0.046	24.481	0.016	1	0.001	0.001	3.181	0.216
site (blocks)	3	0.059	0.02	10.482	0.043	2	0.011	0.006	25.622	0.025
exclusion	1	0.032	0.032	17.399	0.025	1	0.008	0.008	34.749	0.028
year:site	3	0.004	0.001	0.72	0.603	2	<0.001	<0.001	1.106	0.475
site:excl	3	0.034	0.011	6.134	0.085	2	0.002	0.001	5.075	0.165
year:excl	1	0.002	0.002	1.336	0.33	1	<0.001	<0.001	0.292	0.643
Residual	3	0.006	0.002			2	<0.001	<0.001		
R^2-Adj: 0.847						R^2-Adj: 0.894				

Regarding the nutritional quality of the forage, the nMDS results (Fig. 2.3) show that exclusion reduced forage quality. The *E* and *G* plots tended to separate on the first axis of the ordination; plants on *E* plots were more fibrous (higher FND) and had lower protein contents, whereas plants on *G* plots were more suitable to decomposition (higher FNDcel). Livestock exclusion appeared to have no effect on P content, which depended more on site.

Table 2.4. ANOVA table for the thickness of organic layer.

Thickness of organic layer					
	df	SS	MS	*F*	*p*
Site (blocks)	3	0.711	0.237	0.304	0.822
Exclusion	1	37.195	37.195	47.769	0.006
Residuals	3	2.336	0.779		
R^2-Adj: 0.865					

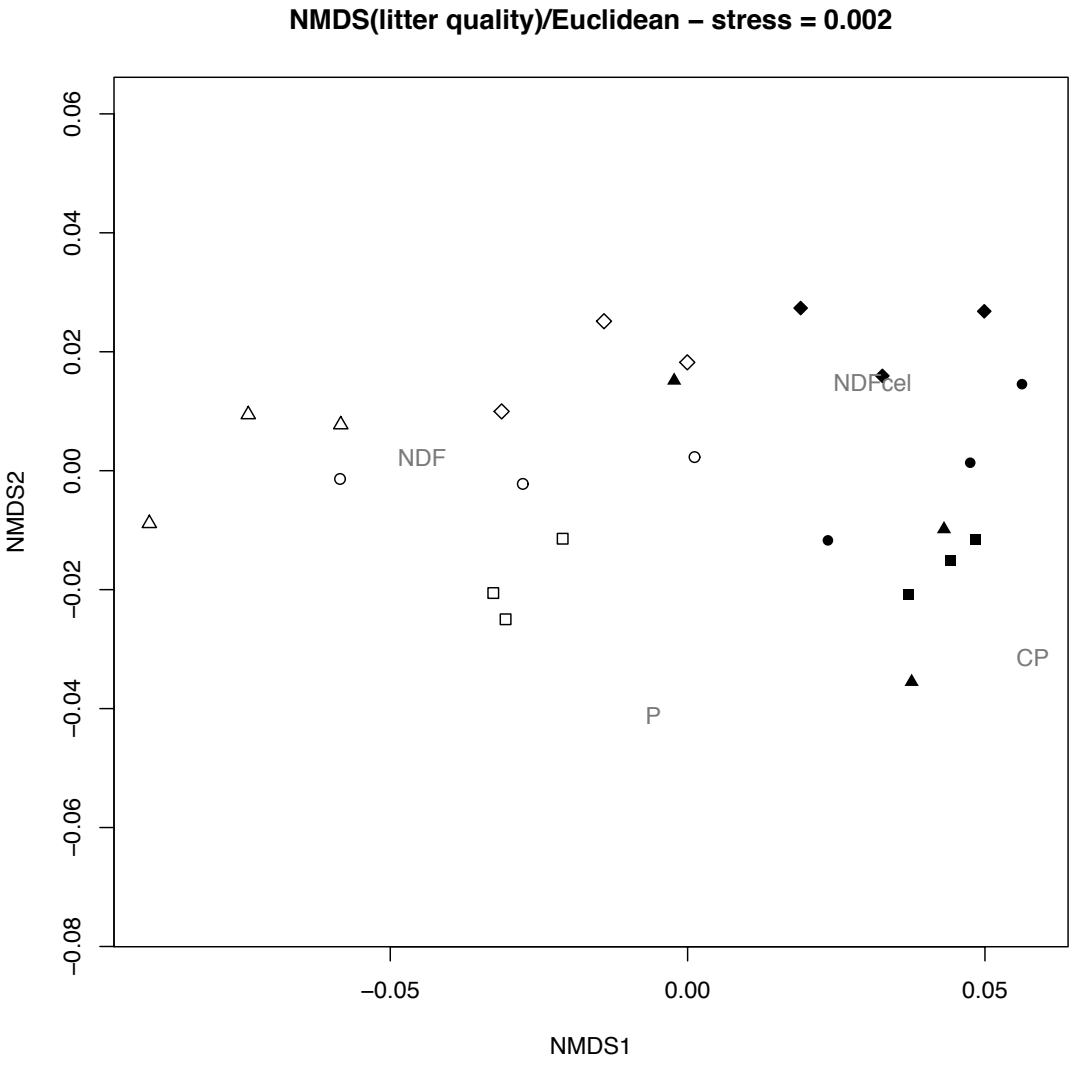

Fig. 2.3. Non-metric multidimensional scaling (nMDS) of experimental units based on forage descriptors. Grazed (*filled* symbols) and excluded (*empty* symbols) plots for Uzkuiti (*squares*), Alotza (*circles*), Oidui (*triangles*), and Igaratza (*diamonds*). Centroids of forage descriptors (*grey* letters) are also displayed: P: phosphorus, CP: crude protein, NDF: neutral detergent fibre, NDFcel: enzymatic digestibility.

2.4. Discussion

In this study, livestock exclusion was experimentally simulated in order to evaluate how grazing modulates forage quality, and to investigate the effects of precipitation and solar radiation on the soil thermal regime and water content (which in turn interacts with soil texture). Although we used a small number of replicates, an effect of grazing exclusion on the measured variables was detectable. In fact, given that the power of a statistical analysis is determined not only by the

number of replicates but also by the effect size and the amount of random variation (Underwood, 1997), the large effect size observed in this experiment compensated for the relative paucity of data.

EFFECT OF GRAZING ON SOIL TEMPERATURE

Our data show that the daily temperature variability in the soils of grazed areas can be as large as seasonal variability in ungrazed areas. To our knowledge, very few studies of grasslands have measured soil temperature both longitudinally and at a high frequency (e.g. Gan et al., 2012; Zhao et al., 2011); when measured, daily variations receive little attention. In the summer, grazing is generally considered to increase the seasonal soil temperature by increasing the amount of radiation reaching the soil (Gan et al., 2012; Risch et al., 2007; Shan et al., 2011; Zhao et al., 2011, 2010), an effect confirmed in our study. However, our data suggest that it should also be considered as increasing short-term temperature variability.

As expected, the thickness of the organic layer increased under grazing abandonment, corroborating the fact that soil in fenced plots is more insulated from the atmosphere. This probably occurs because consumption and trampling by grazers reduces the amount of plant residues returned to the soil, and compacts the organic layer, respectively (Bilotta et al., 2007). Aalto et al. (2013) demonstrated that spatially structured variability in the thickness of the soil organic layer affects soil temperature variability. Our results support this link, and show that the organic layer increases when grazers are excluded.

We observed completely different thermal regimes in the *G* and *E* areas. Insulation made daily temperature variation almost undetectable in the *E* plots, whereas there was remarkable daily temperature variation in the *G* plots. Soil temperature had three main sources of variation: short-term regular variation (i.e. circadian cycle), short-term irregular or unpredictable variation (e.g. cloudy or sunny short periods within days or for whole days), and seasonal variation. Because we had already used a regular component to model daily change in temperature, true seasonal variation, which in our data was only for a few months and not for whole years, was modelled as a trend component. This trend component, corresponding

to seasonal change, was almost the sole source of variation in the *E* plots, as daily temperature was constant and remained unaltered by short-term environmental events. Conversely, the range of short-term variation in the *G* plots was as large as the range of seasonal variation. These differences between grazing regimes were more apparent in the *Oid* plot (i.e. the lower altitude site). Here, productivity was higher, which promoted greater biomass accumulation (thicker organic layer), and vegetation cover was more developed in the *E* plot (Aldezabal et al., 2010).

EFFECT OF GRAZING ON SOIL WATER CONTENT

The effects of grazing on soil water content are very complex, and different responses have been reported for different environments and grazing regimes. Grazing has been shown to increase soil water content (Bremer et al., 2001; Chanasyk and Naeth, 1995; Li et al., 2011), reduce soil water content (Day and Detling, 1994; Donkor et al., 2002), and have no effect on soil water content (Coronato and Bertiller, 1996). Interactions with grazing intensity have also been observed (Gan et al., 2012). The disparity of these responses reflects the complexity of these interactions, which was also detected by our analyses: the water content response to grazing was dependent on environmental factors and soil properties. Soil texture and grazing intensity were confounded in our experiment, and it was impossible to disentangle the potential effects of both variables. However, as grazing intensity was moderate in all cases, the results were interpreted based on the different textures.

In order to illustrate the most important relationships between grazing, environmental factors, and soil properties, a flow diagram is presented in Fig. 2.4 in which the magnitude and relative importance of different processes (e.g. evaporation, transpiration, and infiltration) are indicated using arrows. Although exclusion generally reduced the soil water content, this relationship probably depends on an interaction with grazing, soil texture, and solar radiation (Bremer et al., 2001; Greenwood and McKenzie, 2001; Schrama et al., 2013a). Soil texture was a key factor in understanding the relationship between grazing and soil water content. In the two sandy sites (*Oid* and *Iga*; Table 2.2), exclusion resulted in a decrease in water content, indicating that infiltration in coarse-textured soils is the

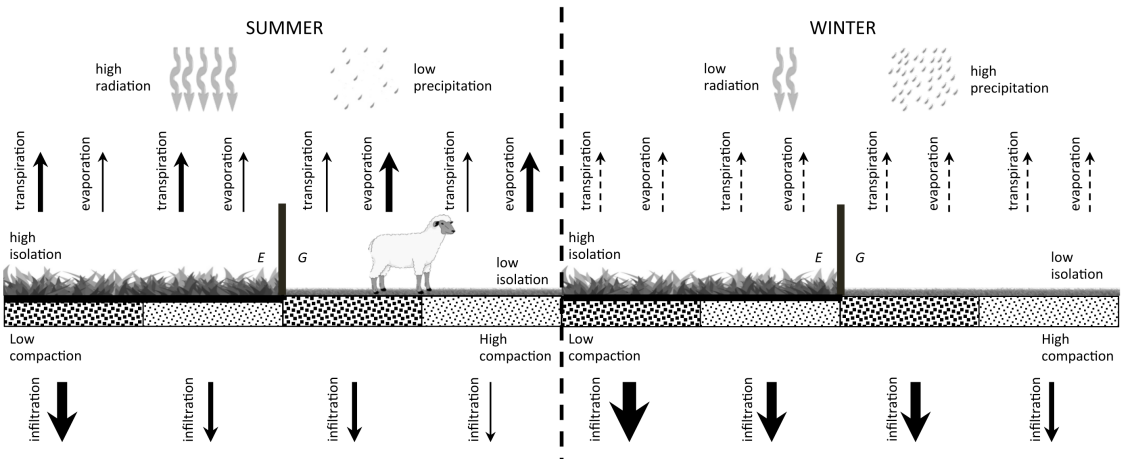

Fig. 2.4. Diagram illustrating the relationships between evaporation, transpiration, and infiltration in different seasons (summer and winter), grazing regimes (grazing [*G*] and exclusion [*E*]), and soil textures (coarse-textured, fine-textured). Different arrow formats indicate the relative importance of a given process; the thicker the arrow, the greater the relative incidence. The dashed arrow indicates the lowest incidence. Note that different arrow formats, although based on existing theory, were subjectively selected in order to graphically present the results obtained.

primary factor associated with water loss, independent of environmental conditions. In this habitat, grazing augmented the water-holding capacity, probably through moderate soil compaction and the reduction of continuous soil pores (Gan et al., 2012; Schrama et al., 2013a). However, in silty-clayey sites, where infiltration is less important, the relationships are more complex; in the winter, when solar radiation and plant activity are very low, transpiration and evaporation are also very low, and water content differences may be determined by infiltration. However, during the summer, when solar radiation and plant activity are high, the relationship between evaporation and transpiration may determine the water content (Bremer et al., 2001). As previously mentioned, grazing increases evaporation and reduces transpiration through defoliation (Bremer et al., 2001). Our results suggest that when higher amounts of solar radiation are received, as was observed in the 2012–2013 summer period (Table 2.1), evaporation in the *G* plots (less insulated) exceeds transpiration in the *E* plots (more plant biomass), resulting in higher water content levels in the excluded areas. In contrast, during periods of low solar radiation, as in the 2011–2012 summer period, which was very cloudy, and unusually low solar radiation was measured (the lowest in the last 11 years, Table 2.1), water content levels were

higher in the *G* plots than in the *E* plots. Under conditions of low solar radiation, higher transpiration in the *E* plots (more plant biomass) was probably more relevant than evaporation in the *G* plots (less insulation), and, consequently, the effect of grazing was reversed.

EFFECT OF GRAZING ON FORAGE QUALITY

Forage quality, as expected (Bardgett et al., 1998; Wardle et al., 2004), was reduced in the *E* plots. This effect can be produced either by the replacement of species with low carbon-nitrogen ratios by more dominant, lower-quality species (Milchunas and Lauenroth, 1993; Semmartin et al., 2004), or by the physiological responses of individual species (Bardgett et al., 1998). The effects observed in the present study were, probably, a result of both mechanisms.

INTEGRATED VIEW

Grazing accelerates soil processes through its effects on soil temperature and fluctuations, soil water content (Butenschoen et al., 2011; Poll et al., 2013; Schrama et al., 2013b), and forage quality (Bardgett and Wardle, 2003), and all these effects were detected in our experiment. Higher soil temperatures accelerate nutrient cycling and microbial activity when there is no limitation of water availability and labile resources (Frey et al., 2008; Poll et al., 2013). Our unpublished data provide evidence of increased microbial enzymatic activity and a decreased metabolic quotient (qCO_2) in the experimental *G* plots. The first is related to faster nutrient cycling and mineralisation, the second, to more efficient resource utilisation (Anderson and Domsch, 2010).

The impact of different mean temperatures on soil processes and microbial communities is well-known (Butenschoen et al., 2011; Dell et al., 2012; Liu, 2013). However, the potential effects of temperature variability on these processes have only been considered in studies at high latitudes, and in the context of freeze-thaw cycles (Yergeau and Kowalchuk, 2008). At such latitudes, freeze-thaw cycles have been reported as increasing denitrification and mineralization (De Luca et al., 1992). Although there are rarely freeze-thaw cycles in temperate mountain grasslands, the large soil temperature variation observed in grazed plots, both on a

daily and a seasonal basis, may act similarly on soil processes, accelerating nutrient cycling and mineralisation. Moreover, these large differences in short-term temperature fluctuations require further research to determine their biological effects. Harsher "continental-like" variations in temperature occur under grazing conditions, whereas under excluded conditions more constant "oceanic-like" variation is observed.

In light of the complexity of the relationships between different grazing intensities and soil factors, the disparity of responses observed in different ecosystems (Gass and Binkley, 2011; Shan et al., 2011; Su et al., 2004; van Wijnen et al., 1999; Wang et al., 2010) is not surprising. In this study, the water content response was the most complex of the measured variables. The effect of grazing was even reversed in the second summer, with a low water content measured in the *G* plots (i.e. less insulated). This indicates that water could become limiting under periods of low precipitation and high solar radiation, with consequences for nutrient cycling. Climatic change predictions of an increased frequency of extreme heat periods (Tank and Konnen, 2003) would accentuate these consequences. However, in the context of global change, a progressive decrease in the number of livestock is expected in Atlantic agro-ecosystems (Rounsevell et al., 2006); this is a phenomenon that should be considered in future research and management.

2.5. Conclusions

The exclusion of grazers retarded nutrient cycling in the soil, by modifying forage quality, soil water content, and, in particular, the soil thermal regime. However, the mechanisms involved are highly complex, and need more investigation in order to be fully understood. Our findings show that future experiments attempting to simulate grazing in productive grasslands will benefit from including in their designs short-term temperature fluctuations, a hitherto neglected but nevertheless important effect of grazing.

Acknowledgements

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CHAPTER 3. Grazing exclusion unleashes competitive plant responses in Atlantic mountain grasslands

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Abstract

Questions: Does the absence of equalizing mechanisms after cessation of grazing unleash strong competitors to create large patches in the community? Do these competitive intraspecific aggregations displace and exclude weaker species, thereby reducing species diversity?

Location: Atlantic grasslands in the Aralar Natural Park, Basque Country, Northern Spain (three field sites located at 42° 59' 9.25'' N, 2° 2' 9.7'' W; 43° 0' 10.6'' N, 2° 5' 22'' W; and 43° 0' 50'' N, 2° 4' 3''W) .

Methods: Large herbivores were experimentally excluded from three sites in a productive semi-natural system of grassland with long history of grazing, using paired grazed plots as experimental controls. After nine years of experimental exclusion, one hundred quadrats were systematically placed in each of the six plots using a spatially explicit layout. Floristic composition and abundance, as well as eight hydrological and chemical soil properties, were measured in each quadrat. The spatial structures created by competitive species were analysed using Redundancy analysis in conjunction with Moran's Eigenvector Maps, disentangling effects exclusively due environmental heterogeneity. Competitive exclusion was further determined using linear regressions between species richness and abundance of competitive species.

Results: Grazing exclusion unleashed competitive species such as *Festuca rubra*, *Agrostis capillaris* or *Trifolium repens*, which became dominant in the exclusion plots and created large spatial patches. Furthermore, a negative linear relationship, consistent across field sites, was observed between species richness and abundance of competitive species in the exclusion plots. This confirmed that grazing acts as an equalizing mechanism that prevents large intraspecific aggregations and hence allows for species coexistence, promoting plant species diversity.

Conclusions: This work demonstrates that experimental cessation of continuous disturbance by herbivores (a powerful equalizing mechanism) in productive grasslands unleashes strong competitors. These competitors, in turn, create large intraspecific spatial aggregations that out-compete weaker species, thereby reducing plant diversity. Future work in contrasting ecosystems, and further integration of grazing theory with other ecological disciplines, may contribute to a

better understanding of the effects of grazing on species interactions and biodiversity.

Key-words: coexistence; competitive exclusion; disturbance; herbivory; plant diversity; productivity; Moran's eigenvector maps; spatial structure.

3.1. Introduction

Almost one hundred years ago, Tansley & Adamson (1925) published the results of the first grazer-exclusion experiment, in which experimental fencing was used to examine the effects of grazer-exclusion (sheep and rabbits) on the productive English chalk grasslands. 11 years following the placement of experimental fencing, Tansley & Adamson (1925) found that the exclusion of sheep and rabbits enhanced the growth and expansion of the more competitive grasses, whereas it resulted in the decline of many weaker species. Since then, the effects of grazing on grassland diversity have been repeatedly tested and widely discussed, leading to significant efforts (Milchunas et al. 1988; Westoby et al. 1989; Cingolani et al. 2005; Oesterheld & Semmartin 2011) that have framed the inconsistent effects of grazing in different ecological and evolutionary contexts.

These efforts allowed for a comprehensive understanding of the wide range of responses by the world's vegetation to grazing. In particular, Milchunas *et al*. (1988) proposed a generalised model founded on the intermediate disturbance hypothesis (Grime 1973; Connell 1978). Based on this model, the effects of grazing on the diversity and species composition of plant communities depend on humidity (or aboveground productivity), evolutionary history and grazing pressure. The generalised model of Milchunas *et al*. (1988) was later modified by Cingolani *et al*. (2005) to include the state-and-transition model of Westoby *et al*. (1989). This modification made the original model of Milchunas *et al*. (1988) more explanatory and applicable—particularly for grazing systems with a short evolutionary history. In these systems, no selection has occurred and grazing is more likely to promote irreversible changes. This model, which is mainly theoretical, has been subsequently confirmed by two meta-analyses (Milchunas &

Lauenroth 1993; Proulx & Mazumder 1998) and several empirical studies (Frank 2005; Bakker et al. 2006; Lezama et al. 2014). According to the model, in productive grasslands with a long evolutionary history of grazing, divergent selection has occurred for traits that increase resistance to grazing and the capacity to compete for light, resulting in a species pool of both short and tall species. Under such conditions, where competition is strongly dependent upon grazing intensity, the relationship between plant diversity and grazing intensity is assumed to be nearly hump-shaped (with maximum diversity at medium grazing intensity) and grazing intensity is known to have strong effects on both species composition and community physiognomy (Milchunas *et al*. 1988; Cingolani *et al*. 2005).

In productive grasslands, where nutrient availability is higher and there are fewer limiting resources (i.e. niche dimensions), niche stabilization mechanisms are expected to be weak (Harpole & Tilman 2007) and competitive species are expected to exclude weaker species by out-competing them for light (Hautier et al. 2009). Under these conditions, an equalizing mechanism, such as disturbance by non-selective shoot herbivory, may play a greater role in species coexistence (Wilson 2011). In fact, grazing reduces species height and lateral spread differences (Deléglise et al. 2011) and consequently, the ability of a species to compete for light and space. Nevertheless, plant communities are affected by both equalizing and stabilizing mechanisms (Chesson 2000). Species coexistence is pluralistic (Adler et al. 2007), and relative contributions of niche vs. neutral processes depend on the species, and the temporal (Stokes & Archer 2010) and spatial (Chase 2014) scales considered.

Grazing mediates spatial heterogeneity in grasslands by modulating plant inter- and intraspecific interactions (Adler et al. 2001; Deléglise et al. 2011; Zhang et al. 2013; Meyers et al. 2014), and in turn, patterns generated by species interactions are directly related to diversity (Chesson 2000). For example, processes generating intraspecific spatial aggregation can lead to competitive exclusion (He & Legendre 2002). Particularly in perennial grasslands, defoliation and trampling by herbivores can reduce clonal extension and aggregation by several

mechanisms: reduction in clonal mobility (Bullock et al. 1994; Tamm et al. 2002), decrease in distance of lateral spread (Smit et al. 2010; Benot et al. 2011), limitation of internode length (Amiaud et al. 2008), and fragmentation of clone patches (Charpentier et al. 1998). Hence, the exclusion of grazing can promote these size-based processes, enhancing interspecific negative interactions relative to intraspecific ones (Zhang et al. 2013) and leading to competitive exclusion of weaker species (Pavlu et al. 2007; Mayer et al. 2009).

Despite increasing awareness regarding the spatial nature of diversity and species interactions (Gardner & Engelhardt 2008), to our knowledge, few experimental works have addressed grazing effects on species interactions and diversity with a spatially explicit design (Adler & Lauenroth 2000; Deléglise et al. 2011; Zhang et al. 2013). Herein, we address this gap of knowledge by conducting a spatially explicit experiment in which large herbivores were excluded from three sites in a productive, semi-natural grassland with long history of grazing. Specifically, we tested the hypothesis that grazing exclusion will favour competitive species, which will form large spatial clusters or patches, excluding weaker species and reducing species diversity. From this hypothesis, the following predictions are derived: (1) after grazing abandonment, disturbance will cease, and in the absence of equalizing mechanisms, competitive species will be released and will create large patches, and (2) these competitive aggregations will displace and exclude weaker species, reducing species diversity. We used an environmental model to control niche processes and a spatial model to capture vegetation patterns, which allowed us to isolate biotic processes derived solely from the termination of equalizing mechanisms after cessation of disturbance by herbivores.

3.2. Materials and Methods

STUDY AREA

Experiments were conducted in three replicate sites in semi-natural grasslands located in Aralar Natural Park (see Fig. S1 in Appendix S1 in Supporting Information): *Site 1* (Igaratza: 42° 59' 9.25'' N, 2° 2' 9.7'' W; 1247 m.a.s.l) (see Fig. S2 in Appendix S1), *Site 2* (Alotza: 43° 0' 10.6'' N, 2° 5' 22'' W; 1223 m.a.s.l) (See

Fig. S3 in Appendix S1), and *Site 3* (Uzkuiti: 43° 0' 50'' N, 2° 4' 3'' W; 1300 m.a.s.l) (see Fig. S4 in Appendix S1). Aralar Natural Park is an 11,000-ha protected area located in the Basque Country (Northern Spain). The area has oceanic climate, with a mean annual temperature of 12.4°C and annual precipitation of >1,400 mm. The area traditionally used by livestock (beef cattle, dairy sheep, and horses) occupies about 2,077 ha (18.9% of the park area), but its usage varies seasonally (from May until November). The primary vegetation type is a highly productive native grassland on a calcareous substrate (Gibbons and Moreno 2002), which contain mainly perennial species (Loidi 1982) and is included in code 6230 of the Habitat Directive (European Commission 2013).

EXPERIMENTAL DESIGN

In order to simulate grazing cessation, three permanent fenced plots (50 m × 50 m each) were installed in May 2005 at the three experimental sites. Next to each exclusion plot (*E* level), we delineated a grazed plot (*G* level), where sheep, cattle and horses were allowed to graze continuously during the vegetative period (from May until November). Hence, we used six field plots (three sites × two treatments): *Site 1 G*, *Site 1 E*, *Site 2 G*, *Site 2 E*, *Site 3 G*, and *Site 3 E*. All three sites were located on relatively flat terrain and, when fences were erected (2005), functional groups composition (graminoids, non-legume forbs and legumes) was not significantly different in E vs. G plots at each field site (see Table S1 in Appendix S1). In each plot, 100 sampling points were located using a spatially explicit layout in which sampling points were located 2 m apart from each other, thus creating plots with an extent of 18 m × 16 m, and a constant spacing of 2 m.

VEGETATION AND SOIL SAMPLING

Sampling was conducted during the growing season of 2014, following nine years of grazer exclusion. At each sampling point, floristic composition and the most relevant soil variables were measured. Floristic composition and structure were measured using two types of abundance metrics in quadrats (overlaid on the sampling points) of 0.5 m × 0.5 m: (i) species frequencies in 49 subquadrats of 0.07 m × 0.07 m each (to avoid underestimating common, but small-sized species) and (ii) species percentage cover (to capture species local densities). Plant

nomenclature follows standard floras (see Table S2 in Appendix S2). The measured soil variables were as follows: pH, water content, and macronutrients, including Kjeldahl total nitrogen (N) (mg L^{-1}), available phosphorus (P) (mg L^{-1}), available potassium (K) (mg L^{-1}), calcium (Ca) (mg L^{-1}), and magnesium (Mg) (mg L^{-1}). In each quadrat, soil water content was measured, using Delta-T SM 150 Soil Moisture Kit, at fixed intervals during the growing season. Mean soil water content (MSWC) (%) was then derived for each quadrat using the area under the curve, which was computed via the R function *trapz*() in package *pracma* (Borchers 2015). Soil analyses were performed following ADAS standard procedures (Jackson et al. 1986). Raw values of measured soil values are in Table S3 in Appendix S2.

STATISTICAL ANALYSIS

All statistical analyses were performed separately for each field plot, and results for exclusion plots were compared to adjacent grazed plots in the same site (pairwise comparisons).

To test the first prediction, which was that competitive species form large patches when grazers are excluded, variation in floristic composition was partitioned in a broad-scale spatial fraction (representing large plant clusters), a medium/fine-scale spatial fraction (representing small plant clusters), and an environmental fraction (intended to partition out patterns generated by environmental constraints, i.e. induced spatial variation) (Peres-Neto et al. 2006). Using environmental models to control niche effects allowed the emergence of equalizing processes (Legendre & Legendre 2012). Variance partitioning was performed using Redundancy Analysis (RDA) (Legendre & Legendre 2012). The response was a Hellinger-transformed (Legendre & Gallagher 2001) frequency species matrix, in which only species occurring in at least 5% of the quadrats were considered. The spatial component was modelled using sets of independent spatial variables constructed using the Moran's eigenvector map (MEM) method (Dray et al. 2006; Legendre & Legendre 2012). MEM spatial variables model positive and negative spatial correlation and were constructed using weighted connectivity matrices (Borcard et al. 2011). The best spatial model for each field plot was selected using

the corrected Akaike information criteria (AICc). Spatial variables were sorted visually into broad and fine/medium-scale structures (Borcard et al. 2011). The environmental component was defined by fitting parsimonius models (one model per field plot) using a double stop forward selection criterion (Blanchet et al. 2008). Variable selection resulted in different models for each plot, possibly because of strong correlations between soil variables (see Figs. S5, S6, S7, S8, S9, S10, and Table S4 in Appendix S2).

To test the second prediction that under herbivore exclusion, large aggregations of competitive species reduce species richness through competitive exclusion of weaker species, the analysis was conducted in three steps. First, species richness was partitioned into a biotic fraction (determined by local abundance of competitive species), an environmental fraction and a spatial fraction, by using RDA. Next, linear models were used to assess the relationship between species richness and the biotic variable, after controlling for spatial autocorrelation and environmental effects. The biotic variable was derived by adding the cover of four abundant, long-spreading, stoloniferous species (*Festuca rubra* L., *Agrostis capillaris* L., *Galium saxatile* L. and *Trifolium repens* L.) and two tussock-forming graminoids (*Deschampsia flexuosa* L. and *Avenula marginata* (Lowe) Holub subsp. *sulcata* (J.Gay ex Delastre) Franco), which create small but very dense monospecific patches. Given that our goal was to control for spatial autocorrelation, and hence avoid inflation of type I errors and conduct valid significance tests (Peres-Neto & Legendre 2010), only the MEMs with positive spatial autocorrelation were used and they were not separated by scale in this case. Before regressing species richness on the biotic variable, to remove spatial autocorrelation and the effects of environmental variables, both variables were tested (regressed) against the environmental and spatial models, respectively. Additional regressions were fitted using the residuals of the previous models, and as such consider only the biotic effects. The model residuals met the assumptions of homogeneity of variance and normality of error. Lastly, the null hypothesis of no effect of E on the estimate of the slope (β) of the regressions between species richness and the biotic variable was tested, using as explanatory variables the

blocking factor *Site* (*Site1*, *Site2*, *Site3*) and fixed factor *Treatment* (*Grazing*, *Exclusion*).

All analyses described above were performed using R version 3.0.2 (R Core Team 2013), and the following specific packages were used: *ade4* (Dray and Dufour 2007), *ggplot2* (Wickham 2009), *gridExtra* (Auguie 2012), *gstat* (Pebesma 2004), *Matrix* (Bates and Maechler 2015), *packfor* (Dray 2013), *permute* (Simpson 2014), *sp* (Pabesma and Bivand 2005), *spacemakeR* (Dray 2013), *spdep* (Bivand 2014), *vegan* (Oksanen *et al*. 2015), and *VennDiagram* (Chen 2014). Supporting information provides the R coding (see Appendix S3) and data (see Appendix S4) to replicate the analysis.

3.3. Results

Six species (*Agrostis capillaris*, *Avenula marginata*, *Deschampsia flexuosa*, *Festuca rubra*, *Galium saxatile*, and *Trifolium repens*) were considered as potentially dominant species, but because of local particularities, not all of them occurred at high densities in all plots. For example, in *Site 1 E* only *F. rubra* occurred at high local densities, with quadrat coverage of up to 90%, whereas in *Site 2 E*, the plot with the greatest co-dominance, all six species occurred at high local densities: *F. rubra* (up to 66%), *T. repens* (33%), *A. capillaris* (63%), *G. saxatile* (34%), *D. flexuosa* (80%) and *A. marginata* (32%) (Table S2).

Table 3.1. Contribution of species to canonical axes. Contributions of species to spatially-structured community composition created by biotic processes at each field plot (first canonical axes of the broad-scaled non-environmental fractions), as deduced from variance partitioning in community composition graphically presented in Figures 1, 2 and 3. Cells with shaded backgrounds indicate the species contributions of greatest magnitude.

Species	Site 1		Site 2		Site 3	
	G	E	G	E	G	E
Achilea millefolium	-0.03	–	–	–	0.4	–
Agrostis capillaris	0.17	-0.13	0.19	0.62	0.04	0.43
Aphanes arvensis	-0.01	–	–	–	–	–
Avenula marginata subsp. sulcata	–	–	–	-0.01	-0.12	0.06
Bellis perennis	-0.3	–	0.03	–	–	–
Campanula scheuchzeri	–	–	0.07	-0.09	-0.08	0.02

Carex caryophyllea	-0.48	-0.03	0.12	–	0.04	-0.02
Cerastium fontanum	0.12	0.13	-0.07	-0.08	-0.51	-0.09
Crocus nudiflorus	0.09	0.08	–	–	–	–
Danthonia decumbens	-0.07	0.25	-0.03	-0.02	0.47	-0.01
Deschampsia flexuosa	–	–	–	-0.14	–	–
Erica vagans	0.05	–	–	–	–	–
Festuca heterophylla	–	–	–	–	-0.13	–
Festuca rubra	-0.03	0.07	0.09	0.05	0.01	0.23
Galium saxatile	0.33	-0.01	0	0.05	-0.24	-0.75
Hieracium pilosella	-0.29	-0.05	0.11	0.04	0.1	–
Hypochoeris radicata	–	0.05	–	0.01	–	–
Jasione laevis	0.32	0.17	-0.59	-0.31	0.45	-0.07
Lotus corniculatus	-0.42	-0.25	–	-0.03	0.1	–
Luzula campestris	-0.05	0.11	-0.11	0	0.02	-0.16
Merendera montana	0	0.01	–	–	–	–
Moenchia erecta	-0.01	–	–	–	–	–
Moehringia trinervia	-0.06	–	–	–	–	–
Poa annua	–	–	0.02	–	0.03	–
Poa pratensis	–	–	–	–	-0.04	0.02
Polygala serpyllifolia	0.02	0.14	0.11	–	-0.02	–
Potentilla erecta	0.12	0.16	0.04	-0.52	-0.01	-0.24
Potentilla montana	-0.1	-0.39	0.2	-0.4	0	–
Ranunculus bulbosus	-0.05	-0.02	0.02	–	0.02	–
Rumex acetosella	0	-0.06	–	–	–	–
Sagina procumbens	-0.07	–	–	–	0.01	–
Stellaria graminea	0.19	-0.74	–	–	–	-0.14
Trifolium repens	0.26	0.14	0.02	0.07	-0.12	-0.28
Veronica arvensis	0	–	0.01	–	–	–
Veronica officinalis	-0.04	0.03	-0.7	-0.21	-0.1	0.03

As expected, spatially structured biotic-induced variation in species composition had greater effects in exclusion plots than in the corresponding grazed plots (Figs. 3.1, 3.2, 3.3; Table 3.1). Specifically, broad-scaled MEMs retained more non-environmental variance (generated by biotic processes) under *E* treatments than in the corresponding *G* treatments in all three sites; it was nearly three times greater in *Site 1* (Fig. 3.1) and approximately twice as great in *Sites 2* (Fig. 3.2) and *3* (Fig. 3.3). The results in *Sites 2 E* and *3 E* meet the prediction that in exclusion plots large patches would appear, which represented intraspecific aggregations of competitive species and the absence of outcompeted species. In *Site 2 E*, the non-environmental broad-scaled structure was mainly determined by the presence of the dominant, long-spreading, stoloniferous species *A. capillaris*, and the absence

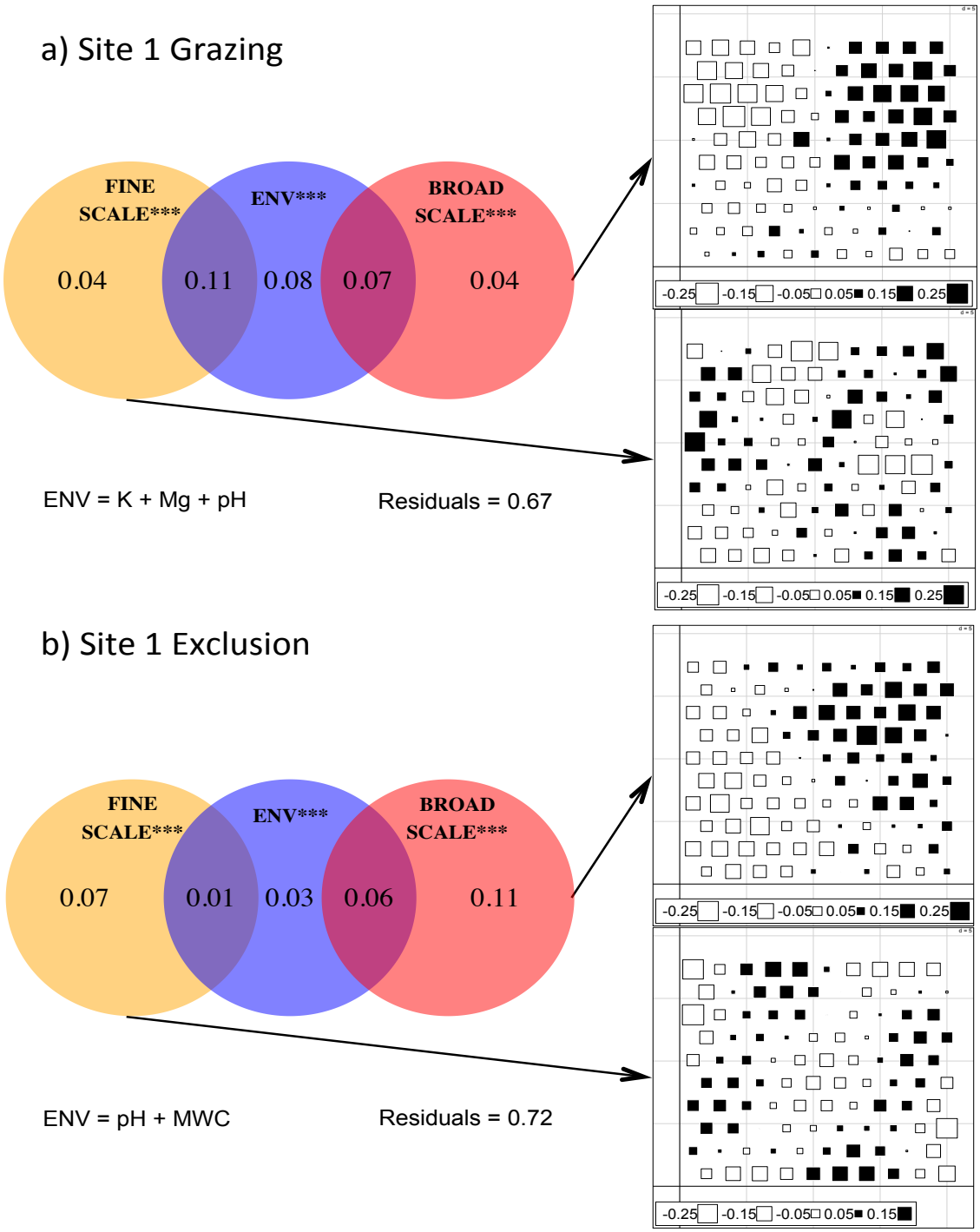

Fig. 3.1. Venn diagrams of variance partitioning of species composition. Venn diagrams corresponding to the RDA variance partitioning of species composition into a broad-scaled spatial fraction (representing large plant patches), a medium/fine-scaled spatial fraction (representing small plant patches) and an environmental fraction for a) *Site 1 Grazing* and b) *Site 1 Exclusion*. Overlap between the spatial fractions and the environmental fraction represent spatially structured environmental variation; non-environmental spatial fractions represent structure in species composition generated by biotic processes. Numbers are adjusted R^2 values. Summary tables of the RDA environmental models are given in Table S5 in Appendix S2.

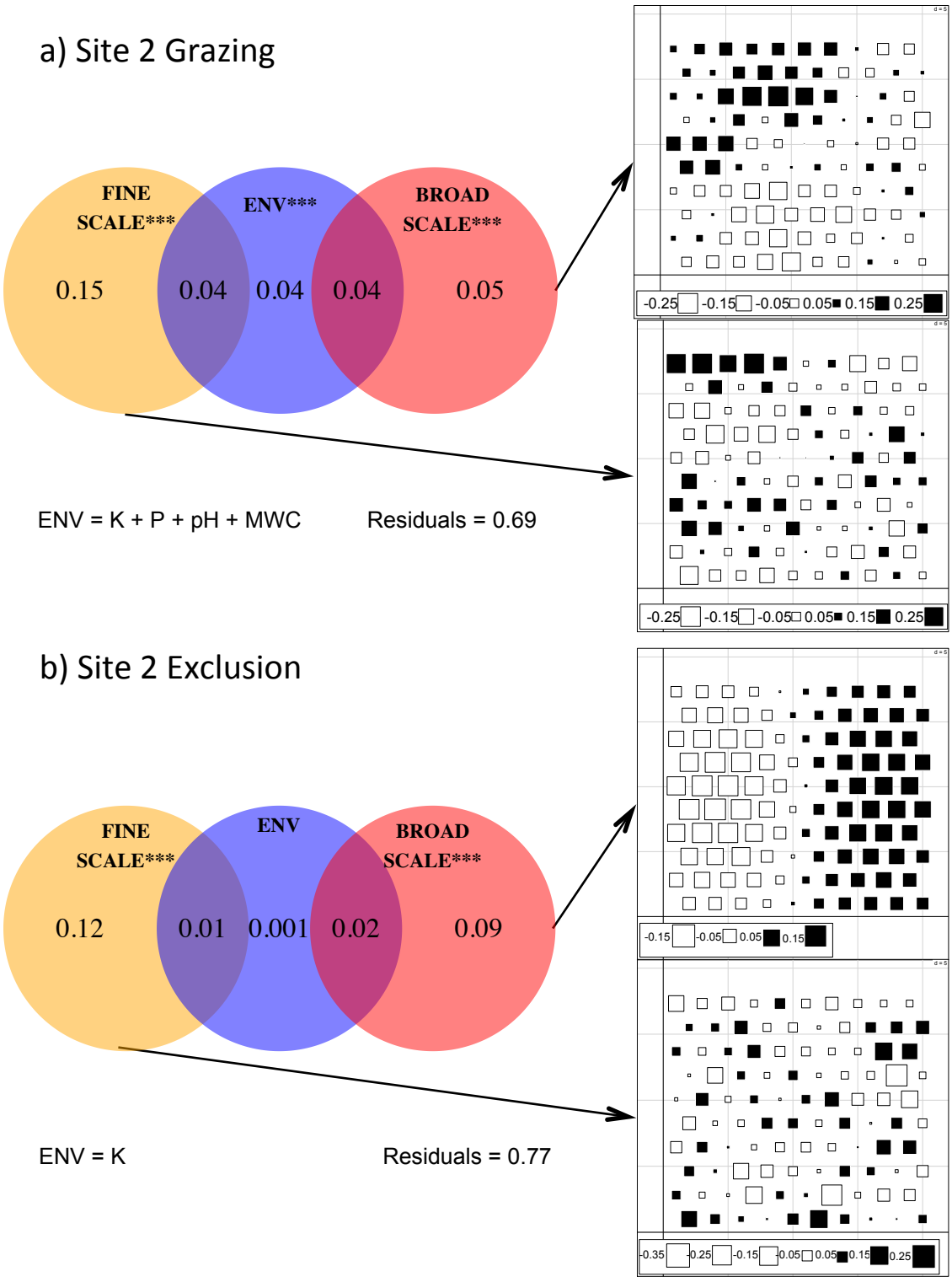

Fig. 3.2. Venn diagrams of variance partitioning of species composition. Venn diagrams corresponding to the RDA variance partitioning of species composition in a) *Site 2 Grazing* and b) *Site 2 Exclusion*. Summary tables of the RDA environmental models are given in Table S5 in Appendix S2.

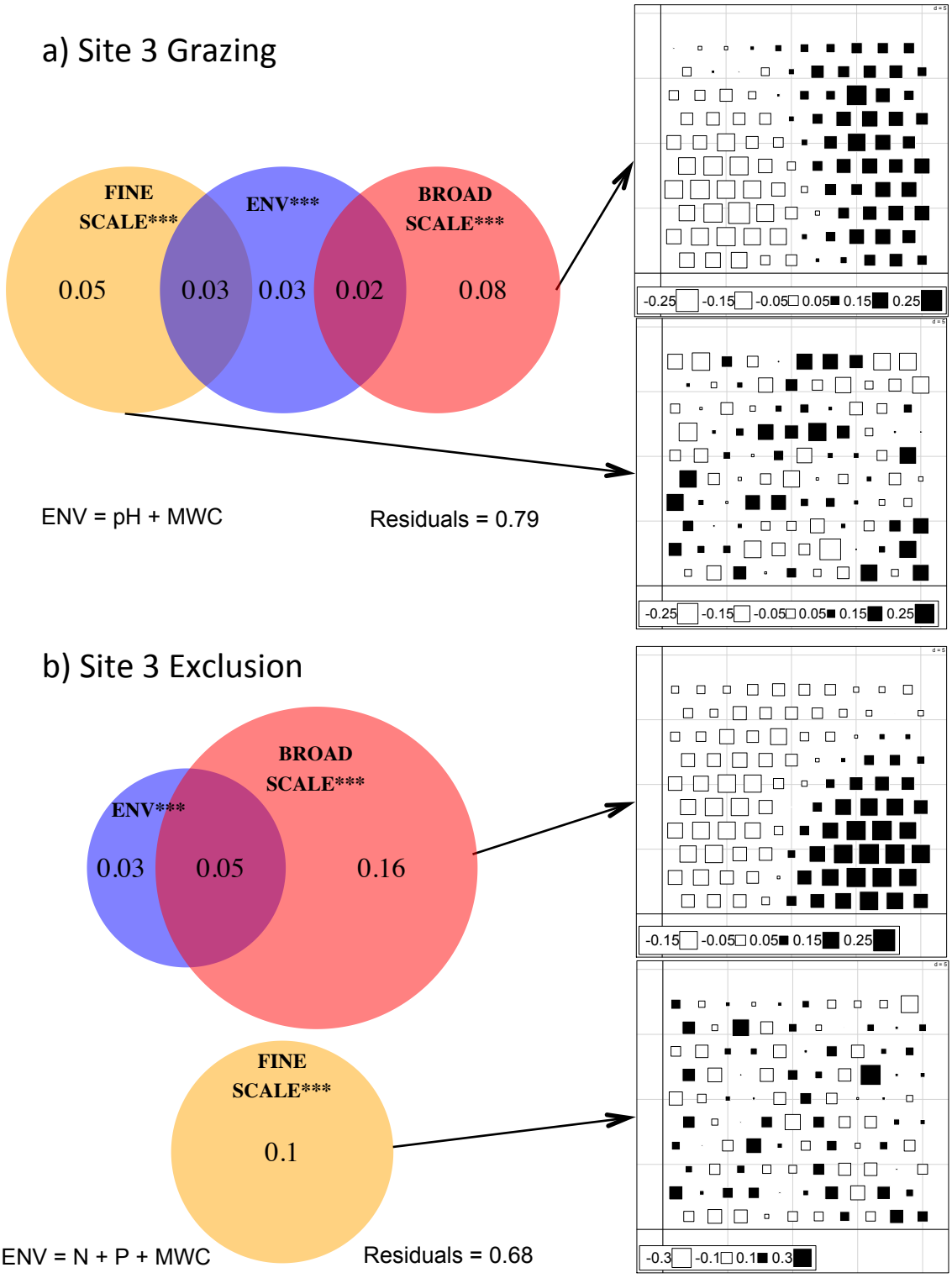

Fig. 3.3. Venn diagrams of variance partitioning of species composition. Venn diagrams corresponding to the RDA variance partitioning of species composition in a) *Site 3 Grazing* and b) *Site 3 Exclusion*. Summary tables of the RDA environmental models are given in Table S5 in Appendix S2.

of *Jasione laevis*, *Potentilla erecta*, *P. montana* and *Veronica officinalis* (Table 3.1). In *Site 3 E* the broad-scaled structure was related to the presence of the dominants *F. rubra* and *A. capillaris,* and the absence of *G. saxatile*, *T. repens,* and *P. erecta* (Table 3.1). In *Site 1 E* and in the three grazed plots, the broad-scaled structure was defined by contrasts of other species (Table 3.1); for example, in *Site 3 G*, the presence of *Danthonia decumbens* and *J. laevis*, and the absence *Cerastium fontanum* and *G. saxatile* defined the broad-scaled structure

Table 3.2. ANOVA table. ANOVA table for the linear model testing the null hypothesis of no effect of grazing exclusion on the estimate of slope (β) in the regressions between species richness and the biotic variable (abundance of competitive species).

Estimate of β					
	df	SS	MS	*F*	*p*
Field site (blocks)	2	0.001	0.0005	2.9208	0.2551
Treatment	1	0.0023	0.0023	13.6356	0.0661
Residuals	2	0.0003	0.0002		
R^2-Adj = 0.77					

As expected, we found that under *E*, competitive species reduced species richness through local patch invasion and subsequent exclusion of weaker species (Figs. 3.4, 3.5, 3.6; Table 3.2). The biotic variable derived by adding the coverage of the most competitive species explained more variance under *E* than under *G* conditions. In *Sites 1 G* (Fig. 3.4a) and *2 G* (Fig. 3.4c), the pure biotic fraction was only 0.01 and models were not significant, whereas in *Sites 1 E* (Fig. 3.4b) and *2 E* (Fig. 3.4d), the purely biotic fraction retained 0.09 and 0.06 variance, respectively. The biotic fraction was significant in both *Sites 3 G* and *3 E*, but in the *E* treatment, it explained 1.8 times more variance than in the *G* treatment (Fig. 3.4e, f). On the other hand, the linear models relating species richness and the corresponding biotic variable showed there was a negative linear relationship between species richness and abundance of competitors in all *E* plots and the *Site 3 G* plot (Fig. 3.5b, d, e, f). Even in *Site 3*, the relationship was stronger in the *E* plot; the regression explained 2.2 times more variance and the slope of the relationship was 2.3 times steeper (Fig. 3.5e, f). There was no significant relationship in *Sites 1 G* and *2 G* (Fig. 3.5a, c). The analysis of the effect of *E* on the slope of the above relationships (i.e. on the estimates, *b*, of the slopes, *β*) provided moderate evidence against the null hypothesis of no effect of *E* ($F_{1,2}$ = 13.6; *p*-value = 0.066) (Table 3.2). The absolute

Fig. 3.4. Venn diagrams of variance partitioning of species richness. Venn diagrams corresponding to the RDA variance partitioning of species richness into a biotic fraction (abundance of competitive species), an environmental fraction and a spatial fraction for all the experimental field plots a), b), c), d), e) and f). The purely *biotic* fraction represents the effect of competitive species after removing the effect of spatial autocorrelation and environmental constraints.

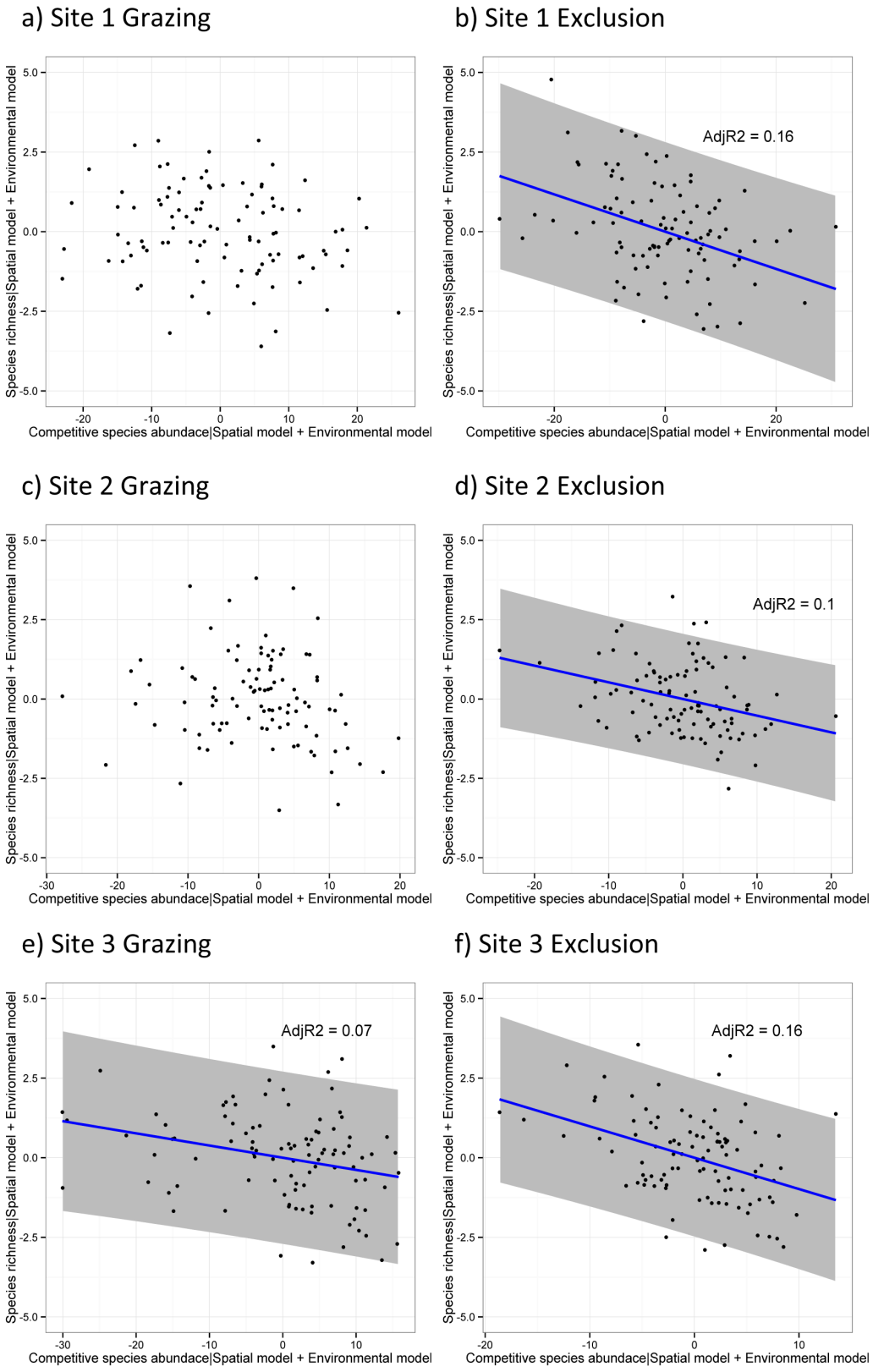

Fig. 3.5. Linear regressions. Linear regressions between species richness and the biotic variable (abundance of competitive species) for all experimental plots a), b), c), d), e) and f). Prior to regressing species richness on the *biotic* variable, the effects of environmental constraints and spatial autocorrelation were cancelled out in both the response (species richness) and the independent biotic variable.

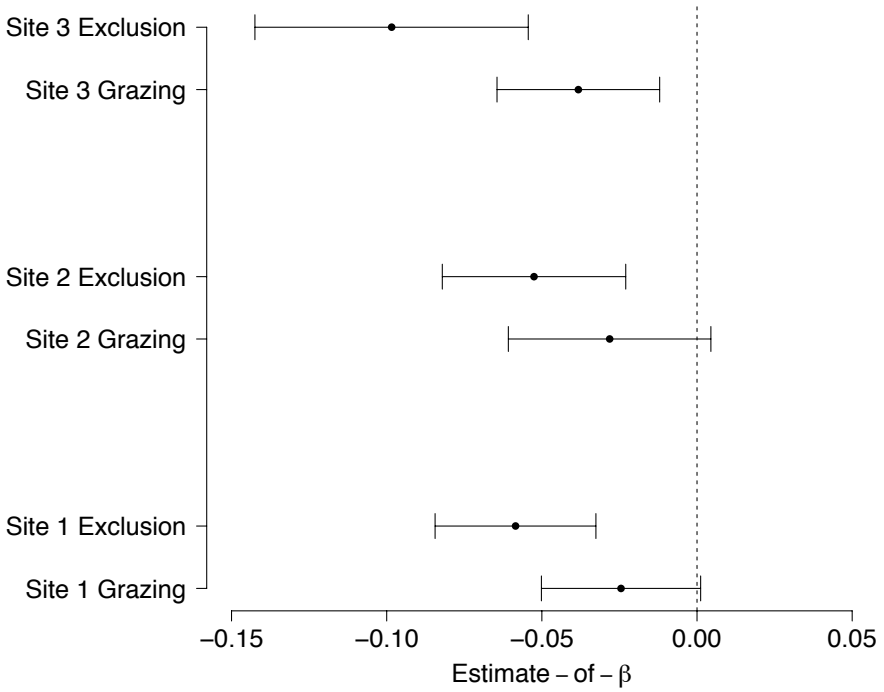

Fig. 3.6. Estimates (*b*) of the slope of linear regressions**.** Estimates (*b*) of the slope parameter (β) with 95% confidence intervals for the regression analyses shown in Fig. 5.

value of the effect size was 0.04 ± 0.01 with a 95% confidence interval of (0.02, 0.06) (Fig. 3.6).

3.4. Discussion

In the productive grasslands of Aralar Natural Park, grazing exclusion (i.e. the suppression of the equalizing mechanism of grazing) unleashed a few long-spreading stoloniferous species, particularly the tall grasses *F. rubra* and *A. capillaris*, which quickly outcompeted the smaller species. These findings are similar to the results published by Tansley & Adamson (1925) in the first known grazer-exclusion experiment, in which tall competitive species were reported to invade and dominate exclusion plots at the expense of smaller and weaker species. All these results fit in the prediction for productive grasslands according to the

generalised model of grazing (Milchunas et al. 1988; Cingolani et al. 2005) and the intermediate disturbance hypothesis (Grime 1973; Connell 1978), where cessation of continuous disturbance by herbivores, i.e. the equalizing mechanism of grazing, leads to competitive exclusion by tall grasses. Although both the grazing-diversity relationship (Milchunas et al. 1988; Milchunas & Lauenroth 1993; Hickman et al. 2004; Lezama et al. 2014) and the ecology of coexistence (Chesson 2000; Adler et al. 2007; Stokes & Archer 2010; Chase 2014) are extensively studied fields, the spatial ecology of grazing has been much less studied (Adler & Lauenroth 2000; Adler et al. 2001; Deléglise et al. 2011; Zhang et al. 2013; Meyers et al. 2014). For this reason, in this work we detail the effects of grazing exclusion on vegetation spatial patterns, as well as the relationship of these patterns with biotic interactions between species, particularly competitive exclusion.

As predicted, the competitive species that dominated the exclusion plots created large spatial patches. For example, *Festuca rubra* and *Agrostis capillaris* were strongly and positively associated with the broad-scaled spatial structures in *Sites 2 E* and *3 E* (Table 3.1). However, in *Site 1 E*, the large patches were not positively associated with any of the hypothetically competitive species, and negative associations primarily drove the canonical axis (Table 3.1). The type of abundance measurements used in the models may explain this apparent inconsistency. Although frequencies provide more detailed spatial information about the distribution of small-sized species (because they reflect the intraquadrat distribution), they "underestimate" the variance of very abundant species that are present in almost all of the sampled subquadrats (see Fig. S10 in Appendix S5). For example, *F. rubra* was the only species in *Site 1 E* with high quadrat coverage (up to 90%), but it was present in almost 100% of the subquadrats (4,897 out of 4,900); therefore, its spatial variance was very small in terms of frequency. Nevertheless, the negative associations of other species with the broad-scaled structures may well be a response to competition with *F. rubra*. In fact, *F. rubra* invaded and excluded less competitive species in *Site 1 E*. When the analyses of the biotic effect on species richness in *Site 1 E* was repeated (Figs 3.4b and 3.5b) using only the abundance of *F. rubra* as the explanatory variable (instead of the sum of the six

potentially competitive species), the results remained essentially unchanged (see Fig. S11 in Appendix S5).

For productive grasslands with abundant resources, in which there are only weak stabilization by niche effects (Harpole & Tilman 2007), ecological theory (Hautier *et al*., 2009) predicts that, in the absence of equalization by disturbance (i.e. grazing), the most competitive species will exclude weaker species via a light-competition mechanism. Because grazing exclusion leads to stronger competitive interactions between species, our results agree with this prediction. A negative linear relationship was observed between species richness and abundance of competitive species in the three studied grasslands (Figs. 3.4, 3.5, 3.6), thus confirming that grazer exclusion can lead to competitive exclusion of weaker plant species (Pavlu et al. 2007; Mayer et al. 2009). These results suggest the presence of both weak (but not null) stabilization by niches and strong equalization by shoot herbivory, in the Aralar Natural Park's highly productive grasslands. Our results, therefore, emphasize the importance of equalizing mechanisms in productive grasslands, but they nevertheless support the pluralistic nature of species coexistence (Adler et al. 2007). Niche effects usually emerge at large spatial scales, when higher habitat heterogeneity is encompassed (Chase 2014), and although given the fine scale of our study, significant environmental models were fit in five out of six field plots. Particularly in *Site 1*, environmental models explained a relatively high amount of variation in community composition (Fig. 3.1). Despite the small spatial extent of the field plots, pH exhibited high variation in *Site 1* (ranged from 4.3 to 7.6) and strongly affected community composition.

The effects of grazer exclusion were more pronounced in *Site 3*; in all the analyses, *Site 3 E* exhibited the strongest evidence of intraspecific aggregation and competitive exclusion, and all species except *F. rubra* and *A. capillaris* were displaced or reduced to very low abundance. The effect of grazing on diversity depends on site productivity (Milchunas & Lauenroth 1993; Proulx & Mazumder 1998; Frank 2005; Bakker et al. 2006; Lezama et al. 2014). Recent studies in these field plots have reported higher MSWC (Odriozola et al. 2014) and aboveground productivity based on the normalized difference vegetation index (NDVI)

measurement, in *Site 3* compared to *Sites 1* and *2* (Aldezabal *et al*. 2010a), as well as a positive relationship between productivity and available moisture (Aldezabal *et al*., 2010b). Moreover, we found that *Site 3* exhibited the highest MSWC (58%) compared to *Site 1 (*38%) and *Site 2* (51%). *Site 3* is facing the sea, whereas the topography of the mountains of the Aralar Natural Park prevents storms from reaching the other two sites directly, particularly *Site 1*. This might explain the recorded measures of MSWC and NDVI. However, patch aggregations and competitive exclusion were also unexpectedly more marked in *Site 3 G* compared to the other grazed plots. The claimed mechanism of competitive exclusion in productive grasslands is based on competition for light (Hautier et al. 2009), which should be neutralised in the presence of strong herbivory. This suggests that a mechanism other than competition for light might be playing a role in competitive exclusion.

Relative abundance and local distribution of species are key features to understanding species diversity (He & Legendre 2002). He and Legendre (2002) proposed that if a mechanism creates regular spatial distributions of species, that mechanism would promote species coexistence, whereas mechanisms creating species aggregations and clumped distributions would impede species coexistence. Our results agree with the prediction that large aggregations of competitive species following grazer exclusion lead to competitive exclusion of weaker species. In contrast to our results, grazer exclusion prevented large intraspecific aggregations in grasslands of the Tibetan Plateau and in North Dakota (Zhang et al. 2013; Meyers et al. 2014). Despite the contrasting mechanism, their results also support the above hypothesis, because large aggregations of overgrazing tolerant species displaced grazing intolerant species, reducing species diversity (Zhang et al. 2013; Meyers et al. 2014). The removal of grazing disturbance led to loss of diversity in our study, whereas the high intensity of grazing disturbance led to loss of diversity in grasslands of the Tibetan Plateau and in North Dakota. Interestingly, at both extremes of the grazing/disturbance intensity axis, where diversity declines (Grime 1973; Connell 1978; Milchunas et al. 1988; Cingolani et al. 2005), the observed process is large intraspecific aggregation of tall competitive vs. overgrazing tolerant species (He & Legendre 2002). Grasslands in the Tibetan

Plateau are overgrazed and have low productivity because of low temperatures and precipitation (Zhang et al. 2013), and in North Dakota, the expansion of invasive species under grazed conditions (Meyers et al. 2014) are indicative of a short evolutionary history of grazing; thus the reduction of diversity under grazing (Milchunas et al. 1988). By contrast, Deléglise *et al*. (2011) did not find differences in intraspecific aggregation of species between grazed areas and long-term exclusion plots. However, in contrast with our own analytical work, their work, and the works of Meyers *et al*. (2014) and Zhang *et al*. (2013), did not explicitly account for the effects of niches by means of environmental models. For this reason, the underlying gradients in environmental variables may have impeded the detection of patterns generated by biotic processes after grazer exclusion.

Grazer exclusion can lead to more marked spatial patterns in local species distribution, thus enhancing spatial heterogeneity (Adler & Lauenroth 2000; Adler et al. 2001). Our results also show higher spatial dependence under grazer exclusion because the purely spatial fractions explained more variance in the *E* plots than in the *G* plots (Figs. 3.1, 3.2, and 3.3). However, the expansion of the few most competitive species, which were in fact responsible for the broad-scaled spatial structures, excluded other species and implied grassland homogenisation and loss of biodiversity.

3.5. Conclusions

In summary, this work illustrated that experimental cessation of continuous disturbance by herbivores, a strong equalizing mechanism, unleashed strong competitors that, in turn, created large intraspecific spatial aggregations and out-competed weaker species, thereby reducing plant diversity. Therefore, we conclude that extensive grazing is an important factor for the maintenance of plant diversity and spatial heterogeneity at fine scales in Atlantic mountain grasslands. Future work in contrasting ecosystems, and the integration of grazing theory with other ecological disciplines, may continue to contribute to a better understanding of the effects of grazing on species interactions and biodiversity.

Acknowledgements

We are indebted to all the people that took part in fieldwork, particularly to Maite Lopez de Arbina, Ana Etxeberria and Maddi Arzak, and to Jose Antonio Irastortza (guard of the Aralar Natural Park) for his assistance in fieldwork and logistical support. We thank Fortin Lab members for useful comments during I.O.'s stay at their laboratory. Soil variables were analysed in Fraisoro Agricultural Laboratories. This study was supported by an FPI-EHU grant to I.O. (4597/2011), and funded by the Basque Government (grant reference: IT299-10), and by the Ministry of Economy and Competitiveness of the Spanish Government (grant reference: AGL2013-48361-C2-1-R).

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3.7. Supplementary material

Appendix S1. Study area, sites details, original functional composition of experimental plots, full species names and species covers. (pp: 81–87)

Appendix S2. Further details about soil variables and environmental model. (pp: 88–98)

Appendix S3. R code. (Available online upon publication) (pp: 99–111)

Appendix S4. Site 3 dataset. (Available online upon publication)

Appendix S5. Frequencies vs. Covers. (pp: 112–114)

Appendix S1. Study area, Sites photographs, original functional composition of experimental plots, full species names and species abundances.

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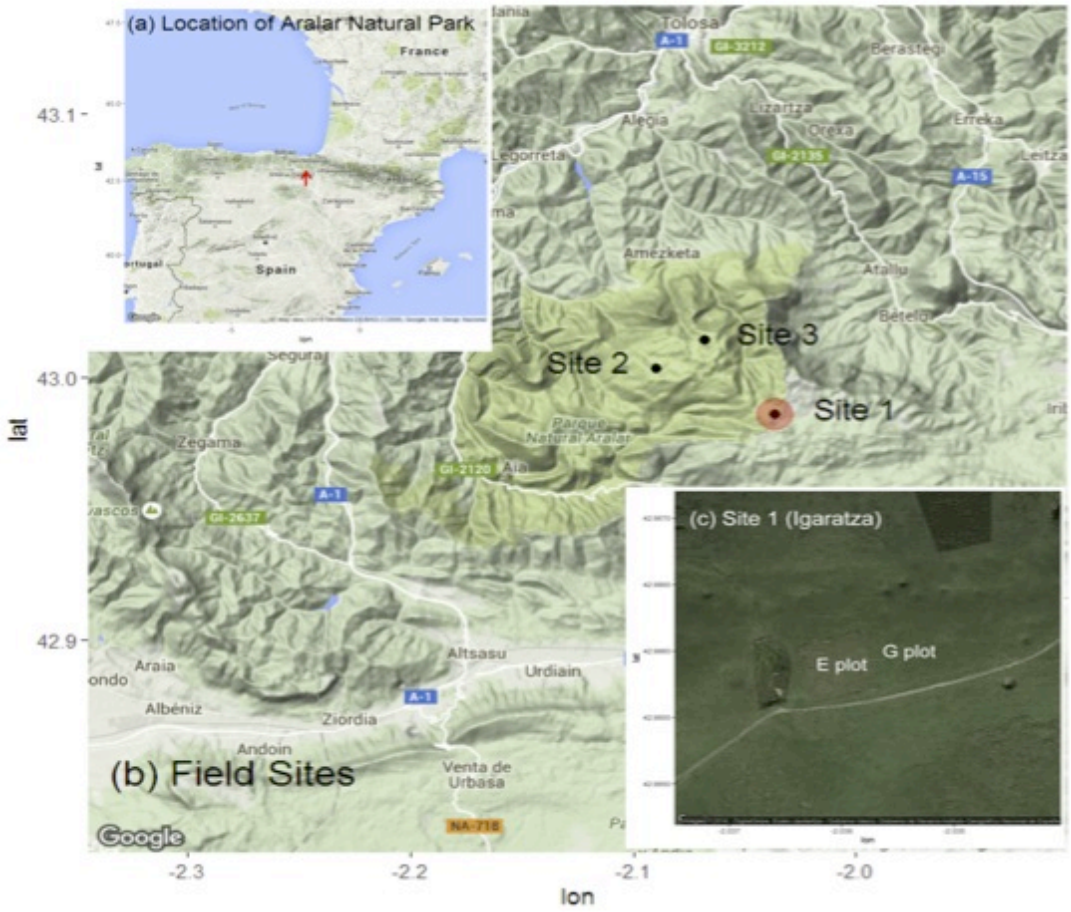

Fig. S1. Sampling design. A grassland system in the Aralar Natural Park, Basque Country, Northern Spain was selected (a), where three field sites were used (b). In each site, two field plots (Grazing and Exclusion) were established in 2005 (c). The environmental variables and the abundance of vascular plant species were measured in each of the six field plots using 100 regularly placed 0.5-m^2 quadrats (adding up to six hundred quadrats). The red circle in (b) indicates the field site (Igaratza-Site 1) for which detail is shown in (c). G plot = Grazing plot (control); E = Exclusion plot (fenced plot).

Fig. S2. Igaratza site (Site 1 in the main text, 42° 59' 9.25'' N, 2° 2' 9.7'' W: 1247 m.a.s.l, Aralar Natural Park, Basque Country, Northern Spain).

Fig. S3. Alotza site (Site 2 in the main text, 43° 0' 10.6'' N, 2° 5' 22'' W: 1223 m.a.s.l, Aralar Natural Park, Basque Country, Northern Spain).

Fig. S4. Uzkuiti (Site 3 in the main text, 43° 0' 50'' N, 2° 4' 3'' W: 1300 m.a.s.l, Aralar Natural Park, Basque Country, Northern Spain).

Table S1. Functional composition of the experimental plots at the time in which herbivores were excluded. The results of Wilcoxon rank tests (*W* statistic) applied to E vs. G plots showed no difference in functional group composition for all three sites. Site 1: *W* = 135; *p-value* = 0.36 (graminoids); *W* = 97; *p-value* = 0.53 (non-legume forbs); *W* = 118.5; *p-value* = 0.82 (legumes). Site 2: *W* = 74; *p-value* = 0.45 (graminoids); *W* = 95; *p-value* = 0.83 (non-legume forbs); *W* = 92; *p-value* = 0.94 (legumes). Site 3: *W* = 156; *p-value* = 0.07 (graminoids); *W* = 100.5; *p-value* = 0.63 (non-legume forbs); *W* = 79.5; *p-value* = 0.18 (legumes).

Site	Treatment	Year	Df	Graminoids (%)		Non-legume forbs (%)		Legumes (%)	
				Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Site 1	G	2005	14	71.5	7.3	26.2	7.1	2.3	2.4
Site 1	E	2005	14	67.7	10.9	27.9	10.5	4.4	7
Site 2	G	2005	14	64	5.6	23.7	9.5	12.3	9
Site 2	E	2005	11	66.5	10.8	22.2	9.1	11.3	6.2
Site 3	G	2005	14	70.6	8	23.1	11.1	6.3	6.4
Site 3	E	2005	14	66.6	7.2	23.2	8.5	10.2	7.3

Table S2. Aralar plant community, Basque Country, Northern Spain. Summary of the species found in 600 0.25 m^2-quadrats (100 quadrats per field plot). Mean Cover (%) was computed for each experimental plot. G: Grazing, E: Exclusion. Symbol – indicates species absence and symbol + indicates species cover < 0.1 (%).

Species	Mean % Cover					
	Site 1		Site 2		Site 3	
	G	E	G	E	G	E
Achillea millefolium L.	0.7	–	–	–	0.4	–
Agrostis capillaris L.	2.8	1.4	12.5	10.4	25.2	35.7
Aphanes arvensis L.	0.1	–	+	–	+	–
Arenaria montana L.	+	–	+	–	–	–
Avenula marginata subsp. sulcata (J.Gay ex Delastre) Franco	–	–	+	0.7	+	0.7
Bellis perennis L.	2.3	+	+	–	+	–
Botrychium lunaria (L.) Sw.	+	+	–	–	–	–
Campanula scheuchzeri Vill.	–	–	0.3	0.2	0.2	0.1
Capsella bursa-pastoris (L.) Medik.	–	–	–	–	+	–
Carex caryophyllea Latourr.	1.2	1	0.4	+	0.3	0.1
Carduus sp. L.	–	–	+	+	–	–
Cerastium fontanum Baumg.	0.7	0.5	1	1	1	0.2
Crocus nudiflorus Sm.	1.8	0.9	–	+	–	–
Cuscuta sp. L.	–	–	–	+	–	–
Danthonia decumbens (L.) DC.	1.8	1.3	1.1	0.1	2.3	0.1
Deschampsia flexuosa (L.) Trin.	–	0.3	–	3	–	+
Erica vagans L.	0.1	0.1	–	–	–	–
Festuca heterophylla Lam.	–	–	–	–	0.2	+
Festuca rubra L.	50.5	57.6	43	39.3	38.4	54.5
Galium saxatile L.	5.1	6.8	13.6	17.4	5.4	1.8
Hieracium pilosella L.	10.6	5.9	0.3	0.1	0.1	+
Hypochoeris radicata L.	0.1	0.3	+	0.2	–	–
Jasione laevis Lam.	7	0.3	2.9	1	0.8	0.1
Koeleria vallesiana (Honck.) Gaudin	–	–	–	+	–	–
Leontodon pyrenaicus Gouan	–	–	+	–	–	–
Lotus corniculatus L.	0.9	1.5	0.1	0.3	0.1	0.1
Luzula campestris (L.) DC. in Lam. & DC.	3.5	1.6	5.8	2.9	3.5	1.1
Merendera montana (Loefl. ex L.) Lange in Willk. & Lange	0.3	0.1	+	–	–	–
Moenchia erecta (L.) G. Gaertn., B. Mey. & Schreb.	+	–	–	–	–	–

Moehringia trinervia (L.) Clairv.	+	–	–	–	+	–
Orchis sp. L	+	–	–	–	–	–
Plantago lanceolata L.	+	0.1	+	–	+	–
Plantago media L.	+	–	–	–	–	–
Poa annua L.	+	–	+	–	1	–
Poa pratensis L.	–	–	–	–	+	0.1
Polygala serpyllifolia Hosé	0.6	0.1	0.1	+	0.1	–
Potentilla erecta (L.) Raeusch.	1.1	5.6	0.9	2.7	1.3	3.1
Potentilla montana Brot.	0.6	3.3	0.2	1.2	+	+
Prunus sp. L.	–	–	–	+	–	–
Ranunculus bulbosus L.	0.1	0.2	+	–	0.1	+
Rumex acetosella L.	0.2	0.4	+	0.1	–	–
Sagina procumbens L.	+	–	–	–	+	–
Scilla verna Huds.	–	+	–	–	–	–
Stellaria graminea L.	0.3	2	–	0.1	–	0.2
Stellaria media (L.) Vill.	–	–	–	–	–	+
Trifolium repens L.	5.2	6.5	11.3	17.3	12.9	0.8
Vaccinium myrtillus L.	–	0.1	–	–	–	–
Veronica arvensis L.	0.3	–	+	–	–	–
Veronica chamaedrys L.	+	–	–	–	–	–
Veronica officinalis L.	0.3	1	1.6	1.4	0.3	0.1
Veronica serpyllifolia L.	+	–	–	–	+	–

Appendix S2. Descriptive statistics of soil variables, relationships among variables, marginal tests and definitive environmental models.

Grazing exclusion unleashes competitive plant responses in Atlantic mountain grasslands by I. Odriozola, G. García-Baquero, M. J. Fortin, N. A. Laskurain and A. Aldezabal.

Table S3. Descriptive statistics of measured soil variables in three grazed (G) and three non-grazed (E) field plots located in three sites of the Aralarmountain range , Basque Country, Northern Spain (100 quadrats per field plot).N: Nitrogen (mg l^{-1}), P: Phosphorous (mg l^{-1}), K: Potassium (mg l^{-1}), Mg: Magnesium (mg l^{-1}), Ca: Calcium (mg l^{-1}), MSWC: Mean Soil Water Content (%).

Soil variables	Site and treatment	Mean	S.d.	Max.	Min.	Range
N	Site 1 G	5,566	454	6,800	4,500	2,300
P	Site 1 G	8	2	14	3	11
K	Site 1 G	65	13	99	36	63
Mg	Site 1 G	64	12	114	36	78
Ca	Site 1 G	1,145	1,138	6,380	284	6,096
pH	Site 1 G	5.1	0.6	7.4	4.5	2.9
MSWC	Site 1 G	37	4	49	27	22
N	Site 1 E	5,814	485	7,200	4,700	2,500
P	Site 1 E	9	2	22	5	17
K	Site 1 E	60	10	89	32	57
Mg	Site 1 E	66	12	114	42	72
Ca	Site 1 E	985	1,164	6,070	222	5,848
pH	Site 1 E	4.9	0.6	7.6	4.3	3.3
MSWC	Site 1 E	38	4	48	28	20
N	Site 2 G	7,529	808	9,700	5,700	4,000
P	Site 2 G	9	3	18	5	13
K	Site 2 G	110	20	205	74	131
Mg	Site 2 G	83	14	126	49	77
Ca	Site 2 G	370	80	798	216	582

pH	Site 2 G	4.6	0.1	4.9	4.3	0.6
MSWC	Site 2 G	48	4	59	36	23
N	Site 2 E	6,260	502	7,500	4,900	2,600
P	Site 2 E	7	1	11	4	7
K	Site 2 E	89	16	129	46	83
Mg	Site 2 E	75	10	114	51	63
Ca	Site 2 E	330	69	652	207	445
pH	Site 2 E	4.6	0.1	5.2	4.4	0.8
MSWC	Site 2 E	51	5	63	35	28
N	Site 3 G	6,507	560	7,900	5,200	2,700
P	Site 3 G	8	3	23	4	19
K	Site 3 G	100	29	289	46	243
Mg	Site 3 G	65	16	136	39	97
Ca	Site 3 G	304	114	883	158	725
pH	Site 3 G	4.6	0.2	5.3	4.2	1.1
MSWC	Site 3 G	54	6	65	35	30
N	Site 3 E	6,777	415	8,000	5,400	2,600
P	Site 3 E	9	3	32	4	28
K	Site 3 E	117	20	193	82	111
Mg	Site 3 E	64	8	89	45	44
Ca	Site 3 E	259	109	1,110	168	942
pH	Site 3 E	4.6	0.2	5.4	4.2	1.2
MSWC	Site 3 E	58	4	67	47	20

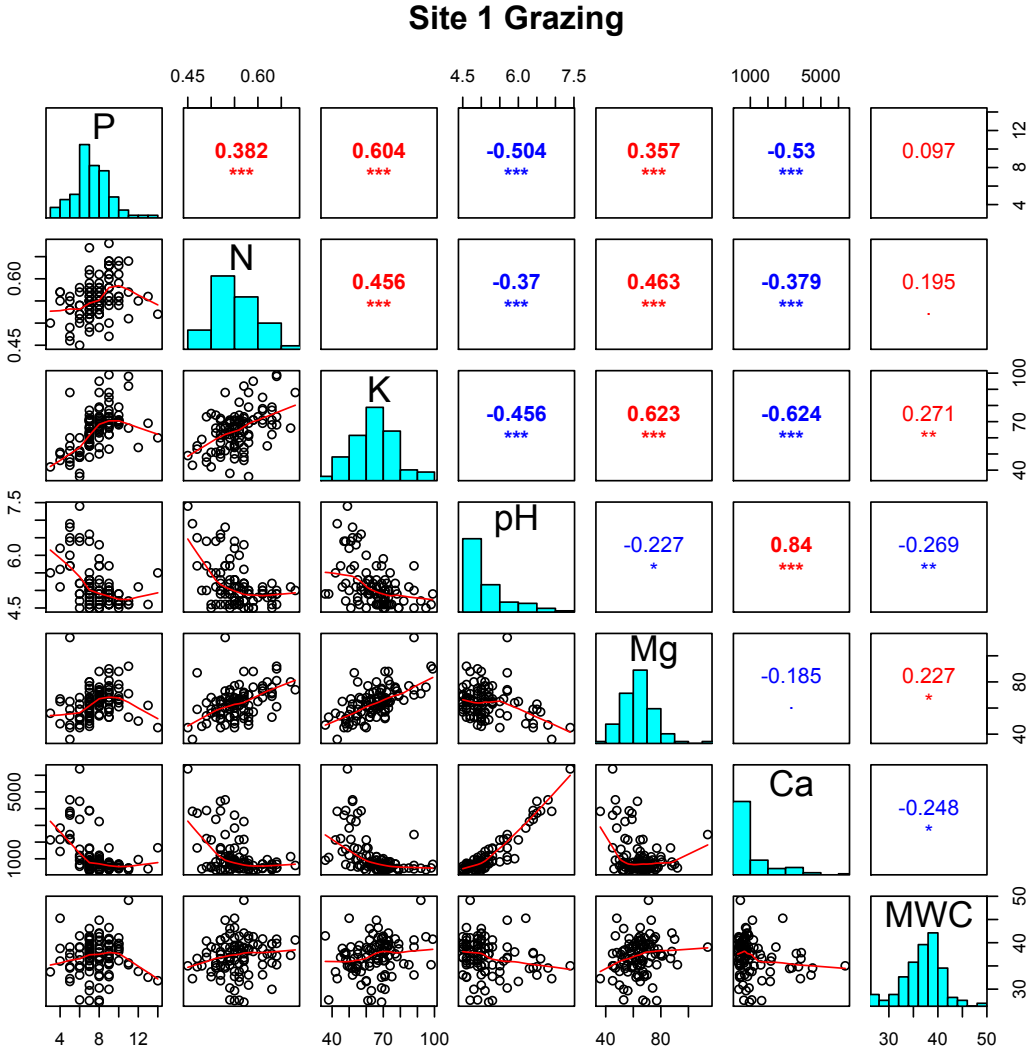

Fig. S5. Relationships between soil variables in Site 1 Grazing. *Spearman* correlation tests (upper panel), histograms (diagonal panel) and dispersion plots with smoothers (lower panel) are displayed (see Table S2).

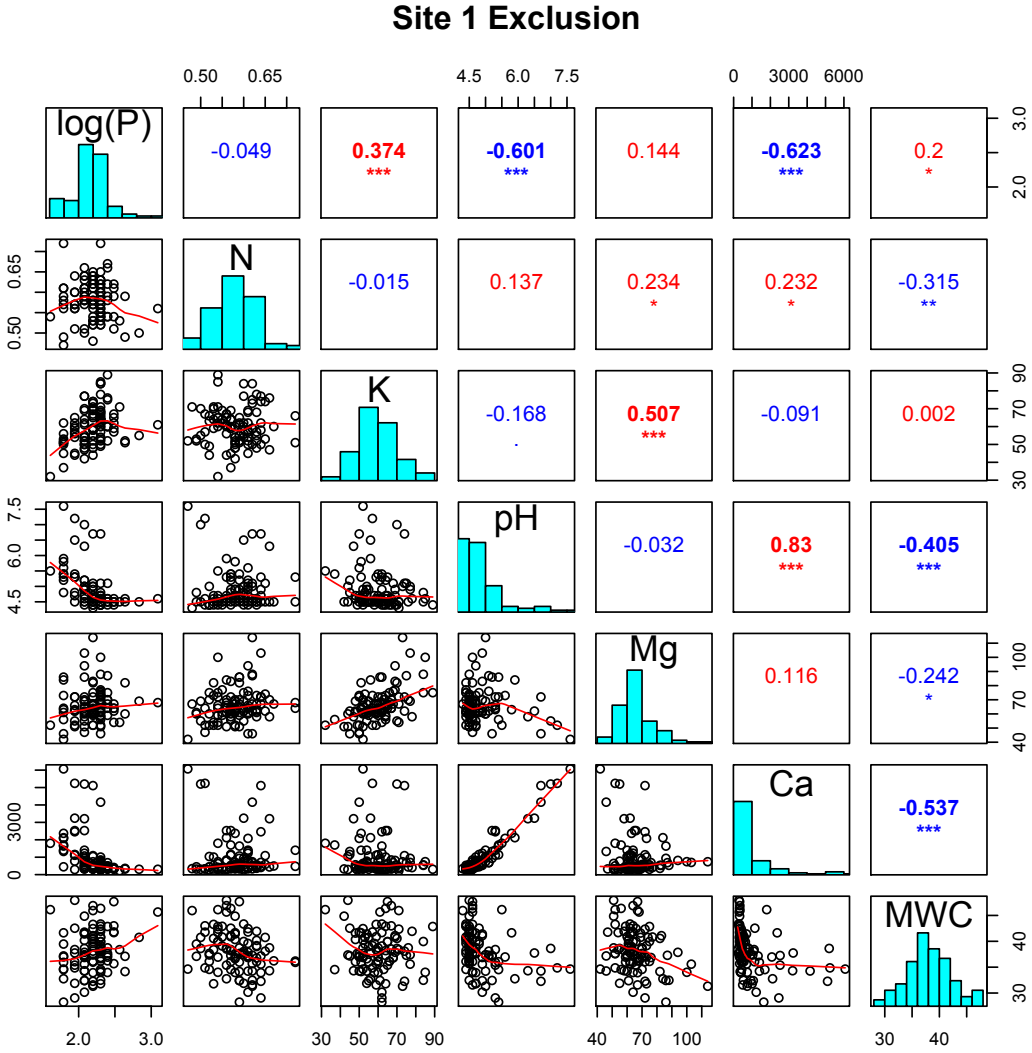

Fig. S6. Relationships between soil variables in Site 1 Exclusion. *Spearman* correlation tests (upper panel), histograms (diagonal panel) and dispersion plots with smoothers (lower panel) are displayed (see Table S2).

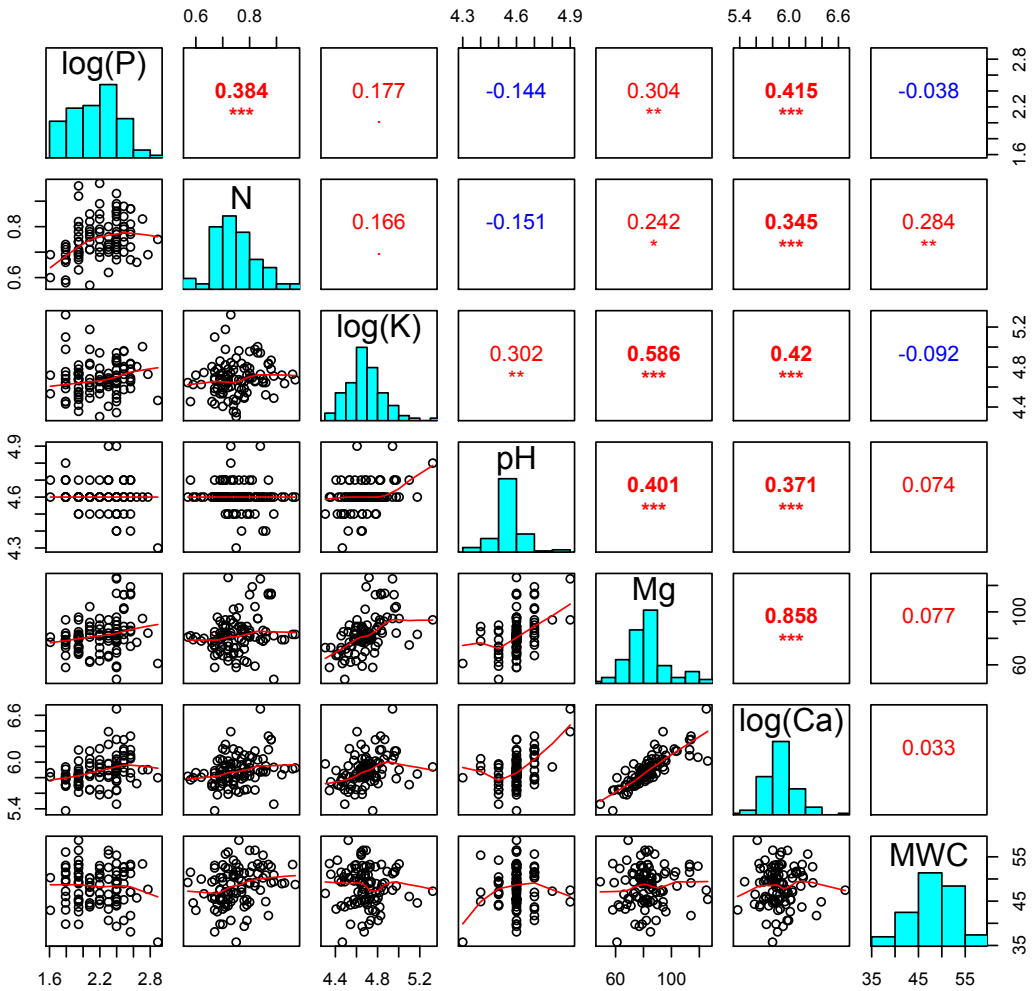

Fig. S7. Relationships between soil variables in Site 2 Grazing. *Spearman* correlation tests (upper panel), histograms (diagonal panel) and dispersion plots with smoothers (lower panel) are displayed. (see Table S2)

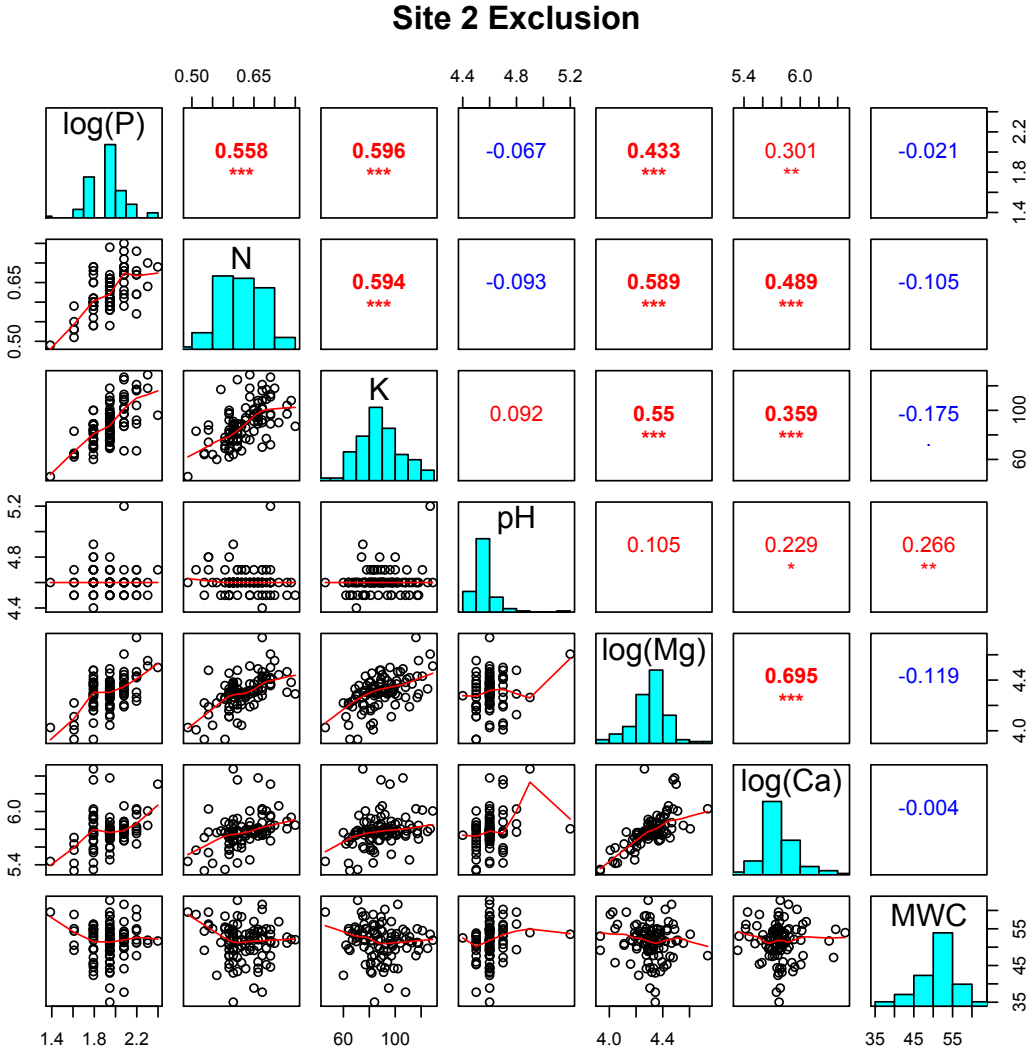

Fig. S8. Relationships between soil variables in Site 2 Exclusion. *Spearman* correlation tests (upper panel), histograms (diagonal panel) and dispersion plots with smoothers (lower panel) are displayed. (see Table S2)

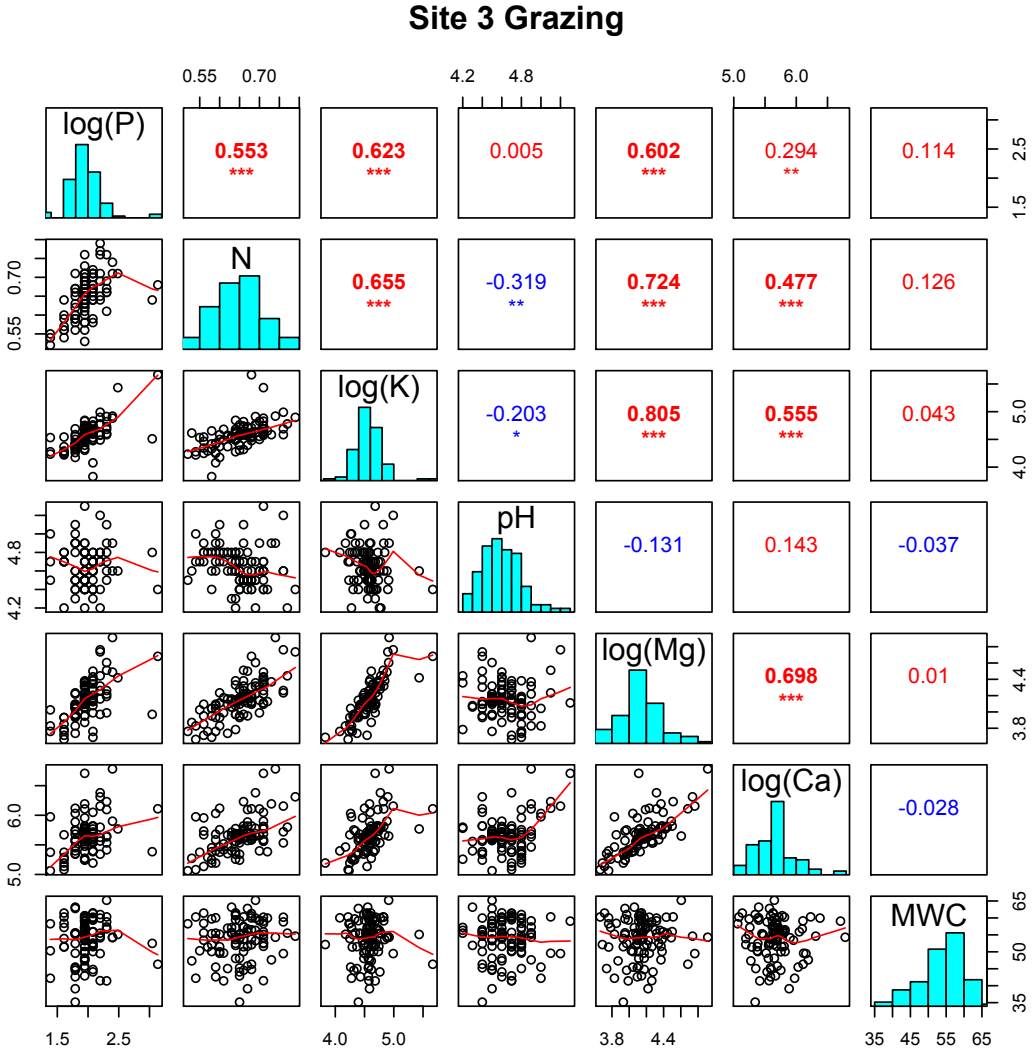

Fig. S9. Relationships between soil variables in Site 3 Grazing. *Spearman* correlation tests (upper panel), histograms (diagonal panel) and dispersion plots with smoothers (lower panel) are displayed. (see Table S2)

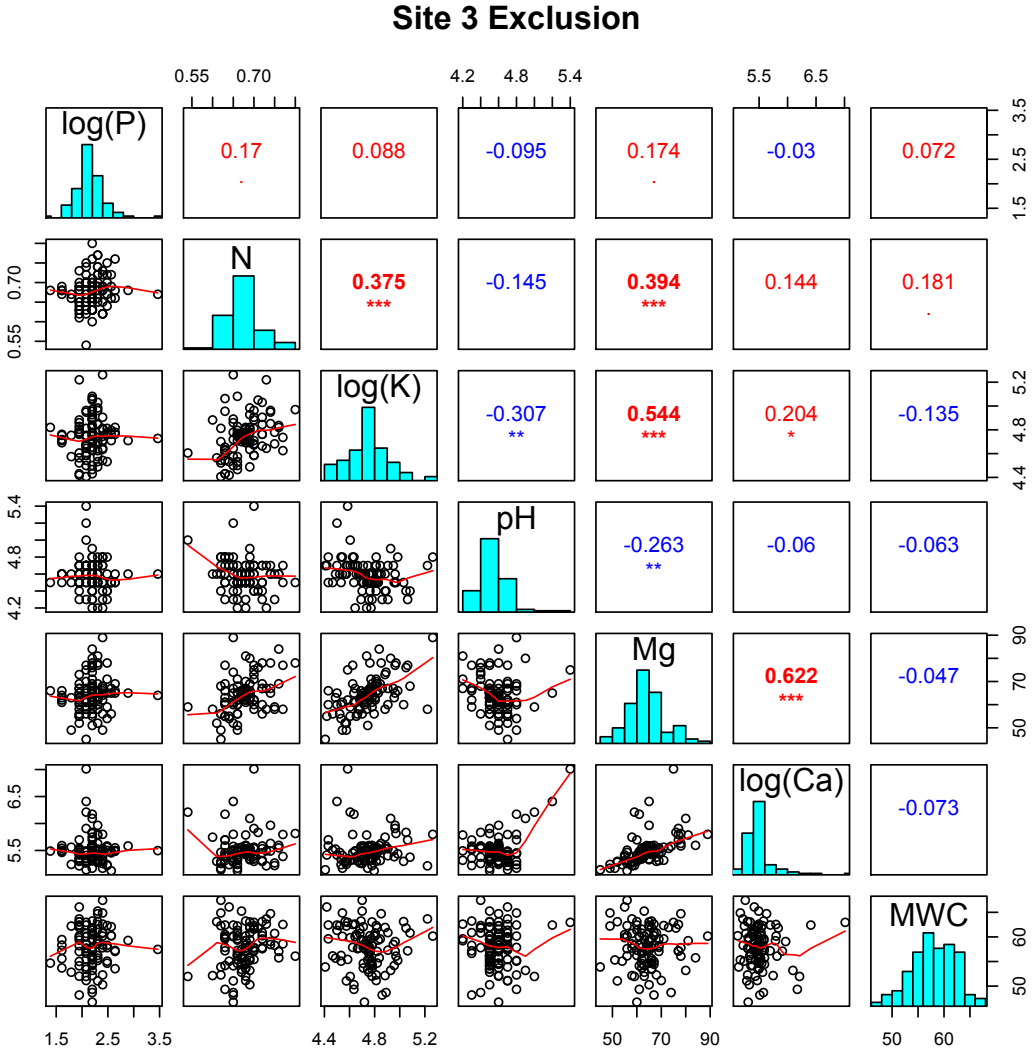

Fig. S10. Relationships between soil variables in Site 3 Exclusion. *Spearman* correlation tests (upper panel), histograms (diagonal panel) and dispersion plots with smoothers (lower panel) are displayed. (see Table S2)

Table S4. Marginal tests results from direct RDAs fitting environmental descriptors to explain species composition in the six field plots located in the Aralar range, Northern Spain. In all cases the response is a Hellinger transformed species matrix. All tests are based in a permutation procedure that used 999 permutations. In complex models (Figs. 1-3 of the main text), the amount of variation explained by each component depends on the other components.

Explanatory variable	Site–Treatment combinaton	df_{Model}	$df_{Residual}$	Adjusted R^2	*F*-value	*p*-value
N	Site 1 G	1	98	0.04	5.174	**0.001**
P	Site 1 G	1	98	0.089	10.711	**0.001**
K	Site 1 G	1	98	0.106	12.798	**0.001**
Mg	Site 1 G	1	98	0.021	3.088	**0.016**
Ca	Site 1 G	1	98	0.244	33.021	**0.001**
pH	Site 1 G	1	98	0.235	31.421	**0.001**
MWC	Site 1 G	1	98	0.021	3.103	**0.014**
N	Site 1 E	1	98	0.007	1.653	0.09
log(P)	Site 1 E	1	98	0.039	5.002	**0.001**
K	Site 1 E	1	98	0.001	1.148	0.313
Mg	Site 1 E	1	98	0.001	1.104	0.335
Ca	Site 1 E	1	98	0.077	9.215	**0.001**
pH	Site 1 E	1	98	0.085	10.213	**0.001**
MWC	Site 1 E	1	98	0.038	4.95	**0.001**
N	Site 2 G	1	98	0.003	1.348	0.181
P	Site 2 G	1	98	0.042	5.38	**0.001**
K	Site 2 G	1	98	0.038	4.868	**0.002**
Mg	Site 2 G	1	98	0.024	3.41	**0.006**
Ca	Site 2 G	1	98	0.025	3.591	**0.002**
pH	Site 2 G	1	98	0.032	4.288	**0.002**
MWC	Site 2 G	1	98	0.023	3.279	**0.003**
N	Site 2 E	1	98	0	0.697	0.73

P	Site 2 E	1	98	0.027	3.705	**0.003**
K	Site 2 E	1	98	0.026	3.665	**0.001**
Mg	Site 2 E	1	98	0.001	1.136	0.311
Ca	Site 2 E	1	98	0	0.577	0.794
pH	Site 2 E	1	98	0.001	1.114	0.341
MWC	Site 2 E	1	98	0	0.574	0.811
N	Site 3 G	1	98	0.012	2.243	**0.033**
P	Site 3 G	1	98	0	0.935	0.46
K	Site 3 G	1	98	0	0.956	0.464
Mg	Site 3 G	1	98	0	0.86	0.539
Ca	Site 3 G	1	98	0	0.74	0.654
pH	Site 3 G	1	98	0.023	3.325	**0.006**
MWC	Site 3 G	1	98	0.072	8.658	**0.001**
N	Site 3 E	1	98	0.017	2.742	**0.021**
P	Site 3 E	1	98	0.016	2.614	**0.029**
K	Site 3 E	1	98	0	0.776	0.538
Mg	Site 3 E	1	98	0	0.376	0.918
Ca	Site 3 E	1	98	0.003	1.281	0.268
pH	Site 3 E	1	98	0.001	1.148	0.297
MWC	Site 3 E	1	98	0.046	5.735	**0.001**

Table S5. Summary tables of RDA environmental models presented in Figs. 1-3 of the main text. Variables were selected by a forward selection procedure (see Materials and Methods in the main text). In all cases the response is a Hellinger transformed species matrix. All tests are based in a permutation procedure that used 999 permutations.

	df	Variance	*F*-value	*p*-value
Site 1 G				
K	1	0.024	15.218	**0.001**
Mg	1	0.003	1.81	0.088
pH	1	0.033	20.788	**0.001**
Residual	96			
R^2-Adj	0.26			
Site 1 E				
MWC	1	0.01	5.194	**0.001**
pH	1	0.014	7.383	**0.001**
Residual	97			
R^2-Adj	0.184			
Site 2 G				
K	1	0.004	5.162	**0.001**
P	1	0.004	5.327	**0.001**
pH	1	0.002	3.187	**0.006**
MWC	1	0.002	2.092	**0.044**
Residual	95	0.072		
R^2-Adj	0.106			
Site 2 E				
K	1	0.004	3.624	**0.002**
Residual	98	0.128		
R^2-Adj	0.026			
Site 3 G				
MWC	1	0.004	3.468	**0.001**
pH	1	0.009	8.334	**0.001**
Residual	97			
R^2-Adj	0.09			
Site 3 E				
N	1	0.004	2.866	**0.011**
P	1	0.004	3.25	**0.01**
MWC	1	0.007	4.952	**0.002**
Residual	96	0.128		
R^2-Adj	0.075			

Appendix S3. R code.

Grazing exclusion unleashes competitive plant responses in Atlantic mountain grasslands by I. Odriozola, G. García-Baquero, M. J. Fortin, N. A. Laskurain and A. Aldezabal.

```
##################################################################################################
## Manuscript title: Grazing exclusion unleashes competitive plant responses in                 ##
## Atlantic mountain grasslands                                                                  ##
## Authors: I. Odriozola, G. García-Baquero, M.-J. Fortin, N.A. Laskurain & A. Aldezabal         ##
## E-mail address: inaki.odriozola@ehu.eus                                                       ##
##################################################################################################

##################################################################################################
## We are indebted to Borcard et al., (2011) "Numerical Ecology with R", for their very         ##
## useful code and good explanations.                                                            ##
##################################################################################################

##################################################################################################
## Large herbivores were exluded in 2005 from three productive semi-natural grasslands in       ##
## the Aralar mountain range, to test the hypothesis that grazing exclusion unleashes           ##
## competitive species which form large spatial patches/clusters that, in turn, out-compete##
## weaker species. Sampling was carried out in 2014, after nine years of fencing.               ##
##                                                                                               ##
## Two specific questions are addressed in the following code:   1) Do competitive species      ##
## form big patches when grazers are excluded?    2) Do those competitive species reduce       ##
## species richness by invading local patches and excluding weaker species under grazing        ##
## exclusion?                                                                                    ##
##                                                                                               ##
## This script is divided into three sections: the first section refers to the first            ##
## specific question and the other two address the second question.                              ##
##                                                                                               ##
## As all analyses are repeated in each site and treatment, here is reported just the           ##
## coding for one of the sites. Please find the complete dataset in Dryad                        ##
##################################################################################################



# Set up working directory and load the required packages
setwd("~/Documents/BIOLOGIA/TESIA/Publikazioak/Odriozola_etal_2016/MS/Limatuak/PPEES/APP/APP_S4")
# Change as required.
library(vegan)
library(packfor) # install.packages("packfor", repos="http://R-Forge.R-project.org")
library(VennDiagram)
library(gridExtra)
library(ggplot2)
library(tripack)
library(spacemakeR) # install.packages("spacemakeR", repos="http://R-Forge.R-project.org")
library(ade4)
library(Matrix)
library(spdep)
library(gstat)

# Import the dataset, which consists of frequencies of 49 grassland species found in 100
# 1-sq.m quadrats (these frequencies are the sum of the presences recorded in 49 0.07 m x 0.07 m
# miniquadrats), 7 environmental variables, and 2 geographical coordinates (Cartesian
# coordinates, X and Y), surveyed in a seasonally grazed plot and a permanently excluded
# plot in a productive semi-natural grassland in the Aralar Natural Park (Basque Country,
# Northern Spain).
d.fr<-read.table("PPEES-A4.txt",h=T)

## Create separate data sets for spa, env, community and biot in Grazing and Exclusion.
d.fr.g<-subset(d.fr,Treatment=="G")   #Data frame for Grazing
d.fr.e<-subset(d.fr,Treatment=="E")   #Data.frame for Exclusion
spa<-data.frame(d.fr.g[,54:55])       #Spatial data frame is the same for Grazing and Exclusion
community.g<-d.fr.g[,3:53]                    #Community data set for Grazing. Abundance=Frequencies
 in subplots
community.e<-d.fr.e[,3:53]                    #Community data set for Exclusion. Abundance =
 Frequencies in subplots
```

```
env.g<-d.fr.g[,56:62]                #Environmental data set for Grazing.
env.e<-d.fr.e[,56:62]                #Environmental data set for Exclusion.

## Explore distributions of environmental variables and relationships between them.
## Graphical representation of scatterplots, histograms and Spearman correlations
source('panelutils.R', chdir = TRUE)  #Borcard et al., (2011)

pairs(env.g, upper.panel=panel.cor,
 diag.panel=panel.hist,lower.panel=panel.smooth,main="Grazing",method="spearman")
#In env.g P, K, Mg and Ca have skewed distributions.

pairs(env.e, upper.panel=panel.cor,
 diag.panel=panel.hist,lower.panel=panel.smooth,main="Exclusion",method="spearman")
#In env.e P, K and Ca have skewed distributions.

##Transform environmental variables with skewed distributions to normalize.

env.g$P<-log(env.g$P)
env.g$K<-log(env.g$K)
env.g$Mg<-log(env.g$Mg)
env.g$Ca<-log(env.g$Ca)
env.e$P<-log(env.e$P)
env.e$K<-log(env.e$K)
env.e$Ca<-log(env.e$Ca)

biotic.g<-rowSums(d.fr.g[,63:68])            #Biotic variable for Grazing. Sum of % covers of the
 competitive species
biotic.e<-rowSums(d.fr.e[,63:68])            #Biotic variable for Exclusion. Sum of % covers of the
 competitive species


##Hellinger transform the plant species data and remove the species that are present <5% quadrats
comm.g.hel<-decostand(community.g,"hellinger")
comm.e.hel<-decostand(community.e,"hellinger")
comm.g.h<-comm.g.hel[,colSums(decostand(comm.g.hel,"pa"))>=5]       ##Definitive community dataset
 for Grazing
comm.e.h<-comm.e.hel[,colSums(decostand(comm.e.hel,"pa"))>=5]       ##Definitive community dataset
 for Exclusion


#############################################################################################################
## Specific analyses to answer the 1st question: "Do competitive species form big patches when     ##
## grazers are excluded?"                                                                          ##
#############################################################################################################

#### The procedure for GRAZING ####

# MEM analysis of the comm.g.h matrix
# ***************************************

# Selection of an optimal spatial weighting matrix.
# Delaunay triangulation weighted by a function of distance.
# Distances are ranged to maximum 1 and raised to power alpha
f2 <- function(D, dmax, y) { 1 - (D/dmax)^y }

# Create a connectivity matrix based on a distance (radius around points).
# The next multivariate variogram of the spe data (with 12 distance
# classes) is used to assess the relevant distances

(spe.vario <- variogmultiv(comm.g.h, spa, nclass = 12))
quartz(title="Multivariate variogram, comm.g.h") # For Mac
windows(title="Multivariate variogram, comm.g.h") # For PC
plot(spe.vario$d, spe.vario$var, ty='b', pch=20, xlab="Distance",
 ylab="C(distance)")
# The variance increases from nclass 0 to 10

# Search the shortest distance that keep all quadrats connected
xy.d1 <- dist(spa)
spanning <- spantree(xy.d1)
dmin <- max(spanning$dist)
dmin
# 2.070841

# Construction of 10 neighbourhood matrices (class nb)
# Vector of 10 threshold distances (from dmin = 2.070841 up to 20, as indicated
```

```
# by the multivariate variogram, in which a monotonic increase is seen up to 20 m)
(thresh10 <- seq(give.thresh(dist(spa)), 20, le = 10))
#[1]  2.070841  4.062970  6.055099  8.047227 10.039356 12.031485 14.023614 16.015742
#[9] 18.007871 20.000000

# Create 10 neighbourhood matrices (each matrix contains all connexions with lengths < = the
 threshold value)
list10nb <- lapply(thresh10, dnearneigh, x=as.matrix(spa), d1=0)

# Display the first rows of the first neighbourhood matrix
print(listw2mat(nb2listw(list10nb[[1]], style="B"))[1:10,1:10], digits=1)

# Weight the connections by the complement of the power of the distances,
# 1-(d/dmax)^y
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=comm.g.h, f=f2,
 y=2:10, dmax=max(unlist(nbdists(x, as.matrix(spa)))),
 xy=as.matrix(spa)))

# Lowest AIC, best model
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,
 na.rm=TRUE))

# Smallest AICc (best model among the possible)
min(spe.f2.minAIC)
# [1] -258.6188

# Number of the best model among the 10 that have been tested
(nb.bestmod <- which.min(spe.f2.minAIC))
# 7

# Actual dmax of best model
(dmax.best <- spe.thresh.f2[nb.bestmod][[1]]$all[1,2])
# 14.02192
#This is the procedure used to get the best dmax (=16 in this case) for the spatial model of each
 plot.
#Some decisions used for the building of the spatial models are somewhat arbitrary.
#As the spatial models were constructed separately for each of the six plots separately,
#we got different best dmax-es, and we used the mode of the results (dmax=8) to obtain comparable
#spatial models across plots.

#Set dmax=8
list10nb <- lapply(8, dnearneigh, x=as.matrix(spa), d1=0)    #thresh value is coerced to 8
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=comm.g.h, f=f2,
 y=2:10, dmax=max(unlist(nbdists(x, as.matrix(spa)))),
 xy=as.matrix(spa)))
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,
 na.rm=TRUE))


# Extraction of the best MEM model
#*********************************
g.MEM.champ <- unlist(spe.thresh.f2[which.min(spe.f2.minAIC)],
 recursive=FALSE)

summary(g.MEM.champ)
# Eigenvalues
g.MEM.champ$best$values

# MEM variables by order of added R2
g.MEM.champ$best$ord

# MEM variables selected in the best model (7 MEM variables)
MEMid <- g.MEM.champ$best$ord[1:which.min(g.MEM.champ$best$AICc)]
sort(MEMid)
#[1]  1  2  3  6  9 30 57 88

# Peruse the 8 MEM spatial variables that constitute the best model
g.MEM.all <- g.MEM.champ$best$vectors
g.MEM.select <- g.MEM.champ$best$vectors[, sort(c(MEMid))]
head(g.MEM.select)
colnames(g.MEM.select) <- c("MEM1","MEM2","MEM3","MEM6","MEM9","MEM30","MEM57","MEM88")
head(g.MEM.select)
#             MEM1        MEM2        MEM3          MEM6        MEM9        MEM30       MEM57
 MEM88
#[1,]  0.066705614 -0.07063411 -0.12105396 -0.166387835  0.20507000  0.061530399  0.01130794 -
```

```
 0.096455533
#[2,]  0.075055119 -0.09619661 -0.13539593 -0.091583108  0.10293611  0.070882390  0.14255640 -
 0.165607868
#[3,]  0.068599702 -0.12021390 -0.12697317  0.003659285 -0.01940609  0.008570923 -0.19678050 -
 0.001463844
#[4,]  0.045856505 -0.13807375 -0.08937403  0.090589903 -0.06384996 -0.035090196 -0.01587691
 0.205042302
#[5,]  0.008413656 -0.14395221 -0.02068093  0.163745872 -0.20781060  0.076771411  0.08213801
 0.143894791
#[6,] -0.031010861 -0.14126761  0.05464997  0.130089458 -0.19586796 -0.105041978 -0.06010546 -
 0.130336511

# Unadjusted R2 of best model
g.R2.MEMbest <- g.MEM.champ$best$R2[which.min(g.MEM.champ$best$AICc)]
g.R2.MEMbest  #0.2417385

# Adjusted R2 of best model
RsquareAdj(g.R2.MEMbest, nrow(comm.g.h), length(MEMid))
# The best spatial model (with 8 MEM variables) explains adj-R^2 = 0.18%
# (R^2 = 0.2154656) of multivariate variation in species composition

# Maps of the 8 significant MEM variables. These spatial variables are next used
# to describe spatially structured variation in species composition
quartz(title="8 MEM variables - comm.g.h")  # For Mac
windows(title="8 MEM variables - comm.g.h")  # For PC
par(mfrow=c(2,4))
for(i in 1:ncol(g.MEM.select)){
 s.value(spa,g.MEM.select[,i], sub=sort(MEMid)[i], csub=2)
}

# 1 and 3 = broad
# the others = fine

# Explain the species matrix
# by means of the broad scaled MEM spatial variables retained in the best MEM model
# ********************************************************************************

# RDA of the spe data constrained by the 3 broad-scaled MEM retained, using vegan
(g.broad.MEM.rda <- rda(comm.g.h~., as.data.frame(g.MEM.select[,1:3])))
(g.broad.MEM.R2a <- RsquareAdj(g.broad.MEM.rda)$adj.r.squared)
# 0.09504006

anova(g.broad.MEM.rda, step = 1000)
# Model: rda(formula = comm.g.h ~ MEM1 + MEM2 + MEM3, data = as.data.frame(g.MEM.select[, 1:3]))
#          Df Variance      F Pr(>F)
#Model     3 0.014197 4.4657  0.001 ***
#Residual 96 0.101730


# Explain the species matrix
# by means of the fine scaled MEM spatial variables retained in the best MEM model
# ********************************************************************************

# RDA of the spe data constrained by the 5 fine-scaled MEM retained, using vegan
(g.fine.MEM.rda <- rda(comm.g.h~., as.data.frame(g.MEM.select[,-c(1:3)])))
(g.fine.MEM.R2a <- RsquareAdj(g.fine.MEM.rda)$adj.r.squared)
# 0.07242837

anova(g.fine.MEM.rda, step = 1000)
# Model: rda(formula = comm.g.h ~ MEM6 + MEM9 + MEM30 + MEM57 + MEM88, data =
 as.data.frame(g.MEM.select[, -c(1:3)]))
#          Df Variance      F Pr(>F)
#Model     5 0.013827 2.5461  0.001 ***
#Residual 94 0.102099


# Partitioning of the multivariate variation in species composition (whole matrix,
# Hellinger-transformed) into three components: environmental variation, Fine-scaled
# spatial variation and Broad-scaled spatial variation as decribed by the MEM spatial
# variables
# ********************************************************************************
#Effect of environmental variables
g.env<-rda(comm.g.h,env.g)
(g.env.R2a<-RsquareAdj(g.env)$adj.r.squared)
#Variables selected using Blanchet (2008) double-stop criteria
```

```
g.env.fwd<-forward.sel(comm.g.h,env.g,adjR2thresh=g.env.R2a,nperm=999)
g.env.sign<-sort(g.env.fwd$order)     ##MWC and pH selected
g.env.red<-env.g[,c(4,7)]             ##Env vraiable to be used in variance partitioning

g.MEM.broad <- g.MEM.select[,1:3]     ##Broad-scale spatial variable to be used in variance
 partitioning
g.MEM.fine <- g.MEM.select[,-c(1:3)]  ##Fine-scale spatial variable to be used in variance
 partitioning

##Partition the variation in Community composition in: ENV, BROAD-SCALE and FINE-SCALE fractions
par(mfrow=c(1,1))
g.varpart<-varpart(comm.g.h,g.env.red, g.MEM.broad,g.MEM.fine)
plot(g.varpart,digits=1)

# Fig.3a in the main text. 1) Build definitive plot of variance partitioning
# and, 2) Plot broad-scaled and fine-scaled community structures that are
# unrelated to environmental variation.
# ***********************************************************************
#Plot variance partitioning
grid.newpage()
venn.plot <- draw.triple.venn(0.08, 0.1, 0.08, 0.02, 0, 0.03, 0, c("ENV***", "BROAD\nSCALE***",
 "FINE\nSCALE***"),fill=c("blue","red","orange"),
euler.d=T,scaled=T,lty="blank",cex=rep(2,7),cat.pos=c(0,0,10),cat.dist=c(-0.05,-0.03,-
 0.1),cat.cex=rep(1.5,3),cat.fontface=rep("bold",3))
grid.text("Residuals = 0.79", x=0.8, y=0.1,gp=gpar(fontsize=20))

#Plot Broad- Fine-scaled fractions
s.value(spa,rda(comm.g.h, g.MEM.broad,cbind(g.env.red,g.MEM.fine))$CCA$u[,1],cleg=1.9)
s.value(spa,rda(comm.g.h, g.MEM.fine,cbind(g.env.red,g.MEM.broad))$CCA$u[,1],cleg=1.9)

##Test significance for:
#Pure env
anova(rda(comm.g.h,g.env.red,cbind(g.MEM.broad,g.MEM.fine)))
#Pure broad-scale
anova(rda(comm.g.h,g.MEM.broad,cbind(g.env.red,g.MEM.fine)))
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(X = comm.g.h, Y = g.MEM.broad, Z = cbind(g.env.red, g.MEM.fine))
#          Df Variance      F Pr(>F)
# Model     3 0.011262 4.0439  0.001 ***
# Residual 89 0.082623

#Pure fine-scale
anova(rda(comm.g.h,g.MEM.fine,cbind(g.env.red,g.MEM.broad)))
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(X = comm.g.h, Y = g.MEM.fine, Z = cbind(g.env.red, g.MEM.broad))
#          Df Variance      F Pr(>F)
# Model     5 0.009712 2.0924  0.001 ***
# Residual 89 0.082623


#### Repeat the procedure for EXCLUSION ####

# MEM analysis of the comm.e.h matrix
# ****************************************
(spe.vario <- variogmultiv(comm.e.h, spa, nclass = 12))
quartz(title="Multivariate variogram, comm.e.h")
windows(title="Multivariate variogram, comm.e.h")
plot(spe.vario$d, spe.vario$var, ty='b', pch=20, xlab="Distance",
 ylab="C(distance)")
# The variance increases from 0 to 16;

# Search the shortest distance that keep all quadrats connected
xy.d1 <- dist(spa)
spanning <- spantree(xy.d1)
dmin <- max(spanning$dist)
dmin
# 2.070841

# Construction of 10 neighbourhood matrices (class nb)
```

```
# Vector of 8 threshold distances (from dmin = 2.070841 up to 16, as indicated
# by the multivariate variogram, in which a monotonic increase is seen up to 16 m)
(thresh10 <- seq(give.thresh(dist(spa)), 16, le = 8))
# [1]  2.070841  4.060721  6.050601  8.040481 10.030360 12.020240 14.010120 16.000000


list10nb <- lapply(thresh10, dnearneigh, x=as.matrix(spa), d1=0)
print(listw2mat(nb2listw(list10nb[[1]], style="B"))[1:10,1:10], digits=1)
# Weight the connections by the complement of the power of the distances,
# 1-(d/dmax)^y
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=comm.e.h, f=f2,
 y=2:10, dmax=max(unlist(nbdists(x, as.matrix(spa)))),
 xy=as.matrix(spa)))

# Lowest AIC, best model
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,
 na.rm=TRUE))

# Smallest AICc (best model among the possible)
min(spe.f2.minAIC)
# [1] -222.931

# Number of the best model among the 10 that have been tested
(nb.bestmod <- which.min(spe.f2.minAIC))
# 4

# Actual dmax of best model
(dmax.best <- spe.thresh.f2[nb.bestmod][[1]]$all[1,2])
# 16
#This is the procedure used to get the best dmax (=16 in this case) for the spatial model of each
 plot. Some decisions
#to build the spatial models are somewhat arbitrary. As the spatial models were constructed for each
 of the six plots
#separately, we got different best dmax-es, and we used the mode of the results (dmax=8) to use a
 comparable
#spatial model across all plots.

#Set dmax=8
list10nb <- lapply(8, dnearneigh, x=as.matrix(spa), d1=0)    #thresh value is coerced to 8
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=comm.e.h, f=f2,
 y=2:10, dmax=max(unlist(nbdists(x, as.matrix(spa)))),
 xy=as.matrix(spa)))
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,
 na.rm=TRUE))

# Extraction of the best MEM model
#*********************************
e.MEM.champ <- unlist(spe.thresh.f2[which.min(spe.f2.minAIC)],
 recursive=FALSE)

summary(e.MEM.champ)
# Eigenvalues
e.MEM.champ$best$values

# MEM variables by order of added R2
e.MEM.champ$best$ord

# MEM variables selected in the best model (7 MEM variables)
MEMid <- e.MEM.champ$best$ord[1:which.min(e.MEM.champ$best$AICc)]
sort(MEMid)
# [1]  1  2  3 12 23 39 49 64 78

# Peruse the 9 MEM spatial variables that constitute the best model
e.MEM.all <- e.MEM.champ$best$vectors
e.MEM.select <- e.MEM.champ$best$vectors[, sort(c(MEMid))]
head(e.MEM.select)
colnames(e.MEM.select) <- c("MEM1","MEM2","MEM3","MEM12","MEM23","MEM39","MEM49","MEM64","MEM78")
head(e.MEM.select)
#              MEM1        MEM2        MEM3        MEM12       MEM23        MEM39       MEM49
 MEM64        MEM78
#[1,]  0.059797803 -0.06284992 -0.10747004  0.234090270  0.02530249  0.131012139 -0.22210407
 0.008922903  0.015335064
#[2,]  0.070557758 -0.08934516 -0.12752302  0.228085824 -0.06110257 -0.054745846 -0.07872587 -
 0.035222189  0.014904654
#[3,]  0.065848248 -0.11320085 -0.12170095  0.194202374  0.01518953 -0.142057022 -0.06765286
```

```
 0.003591373  0.008460910
#[4,]  0.043593052 -0.12971295 -0.08359994  0.122518792  0.04281635  0.054874417 -0.01349822 -
 0.002562068 -0.037669604
#[5,]  0.008228085 -0.13607364 -0.01907636  0.004301988 -0.09520461 -0.066986272 -0.03603446
 0.056134169 -0.003637693
#[6,] -0.029404116 -0.13336792  0.05219894 -0.089473415  0.01539961  0.008432208 -0.12076273 -
 0.123462084  0.006355103

# Unadjusted R2 of best model
R2.MEMbest <- e.MEM.champ$best$R2[which.min(e.MEM.champ$best$AICc)]
R2.MEMbest # 0.3502226

# Adjusted R2 of best model
RsquareAdj(R2.MEMbest, nrow(comm.e.h), length(MEMid))
# The best spatial model (with 9 MEM variables) explains adj-R^2 = 29%
# (0.2852449) of multivariate variation in species composition

# Maps of the 9 significant MEM variables. These spatial variables are next used
# to describe spatially structured variation in species composition
quartz(title="9 MEM variables - comm.e.h")
windows(title="9 MEM variables - comm.e.h")
par(mfrow=c(3,3))
for(i in 1:ncol(e.MEM.select)){
 s.value(spa,e.MEM.select[,i], sub=sort(MEMid)[i], csub=2)
}

# 1:3 = broad
# the others = fine

# Explain the species matrix
# by means of the broad scaled MEM spatial variables retained in the best MEM model
# ************************************************************************************

# RDA of the spe data constrained by the 3 broad-scaled MEM retained, using vegan
(e.broad.MEM.rda <- rda(comm.e.h~., as.data.frame(e.MEM.select[,1:3])))
(e.broad.MEM.R2a <- RsquareAdj(e.broad.MEM.rda)$adj.r.squared)
# 0.1910822

anova(e.broad.MEM.rda, step = 1000)
# Model: rda(formula = comm.e.h ~ MEM1 + MEM2 + MEM3, data = as.data.frame(e.MEM.select[, 1:3]))
#          Df Variance      F Pr(>F)
#Model     3 0.030881 8.7952  0.001 ***
#Residual 96 0.112356


# Explain the species matrix
# by means of the fine scaled MEM spatial variables retained in the best MEM model
# ************************************************************************************

# RDA of the spe data constrained by the 6 fine-scaled MEMs retained, using vegan
(e.fine.MEM.rda <- rda(comm.e.h~., as.data.frame(e.MEM.select[,-c(1:3)])))
(e.fine.MEM.R2a <- RsquareAdj(e.fine.MEM.rda)$adj.r.squared)
# 0.07879727

anova(e.fine.MEM.rda, step = 1000)
# Model: rda(formula = comm.e.h ~ MEM12 + MEM23 + MEM39 + MEM49 + MEM64 + MEM78, data=
 as.data.frame(e.MEM.select
#[, -c(1:3)]))
#          Df Variance      F Pr(>F)
#Model     6 0.019284 2.4114  0.001 ***
#Residual 93 0.123954

# Partitioning of the multivariate variation in species composition (whole matrix,
# Hellinger-transformed) into three components: environmental variation, Fine-scaled
# spatial variation and Broad-scaled spatial variation as decribed by the MEM spatial
# variables
# ************************************************************************************
#Effect of environmental variables
e.env<-rda(comm.e.h,env.e)
(e.env.R2a<-RsquareAdj(e.env)$adj.r.squared)
#Variables selected using Blanchet (2008) double-stop criteria
e.env.fwd<-forward.sel(comm.e.h,env.e,adjR2thresh=e.env.R2a,nperm=999)
e.env.sign<-sort(e.env.fwd$order)     ##MWC, P and N selected
e.env.red<-env.e[,c(1,2,7)]           ##Env vraiable to be used in variance partitioning
```

```
e.MEM.broad <- e.MEM.select[,1:3]     ##Broad-scale spatial variable to be used in variance
 partitioning
e.MEM.fine <- e.MEM.select[,-c(1:3)]  ##Fine-scale spatial variable to be used in variance
 partitioning

##Partition the variation in Community composition in: ENV, BROAD-SCALE and FINE-SCALE fractions
par(mfrow=c(1,1))
e.varpart<-varpart(comm.e.h,e.env.red, e.MEM.broad,e.MEM.fine)
plot(e.varpart,digits=1)

# Fig.3b in the main text. 1) Build definitive plot of variance partitioning
# and, 2) Plot broad-scaled and fine-scaled community structures that are
# unrelated to environmental variation.
# ***********************************************************************
#Plot variance partitioning
grid.newpage()
venn.plot <- draw.triple.venn(0.08, 0.21, 0.1,0.05,0,0,0, c("ENV**","BROAD\nSCALE***",
 "FINE\nSCALE***"),fill=c("blue","red","orange"),euler.d=T,scaled=T,lty="blank",cex=rep(2,7),
cat.cex=rep(1.5,3),cat.pos=c(-30,-0.3,0),cat.dist=c(-0.03,-0.05,-0.07),cat.fontface=rep("bold",3))
grid.text("Residuals = 0.68", x=0.79, y=0.03,gp=gpar(fontsize=20))

#Plot Broad- Fine-scaled fractions
s.value(spa,rda(comm.e.h, e.MEM.broad,cbind(e.env.red,e.MEM.fine))$CCA$u[,1],cleg=2)
s.value(spa,rda(comm.e.h, e.MEM.fine,cbind(e.env.red,e.MEM.broad))$CCA$u[,1],cleg=2)


##Test significance for:
#Pure env
anova(rda(comm.e.h,e.env.red,cbind(e.MEM.broad,e.MEM.fine)))
#Pure broad-scale
anova(rda(comm.e.h,e.MEM.broad,cbind(e.env.red,e.MEM.fine)))
#Pure fine-scale
anova(rda(comm.e.h,e.MEM.fine,cbind(e.env.red,e.MEM.broad)))
# Number of permutations: 999
#
# Model: rda(X = comm.e.h, Y = e.MEM.fine, Z = cbind(e.env.red, e.MEM.broad))
#          Df Variance      F Pr(>F)
# Model     6 0.019224 3.2503  0.001 ***
# Residual 87 0.085763



# ************************************************************************************************
# ************************************************************************************************

##################################################################################################
## Specific analyses to answer the 2nd question: "Do those competitive species reduce specie       ##
## richness invading local patches and excluding weaker species under grazing exclusion?"          ##
##################################################################################################

#Build species richness data sets for quadrats in Grazing and Exclusion
rich.g<-rowSums(decostand(community.g,"pa"))  ##Species richness in Grazing
rich.e<-rowSums(decostand(community.e,"pa"))  ##Species richness in Exclusion

#### The procedure for GRAZING ####

# MEM analysis of species richness
# *********************************
#The procedure used to do the MEM analysis of species richness is similar to the one used with
 species composition.
#The only difference is that in this case the aim is to control for the spatial autocorrelation to
 avoid the inflation
#of Type I error in the models. So only the MEM variables modelling positive spatial autocorrelation
 are considered.

# The connectivity matrix is based on a distance (radius around points).
# Now the response is univariate and a univariate variogram of the richness
# data (with 12 distance classes) is used to assess the relevant distances
rich.g.xy <- data.frame(spa,rich.g)
rich.g.variog <- variogram(rich.g~1,~X+Y,data=rich.g.xy,width= 2.070841,cutoff=25)
plot(rich.g.variog)

(thresh10 <- seq(give.thresh(dist(spa)), 25, le = 12))
list10nb <- lapply(thresh10, dnearneigh, x=as.matrix(spa), d1=0)
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=rich.g, f=f2,y=2:10,MEM.autocor="positive",
```

```
 dmax=max(unlist(nbdists(x, as.matrix(spa)))),xy=as.matrix(spa)))
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,na.rm=TRUE))
min(spe.f2.minAIC)
(nb.bestmod <- which.min(spe.f2.minAIC))
(dmax.best <- spe.thresh.f2[nb.bestmod][[1]]$all[1,2])
#This is the procedure used to get the best dmax (=8 in this case) for the spatial model of each
 plot. Some decisions
#to build the spatial models are somewhat arbitrary. As the spatial models were constructed for each
 of the six plots
#separately, we got different best dmax-es, and we used the mode of the results (dmax=4) to use a
 comparable
#spatial model across all plots.

#Set dmax=4
list10nb <- lapply(4, dnearneigh, x=as.matrix(spa), d1=0)    ##dmax is coerced to be 4
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=rich.g, f=f2,y=2:10,MEM.autocor="positive",
 dmax=max(unlist(nbdists(x, as.matrix(spa)))),xy=as.matrix(spa)))
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,na.rm=TRUE))

# Extraction of the best MEM model
# *******************************
g.MEM.champ <- unlist(spe.thresh.f2[which.min(spe.f2.minAIC)],recursive=FALSE)
summary(g.MEM.champ)

# Eigenvalues
g.MEM.champ$best$values

# MEM variables by order of added R2
g.MEM.champ$best$ord

# MEM variables selected in the best model (8 MEM variables)
g.MEMid <- g.MEM.champ$best$ord[1:which.min(g.MEM.champ$best$AICc)]
sort(g.MEMid)
#[1]  1  6 10 16 26 32 33

# Peruse the 7 MEM spatial variables that constitute the best model
g.MEM.all <- g.MEM.champ$best$vectors
g.MEM.select <- g.MEM.champ$best$vectors[, sort(c(g.MEMid))]
head(g.MEM.select)
colnames(g.MEM.select) <- c("MEM1","MEM6","MEM10","MEM16","MEM26","MEM32","MEM33")
head(g.MEM.select)

g.R2.MEMbest <- g.MEM.champ$best$R2[which.min(g.MEM.champ$best$AICc)]
g.R2.MEMbest
#0.2917266

# Partitioning of the variation in species richness into three components: environmental
# variation, biotic variation (described by the abundance of competitive species) and spatial
# autocorrelation (decribed by the MEM spatial variables).
# ********************************************************************************
#Effect of environmental variables
g.env<-rda(rich.g,env.g)
(g.env.R2a<-RsquareAdj(g.env)$adj.r.squared)
#Variables selected using Blanchet (2008) double-stop criteria
g.env.fwd<-forward.sel(rich.g,env.g,adjR2thresh=g.env.R2a,nperm=999) ##pH + K
g.env.red <- env.g[,c(3,4)]

##Partition the variation in species richness in: BIOTIC, ENV and SPATIAL fractions
g.varpart<-varpart(rich.g,biotic.g,g.env.red,g.MEM.select)
par(mfrow=c(1,1))
plot(g.varpart,digits=1)

# Fig.4e in the main text. Plot the variance partition of species richness
# ************************************************************************
grid.newpage()
venn.plot <- draw.triple.venn(0.15, 0.15, 0.25, 0.05, 0.11, 0.09, 0.04, c("BIOT**", "ENV*",
 "SPA**"),fill=c("green","blue","red"),euler.d=T,scaled=F,lty="blank",cex=rep(2,7),
cat.pos=c(-40,40,0),cat.dist=c(-0.04,-0.04,-0.35),cat.cex=rep(1.5,3),cat.fontface=rep("bold",3))
grid.text("Residuals = 0.67", x=0.83, y=0.05,gp=gpar(fontsize=20))

##Test significance for:
#Pure env
anova(rda(rich.g,g.env.red,cbind(g.MEM.select,biotic.g)))
#Pure biot
anova(rda(rich.g, biotic.g,cbind(g.env.red, g.MEM.select)))
```

```
#Pure spatial
anova(rda(rich.g, g.MEM.select,cbind(g.env.red, biotic.g)))

#### Repeat the procedure for EXCLUSION ####

# MEM analysis of species richness
# ********************************

# The connectivity matrix is based on a distance (radius around points).
# Now the response is univariate and a univariate variogram of the richness
# data (with 12 distance classes) is used to assess the relevant distances

rich.e.xy <- data.frame(spa,rich.e)
rich.e.variog <- variogram(rich.e~1,~X+Y,data=rich.e.xy,width= 2.070841,cutoff=25)
plot(rich.e.variog)

(thresh10 <- seq(give.thresh(dist(spa)), 16, le = 8))
list10nb <- lapply(thresh10, dnearneigh, x=as.matrix(spa), d1=0)
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=rich.e, f=f2,y=2:10,MEM.autocor="positive",
 dmax=max(unlist(nbdists(x, as.matrix(spa)))),xy=as.matrix(spa)))
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,na.rm=TRUE))
min(spe.f2.minAIC)
(nb.bestmod <- which.min(spe.f2.minAIC))
(dmax.best <- spe.thresh.f2[nb.bestmod][[1]]$all[1,2])
#This is the procedure used to get the best dmax (=2 in this case) for the spatial model of each
 plot. Some decisions
#to build the spatial models are somewhat arbitrary. As the spatial models were constructed for each
 of the six plots
#separately, we got different best dmax-es, and we used the mode of the results (dmax=4) to use a
 comparable
#spatial model across all plots.

#Set dmax=4
list10nb <- lapply(4, dnearneigh, x=as.matrix(spa), d1=0)    ##dmax is coerced to be 4
spe.thresh.f2 <- lapply(list10nb, function(x) test.W(x, Y=rich.e, f=f2,y=2:10,MEM.autocor="positive",
 dmax=max(unlist(nbdists(x, as.matrix(spa)))),xy=as.matrix(spa)))
spe.f2.minAIC <- sapply(spe.thresh.f2, function(x) min(x$best$AICc,na.rm=TRUE))

# Extraction of the best MEM model
#*********************************
e.MEM.champ <- unlist(spe.thresh.f2[which.min(spe.f2.minAIC)],recursive=FALSE)
summary(e.MEM.champ)

# Eigenvalues
e.MEM.champ$best$values

# MEM variables by order of added R2
e.MEM.champ$best$ord

# MEM variables selected in the best model (8 MEM variables)
e.MEMid <- e.MEM.champ$best$ord[1:which.min(e.MEM.champ$best$AICc)]
sort(e.MEMid)
#[1]  1  2  3  4  6  7  8  9 22 33 34

# Peruse the 11 MEM spatial variables that constitute the best model
e.MEM.all <- e.MEM.champ$best$vectors
e.MEM.select <- e.MEM.champ$best$vectors[, sort(c(e.MEMid))]
head(e.MEM.select)
colnames(e.MEM.select) <-
 c("MEM1","MEM2","MEM3","MEM4","MEM6","MEM7","MEM8","MEM9","MEM22","MEM33","MEM34")
head(e.MEM.select)

e.R2.MEMbest <- e.MEM.champ$best$R2[which.min(e.MEM.champ$best$AICc)]
e.R2.MEMbest
#0.374876

# Partitioning of the variation in species richness into three components: environmental
# variation, biotic variation (described by the abundance of competitive species) and spatial
# autocorrelation (decribed by the MEM spatial variables).
# ******************************************************************************
#Effect of environmental variables
e.env<-rda(rich.e,env.e)
(e.env.R2a<-RsquareAdj(e.env)$adj.r.squared)
#Variables selected using Blanchet (2008) double-stop criteria
e.env.fwd<-forward.sel(rich.e,env.e,adjR2thresh=e.env.R2a,nperm=999) ##MWC + N
```

```
e.env.red <- env.e[,c(2,7)]

##Partition the variation in species richness in: BIOTIC, ENV and SPATIAL fractions
e.varpart<-varpart(rich.e,biotic.e,e.env.red,e.MEM.select)
par(mfrow=c(1,1))
plot(e.varpart,digits=1)

# Fig.4e in the main text. Plot the variance partition of species richness
# ************************************************************************
grid.newpage()
venn.plot <- draw.triple.venn(0.21, 0.13, 0.29, 0.06, 0.03, 0.09, 0.03, c("BIOT***", "ENV**",
 "SPA***"),fill=c("green","blue","red"),euler.d=T,scaled=F,lty="blank",cex=rep(2,7),
cat.pos=c(-40,40,4),cat.dist=c(-0.01,-0.04,-0.05),cat.cex=rep(1.5,3),cat.fontface=rep("bold",3))
grid.text("Residuals = 0.52", x=0.8, y=0.05,gp=gpar(fontsize=20))


##Test significance for:
#Pure env
anova(rda(rich.e,e.env.red,cbind(e.MEM.select,biotic.e)))
# Model: rda(X = rich.e, Y = e.env.red, Z = cbind(e.MEM.select, biotic.e))
#          Df Variance      F Pr(>F)
# Model     2  0.24812 6.8011  0.002 **
# Residual 85  1.55050

#Pure biot
anova(rda(rich.e, biotic.e,cbind(e.env.red, e.MEM.select)))
# Number of permutations: 999
#
# Model: rda(X = rich.e, Y = biotic.e, Z = cbind(e.env.red, e.MEM.select))
#          Df Variance      F Pr(>F)
# Model     1  0.30369 16.648  0.002 **
# Residual 85  1.55050

#Pure spatial
anova(rda(rich.e, e.MEM.select,cbind(e.env.red, biotic.e)))
# Number of permutations: 999
#
# Model: rda(X = rich.e, Y = e.MEM.select, Z = cbind(e.env.red, biotic.e))
#          Df Variance      F Pr(>F)
# Model    11  0.88719 4.4215  0.001 ***
# Residual 85  1.55050


# Linear models (LM) relating species richness to the biotic variable (abundance of competitors).
# Before fitting the models the effect of MEM variables and environmental variables on the response
# is partialled out, so that the analysis assesses only the effect of biotic interactions.
# The inflation of type I error due to spatial autocorrelation and niche effects are prevented.
#*******************************************************************************

#### The procedure for GRAZING ####

#Create response and explanatory variables for posterior LM
g.resid<-resid(lm(rich.g~.,data=data.frame(g.env.red,g.MEM.select)))
#Sp. richness after partialling out the effects of spatial and environmental variables
g.biot.resid<-resid(lm(biotic.g~.,data=data.frame(g.env.red,g.MEM.select)))
#Biotic variable after partialling out the effects of spatial and environmental variables

g.biot.lm<-lm(g.resid~g.biot.resid,2) #Fit the model between sp. richness and the biotic variable.
summary(g.biot.lm)     #Model summary
#Coefficients:
#               Estimate Std. Error t value Pr(>|t|)
#(Intercept)  -4.878e-17  1.368e-01   0.000  1.00000
#g.biot.resid -3.825e-02  1.337e-02  -2.862  0.00515 **
#---
#Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
#
#Residual standard error: 1.368 on 98 degrees of freedom
#Multiple R-squared:  0.07711, Adjusted R-squared:  0.06769
#F-statistic: 8.188 on 1 and 98 DF,  p-value: 0.005154

##There is a significant negative relationship between richness and abundance of competitors.

#Plot residuals to validate the model
quartz()
windows()
```

```
par(mfrow=c(2,2))
plot(g.biot.lm)#The model is tecnically OK.

#Create the necessary objects to plot the models.
g.data<-data.frame(g.resid,g.biot.resid)      #Create an object including response and explanatory
 variables
g.xs<-seq(range(g.biot.resid)[1],range(g.biot.resid)[2],l=100)
#Create new explanatory data using the observed range, to do a smooth prediction with the model.
g.pred<-
 data.frame(predict(g.biot.lm,interval="prediction",level=0.95,newdata=data.frame(g.biot.resid=g.xs))
 )
#Predict with the model using new explanatory data.

#### Repeat the procedure for EXCLUSION ####

#Create response and explanatory variables for posterior LM
e.resid<-resid(lm(rich.e~.,data=data.frame(e.env.red,e.MEM.select)))
#Sp. richness after partialling out the effects of spatial and environmental variables
e.biot.resid<-resid(lm(biotic.e~.,data=data.frame(e.env.red,e.MEM.select)))
#Biotic variable after partialling out the effects of spatial and environmental variables

e.biot.lm<-lm(e.resid~e.biot.resid,2) #Fit the model between sp. richness and the biotic variable.
summary(e.biot.lm)     #Model summary
#Coefficients:
#               Estimate Std. Error t value Pr(>|t|)
#(Intercept)  -8.410e-17  1.252e-01   0.000        1
#e.biot.resid -9.842e-02  2.247e-02  -4.381 2.96e-05 ***
#---
#Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
#
#Residual standard error: 1.252 on 98 degrees of freedom
#Multiple R-squared:  0.1638,  Adjusted R-squared:  0.1553
#F-statistic: 19.19 on 1 and 98 DF,  p-value: 2.963e-05

##There is a significant negative relationship between richness and abundance of competitors.

#Plot residuals to validate the model
quartz()
windows()
par(mfrow=c(2,2))
plot(e.biot.lm)#The model is tecnically OK.

#Create the necessary objects to plot the models.
e.data<-data.frame(e.resid,e.biot.resid)
#Create an object including response and explanatory variables
e.xs<-seq(range(e.biot.resid)[1],range(e.biot.resid)[2],l=100)
#Create new explanatory data using the observed range, to do a smooth prediction with the model.
e.pred<-
 data.frame(predict(e.biot.lm,interval="prediction",level=0.95,newdata=data.frame(e.biot.resid=e.xs))
 )
#Predict with the model using new explanatory data.

# Fig.4e in the main text. Plot the data and the linear models (LM) for GRAZING and EXCLUSION
#*********************************************************************************************
quartz(width=10,height=5)
windows(width=10,height=5)
p1<-ggplot(g.pred, aes(x = g.xs, y = g.resid)) + ylim(-5,5) +
 theme_bw() +
 geom_ribbon(aes(ymin = lwr, ymax = upr),fill = "grey") +
 geom_point(data=g.data,aes(x = g.biot.resid, y = g.resid))  +
 geom_line(aes(y = fit), colour = "blue", size = 1) + geom_text(label="AdjR2 = 0.07",x=6,y=4) +
 xlab("r(Abundace of competitive sp.)") +
 ylab("r(Sp. richness)")
p2<-ggplot(e.pred, aes(x = e.xs, y = e.resid)) + ylim(-5,5) +
 theme_bw() +
 geom_ribbon(aes(ymin = lwr, ymax = upr),fill = "grey") +
 geom_point(data=e.data,aes(x = e.biot.resid, y = e.resid))  +
 geom_line(aes(y = fit), colour = "blue", size = 1) + geom_text(label="AdjR2 = 0.16",x=6,y=4) +
 xlab("r(Abundace of competitive sp.)") +
 ylab("r(Sp. richness)")
grid.arrange(p1,p2,ncol=2)

#Test of the null hypothesis of no effect of E on the estimate of the slope (β) in the regressions
#between species richness and the biotic variable, using as explanatory variables the blocking factor
#Site (Site1, Site2, Site3) and fixed factor Treatment (Grazing, Exclusion).
```

```
#*************************************************************************************************

slope <- c(-2.449e-02,-5.849414e-02,-2.817e-02,-5.252930e-02,-3.824572e-02,-9.842383e-02)
#The above regressions are only for Site 3, the other regressions are not presented in this script.
Site <- c("site1","site1","Site2","Site2","Site3","Site3")
Treatment <- c("G","E","G","E","G","E")
sl.lm <- lm(slope~Site+Treatment)

anova(sl.lm)   #ANOVA table of the analysis. There is moderate evidence that Grazing exclusion
 enhances the negative relationship.
#Response: slope
#          Df     Sum Sq    Mean Sq F value  Pr(>F)
#Site       2 0.00100334 0.00050167  2.9208 0.25505
#Treatment  1 0.00234202 0.00234202 13.6356 0.06614 .
#Residuals  2 0.00034351 0.00017176
#---
#Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

summary(sl.lm) #Summary table of the test
# Fig.6 in the main text. Plot of the estimates of the slopes and their confidence intervals
#*************************************************************************************************

quartz(width=7,height=7)
windows(width=7,height=7)
y <- c(1,1.5,3,3.5,5,5.5)      #Just for the plot
lci <- slope-1.96*c(1.309e-02,1.322e-02,1.665e-02,1.508e-02,1.337e-02,2.247e-02)
#Lower confidence interval of the estimate of the slope
uci <- slope+1.96*c(1.309e-02,1.322e-02,1.665e-02,1.508e-02,1.337e-02,2.247e-02)
#Upper confidence interval of the estimate of the slope
par(mar=c(5,8,4,2))
plot(slope,y,type="n",axes=F,xlim=c(-0.15,0.05),ylab="",xlab=expression(Estimate-of-beta))
axis(1)
axis(2,at=y,labels=c("Site 1 Grazing","Site 1 Exclusion","Site 2 Grazing","Site 2 Exclusion","Site 3
 Grazing","Site 3 Exclusion"),las=1)
points(slope,y,pch=16)
segments(x0=lci,y0=y,x1=uci)
segments(x0=lci,y0=y-0.1,y1=y+0.1)
segments(x0=uci,y0=y-0.1,y1=y+0.1)
lines(x=rep(0,6),y=seq(-1,6.2,l=6),lty="dashed")
```

Appendix S5. Illustration of the differences between measuring species abundances in frequencies and covers, and analyses demonstrating that *Festuca rubra* excludes weaker species in *Site 1 Exclusion*.

Grazing exclusion unleashes competitive plant responses in Atlantic mountain grasslands by I. Odriozola, G. García-Baquero, M. J. Fortin, N. A. Laskurain and A. Aldezabal.

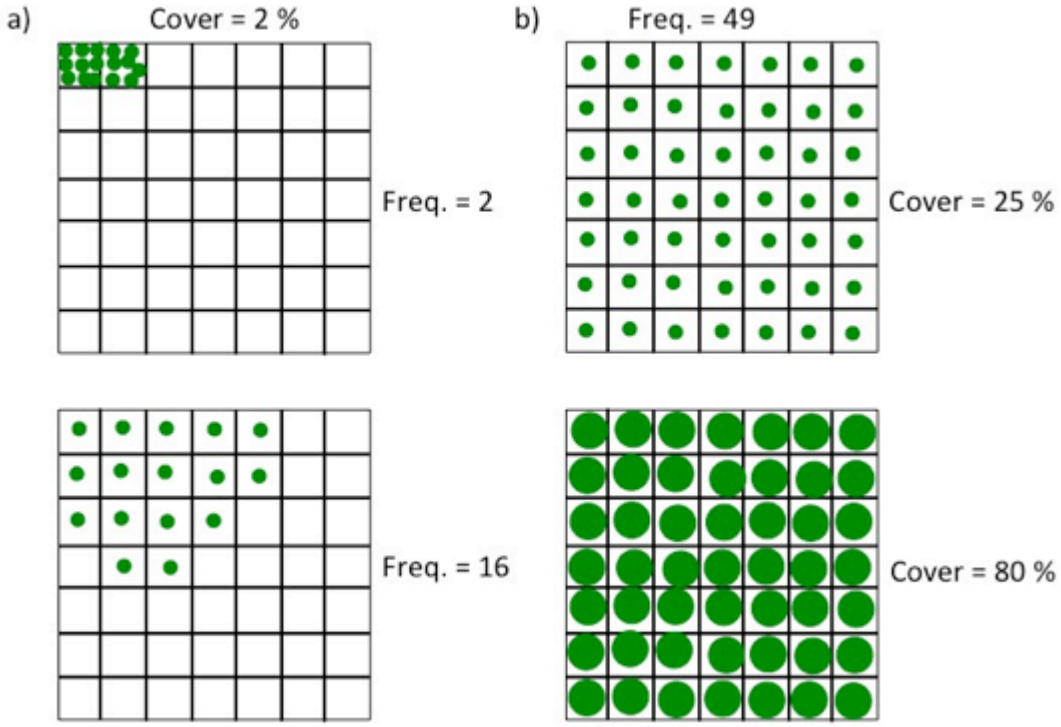

Fig. S11. Illustration of the differences between the two types of abundances –% cover and frequencies– used in this work: a) the same cover can represent different frequencies; b) the same frequency can represent different covers.

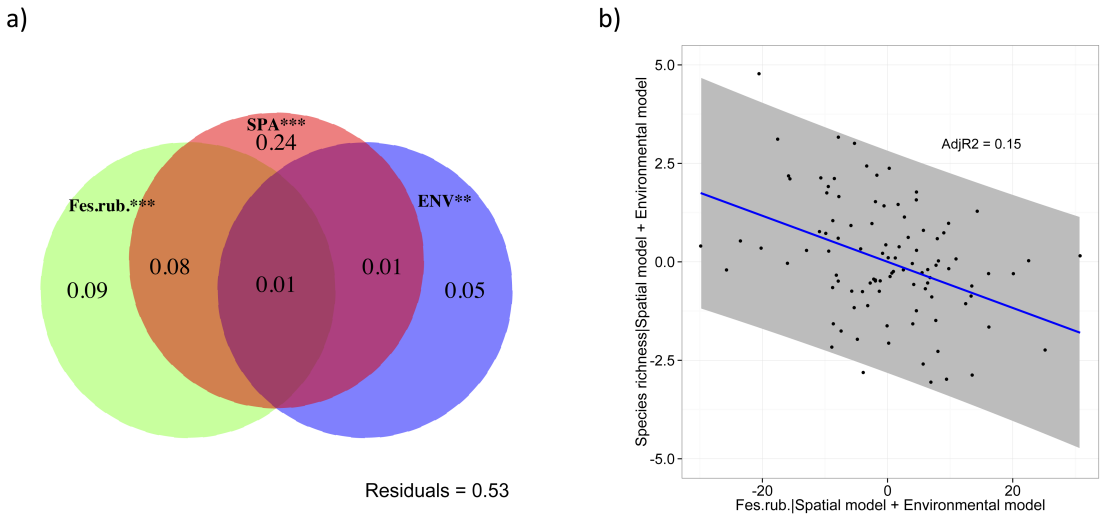

Fig. S12. Effect of the abundance of *Festuca rubra* instead of the sum of the six potential competitive species on species richness in *Site 1 Exclusion*: a) RDA variance partitioning of species richness into a *biotic* fraction –abundance of *Festuca rubra*–, an environmental fraction and a spatial fraction, as in the Fig. 4b of the main text; b) Linear regression between species richness and the abundance of *Festuca rubra*, as in the Fig. 5b of the main text.

CHAPTER 4. Patterns of species relatedness created by competitive exclusion depend on niche stabilization: evidence from Atlantic grasslands

Odriozola, I., García-Baquero, G., Etxeberria, A. & Aldezabal, A.

Summary

1. It is commonly assumed that closely related species share more similar niches than do distantly related species, thus limiting the ability of closely related species to coexist and leading to patterns of phylogenetic over-dispersion. On the contrary, recent theoretical developments argue that competitive exclusion may lead to patterns of either over-dispersion, clustering or randomness, depending on the relative importance of niche differences and interspecific competitive ability differences.

2. In this study, we utilized semi-natural communities (from grasslands) *in situ* to test the hypothesis that the pattern of species relatedness generated by competitive exclusion depends on niche stabilization.

3. Instead of inferring processes from observed patterns, we experimentally manipulated grassland plots to test the effects of competitive exclusion. We compared grazed plots (in which grazing functioned as an equalizing mechanism and suppressed above-ground competition) with neighbouring plots experimentally excluded from grazing over the course of nine years (in which above-ground competition was not prevented). We used an extended version of RLQ ordination that accounted for both space and phylogeny as well as pattern analysis based on the standardized effect size of community mean pairwise distances to analyse data.

4. As expected, weak levels of niche stabilization allowed competitive exclusion to generate phylogenetic clustering (under-dispersion). This clustering occurred because phylogenetically structured traits as plant canopy height and the capacity for lateral spread conferred superior competitive ability to grasses that outcompeted species in all dicot branches. Moreover, as predicted, moderate levels of niche stabilization, allowed competitive exclusion to result in a *random* pattern of phylogenetic species assembly (i.e. neither under-dispersion nor over-dispersion). This occurred because niche differences partially counter-balanced competitive exclusion by superior competitors in the grass family.

5. *Synthesis.* This study, focused on plant communities in a semi-natural grassland, to our knowledge, represents the first field-based experiments providing evidence that patterns of species relatedness created by competitive exclusion depend on niche stabilization. The results of our experiment confirm that competitive

exclusion may cause differential patterns of phylogenetic assembly, which demonstrates that inferring processes regarding mutually excluding environmental filtering or competitive exclusion from observed patterns, may lead to erroneous conclusions.

Keywords: biotic interactions, community assembly, competition-relatedness hypothesis, phylogenetic clustering, phylogenetic over-dispersion, plant traits, RLQ analysis, species coexistence.

4.1. Introduction

Several ecological processes (speciation, dispersal, environmental factors, and biotic interactions) are implicated in the fundamental question of how species assemble and coexist (Götzenberger *et al.* 2012). However, competitive interactions and their potential stabilization driven by niches (Chesson 2000) are perhaps the most important processes occurring at local (fine) scales (HilleRisLambers *et al.* 2012). In order to understand the effect of competitive interactions on species assembly, the so-called competition-relatedness hypothesis (CRH) (Cahill *et al.* 2008) has often been proposed (Webb *et al.* 2002; Dayan & Simberloff 2005; Cavender-Bares *et al.* 2009). This hypothesis, critically reviewed by Mayfield & Levine (2010), proposes that closely related taxa likely share more similar niches compared to distantly related counterparts, limiting their ability to coexist.

Furthermore, several authors (Webb *et al.* 2002; Swenson *et al.* 2006; Cavender-Bares *et al.* 2009) have suggested that under the CRH, and in the presence of phylogenetic niche conservatism, competitive exclusion would create patterns of phylogenetic over-dispersion (or repulsion) in local communities. Thus, if the observed species maintain ancestral traits relevant to competition, strong interspecific competition should lead to patterns in which closely related species co-exist less often than expected by chance (phylogenetic over-dispersion). By contrast, under the assumption of phylogenetic niche conservatism, the dominant processes of environmental filtering would lead to phylogenetic under-dispersion

or clustering, as closely related species would share the necessary traits to simultaneously exist in a given environment. Importantly, assuming that the assembly processes can be inferred from observed patterns of species co-occurrence, which is not always true (García-Baquero & Crujeiras 2015), and that the CRH holds, several authors (Swenson *et al.* 2006; Helmus *et al.* 2007; Mayfield & Levine 2010) proposed that the phylogenetic patterns of biological communities can be employed to determine if processes of competitive exclusion (indicated by phylogenetic over-dispersion) or environmental filtering (indicated by under-dispersion) drove the assembly of a given community.

As reviewed by Mayfield & Levine (2010), the empirical evidence supporting the CRH in plant systems is not uniform. A meta-analysis using five multi-species experiments involving 142 vascular plant species provided weak support for CRH (Cahill *et al.* 2008). Therefore, Mayfield & Levine (2010), and later HilleRisLambers *et al.* (2012), used Chesson's (2000) coexistence theory to propose an updated theoretical framework to predict the effects of competitive exclusion on phylogenetic community patterns. In particular, the authors (Mayfield & Levine 2010; HilleRisLambers *et al.* 2012) argued that it is both the level of niche stabilization and the strength of interspecific competitive ability differences (as measured by relevant traits) that determines the impact of competitive exclusion on phylogenetic patterns. Essential differences in niches, if positively correlated with phylogenetic distance (i.e. strong stabilization) cause closely related species (with higher niche overlap) to compete more intensely than with distantly related organisms, favouring the coexistence of distantly related taxa and creating patterns of phylogenetic over-dispersion. However, key differences in competitive ability positively correlated with phylogenetic distance would cause closely related species (which share traits that confer competitive ability) to coexist more often than expected by chance, and thereby create patterns of phylogenetic clustering (Mayfield & Levine 2010). Therefore, in the presence of phylogenetically conserved differences in competitive ability, the expected pattern of species relatedness would be either: 1) clustered (in the absence of stabilization driven by niches) or 2) clustered, over-dispersed, or *random* (depending on the relative influence of the niche vs. differences in competitive ability) (Mayfield & Levine

2010).

Mayfield & Levine's (2010) theoretical framework was previously used in observational (Muscarella *et al.* 2016), *in silico* (Herben & Goldberg 2014), field-based experimental (Bennett *et al.* 2013) and common garden-based studies (Godoy, Kraft & Levine 2014). Bennet *et al.* (2013) and Godoy *et al.* (2014) tested the effect of phylogenetic relatedness on species assembly patterns resulting from competitive exclusion, without testing for the potential effects of stabilization by plant niches. They showed that strong competition did not always lead to patterns of phylogenetic over-dispersion (Bennett *et al.* 2013) and that differences in competitive ability can be phylogenetically structured (Godoy *et al.* 2014). These studies confirmed that empirical approaches to infer assembly processes from observed patterns of species co-occurrence produce indecisive answers to the fundamental question regarding how species assemble and coexist. Therefore, the potential effects of plant niche stabilization on competitive exclusion and associated patterns appear to remain untested.

HilleRisLambers *et al.* (2012) proposed that the implementation of research programs ideally based on the combination of three different strategies: experimental manipulation of the abiotic or biotic environment, assessment of trait-phylogeny-environment relationships, and examination of frequency-dependent population growth. In this study, we tested the hypothesis that the pattern of species relatedness generated by competitive exclusion depends on niche stabilization, a thus far untested central conjecture of Mayfield & Levine (2010). Our experimental research uses entire plant communities (Atlantic grasslands) and combines the first two strategies proposed by HilleRisLambers *et al.* (2012). First, we erected grazer-exclusion fences (exclusion (E) plots) nine years ago, at two sites differing in strength of niche stabilization (very weak in one sites vs. moderately strong in the other, Odriozola *et al*. 2016), in a highly productive Atlantic grassland. Simultaneously, we established paired grazing (G) plots. In the G plots, grazing acted as an equalizing mechanism (Chesson 2000) preventing strong competitors from out-competing the weakest species. The comparison between G and E (where competition was not prevented by grazing)

across sites allowed us to examine whether the effects of competitive exclusion depended on niche stabilization (Mayfield & Levine 2010). Additionally, using the extended version of the **RLQ** ordination proposed by Pavoine *et al.* (2011), we were able to link spatially structured environmental variables with phylogenetically structured species traits using a biological matrix of species abundance. In order to test for phylogenetic signals of traits contributing to competitive ability, we considered the functional traits of canopy height and clonality, which have proven to be relevant to competition in fertile grasslands (Craine *et al.* 2001; Grime 2001; Gough *et al.* 2012; Dickson *et al.* 2014). We derived the following predictions: 1) at the site with very weak niche stabilization, species from clades with superior competitive abilities –conferred by the plant height and degree of vegetative spread– will out-compete species in weaker clades, resulting in a phylogenetically clustered subset of species; and 2) at the site with moderately strong plant niche stabilization, competitive exclusion by species in competitively superior clades will be counter-balanced by niche differences, resulting in the presence of a random subset of species.

4.2. Materials and Methods

STUDY AREA

Experiments were conducted at two field sites in a semi-natural grassland system located in Aralar Natural Park: Site 1 (Uzkuiti: 43° 0' 50'' N, 2° 4' 3'' W: 1300 m.a.s.l) and Site 2 (Igaratza: 42° 59' 9.25'' N, 2° 2' 9.7'' W: 1247 m.a.s.l). Aralar Natural Park is an 11,000-ha protected area located in the Basque Country (Northern Iberian Peninsula). The area has oceanic climate, with a mean annual temperature of 12.4°C and annual precipitation of >1,400 mm. The area was traditionally used by livestock (beef cattle, dairy sheep, and horses) and occupies approximately 2,077-ha (18.9% of the park area); however, its usage varies seasonally (from May until November). The primary vegetation type is a highly productive (mean above-ground net primary productivity of 3.37 t Dry Mass ha^{-1} $year^{-1}$ with a standard error of 0.88) native grassland on a calcareous substrate (Gibbons and Moreno 2002), which contains mainly perennial species (Loidi 1982) and corresponds to the priority habitat "species-rich *Nardus* grasslands" (code

6230) of the Habitat Directive (92/43/EEC, European Commission 2013).

EXPERIMENTAL DESIGN

In order to simulate grazing cessation, and allow for above-ground competition, a permanent fenced exclusion (E) plot (50 m × 50 m) was established in May 2005 at each of the two field sites. Next to each E plot, we delineated a grazed plot (G), where sheep, cattle, and horses were allowed to graze continuously during the vegetative period (May–November). Both sites were located on relatively flat terrain and, when fences were erected (2005), functional group composition (including graminoids, non-legume forbs, and legumes) based on relative abundances was not significantly different between E and G plots at either field site (Odriozola *et al*. 2016). In each plot, 100 sampling units (quadrats) were delineated in a layout in which quadrats were located 2 m apart, creating plots of 18 m × 16 m. At the field sites, soil water content and pH were shown to significantly affect floristic composition (Odriozola *et al.* 2016). However, the sites differ in the amount of multivariate variation in species composition explained by either pH or soil water content or both parameters. At Site 1, these environmental variables only explained R^2 = 8% regarding the species composition (demonstrating a very weak niche stabilization effect). At Site 2, pH and soil water content explained R^2 = 26% in respect to species composition (demonstrating a moderately strong niche stabilization effect) (Odriozola *et al*. 2016). Plant segregation along hydrological niches is a well-documented process across continents and ecosystems (Silvertown *et al.* 1999; Silvertown, Araya & Gowing 2015; García-Baquero *et al.* 2016). On the other hand, species turnover along pH gradients is also well documented, as pH affects edaphic factors like availability of different nitrogen forms (Bartelheimer & Poschlod 2013) or solubility of toxic compounds (Poschenrieder *et al.* 2008). Moreover, pH exhibited high local, fine scale heterogeneity at Site 2, but not Site 1, creating a pH gradient along which grasses, forbs, and sedges vary in abundance (Fig. 4.1). Soil pH is, in turn, negatively correlated with soil nitrogen and phosphorus and positively correlated with calcium and magnesium (Odriozola *et al*. 2016).

VEGETATION AND SOIL SAMPLING

Sampling was conducted during the growing season of 2014, after nine years of grazer exclusion. In each quadrat, floristic composition and soil properties were measured. Floristic composition and structure were measured using species percentage cover. In each quadrat, pH was measured using standard protocols (Jackson, Farrington & Henderson 1986), and soil water content was measured using a Delta-T SM 150 Soil Moisture Kit, (Delta-T Devices, Cambridge, UK) at fixed intervals during the growing season. Mean soil water content (MSWC) (%) was then derived for each quadrat using the area under the curve, which was computed with the R function trapz in the pracma package (Borchers 2015).

PHYLOGENIES AND TRAITS

The phylogenetic trees (Fig. 4.2) were obtained using Phylomatic software (Webb & Donoghue 2005), with the latest APG (AGP III 2009) classification. The bladj algorithm in the Phylocom software (Webb, Ackerly & Kembel 2008) was used to assign branch lengths to the trees.

Three traits related with plant competitive ability were considered in this study: canopy height (H), clonal growth organs (CGO) and lateral spread (LS). H was selected as an indicator of capacity to compete for light, and CGO and LS were selected as indicators of a capacity to compete for space, since the combination of height and clonality drives competitive exclusion in fertile grasslands (Gough *et al.* 2012; Dickson *et al.* 2014). The data for the H trait were collected from field measurements as well as the LEDA trait database (Kleyer *et al.* 2008). Field measurements were conducted following standard protocols (Cornelissen *et al.* 2003). CGO is a multi-choice (root splitters, adventitious roots, bulbs, rhizomes, and stolons) categorical trait and was obtained from the LEDA (Kleyer *et al.* 2008) and Ecoflora of British Isles (Fitter & Peat 1994) databases. The ordinal trait LS was defined following the Ecoflora database (Fitter & Peat 1994): 1) non-clonal and short creeping clonal species (capacity to either not form patches or only small patches), 2) tussock forming species (capacity to form medium size patches), and 3) long creeping stoloniferous and rhizomatous species (capacity to form large patches).

ASSEMBLING THE DATASET

For each site, we used a five-matrix dataset (Pavoine *et al.* 2011). A first matrix with species composition (**L**), where each element represented the abundance (cover) of a species in a sample unit. Also, for each site we included a spatial matrix **S** that specified the Cartesian geographic coordinates (X, Y) for the sample units, and an environment matrix (**E**), including values for pH and MSWC as the sample units. A fourth trait matrix (**T**) described average trait values for the species found at the sites (H, LS, and CGO). Finally, the phylogenetic trees (Fig. 4.2) were used to derive a phylogenetic matrix (**P**) for each site, where each element represented pairwise phylogenetic distances among species.

DATA ANALYSIS

To test the null hypothesis of no effect of pH on the abundance of main plant groups, Generalised Additive Models (GAM) were used (Wood 2006), through the mgcv package (Wood 2011) in R software version 3.2.2 (R Core Team 2015). To evaluate if the biological traits had a phylogenetic signal, we used a root-skewness test (Pavoine, Baguette & Bonsall 2010). Data analysis then used an extended version of the so-called **RLQ** ordination (Dodélec *et al.* 1996). **RLQ** ordination allows the simultaneous ordination of three tables; linking a trait matrix **R**, weighted by species abundance matrix **L**, to an environmental matrix **Q**. The extended version of **RLQ** used here includes not only environmental and species traits but also space and phylogeny (Pavoine *et al.* 2011). We therefore related the spatially structured environmental variables in matrix **R** (which is a matrix that combines environmental descriptors and spatial variables from the above matrices **E** and **S**, respectively) with phylogenetically structured species traits in matrix **Q** (which is a matrix that combine species traits and phylogenetic variables described by the above matrices **T** and **P**, respectively), via community composition and abundance (as described by matrix **L**). The functions described by Pavoine *et al*. (2011) were implemented using the ade4 package in R software version 3.2.2 (Dray & Dufour 2007). Finally, we tested for patterns of phylogenetic assembly using the ses.mpd function in the R package picante (Kembel *et al.* 2010).

R code and data sufficient to replicate our analyses are provided in Appendices S1 and S2 of the supplementary material, respectively.

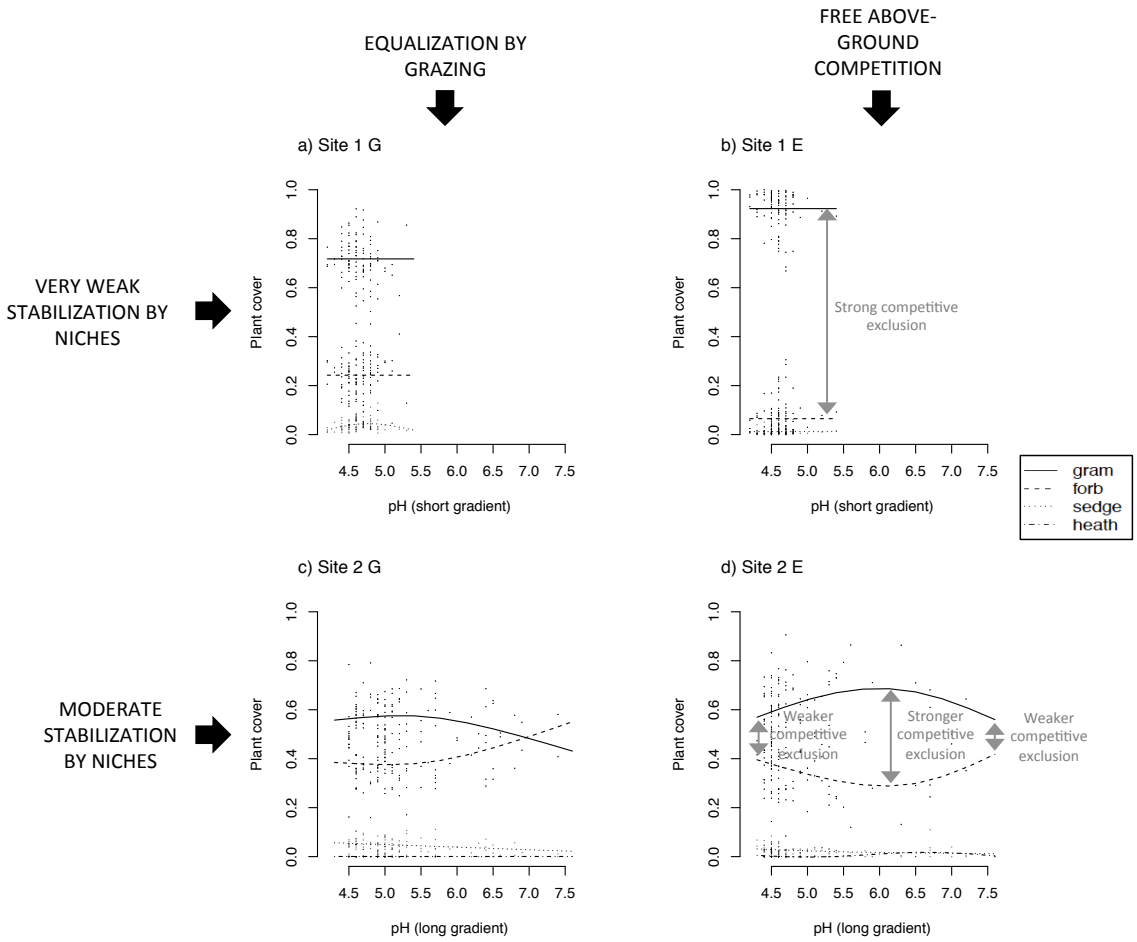

Fig. 4.1. Generalised Additive Models (GAM) relating the main plant groups (grasses, forbs, sedges, and heaths) and pH to a) Site 1 grazing, b) Site 1 exclusion, c) Site 2 grazing, and d) Site 2 exclusion. At Site 1, there was a short pH gradient, which had either a null or very weak effect on stabilizing competition. At Site 2, there was a longer pH gradient, moderately stabilizing competitive exclusion.

4.3. Results

Plant height and clonality (assessed by traits H, CGO, and LS) were found to be significantly phylogenetically structured, although the phylogenetic signal for LS was not significant at Site 2 (Table 4.1). At Site 1, pH exhibited a short gradient and had no effect on the abundance of plant groups. Consequently, in the absence of pH niche stabilization, competitive exclusion by grasses was constant and strong across the gradient (Fig. 4.1a, b and Table 4.2). Alternatively, pH exhibited a long gradient at Site 2 and plant groups, in turn, segregated along the pH gradient,

resulting in stronger competitive exclusion by grasses found at medium pH values, and weaker competitive exclusion at the highest and lowest pH values (Fig. 4.1c, d and Table 2).

The extended **RLQ** plots relating spatially structured environment with phylogenetically structured plant traits (Figs 4.3-4.6), which are remarkably rich in information, are interpreted here regarding only the main objective of the study, which was to assess whether plant segregation into niches counter-balanced competitive exclusion. Treatment (G vs. E) was not included in the environmental matrix, so its effect was identified in the space-based maps (Figs 4.3a, 4.4a, 4.5a and 4.6a) representing biotically induced spatial structure according to trait distribution. At Site 1, where plant segregation into niches was very weak, the first canonical axis explains 59% of the total variance (Fig. 4.3), and shows that, under

Table 4.1. Summary of the results from tests for phylogenetic signals on traits related to competitive ability.

Sites	Traits	Deviation from theoretical values	*P*-value
Site 1	Canopy height	-2.89	0.009
	Clonal growth organs	-3.209	0.003
	Lateral spread	-1.78	0.045
Site 2	Canopy height	-2.235	0.015
	Clonal growth organs	-3.535	0.003
	Lateral spread	-0.772	0.209

Table 4.2. Summary table of generalised linear model (GAM) analyses between plant groups (grasses, forbs, sedges, and heaths) and pH, at the two field sites (Site 1 and Site 2) under the two grazing regimes (grazing and exclusion).

Site and treatment	Response (plant group % cover)	R2 (adj.)	*F* (edf)	*p*-value
Site 1 G	Grasses	0.01	2.12 (1)	0.149
Site 1 G	Forbs	0	1.4 (1)	0.24
Site 1 G	Sedges	0.07	3.68 (2.02)	0.024
Site 1 E	Grasses	0.02	2.1 (1.11)	0.142
Site 1 E	Forbs	0.03	2.09 (1.2)	0.139
Site 1 E	Sedges	0	0.04 (1)	0.849
Site 2 G	Grasses	0.04	2.57 (1.82)	0.078
Site 2 G	Forbs	0.07	3.81 (1.83)	0.023
Site 2 G	Sedges	0.06	7.66 (1)	0.007
Site 2 G	Heaths	0	0.34 (1)	0.561
Site 2 E	Grasses	0.08	3.87 (2.02)	0.02
Site 2 E	Forbs	0.06	3 (2)	0.048
Site 2 E	Sedges	0.08	5.76 (1.55)	0.006
Site 2 E	Heaths	0.12	3.52 (3.11)	0.005

grazing exclusion (above-ground competition), tall (high H) grasses with a tendency to spread laterally (high LS) dominated the community, displacing species in all other clades and competitively excluding many of them (Fig. 4.3a, b, d, e). Root splitters and species with adventitious roots are related to the positive end of the axis, and many species in the community have rhizomes and stolons (Fig. 4.3a, c). The same axis shows a weak correlation with soil variables, which was negative in respect to pH and positive with MSWC (Fig. 4.3a, f). At Site 1, Axis 2 (Fig. 4.4), which explains 29% of total variance, represents a contrast between *Festuca rubra* and *Agrostis capillaris*; both species exclude each other at very local scales, and the contrast is more pronounced in the exclusion plot (Fig. 4.4a, b). Under *Agrostis* dominance, few species (e.g. *Luzula campestris* and *Trifolium repens*) generally survive, whereas under *Festuca* dominance, whole branches (of species belonging to the families *Ranunculaceae*, *Rosaceae*, and *Fabaceae*) coexist (Fig. 4.4b). Trait differences are not relevant in respect to this axis as none of the traits or soil variables are correlated to the axis (Fig. 4.4c, d, e, f).

The first axis of the ordination for Site 2 (accounting for 46% of total variance) (Fig. 4.5), the site with moderately strong plant niche stabilization, represents a process similar to that described by the first axis of Site 1, in which grasses with traits conferring competitive ability exclude inferior competitors. Although tall species with the capacity to spread laterally tend to dominate the E plot, in contrast to Site 1, patches of short species with low vegetative spread are present (Fig. 5a, b, c, d, e). Unexpectedly, soil variables were found to be very weakly correlated with the axis (Fig. 4.5f). Lastly, the second axisof the ordination for Site 2 (19% of the variance) is related to long creeping stoloniferous and rhizomatous species with the capacity to spread laterally, and the location of white squares in the grazing plot indicate the prevalence of these species under grazing conditions (Fig. 4.5a, b, c, e). This axis is uncorrelated with H and soil variables (Fig. 4.5d, f).

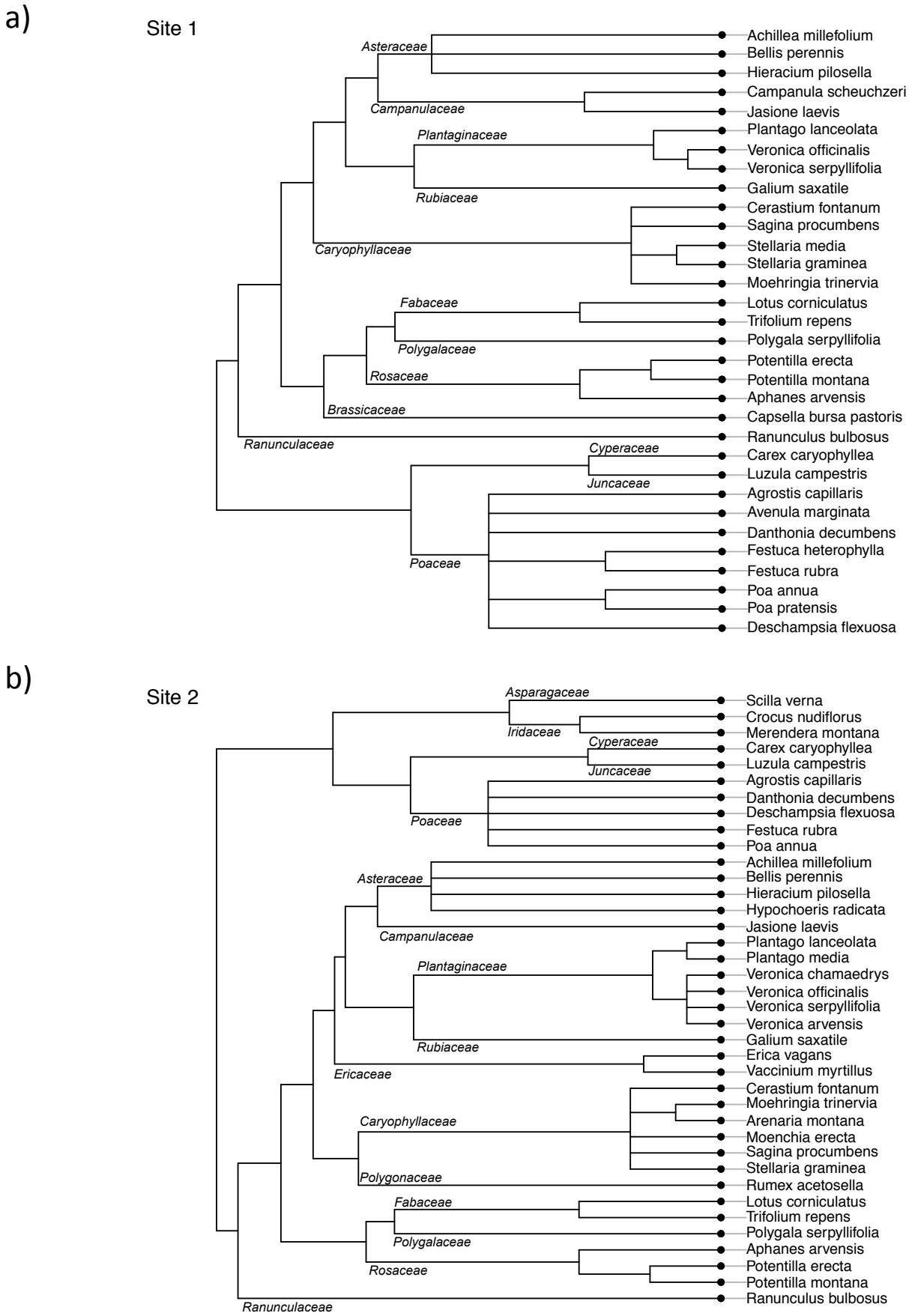

Fig. 4.2. Phylogenetic trees for a) Site 1, and b) Site 2.

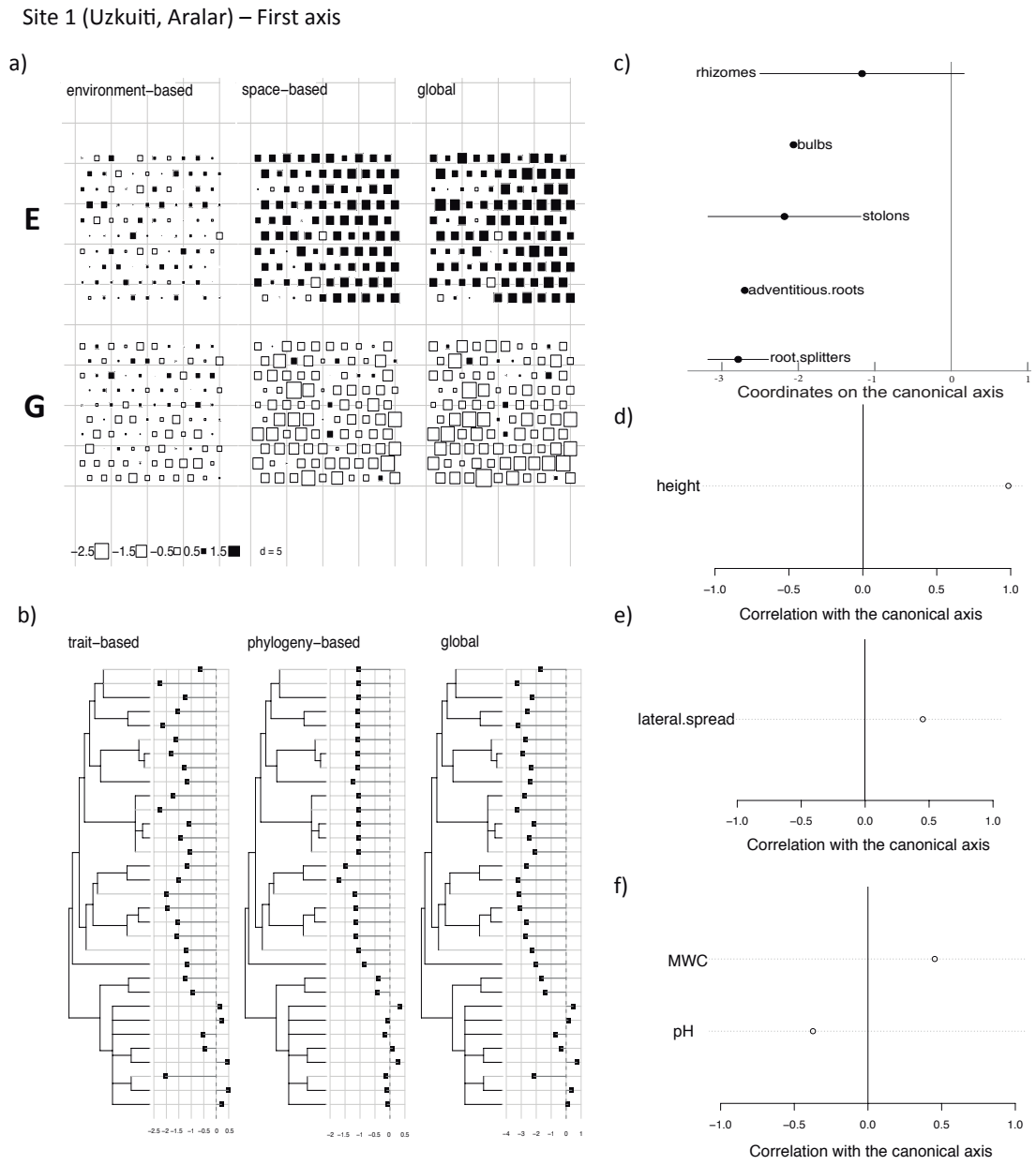

Fig. 4.3. Results of the extended **RLQ** analysis for the first axis in Site 1. a) The global coordinates of the sites are defined as the sum of a combination of the soil descriptors and a combination of spatial variables. The quadrats are located in their geographical location in the 20 m × 40 m area. The 100 quadrats above correspond to the grazer-exclusion plot (E) and the 100 quadrats below to the grazing plot (G). The size of the squares is proportional to the absolute values of site coordinates; white indicate a negative coordinate and black a positive coordinate. b) The coordinates of the species are defined as the sum of a combination of trait variables and a combination of phylogenetic variables. The coordinates are given by a Cleveland dot plot next to the phylogenetic tree (See Fig. 4.2 for species names). Branch tips in grey indicate species present in the G plot that has been competitively excluded from the E plot. c) For the multi-choice, categorical trait, clonal growth organs (CGO), the attributes are located at the average coordinates of the species that posses them. For a given attribute, the standard deviation of the scores of the species that posses this attribute is given by the length of a segment. d) For the ordinal

trait lateral spread (LS), Spearman correlation (based on ranks) between the trait and coordinates of species on the canonical axis is given. e) For the numerical trait canopy height (H), Pearson correlation (based on raw data) between the trait and coordinates of species on the canonical axis is given. f) For numeric environmental variables, Pearson correlations (based on raw data) between the variables and the coordinates of the sites on the canonical axis are given. As a synthetic interpretation, quadrats in E plot weakly tended to be more acidic and to have more MSWC. Additionally, in these quadrats, species from the grass family, which in turn are tall and spread laterally, are dominant, and exclude sedges and dicots across all families.

In order to obtain an overall view, we synthesized our findings under Mayfield and Levine's (2010) theoretical framework (Fig. 7). The first axis of the ordination for Site 1 represents strong levels of competitive exclusion, due to differences in competitive ability between grasses and other clades, as well as weak niche stabilization. In contrast, the first axis of Site 2 represents moderate competitive exclusion because competitive exclusion by superior grasses is counterbalanced by moderately strong niche stabilization. The second axis of Site 1 represents two species with similar competitive abilities, excluding each other locally. Lastly, the second axis of the Site 2 represents species from all clades with capacity to spread laterally, locally excluding other species.

Finally, we tested for the patterns of species assembly generated by competitive exclusion. We found moderate evidence (observed value of the statistic = 501.71 < expected value = 504.91; *p*-value = 0.055) against the null hypothesis of random phylogenetic assembly in the exclusion plot of Site 1 (characterized by very weak plant segregation into niches). Since the observed value is significantly less than the expected value, the observed pattern corresponds to phylogenetic clustering or under-dispersion. The null hypothesis of random assembly in Site 2 exclusion was not rejected (observed value = 515.28 > expected value = 510.27; *p*-value = 0.995).

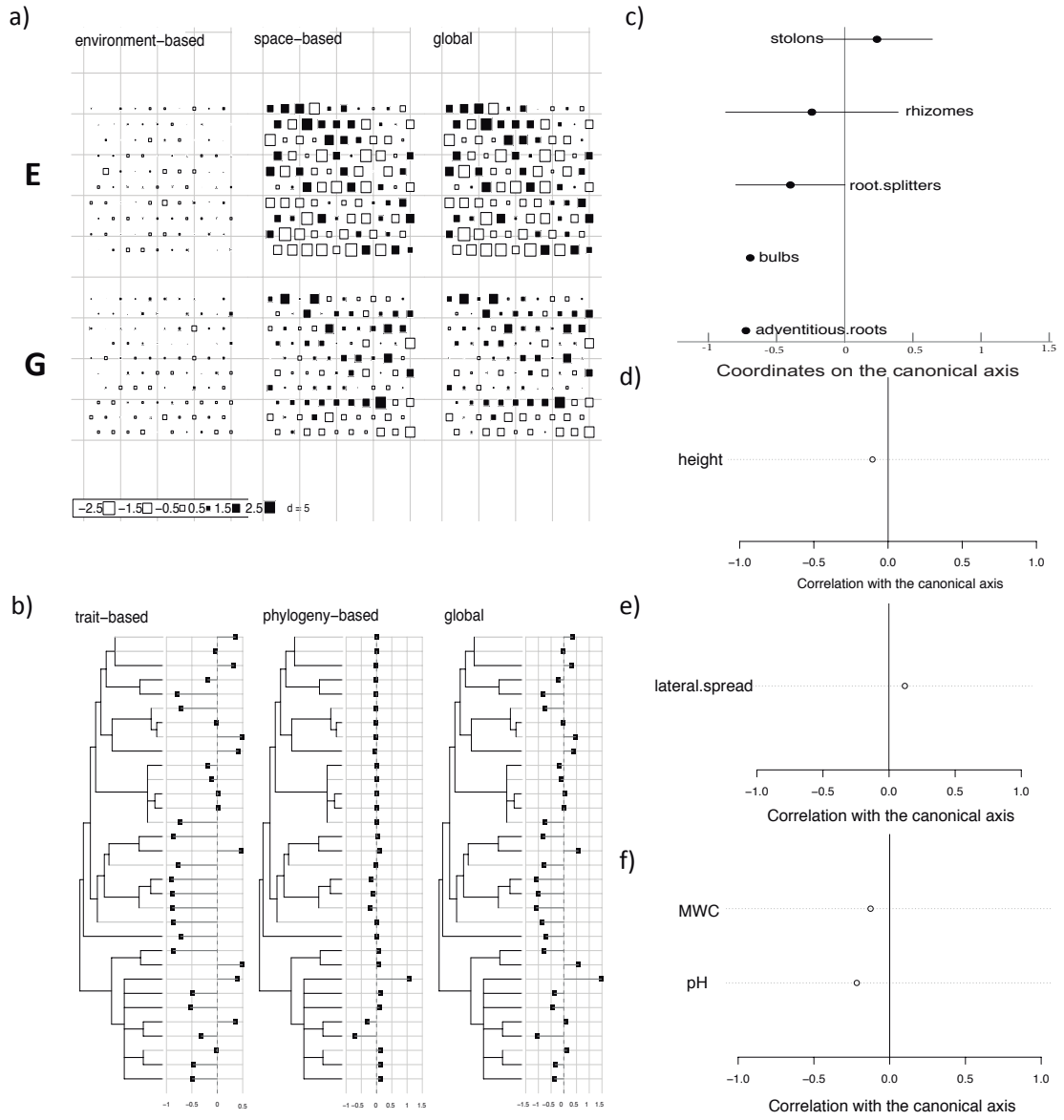

Fig. 4.4. Results of the extended **RLQ** analysis for the second axis in Site 1. a) The global coordinates of the sites are defined as the sum of a combination of the soil descriptors and a combination of spatial variables. The 100 quadrats above correspond to the grazer-exclusion plot (E) and 100 quadrats below to the grazing (G) plot. b) Coordinates of species are the combination of traits and phylogenetic variables. Species coordinates are given by Cleveland dot plots next to the phylogenetic tree (See Fig. 4.2 for species names). Branch tips in grey indicate species present in the G plot that has been competitively excluded from the E plot. c) Attributes of clonal growth organs (CGO) are located at the average coordinates of the species that posses them. d) Spearman correlation between lateral spread (LS) and coordinates of species on the canonical axis. e) Pearson correlation between canopy height (H) and coordinates of species on the canonical axis. f) Pearson correlations between the environmental variables and the coordinates of the sites on the canonical axis. More information and interpretation details are given in Fig. 4.3 legend in the main text.

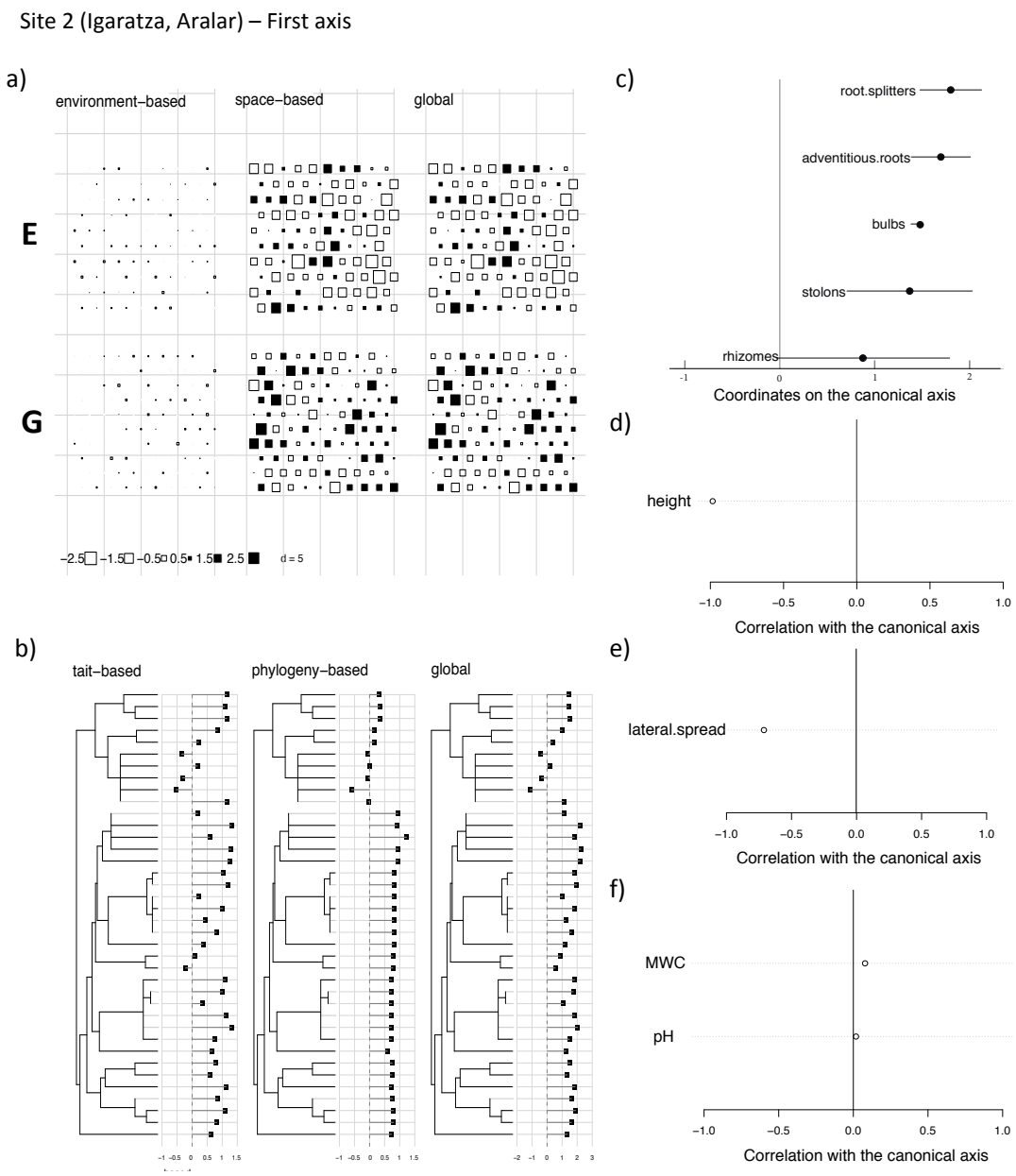

Fig. 5. Results of the extended **RLQ** analysis for the first axis in Site 2. a) Global coordinates of the sites as the sum of the combination of environmental and spatial variables. The 100 quadrats above correspond to the grazer-exclusion plot (E) and the 100 quadrats below to the grazing (G) plot. b) Coordinates of species are the combination of traits and phylogenetic variables. Species coordinates are given by Cleveland dot plots next to the phylogenetic tree (See Fig. 4.2 for species names). Branch tips in grey indicate species present in the G plot that has been competitively excluded from the E plot. c) Attributes of clonal growth organs (CGO) are located at the average coordinates of the species that posses them. d) Spearman correlation between lateral spread (LS) and coordinates of species on the canonical axis. e) Pearson correlation between canopy height (H) and coordinates of species on the canonical axis. f) Pearson correlations between the environmental variables and the coordinates of the sites on the canonical axis. More information and interpretation details are given in Fig. 4.3 legend.

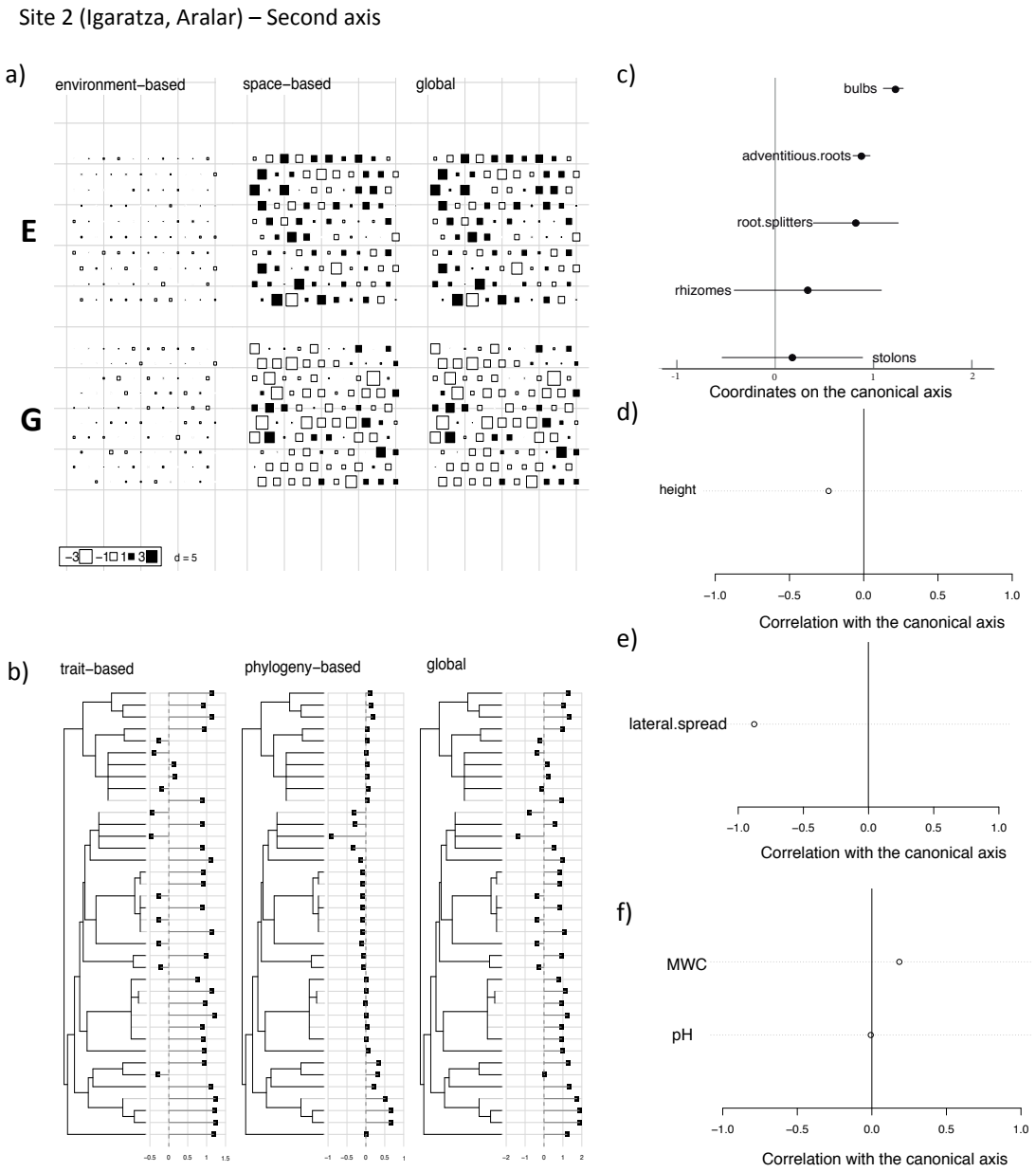

Fig. 6. Results of the extended **RLQ** analysis for the second axis in Site 2. a) Global coordinates of the sites as the sum of the combination of environmental and spatial variables. The100 quadrats above correspond to the grazer-exclusion plot (E) and the 100 quadrats below to the grazing (G) plot. b) Coordinates of species are the combination of traits and phylogenetic variables. Species coordinates are given by Cleveland dot plots next to the phylogenetic tree (See Fig. 4.2 for species names). Branch tips in grey indicate species present in the G plot that has been competitively excluded from the E plot. c) Attributes of clonal growth organs (CGO) are located at the average coordinates of the species that posses them. d) Spearman correlation between lateral spread (LS) and coordinates of species on the canonical axis. e) Pearson correlation between canopy height (H) and coordinates of species on the canonical axis. f) Pearson correlations between the environmental variables and the coordinates of the sites on the canonical axis. More information and interpretation details are given in Fig. 4.3 legend in the main text.

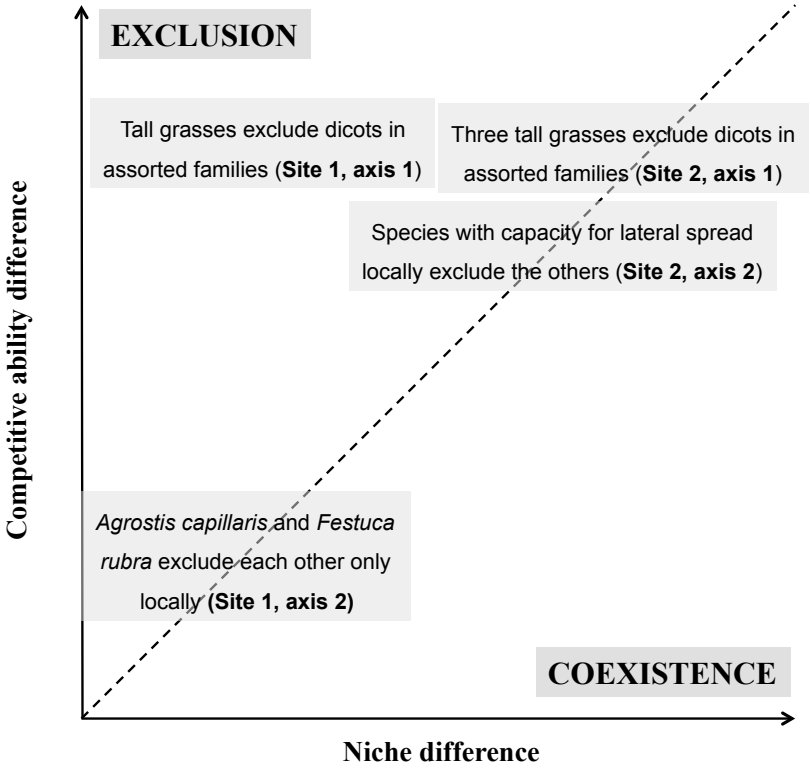

Fig. 7. The four **RLQ** axes (Figs 4.3–4.6) interpreted as in Mayfield and Levine (2010). Coexistence occurs when niche differences exceed competitive ability differences. Site 1, Axis 1: Competitive grasses excluded inferior dicots in the absence of niche differences. Site 1, Axis 2: Similar competitors excluded each other only locally. Site 2, Axis 1: Competitive grasses excluded inferior dicots, but the process was counter-balanced by differences across niche differences. Site 2, Axis 2: Species with the capacity to spread laterally excluded others locally.

4.4. Discussion

Mayfield & Levine (2010; as discussed in HilleRisLambers *et al*. 2012) applied Chesson's (Chesson 2000) theoretical framework to argue that the pattern of species relatedness created by competitive exclusion in plant communities depend on both the importance of plant niche differences and the presence of species' tendency to retain ancestral traits (i.e. phylogenetic signal) that confers competition ability. Experimental manipulation of the equalizing mechanism of grazing (and hence above-ground competition), together with the use of Pavoine *et al*.'s (2011) ordination techniques (which combines information on species traits, phylogeny, environment and space), allowed us to show that the effects of competitive exclusion on the pattern of species relatedness in Atlantic grasslands

depend in fact on stabilization by niches. Our empirical results reveal that competitive exclusion may lead to differing patterns of phylogenetic assembly, supporting Mayfield & Levine's (2010) proposition that inferring the mutually excluding processes of environmental filtering or competitive exclusion from observed patterns may lead to erroneous conclusions. Previous studies have investigated the effects of phylogenetic relatedness on the species assembly patterns resulting from competition (Bennett *et al.* 2013; Godoy *et al.* 2014). However, to our knowledge, this study, using whole natural plant communities, is the first field-based experimental work providing evidence consistent with the notion that the pattern of species relatedness created by competitive exclusion depends on niche stabilization.

PHYLOGENETIC CLUSTERING IN ABSENCE OF NICHE STABILIZATION

In this study, the traits conferring competitive ability differences (height and clonality) had significant phylogenetic structure (phylogenetic signal). Grasses at both sites had superior canopy heights and capacity to spread laterally, a combination that provides competitive ability in fertile grasslands (Craine *et al.* 2001; Grime 2001; Gough *et al.* 2012; Dickson *et al.* 2014). As predicted, and as theorised by Mayfield & Levine (2010), in Site 1 E plot –i.e. in the absence of equalization by large grazers and hence with free above-ground competition, and in the presence of very weak stabilization by niches– the superiority of grasses compared to more distantly related species allowed them to coexist more often than species in other clades, resulting in a subset with an over-representation of grasses (i.e. phylogenetic clustering). Our results are opposed to Bennet *et al*.'s (2013) suggestion that strong, size asymmetric aboveground competition (which was observed in our study) is likely to lead to phylogenetic over-dispersion. Bennet *et al*. (2013) assumed that niche differences are expected to be conserved, whereas competitive ability differences are likely to converge (Grime 2006). However, as shown by Godoy *et al*. (2014) with experimental plant communities, and as confirmed here using semi-natural plant communities, competitive ability differences can be conserved.

Interestingly, the methods described by Pavoine *et al*. (2011) allowed us to detect simultaneous secondary processes. As shown by the second axis in Site 1 (Fig. 4.4), two closely related species with similar competitive abilities (*Festuca rubra* and *Agrostis capillaris*) exclude each other at a local (fine) scale. This competition creates a noticeable checkerboard pattern, particularly in the E plot. In the main process (first axis), the two main species together collaborate to exclude weaker and more distantly related species. Although the largest geographic distances (approximately 44 m) in our plots are still local, the observed pattern agrees with other studies showing that phylogenetic clustering increases with spatial scale in plant communities (Cavender-Bares *et al.* 2009; Vamosi *et al.* 2009). However, here we show that the underlying process creating this pattern is not necessarily due to competition at the finest scales and environmental filtering (Cavender-Bares *et al.* 2009). At Site 1 E plot, competitive grasses impose a strong biotic filter at plot scale, and only species with the required traits may survive (i.e. tall grasses with vegetative spread); then, the remaining species with similar traits repel each other at the finest scales.

RANDOMNESS UNDER NICHE STABILIZATION

As expected under Mayfield & Levine's (2010) theoretical framework, competitive exclusion resulted in a subset of species with a random phylogenetic pattern at Site 2, where moderately strong stabilization by niches is present. Similar to Site 1, the first axis in Site 2 still represents a contrast between tall species (i.e. grasses) with the capacity to spread laterally and species in other tree branches that lack these traits. Although competitive grasses tended to dominate the E plot, patches with species lacking those traits were also present (Fig. 4.5a). This patchiness may represent niche stabilization, with competitive grasses strongly dominating at medium values of the pH gradient (white squares in Fig. 4.5a) and competitively weaker species finding their opportunity to survive at both extremes of the pH gradient (black squares in Fig. 4.5a). Patterns observed in our results are compatible with studies claiming that processes generating patterns of over-dispersion and clustering, when acting together, may create an overall random pattern (Soliveres, Torices & Maestre 2012; García-Baquero & Crujeiras 2015). We conjecture that the superior competitive ability of grasses over other clades

contributes to phylogenetic clustering at Site 2. At the same time, segregation along the niche may contribute to over-dispersion inducing more intense competition between grasses in medium pH values (where they are more abundant), between sedges in the lowest values (where they are more abundant) and between forbs in the highest values (where they are more abundant).

On the other hand, although traits conferring competitive abilities have phylogenetic signals at both field sites, in Site 1, the traits were phylogenetically clustered in the grass family (competitive characteristics are exclusively in the family *Poaceae*), whereas in Site 2, the grass (*Poaceae*) and heath (*Ericaceae*) families shared these characteristics. These phylogenetic patterns generated by pre-existing floristic compositions may contribute to an enhanced phylogenetic clustering after competitive exclusion in Site 1 E plot. Lastly, the annual species *Poa annua* was found in both sites among the competitive grasses; *P. annua* does not have the same competitive characteristics and it is indeed competitively excluded from the E plots in both sites. This exception did not have a strong influence in the generation of the phylogenetic pattern of species assembly at Site 1, where 8 out of 10 monocot species are in the grass family, but it may have a stronger influence at Site 2, where only 5 out of 10 monocot species are in the grass family.

LIMITATIONS OF THE **RLQ** ANALYSIS

An unexpected result of the **RLQ** analysis was that soil descriptors were weakly related to the canonical axes at both sites, when soil variables were shown to significantly affect to both species composition and the abundance of functional groups (Odriozola *et al*. 2016; Fig. 4.1). A limitation of the **RLQ** ordination framework is that it assumes that the relationships between the different components are monotonic. As many species are predicted to show non-linear, unimodal relationships with respect to environmental gradients (Whittaker 1967; Austin 1987; Palmer & Dixon 1990), that is not a sensible assumption. For example García-Baquero, Silvertown *et al.* (2016) found that the relationship between soil water and species' presence at a fine scale had a hump-shaped form for about 35% of the tested species. Many studies failed to detect plant niches by assuming linear

responses (Austin 2007). That may well be our case when using extended **RLQ** ordination, because by relaxing the assumption of linearity through GAM modelling, we observed the presence of non-monotonic relationships (Fig. 4.1). Complex relationships of species with pH gradients, similar to the one found in our study, have been previously reported (Jansen & Oksanen 2013).

FUTURE PERSPECTIVES

The use of an Atlantic highly productive grassland as test community has the advantage of producing results in a relatively short period of time (nine years). However, it seems self-evident that further experimental exploration of the effects of competitive exclusion on assembly processes and phylogenetic patterns will benefit from using other, biogeographically and ecologically different, study systems. It seems likely that pre-existent datasets from experiments similar to ours may be re-analysed using the excellently-suited Pavoine *et al.*'s (2011) techniques, which would provide further evidence on the topic (but see the limitations above).

Because our experiment used pre-existent semi-natural grassland communities, we did not experimentally manipulate the initial species composition found in the test communities. One consequence was that we were unable to experimentally examine Mayfield & Levine's (2010) proposition that the phylogenetic patterns resulting from competitive exclusion in plant communities depends on the presence of a phylogenetic signal among the traits that confer competition ability (Mayfield & Levine 2010; HilleRisLambers *et al.* 2012). For this reason, we believe that future research will benefit not only from field experiments in which systems other than productive grasslands are used, but also from mesocosm experiments. In these types of experiments the scientist may exert a more complete control over the conditions tested, hence the use of mesocosms may offer the possibility to analyse the effect of competitive exclusion on assembly processes and phylogenetic patterns using experimental designs in which both progressively increasing degrees of niche stabilization and the presence/absence of species' tendency to retain ancestral traits that confer competition ability are tested.

4.5. Conclusions

Experimentally manipulating two semi-natural whole communities with differing degrees of stabilization by niches, we have shown that the effect of competitive exclusion on the pattern of species relatedness in Atlantic grasslands depends on stabilization by niches. As grasses with superior competitive ability excluded short species in all main phylogenetic tree branches, competitive exclusion under very weak stabilization by niches led to a phylogenetically clustered subset of species. On the other hand, under moderate stabilization by niches competitive ability differences were counter-balanced by niche differences, resulting in a *random* phylogenetic pattern (i.e. a pattern that corresponds to neither over-dispersion nor under-dispersion) of species assembly. We suggest that the development of statistical approaches capable of examining relationships between space, environment, species traits and phylogeny, similar to the extended RLQ analysis, but better suited to capture non-linear relationships between species (or traits) and environment, will benefit future analysis of datasets similar to ours. Finally, we believe that future research will benefit from field experiments in which systems other than productive grasslands are used. Similarly, we propose future mesocosm experiments, with experimental control on initial species, in which progressively increasing degrees of niche stabilization are tested.

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4.7. Supplementary material

Appendix S1. R code. (Available online upon publication) (pp: 147–152)

Appendix S2. Site 1 dataset. (Available online upon publication)

Appendix S1. R code.

Patterns of species relatedness created by competitive exclusion depend on niche stabilization: evidence from Atlantic grasslands by by I. Odriozola, G. García-Baquero, A. Etxeberria and A. Aldezabal.

```
##############################################################################################
## Manuscript title: The pattern of species relatedness induced by competitive exclusion   ##
## exclusion depends on stabilization by niches: evidence from Atlantic grasslands.        ##
##                                                                                          ##
## Authors: I. Odriozola, G. García-Baquero, A. Etxeberria & A. Aldezabal                   ##
## E-mail address: inaki.odriozola@ehu.eus                                                  ##
##############################################################################################

##############################################################################################
## We are indebted to Pavoine et al. (2011) [1] "Linking patterns in phylogeny, traits,    ##
## abiotic variables and space: a novel approach to linking environmental filtering and    ##
## plant community assembly", for their very useful code                                    ##
##############################################################################################

##############################################################################################
## Large herbivores were exluded in 2005 from two field sites with contrasting stabilization##
## by plant niches in a productive semi-natural grassland in the Aralar mountain range. We  ##
## test test the hypothesis that after removal of the equalizing mechanism of grazing, the  ##
## pattern of species relatedness generated by competitive exclusion depends on             ##
## stabilization by niches.                                                                 ##
##                                                                                          ##
## Since all analyses are repeated in both sites, we report here the coding for just one of ##
## the sites is reported . Please find the complete dataset in Dryad                        ##
##############################################################################################



## 1. Set up working directory and load the required packages
## *********************************************************
setwd("~/Documents/BIOLOGIA/TESIA/Publikazioak/Grazing_phylo/APP/APP_2") # Change as required.
source("JEC_1743_sm_apps5.txt") # load additional functions from Appendix S5 in Pavoine et al. (2011)
 [1]
library(ade4)
library(ape)
require(phytools)
library(vegan)
library(spdep)
library(mgcv)



## 2. Load and prepare data for analysis
## *************************************
load("site1_data.RData")
Treatment <-  site1_data$env[,1]       # Grazing regime: Grazing (G) vs. Exclusion (E)
comp <- site1_data$comp                                  # Floristic composition matrix (Abundance
 in % cover)
env <- site1_data$env[,-1]                       # Environmental matrix
trait <- site1_data$trait                          # Matrix of functional traits of species
phy <- site1_data$phy                                 # Phylogenetic tree constructed with Phylomatic
 V3 + Phylocom
spa <- site1_data$spa                                 # Matrix of X Y spatial coordinates

# Change the phylogenetic tree (an object of the class "phylo") into an object of the class "phylog",
 used by ade4
phylog <- newick2phylog(write.tree(phy)) # => "phylog" class

## Trait preparation for posterior analysis
# Clonal Growth Organ (CGO). A multichoice binary trait
tabBinary <- prep.binary(trait[,3:8],6)

# Canopy Height (H). A quantitative trait
tabQuantitative <- data.frame(H=trait[,2])

# Lateral spread (LS). An ordinal trait
tabOrdinal <- data.frame(LS=trait[,9])
```

```
rownames(tabBinary) <- trait$sp                       # Identify trait values with corresponding
 species
rownames(tabQuantitative) <- trait$sp # Identify trait values with corresponding species
rownames(tabOrdinal) <- trait$sp               # Identify trait values with corresponding species



## 3. Test for the relatinship between pH and the abundance of main plant groups
## (Fig. 1 in main text)
## ************************************************************************
gram <- apply(comp[,c(1:8)],1,sum)           # Sum of the % cover of grasses
sedge <- apply(comp[,c(9,10)],1,sum)  # Sum of the % cover of sedges
forb <- apply(comp[,c(11:32)],1,sum)  # Sum of the % cover of forbs
gram.g <- gram[1:100]                                    # Grass % covers corresponding to
 quadrats in G plot
gram.e <- gram[101:200]                                         # Grass % covers corresponding to
 quadrats in E plot
sedge.g <- sedge[1:100]               # Same for sedges (G)
sedge.e <- sedge[101:200]             # Same for sedges (E)
forb.g <- forb[1:100]                 # Same for forbs (G)
forb.e <- forb[101:200]               # Same for forbs (E)

env.g <- env[1:100,]                                     # Env variable's values for the G plot
env.e <- env[101:200,]                                  # Env variable's values for the E plot

## Fit models using GAM. We used penalisation splines as base splines (bs = "ps").
## To set the number of knots (k = 4), we used the next rule of thumb: choose k as
## the number of unique values of the explanatory variable (pH) divided by 4.
gram.gam.g <- gam(gram.g~s(pH,bs="ps",k=4,m=2),method="REML",data=env.g)
forb.gam.g <- gam(forb.g~s(pH,bs="ps",k=4,m=2),method="REML",data=env.g)
sedge.gam.g <- gam(sedge.g~s(pH,bs="ps",k=4,m=2),method="REML",data=env.g)
summary(gram.gam.g);summary(forb.gam.g);summary(sedge.gam.g)
# The null hypothesis of no effect of pH is rejected only for sedges.

gram.gam.e <- gam(gram.e~s(pH,bs="ps",k=4,m=2),method="REML",data=env.e)
forb.gam.e <- gam(forb.e~s(pH,bs="ps",k=4,m=2),method="REML",data=env.e)
sedge.gam.e <- gam(sedge.e~s(pH,bs="ps",k=4,m=2),method="REML",data=env.e)
summary(gram.gam.e);summary(forb.gam.e);summary(sedge.gam.e)
# The null hypothesis of no effect of pH is not rejected for any group.

seq.pH.g <- seq(4.2,5.3,l=20)  # Sequency of pH to be used in predictions with .g models.
seq.pH.e <- seq(4.2,5.3,l=20)  # Sequency of pH to be used in predictions with .e models.

## The smoother of pH is changed by 1 in cases of no significant effect.
gram.gam.g <- gam(gram.g~1,method="REML",data=env.g)
forb.gam.g <- gam(forb.g~1,method="REML",data=env.g)
sedge.gam.e <- gam(sedge.e~1,method="REML",data=env.e)
gram.gam.e <- gam(gram.e~1,method="REML",data=env.e)
forb.gam.e <- gam(forb.e~1,method="REML",data=env.e)

## Predict the lines to be plotted, using the above fitted models.
gram.pred.g <- predict(gram.gam.g,type="response",newdata=data.frame(pH=seq.pH.g))
forb.pred.g <- predict(forb.gam.g,type="response",newdata=data.frame(pH=seq.pH.g))
sedge.pred.g <- predict(sedge.gam.g,type="response",newdata=data.frame(pH=seq.pH.g))
gram.pred.e <- predict(gram.gam.e,type="response",newdata=data.frame(pH=seq.pH.e))
forb.pred.e <- predict(forb.gam.e,type="response",newdata=data.frame(pH=seq.pH.e))
sedge.pred.e <- predict(sedge.gam.e,type="response",newdata=data.frame(pH=seq.pH.e))

## Plot the subfigure a) of Fig. 1 in main text. xlim is set as the whole range of pH
## considering both field sites.
plot(gram.pred.g~seq.pH.g,ylim=c(0,1),xlim=c(4.2,7.6),type="n",axes=F,ylab="Plant cover",xlab="pH
 (short gradient)",main="a) Site 1 G",font.main=1)
axis(1)
axis(2)
lines(gram.pred.g~seq.pH.g)
points(gram.g~env.g$pH,pch=3,cex=0.02)
lines(forb.pred.g~seq.pH.g,lty=2)
points(forb.g~ env.g$pH,pch=4,cex=0.02)
lines(sedge.pred.g~seq.pH.g,lty=3)
points(sedge.g~ env.g$pH,pch=0,cex=0.02)
legend("right",legend=c("gram","forb","sedge"),lty=c(1,2,3))

## Plot the subfigure b) of Fig. 1 in main text. xlim is set as the whole range of pH
## considering both field sites.
plot(gram.pred.e~seq.pH.e,ylim=c(0,1),xlim=c(4.2,7.6),type="n",axes=F,ylab="Plant cover",xlab="pH
```

```
 (short gradient)",main="b) Site 1 E",font.main=1)
axis(1)
axis(2)
lines(gram.pred.e~seq.pH.e)
points(gram.e~env.e$pH,pch=3,cex=0.02)
lines(forb.pred.e~seq.pH.e,lty=2)
points(forb.e~ env.e$pH,pch=4,cex=0.02)
lines(sedge.pred.e~seq.pH.e,lty=3)
points(sedge.e~ env.e$pH,pch=0,cex=0.02)
legend("right",legend=c("gram","forb","sedge"),lty=c(1,2,3))



## 4. Fig.2a in the main text. Plot of phylogenetic tree for Site1
## ************************************************************
quartz(title="Phylogenetic tree for Site 1") # For Mac
windows(title="Phylogenetic tree for Site 1") # For PC
plot(phylog, f.phylog = 0.70, cleaves = 0.6, clabel.l = 0.70, clabel.n = 0.70, f = 0.80,
sub = "Phylogenetic tree for Site 1",csub = 0.95, possub = "topleft")



## 5. Correspondence analysis of the floristic composition matrix
## ************************************************************
coacomp <- dudi.coa(comp, scan = FALSE, nf = 31)
summary(coacomp)



## 6. Spatial analysis
## *******************
# Create the Gabriel graph
nb1 <- graph2nb(gabrielneigh(as.matrix(spa)), sym = T)
nb1

lw1 <- nb2listw(nb1) # gives a neighbours list with spatial weights (i.e. matrix W)
lw1

# plot the graph
par(mfrow=c(1,1))
plot(nb1, spa, pch = 21, bg = 'red')
title(main="Gabriel Graph")
class(nb1)
# [1] "nb"
# This is the Gabriel graph -the graph that in this case defines which points
# are connected

# The matrix of spatial variables is obtained as the eigenvectors of a
# neighbour matrix. This matrix is analysed by principal component analysis.
nb1.neigh <- nb2neig(nb1)
vecspa <- scores.neig(nb1.neigh)
pcaspa <- dudi.pca(vecspa, coacomp$lw, scan = FALSE, nf = 199)
summary(pcaspa)



## 7. PCA analysis of the env matrix
## *********************************
pcaenv <- dudi.pca(env, row.w=coacomp$lw, scannf = FALSE, nf = 7,scale=T)
summary(pcaenv)



## 8. The distances between species based on their biological traits, analyzed by PCoA.
##*************************************************************************************
# Distance matrices for CGO, H and LS separately
listdis <- ldist.ktab(ktab.list.df(list(tabBinary,tabQuantitative,tabOrdinal)), c("B","Q","O"), scan
 = TRUE)
5
1
1
# select: 5, 1, 1
# Select an integer (1-10): 5 (5 = CZEKANOWSKI (1913) or SORENSEN (1948) S7 coefficient of GOWER &
 LEGENDRE)
# Select an integer (1-2): 1 (1 = ranked variables treated as quantitative variables)
```

```
# Select an integer (1-2): 1 (1 = Euclidean)
names(listdis)
summary(listdis)

# Distance matrices for CGO, H and LS together
disT<- dist.ktab(ktab.list.df(list(tabBinary,tabQuantitative,tabOrdinal)),c("B","Q","O"), scan =TRUE)
5
1
1
#select: 5, 1
# Select an integer (1-10): 5 (5 = CZEKANOWSKI (1913) or SORENSEN (1948) S7 coefficient of GOWER &
 LEGENDRE)
# Select an integer (1-2): 1 (1 = ranked variables treated as quantitative variables)
# Select an integer (1-2): 1 (1 = Euclidean)
pcotraits <- dudi.pco(disT, coacomp$cw, full = TRUE)
summary(pcotraits)



## 9. The distances between species based on their phylogenetic relatedness, analysed by PCoA.
## ********************************************************************************************
pcophy <- dudi.pco(as.dist(as.matrix(phylog$Wdist)[names(comp),names(comp)]),coacomp$cw, full = TRUE)
summary(pcophy)



## 10. Tests for phylogenetic signals in traits
## ********************************************
#Function rtest.decdiv() in appendix S5, Pavoine et al., 2011. [1]
#All traits together
phystot <- rtest.decdiv(phylog, rep(1, 32),
 as.dist(as.matrix(disT)[names(phylog$leaves),names(phylog$leaves)]),
nrep = 999, vranking = "droot", optiontest = "less",ties.method = "average", option = 3)
phystot
# Monte-Carlo test
# Call: [1] "rtest.decdiv"
#
# Observation: 0.4355755
#
# Based on 999 replicates
# Simulated p-value: 0.004
# Alternative hypothesis: less
#
# Std.Obs.stat3.droot         Expectation            Variance
#        -3.372630956         0.542579496         0.001006613 #
#
# The combination of the traits CGO, H and LS has significant phylogenetic signal

# CGO:
physB1 <- rtest.decdiv(phylog, rep(1, 32), as.dist(as.matrix(listdis$B1)[names(phylog$leaves),
names(phylog$leaves)]), nrep = 999, vranking = "droot", optiontest = "less", ties.method = "average",
 option = 3)
physB1
# Monte-Carlo test
# Call: [1] "rtest.decdiv"
#
# Observation: 0.4563324
#
# Based on 999 replicates
# Simulated p-value: 0.006
# Alternative hypothesis: less
#
# Std.Obs.stat3.droot         Expectation            Variance
#       -2.8559446154        0.5412071088        0.0008831965
#
# CGO trait has significant phylogenetic signal

# H
physH <- rtest.decdiv(phylog, rep(1, 32), as.dist(as.matrix(listdis$B1)[names(phylog$leaves),
 names(phylog$leaves)]),
nrep = 999, vranking = "droot", optiontest = "less", ties.method = "average", option = 3)
physH
# Monte-Carlo test
# Call: [1] "rtest.decdiv"
#
```

```
# Observation: 0.4563324
#
# Based on 999 replicates
# Simulated p-value: 0.009
# Alternative hypothesis: less
#
# Std.Obs.stat3.droot         Expectation            Variance
#       -2.8588687151        0.5417418819        0.0008925325
# H trait has significant phylogenetic signal

# LS
physLS <- rtest.decdiv(phylog, rep(1, 32), as.dist(as.matrix(listdis$LS)[names(phylog$leaves),
 names(phylog$leaves)]),
nrep = 999, vranking = "droot", optiontest = "less", ties.method = "average", option = 3)
physLS
# Monte-Carlo test
# Call: [1] "rtest.decdiv"
#
# Observation: 0.4407731
#
# Based on 999 replicates
# Simulated p-value: 0.044
# Alternative hypothesis: less
#
# Std.Obs.stat3.droot         Expectation            Variance
#        -1.942054447         0.546629430         0.002971058
# LS trait has significant phylogenetic signal



## 11. The RLQ analysis:
## *********************
rlqmix <- rlqESLTP(pcaenv, pcaspa, coacomp, pcotraits, pcophy, scan = F, nf = 2)
barplot(rlqmix$eig)
rlqmix$eig[1]/sum(rlqmix$eig)
# [1] 0.5895352
rlqmix$eig[2]/sum(rlqmix$eig)
# [1] 0.2948567

# Figs 3a and S3a in the main text
plot(rlqmix, xy=spa, ax=1, wh="S")
plot(rlqmix, xy=spa, ax=2, wh="S")

# Figs 3b and S3b in the main text
plot(rlqmix, phy=phylog, ax=1, wh="P")
plot(rlqmix, phy=phylog, ax=2, wh="P")

# Figs 3c and S3c in the main text
plot(rlqmix, traits=tabBinary[,-c(6)], ax=1, type="B", wh="T")
# This works with R version 2.10 and ade4 version 1.4-14

plot(rlqmix, traits=tabBinary[,-c(6)], ax=2, type="B", wh="T")
# This works with R version 2.10 and ade4 version 1.4-14

# Figs 3d and S3d in the main text
plot(rlqmix, traits=tabQuantitative, ax=1, type="Q", wh="T")
plot(rlqmix, traits=tabQuantitative, ax=2, type="Q", wh="T")

# Figs 3e and S3e in the main text
plot(rlqmix, traits=tabOrdinal, ax=1, type="O", wh="T")
plot(rlqmix, traits=tabOrdinal, ax=2, type="O", wh="T")

# Fig 3c in the main text
plot(rlqmix, env=pcaenv$tab , ax=1, type="Q", wh="E")
plot(rlqmix, env=pcaenv$tab , ax=2, type="Q", wh="E")



## 12. Test of phylogenetic pattern
##*********************************
# Test of the null hypothesis of random phylogenetic pattern after competitive
# exclusion in plot E (G plot is used as control plot)
library(picante)

site1_com <- aggregate(comp,by=list(Treatment),mean)[,-1]
```

```
rownames(site1_com) <- c("E","G")

# Phylogenetic tree, against which composition in Site1 E will be tested
phydist <- cophenetic(phy)

clus.test <- ses.mpd(site1_com, phydist, null.model = "independentswap",
 abundance.weighted = FALSE, runs = 99)
clus.test[-2,]
#   ntaxa  mpd.obs mpd.rand.mean mpd.rand.sd mpd.obs.rank mpd.obs.z mpd.obs.p runs
# E    22 501.7129      504.9107    2.147563          5.5 -1.489016     0.055   99

# There is moderate evidence for phylogenetic clustering in Site 1 E


##****************************REFERENCES*****************************
## [1] Pavoine, S. et al. 2011. Journal of Ecology 2011, 99: 165-175
```

CHAPTER 5. General Discussion

5.1. Overview of results

In this dissertation, the experimental simulated cessation of mixed grazing (sheep, horses, and cattle) has been reported, and its consequences on soil processes and plant diversity in the temperate grassland system of the Aralar Natural Park have been studied. After nine years of grazing exclusion, significant changes occurred in soil and forage properties (Chapter 2), plant diversity, and community physiognomy (Chapter 3). Diversity loss from competitive exclusion was more pronounced under weak niche stabilisation (i.e. homogeneous fine-scale soil environment), whereas competitive exclusion was buffered under moderate niche stabilisation (i.e. heterogeneous fine-scale soil environment) (Chapter 4).

Grassland productivity is the main predictor of grazing effects on soil processes (Bardgett & Wardle 2003; Schrama et al. 2013) and plant diversity (Milchunas et al. 1988; Cingolani et al. 2005). As expected for highly productive grasslands, such as the Atlantic temperate grasslands studied here, grazer exclusion retarded nutrient cycling and mineralisation, and reduced resource use efficiency by microorganisms (Aldezabal et al. 2015). This occurred because the exclusion of grazers reduced forage quality, and the mean and variance of soil temperature (Chapter 2). In the absence of an equalising mechanism provided by grazers, competitive species displaced competitively inferior species (Chapters 3). Competitive species were mostly grasses (Chapter 4) with higher C/N ratios (i.e. lower forage quality) than subordinate species (unpublished data), which were mostly dicots (Chapter 4). Additionally, many species showed higher C/N ratios in the exclusion plots than their conspecifics in grazing plots (unpublished data). Moreover, the absence of defoliation and trampling by herbivores resulted in rapid biomass accumulation in the exclusion plots, which provided insulation to the soil and reduced the mean and variance summer soil temperatures (Chapter 2).

Regarding plant diversity, in absence of equalizing mechanisms provided by grazers, competitive species created large intraspecific patches and out-competed species with inferior competitive abilities, thereby reducing plant diversity (Chapter 3). The dominance of tall grasses that spread laterally at the expense of species without those characteristics (Chapter 4) resulted in the spatial

homogenisation of plant diversity, and loss of species (Chapter 3) and phylogenetic diversity (Chapter 4). As expected in productive grasslands, the plant traits canopy height and clonality were key features for competitive exclusion (Chapter 4); as predicted, equalisation by grazing was crucial for species coexistence (Chapters 3 and 4). In the presence of herbivores, plant species possessing the above competitive traits remained under control because they were unable to fully develop those traits. After cessation of grazing, these plant species were uncontrolled and became dominant in the community in a relatively short time (nine years). Lastly, given that niche stabilisation is expected to be weak in highly productive grasslands, and that biotic processes predominate at fine spatial scales, the (moderate) niche stabilisation that exerted soil pH heterogeneity in Igaratza is significant (Chapters 3 and 4). This stabilisation buffered competitive exclusion and prevented the loss of species and phylogenetic diversity (Chapter 4). Under weak niche stabilisation, grasses with superior competitive ability out-competed dicots in all branches in the phylogenetic tree, resulting in an important decline of species and phylogenetic diversity (i.e. subset of species with a clustered phylogenetic pattern). However, under moderately strong niche stabilisation, competitive exclusion by superior species was counter-balanced by niche stabilisation, and resulted in a less important loss of species and phylogenetic diversity (i.e. subset of species with a random phylogenetic pattern).

5.2. Future of Atlantic grasslands

The results presented in this dissertation show that the future of Atlantic grasslands strongly depends on the continuity of the presence of large domestic herbivores. With most wild large herbivores extinct in Europe, two main approaches are used for the conservation of open habitats: rewilding by large herbivores and the maintenance of extensive livestock farming (Sutherland 2002). An interesting example of rewilding is Oostvaardersplassen in the Netherlands: allowing fluctuations of water level in the area and reincorporating the wild herbivore community have successfully created a wood-pasture landscape (Smit et al. 2015). However, in regions like the Basque mountains, where traditional livestock farming still occurs, the sustainable management of this system with

highly traditional and societal values would conserve its semi-natural grasslands. As stated in the General Introduction, the primary sector is not quantitatively important in the Atlantic Basque Country as it produces less than 1% GDP (EUSTAT 2011) and its potential to develop is limited due to the abrupt orography of the country. However, locally manufactured products with high gastronomic quality and acceptance have societal and economic relevance in the Basque Country, e.g. Cheese Day which is celebrated annually in Idiazabal Village (Idiazabalturismo.com 2016); the International Cheese Awards, Donostia, 2016 (Gff.co.uk 2016); high quality gastronomy is one of the main touristic attractions in the Basque Country; and the presence of a culinary university with international recognition (Basque Culinary Center) (Bculinary.com 2016). Although further reflection is beyond the scope of this dissertation, it is clear that the intensification of livestock farming would have ecological, societal, and economic implications.

5.3. Perspectives

Sutherland et al. (2013) identified the consequences of spatio-temporal heterogeneity for biodiversity as one of the main fundamental ecological questions requiring further research. Since highly productive Atlantic grasslands respond quickly to experimentally induced changes (e.g. herbivore exclusion), they may constitute useful study areas to assess the spatio-temporal dynamics of biodiversity or other processes in response to induced experimental changes. Most of the work presented in this dissertation has been cross-sectional. However, limited longitudinal analyses were included (Chapter 2), e.g. longitudinal measurement of temperature has shown considerable soil temperature fluctuations under grazing, a previously neglected but important effect of grazing. The consequences of this require further research.

The relatively recent merging of phylogenetic biology and community ecology (Webb et al. 2002; Cavender-Bares et al. 2009) has brought new perspective to species assembly processes. Currently, one of the main limitations for predicting the effects of herbivory on grassland composition is the difficulty to estimate the evolutionary history of grazing in grassland systems (Oesterheld & Semmartin

2011). The combination of functional traits and phylogenies may provide a useful tool to understand the adaptations of plant communities to historical events. Cingolani et al. (2005) postulated that in productive grasslands with long evolutionary history, grazer density is assumed to have fluctuated widely. This would create pressure to tolerate high grazing intensity and dominate the canopy in periods or areas that grazing intensity is not sufficient to neutralise the competition for light and space. An interesting hypothesis to be tested in the future is whether those fluctuations resulted in phylogenetic conservatism of traits conferring capacity to tolerate grazing and capacity to dominate the canopy. Results in Chapter 4 agree with this hypothesis: most species have vegetative growth, an evolutionary adaptation to tolerate grazing. Moreover, canopy height and capacity to spread laterally are phylogenetically conserved, and the both are necessary to dominate the canopy in fertile grasslands. In grasslands with different characteristics, other pressures may result in the phylogenetic conservatism of other traits like physical defences to herbivory or belowground adaptations to resource partitioning.

A future grazer-exclusion experiment could be interesting in this study area. Grazer exclusion fences would be placed along a niche stabilisation gradient: from dense pasture with deep soil, where stabilisation is possibly very weak, to stony pasture with shallow soils, where strong pH heterogeneity (created by the proximity of calcareous substrate) and water stress (due to water loss by infiltration) possibly exert strong niche stabilisation. First, it would be of useful to assess whether conserved traits shift along the stabilisation gradient. For example, phylogenetic conservatism of traits conferring canopy dominance would be expected in dense pasture; in contrast, conservation of traits conferring belowground adaptations to resource acquisition would be expected in stony pasture. A comparison between grazing and exclusion plots could determine the consequences of niche stabilisation and competition type. Additionally, a spatially explicit sampling design combined with annual sampling would assess the spatio-temporal dynamics of biodiversity under the established experimental conditions. Lastly, working with pre-existing natural plant communities prevents the manipulation of original species composition. A mesocosm experiment, coupled

with the above field experiment, would be valuable. Such an experiment could determine the effect of competitive exclusion on assembly processes and phylogenetic patterns using experimental designs in which increasing degrees of niche stabilisation and the ability of species to retain ancestral traits that confer competition ability are tested.

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CHAPTER 6. General Conclusions

1. The exclusion of large herbivores retarded nutrient cycling in the soil by modifying soil water content and forage quality; but, particularly, herbivore exclusion led to dramatic changes in the soil thermal regime.

2. Grazing exclusion resulted in the replacement of species with low C/N ratios by other dominant, lower quality species. Additionally, grazing induced higher quality regrowth in individual species. Moreover, litter biomass accumulation in the absence of herbivores created a thicker organic layer that provided insulation to the soil. This resulted in lower mean summer temperatures and minimal temperature fluctuations. The combination of higher quality resources and higher temperatures, generally without water limitations, resulted in enhanced nutrient cycling and mineralisation in grazed areas.

3. Cessation of disturbance by herbivores, a strong equalising mechanism, stimulated plant species with superior competitive ability that, created large intraspecific spatial aggregations and out-competed weaker species, and therefore reduced plant species diversity.

4. Cessation of an equalising mechanism by herbivory led to strong competitive exclusion in the field plot with the weakest niche stabilisation. In contrast, competitive exclusion was buffered by niche stabilisation in a field plot with moderately strong niche stabilisation.

5. Traits conferring competitive ability to species (i.e. canopy height and clonality) were phylogenetically conserved in the studied grasslands. Species in the grass family (Poaceae) generally showed superior competitive abilities. Under weak stabilisation, strong competitive exclusion resulted in a phylogenetically clustered species subset, with the over-representation of grasses (i.e. an important loss of phylogenetic diversity). However, when competitive ability differences were buffered by niche stabilisation, competitive exclusion resulted in a phylogenetically random subset of species (i.e. lower loss of phylogenetic diversity).

6. Since 10 years of grazer exclusion retarded nutrient cycling and mineralisation and resulted in the loss of spatial heterogeneity of floristic composition and plant diversity in the field plots, the overall conclusion is that traditional grazing by mixed livestock (sheep, cattle, and horses) is a key factor for maintaining soil function and plant diversity in Atlantic grasslands.