UNIVERSITAT DE BARCELONA

Distribution and dynamics of multiple components of plant diversity in a high mountain area: the Ordesa-Monte Perdido National Park

Distribución y dinámica temporal de los diferentes componentes de la diversidad vegetal en la alta montaña: el Parque Nacional de Ordesa y Monte Perdido

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Doctoral Thesis

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Memòria presentada per Iker Pardo Guereño per optar al grau de doctor per la Universitat de Barcelona.

Programa de Doctorat en Biodiversitat

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Doctorand	Directora de tesi	Tutor de tesi
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Ama, zuri eskainia

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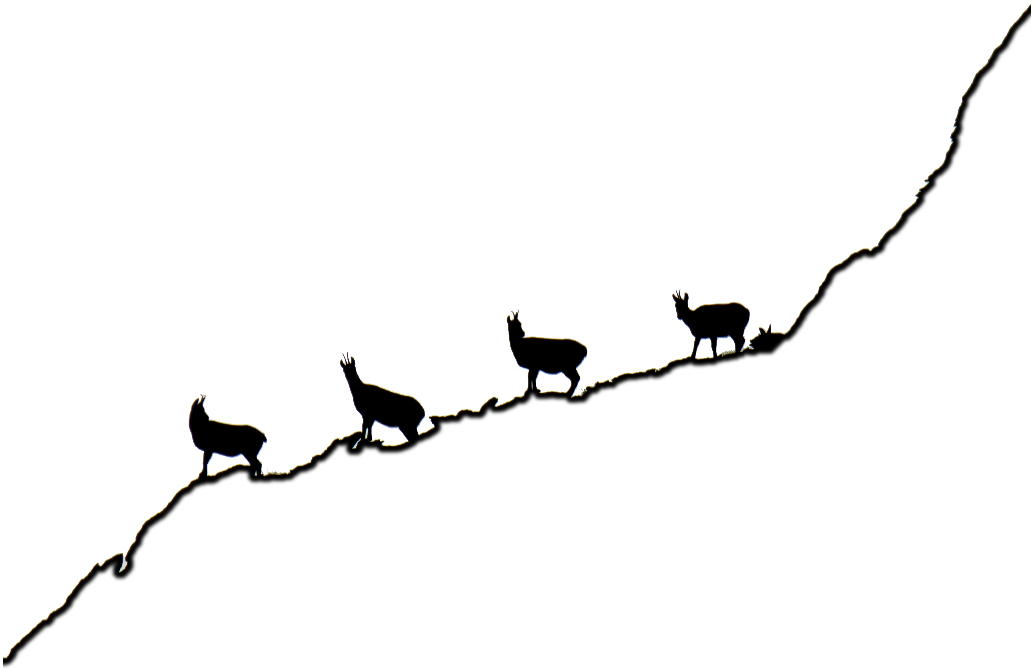

Tesia bidaia luzea da, eta horrelakoetan helburua baino aberasgarriagoa da bidean zehar ikasitakoa, jasotako eskarmentu eta ezagututako pertsonak. Lerrotara ekarri nahiko nituzke, beraz, bidaide izan ditudan lagun guztiak, nire eskerrik beroenak agertzearren.

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Instituto Pirenaico de Ecología-ko Jaka zein Zaragozako egoitzak kanpamentu basea izan dira urteotan zehar. IPEko parte diren teknikari, administrari, zuzendari eta ikerlariei nire esker on zintzoa luzatu nahi nieke. Baina baldin bada IPEko inor tesi hau posible egin duenik, hori *Bego* da. Zuri, langile nekaezin, inork ez bezala zientzia bizi-sentitu-maitatzen duzun horri, mila esker gauza ñimiñoak sekretu handien gordeleku direla erakusteagatik, eta batez ere, horren leku ederrenean ikerkuntzan aritzeko aukera paregabea eskaintzeagatik. Dena den, tesi hau ez litzateke posible orain dela urte andana zenbait ikerlariek egindako lan mardulik gabe. *Arantza*, *Chechu*, *Daniel* eta *Ricardo*, mila esker orain dela hamarkada luze pare bat (nik zazpi bat urte nituenean!) hasitako lana nire eskutan uzteagatik, haatik eta zuen etengabeko laguntza eta aholkuengatik. Zinez, plazer handia da zuekin guztiekin lan egitea!

Orobat, aipamen berezia dagokie hamarkadetan zehar landareen inguruko informazioa jaso duten naturzale zein ikerlari guztiei, eta bereziki *Dr. P. Montserrat*i, Jakako herbarioa hein handi batean bere lan agortezinaren isla delako. Noski informazio hori txukundu eta atondu duten langileak ere goraipatu nahiko nituzke. Zeharka izan bada ere, tesi hau burutzeko funtsezkoa izan da *Federico Fillat*ek hamarkadetan zehar egindako ekarpena. Tesi honetarako hirugarren informazio iturria ordea, neronek mendian jasotakoa da. Afera horretan, egunsentitik iluntzeraino nirekin aritu diren guztiei – *Jesús*, *Juanlu*, *Marc*, *Mónica*, *María Jarne*, *María Pata, Pedro Sanchez* (poa pooa poooa), *Pedro Bravo*, *Isabel*, *Xabi* eta, bai *Maider* zu ere – mila esker. Halaber, eskerrak luzatu nahiko nizkieke mendiko lana egiteko logistikan lagundu nauten guztiei, hala Ordesa eta Monte Perdidoko Parkeko kudeatzailei, *Elena Villagrasa* tarteko, nola Nerineko Palacio etxekoei, eta baita Torlako aterpean bildutako zientzialari jator guztiei (besteak beste *Nacho*, *Fernando*, *Ramón Antor*).

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Iker
Iruñean, 2015eko urriaren 7an

General Introduction

The multiple facets of biodiversity

In the Convention on Biological Diversity held in 1992, biodiversity was defined as “the variation of genes (diversity within species), taxonomic (between species) and ecosystem functionalities (of ecosystems)”. Despite its multifaceted essence, biodiversity has traditionally been measured as “number of species”, or by means of diversity indices that also include species' abundance (Magurran & McGill 2011). However, it is intuitive that two given assemblages with the same number of species and similar abundances are not necessarily equally diverse, because the amount of different features will probably be higher in one of them, but how different are these assemblages considering this new perspective? How can we measure such difference?

The difference between species is determined by traits, i.e. any physical, biochemical, behavioral or phenological feature of an individual that may potentially affects species' performance (Cadotte, Carscadden & Mirotchnick 2011). It can be characterized along a single trait axis (Garnier *et al.* 2001) or within a multivariate trait space (Petchey & Gaston 2002). However, the resulting difference will be dependent on the chosen trait(s) (Vellend *et al.* 2011). In addition, not all traits that may potentially contribute to such difference are measurable, and many are probably still unknown, which may result in a partial characterization of species' differences (Cadotte, Cardinale & Oakley 2008). Phylogenetic diversity (PD) was proposed as an integrative metric of diversity to account for feature differences between species (Erwin 1991; Faith 1992).

The use of PD relies on the assumption that trait differences among species are retained in the tree of life through the pattern of the common ancestry and under homogeneous rates of trait evolution (Brownian motion; Felsenstein 1985; Harvey & Purvis 1991). Thus, it is assumed that the phenotypic differences between species are proportional to their phylogenetic distance to a common ancestor. However, not all traits follow such evolutionary pattern (Gravel *et al.* 2012; Kelly, Grenyer & Scotland 2014). For instance, trait differences in sympatric species can rapidly evolve to take advantage of ecological opportunities. Likewise, distantly related lineages can present similar trait due to adaptation to environmental conditions (e.g. the case of succulent plants; see also (Blomberg, Garland Jr & Ives 2003; Fukami *et al.* 2007; Prinzing *et al.* 2008). Indeed, trait differences between species are the product of the interaction between the environment they live in, and their evolutionary history (Freckleton & Jetz 2009). Notwithstanding this fact, the overall phenotypic differentiation (i.e. the difference regarding a large number of traits) between species is often better estimated with the phylogenetic distance than by measuring any set of traits (Cavender-Bares *et al.* 2009; Vellend *et al.* 2011).

Scientists have long recognized the importance of accounting for differences among species for biodiversity conservation (Vane-Wright, Humphries & Williams 1991). However, the transition from theory to conservation practice is still to be made (Winter, Devictor & Schweiger 2013). Increasing availability of genetic and trait data, along with the emergence of computer tools and metrics of diversity (Cornelissen *et al.* 2003; Schweiger *et al.* 2008; Cavender-Bares *et al.* 2009) provide us now with a unique opportunity to bridge this gap (e.g. Isaac *et al.* 2007). If all traits are equally important, then assemblages of species with the highest trait diversity will make a larger contribution to the option value (i.e. future uses and benefits) of biodiversity (Vane-Wright *et al.* 1991; Faith 1992, 1994; Polasky *et al.* 2001). When such difference is measured in terms of PD, the most diverse species assemblage will contribute the most to preserve the evolutionary potential of biodiversity (Vázquez & Gittleman 1998; Mace, Gittleman & Purvis 2003; Forest *et al.* 2007), as well as to predict its potential loss (Nee 1997; Purvis & Hector 2000; Thuiller *e*t al. 2011).

Differences among species are also linked to ecosystem functioning (Walker, Kinzig & Langridge 1999; Loreau 2001; Díaz & Cabido 2001). Traits determine how species interact with environment as well as their influence on ecosystem level processes (e.g. resistance to, or resilience after perturbations, primary production, etc.; Chapin III *et al.* 2000; Hooper *et al.* 2005; Cadotte *et al.* 2011; Isbell *et al.* 2011). Functional diversity (FD), defined as “the value and range of those species and species' traits that influence ecosystem functioning” (Tilman *et al.* 2001), is related to ecosystem functioning through the complementary use of resources by functionally dissimilar species (i.e. niche partitioning; Chapin III *et al.* 2000; Naeem & Wright 2003). Therefore, the explanatory power of FD for ecosystem function relies on how strongly traits are related to the function(s) of interest (Walker *et al.* 1999; Lavorel & Garnier 2002; Petchey & Gaston 2006). The knowledge about the functionally relevant traits is relatively abundant in the literature, and it may come from both observational and experimental studies, as well as theoretical models, on ecosystem functioning (Petchey & Gaston 2006). When the functional traits of interest are phylogenetically conserved, then PD may also be a good predictor of ecosystem functioning (e.g. Maherali & Klironomos 2007; Flynn *et al.* 2011; Srivastava *et al.* 2012), and the best possible proxy when the set of traits involved in ecosystem processes are complex or immeasurable (Crozier 1997; Cadotte *et al.* 2009; Mouquet *et al.* 2012). However, there is no reason to consider

PD and FD as mutually exclusive. Both measures may be used indeed in a complementary fashion to better characterize the diversity of species assemblages (Cadotte, Albert & Walker 2013).

In this thesis we focus on taxonomic (species and subspecies), phylogenetic and functional diversity components of vascular plants in a high mountain area. Plants constitute a mega-diverse taxonomic group with a far-reaching conservation value. They offer many different sources of habitat and food for multiple organisms, and are central for the human well-being, being involved in all existing ecosystem services listed in the Millennium Ecosystem Assessment (2005). Still, plants are often given less consideration in assessing protected areas than other less abundant groups (e.g. vertebrates; Kier *et al.* 2005), and they are underrepresented in red lists (Vié *et al.* 2008).

Uneven distribution of biodiversity and conservation challenges

After several decades of data collection, abundant information about species distribution is now available in atlas, herbaria, museums and unpublished studies. This information, though taxonomically incomplete and geographically biased (Kier *et al.* 2005; Robertson, Cumming & Erasmus 2010; Pimm *et al.* 2014), is the basis of our knowledge about the geographic patterns of biodiversity. Biodiversity maps are, for example, invaluable for studying causes of latitudinal and elevational gradients of biodiversity, but also to identify which areas (e.g. ecoregions, biomes, habitats) contribute the most to global, regional and local biodiversity (Barthlott, Lauer & Placke 1996; Myers *et al.* 2000; Murray-Smith *et al.* 2009). This information is central for the practice of conservation, because scientists and managers face the challenge of identifying priority areas where to allocate finite resources.

Scientists have sought for multiple ways to identify priority areas for conservation. An approach that has been at the forefront of conservation policy and thinking is the identification of hotspots (Brummitt & Lughadha 2003): exceptionally diverse areas in terms of species richness (SR), species endemism or threatened species (Myers 1988; Prendergast *et al.* 1993; Reid 1998; Myers *et al.* 2000). Systematic conservation planning (*sensu* Margules & Pressey 2000) have also directed the focus towards other criteria for prioritization such as complementarity, i.e. the gain in representativeness of biodiversity when a site is added to an existing set of areas

(Kirkpatrick 1983; Margules, Nicholls & Pressey 1988), or irreplaceability, defined as the extent of spatial options available for the conservation of a given biodiversity feature within a region (Pressey *et al.* 1993). The *pros* and *cons* of one or another approach have been intensely discussed elsewhere (Williams *et al.* 1996; Jepson & Canney 2001; Brummitt & Lughadha 2003; Kareiva & Marvier 2003). However, a common aspect of these approaches is that their conservation targets are often formulated at species levels. Thereby, if SR is not a good surrogate for other diversity components, the selected set of areas will present large gaps in terms of multifaceted diversity (Possingham & Wilson 2005; Orme *et al.* 2005; Ceballos & Ehrlich 2006).

The surrogacy between diversity components has often been studied by means of the spatial congruence of different types of hotspots. Prendergast and colleagues (1993) evidenced that hotspots of SR and those defined on the basis of the abundance of rare species were not necessarily congruent, a pattern that was extended to hotspots of endemic and threatened species in subsequent studies (Bonn, Rodrigues & Gaston 2002; Orme *et al.* 2005; Ceballos & Ehrlich 2006; López-López *et al.* 2011; but see Kier et al. 1997). The congruence among SR, PD and FD has been less thoroughly studied so far. Rodrigues and Gaston (2002) found that SR was a good surrogate for PD, and they suggested that this pattern may be general. While a few studies have provided support for this (Sechrest *et al.* 2002; López-Osorio & Miranda-Esquivel 2010), many others have not (Devictor *et al.* 2010; Prado *et al.* 2010; Mouillot *et al.* 2011; Taberlet *et al.* 2012), which suggest that the mismatch among diversity components may be the norm. Almost without exception, these studies were carried out at global to regional scales, it is therefore unknown whether this conflict persists at fine spatial scales at which most management actions are implemented (Schwartz 1999; Laguna *et al.* 2004; Harris, Jenkins & Pimm 2005).

Biodiversity through time

Distributional databases provide a static picture of the biodiversity. However, all organizational levels of biodiversity (from genes to landscape) experience temporal changes owing to natural and anthropogenic causes. Global biodiversity is now changing at extremely rapid rates due to human activity (Pimm *et al.* 2014), and current extinction levels are only comparable to the ones recorded in past five major extinctions (Barnosky *et al.* 2011). Land-use changes and climate warming are the major drivers of global change in terrestrial ecosystems (Vitousek *et al.* 1997; Sala *et al.* 2000), and evidences of their impact on species' abundance, distribution, phenology, physiology and interactions with other species are relatively frequent (Chapin III *et al.* 2000; Walther *et al.* 2002; Parmesan 2006; Walther 2010). Since the impact of global change will probably intensify in the next decades (Millennium Ecosystem Assessment 2005; IPCC 2014), it is urgent to increase our understanding about where, how, and to what extent these impacts are affecting biodiversity, so that it would be possible to develop timely management actions to alleviate their potential ecological and social consequences (Chapin III *et al.* 2000).

Although a mechanistic understanding of the biological consequences of global change is still lacking (but see the cascading model proposed by Hughes 2000), a large amount of scientific work has focused on predictive assessments (e.g. Peterson *et al.* 2002; Thuiller *et al.* 2005; Engler *et al.* 2011). In recent years, species distribution models (SDMs) have been increasingly used to forecast the potential impacts of global change on biodiversity (Brotons 2014). This method links current species occurrences to environmental variables (mainly climate, but also topography, land use, etc.) to predict its probability of presence at other sites, times and/or environmental conditions (Guisan & Zimmermann 2000; Guisan & Thuiller 2005). Despite continuous improvements in SDMs (see Elith & Leathwick 2009), they still do not account realistically for biological features of species such as dispersal, demographic performance, physiology, evolutionary response of species, and in particular, the effect of biotic interactions that are able to modify predicted individual abundances of coexisting species (Lavergne *et al.* 2010; Van der Putten, Macel & Visser 2010; Wisz *et al.* 2013; Valiente-Banuet *et al.* 2015). Failure to account for these aspects clearly confines the reliability of SDMs for predictive assessments (Kissling *et al.* 2012; Wisz *et al.* 2013; Svenning *et al.* 2014). Not less important is that a realistic validation of future species distribution is, at best, limited by the scarcity of empirical data (Guisan & Theurillat 2005; Guisan & Thuiller 2005). Most probably, the urgency to anticipate the future impacts of biodiversity might have led us to put the cart before the horse.

Gathering empirical data is not an easy task either. The temporal response of communities (the very end of the cascading model proposed by Hughes 2000) to environmental change is not always linear, and it may lag long time periods (Harrison, Damschen & Grace 2010; Bertrand *et al.* 2011). This is particularly the case in plant communities dominated by long-lived species (Morris *et al.* 2008), and/or with abundant clonal species (Steinger, Körner & Schmid 1996), whose slow dynamics may hide a deterministic trend towards extinction due to habitat loss or fragmentation ("extinction debt"; Tilman *et al.* 1994). While the modification of habitat drives some species to extinction, it creates suitable habitat for others, but time-lags between habitat generation and colonization of species ("colonization credits") are also possible (Piqueray *et al.* 2011). These lagged responses hamper the utility of short-term empirical evidences to anticipate future diversity changes, and can also lead to misleading management decisions (see Vellend *et al.* 2013). A long-term perspective is also needed to disentangle the directional response of ecological communities to global change from the stochastic fluctuations they exhibit due to natural causes (Magurran *et al.* 2010).

Long-term studies are conventionally defined as those long enough to encompass processes that structure the ecosystem under study (Strayer et al. 1986). Revisitation studies, which use historical vegetations surveys as a reference of initial conditions, are often carried out to investigate the long-term response of communities to global change (e.g. Lavergne, Molina & Debussche 2006; Vittoz *et al.* 2008; Wilson & Nilsson 2009; Harrison *et al.* 2010; Kapfer *et al.* 2011). However, the data obtained from resurveys is too often subjected to strong differences in the sampling effort, taxonomic uncertainties (Vellend *et al.* 2013), and inter-observed differences (Klimeš 2003), which may difficult its interpretation (Vittoz & Guisan 2007). Relocating original site and determining the exact area where the first survey was done is also challenging (Chytrý *et al.* 2014), and can result in an overestimation of local extinction and colonization of species over time (Nilsson & Nilsson 1985; Kullman 2010). Although most of these pitfalls can be partially overcome (see Vellend *et al.* 2013), the interpretation of this kind of data is necessarily open to criticism.

When vegetation resurveys are conducted in permanently marked plots or transects, the comparison between historical and contemporary data is much more accurate. Still, ascribing the nature (transient *vs.* directional) of reported changes may be as problematic as in plot-less revisitation studies (Table 1). Monitoring of communities across successive years is therefore needed to tease out the nature of temporal changes in communities. Unfortunately, the long-term monitoring of plants communities is rather uncommon, and often suffers from extremely poor replication due to logistic reasons (see Strayer et al. 1986; Lindenmayer *et al.* 2012).

Another critical point of revisitation studies is how to attribute observed changes to environmental causes. In most cases, drivers of change are inferred indirectly through the analysis of particular species traits, because detailed environmental records of initial conditions are often lacking (but see McGovern *et al.* 2011). A more definitive evidence of the effects of drivers can be achieved with experimental manipulations (e.g. simulated warming or herbivory exclusion), where the effect of confounding factors can be ruled out (e.g. Rawes 1981; Price & Waser 2000; Klanderud 2008; Olsen & Klanderud 2014). However, experimental studies are often conducted at local scale and without a long-term perspective, making results difficult to generalize to natural conditions (Watkinson & Ormerod 2001).

Mountains as 'natural laboratories' for the study of biodiversity distribution and its response to global change

Mountain ranges usually harbor higher diversity than surrounding lowlands regions. Some mountains are well recognized indeed as centres of biodiversity, such as the ones in the Mediterranean region (Médail & Quezél 1999). Besides, some mountains have also disproportionate abundance of endemic species (e.g. 36% in Sierra Nevada; Blanca *et al.* 2002) due to their isolation and complex evolutionary history (Hewitt 2000; Jansson 2003). Aside from their biological value, mountain ecosystems deliver multiple regulating and cultural services to human populations (energy, food and water), sometimes far away from ranges (Beniston 2006). Understanding the effects of global change on mountain is therefore not only a biological conservation affair, but a priority for human well-being. Although mountain ranges strongly differ in elevation, extension, topographic complexity, geographic heterogeneity, current climate and historical constrains worldwide (Nagy & Grabherr 2009), they share some common features (elevational gradients, spatial heterogeneity and vulnerability to global changes) that make them excellent “natural laboratories” for the study of the spatial distribution of biodiversity, and its temporal response to global drivers of change (Körner 2007a; Sanders & Rahbek 2012).

The elevational arrangement of vegetation in mountains evidences the predominant role of climate on plant distribution (Körner 2007a; Nagy & Grabherr 2009). Temperature, the vegetative growing period, and other related variables decrease with increasing elevation, whereas precipitation and moisture increase from lowlands to summits. Therefore, the climatic gradient along elevation actually represent two opposite gradients, and it is not surprising that most species often concentrate at intermediate elevations (Rahbek 1995; Körner 2007a). Some of the hypotheses that have been formulated to explain the hump-shaped pattern of SR along elevation are the mid-domain effect (overlap in the distribution of species ranges along bounded domains at the lower and upper elevations; Colwell & Hurtt (1994), and the mass effect (a peak of SR at ecotonal positions promoted by the feedback between adjacent communities; Shmida & Wilson 1985; Grytnes 2003). A monotonic decrease in SR with elevation is also possible (Rahbek et al. 1995, Panyotis et al. 2015), which may mirror the differences in water-energy relationships along elevation (Currie *et al.* 2004; Beck & Chey 2008), the reduction of available area along the elevational gradient, or the effects of land use (Austrheim 2002; Nogués-Bravo *et al.* 2008).

The relationship between elevation and other diversity components than SR has received much less attention. The increase of plant endemism with elevation is linked to the isolation and speciation at high elevation areas during Pleistocene glaciations (Tribsch 2004). Regarding PD, Bryant *et al.* (2008) found an unimodal distribution with a peak at intermediate elevations. In contrast, Ndiribe *et al.* (2014) did not find substantial differences in the phylogenetic structure of plant communities along elevation, but within lineages. In a study conducted in the Alps, de Bello *et al.* (2013) showed that FD diminished with the net effect of elevation (residuals of the regression between elevation and temperature), whereas it did increase with temperature. Taken together, these studies evidence that biodiversity patterns are not shaped by a single variable, but they also underpin the predominant role of climate along the elevational gradient (Lomolino 2008). This fact makes mountain ecosystems, and hence their biodiversity, particularly vulnerable to the impact of climate warming (Diaz, Grosjean & Graumlich 2003).

Current trends of climate warming are higher in mountains than the global mean (Böhm *et al.* 2001), and it is predicted that temperatures will continue increasing throughout the XXI century (IPCC 2014). Aside from increasing temperatures, precipitation and the pattern of snow deposition have changed since mid-twentieth century, whereas extreme events have increased in mountains (Beniston 2003; López-Moreno 2005; López-Moreno *et al.* 2010b). These changes will translate into a progressive loss of harsh environmental conditions, and an increase in the length of growing periods for plants at high elevation (Theurillat & Guisan 2001).

At low-to-intermediate elevation, the effect of land-use change in vegetation is more apparent than that of climate warming (Tasser & Tappeiner 2002). In many European mountains, the landscape has long been shaped by human activity (Chocarro *et al.* 1990; Fillat *et al.* 2008; Nagy & Grabherr 2009; Schwoerer *et al.* 2015). Lowlands and hillsides were often deforested for timber extraction, while subalpine treelines were lowered to increase areas for summer pasturing (Cernusca *et al.* 1996; Fillat *et al.* 2008; Chemini & Rizzoli 2014). However, socio-economic shift of rural societies throughout the XX century led to a dramatic changes in the traditional use of the territory: an overall extensification or land abandonment, and localized intensification of agro-pastoral systems in valleys (Cernusca *et al.* 1996; Tasser & Tappeiner 2002; Chemini & Rizzoli 2014). These shifts in land-use have triggered changes in land-cover (Tasser & Tappeiner 2002; Lasanta-Martínez, Vicente-Serrano & Cuadrat-Prats 2005), and large changes in plant diversity are expected.

Global change impacts on mountain biodiversity: predictions vs. observation

Many generalities have been crafted about the potential effects of climate warming in mountain plant diversity (Theurillat & Guisan 2001; Crawford 2008). It is both expected and predicted that species will track increasing temperatures by shifting upwards (Hughes 2000; Theurillat & Guisan 2001; Dirnböck, Dullinger & Grabherr 2003; Walther 2003). A large number of studies have provided support for the 'upwards migration hypothesis' worldwide (Grabherr, Gottfried & Pauli 1994; Lenoir *et al.* 2008; Chen *et al.* 2011; Pauli *et al.* 2012; Morueta-Holme *et al.* 2015), whereas a few others have found downwards migrations of species, or no change in their elevational range (Lenoir *et al.* 2010).

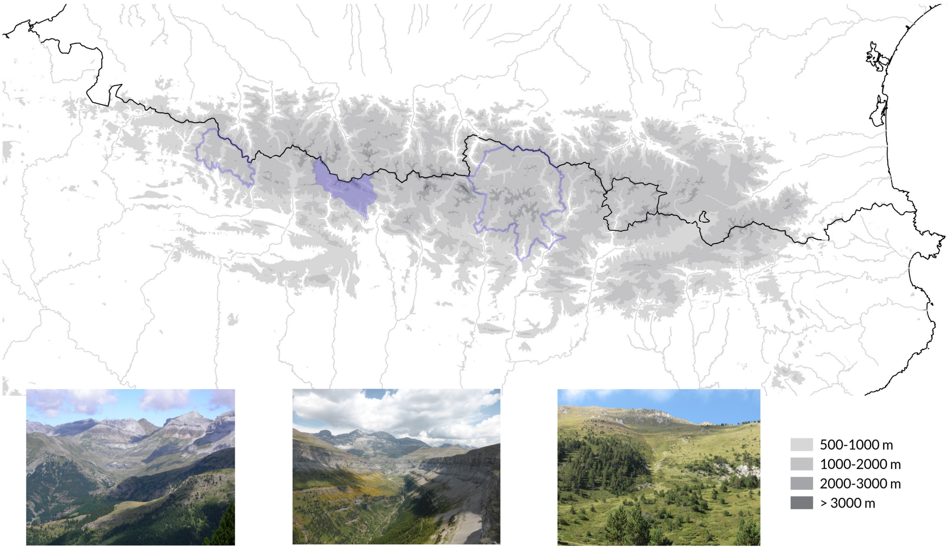
Plant species occurring in summits are though to be particularly vulnerable to climate warming because of their progressive loss of suitable habitat and impossibility to migrate upwards (Grabherr *et al.* 1994; Theurillat & Guisan 2001; Dirnböck *et al.* 2003). In contrast, species with higher thermal affinities may increase in abundance at higher elevations at the expense of cold-adapted ones ('Thermophiliation hypothesis'; Gottfried *et al.* 2012). While several studies have provided empirical support for this hypothesis at both local and continental scales (Keller, Kienast & Beniston 2000; Parolo & Rossi 2008; Erschbamer *et al.* 2008; Gottfried *et al.* 2012), Grytnes and colleagues (2014) have demonstrated that species increasing at high elevation are not particularly warmth demanding. Irrespective of the thermal affinities of species, the balance between species gains and losses over the last decades has been positive in most studied summits (Grabherr *et al.* 1994; Klanderud & Birks 2003; Pauli *et al.* 2012; Grytnes *et al.* 2014).

Another widespread expectation is that bioclimatic boundaries will shift upwards with increasing temperatures (Theurillat & Guisan 2001; Peñuelas & Boada 2003; Körner 2007b). One of the most recurrent monitors of such climatic effect is the subalpine-alpine treeline ecotone. Undisturbed treelines represent transitions from the subalpine forest limit to the alpine zone, where tree growth and recruitment is determined climatically. Therefore, upward shifts of treelines in response to increasing temperatures are expected (Grace, Berninger & Nagy 2002). Some treelines have advanced upslope, whereas many others have experienced tree growth and densification in response to climate warming (Kullman 2002; Camarero & Gutiérrez 2004; Harsch *et al.* 2009; Mathisen *et al.* 2013). Where the position of treeline was historically lowered by human activity, the response of treeline to climate warming may be confounded with, or overridden by, the effect of land-use change (Hofgaard 1997; Gehrig-Fasel, Guisan & Zimmermann 2007). Whatever the reason, either the upwards advance of treelines or their densification are expected to trigger important structural changes (e.g. habitat fragmentation) in adjacent alpine grasslands (Theurillat & Guisan 2001; Dirnböck *et al.* 2003; Becker *et al.* 2007). However, how the field-layer vegetation will respond to such changes is still understudied (Illerbrun & Roland 2011).

Regarding land-use changes, the generalized decline in livestock grazing is expected to have strong impacts on plant diversity and composition of grasslands (Milchunas, Sala & Lauenroth 1988; Olff & Ritchie 1998; Vesk & Westoby 2001; Tasser & Tappeiner 2002). Comparisons between historical and contemporary aerial photographs have revealed large changes in vegetation cover below the treeline: some open areas formerly used for livestock grazing have been reforested (Gehrig-Fasel *et al.* 2007; Gellrich *et al.* 2007; Améztegui, Brotons & Coll 2010; Gartzia, Alados & Pérez-Cabello 2014), whereas many others have been colonized by shrubs following ecological succession after abandonment (Komac, Alados & Camarero 2011). Land abandonment and grazing reduction have also affected plant diversity and/or composition of grasslands as well as their foraging quality below the treeline (e.g. Krahulec *et al.* 2001; Dullinger *et al.* 2003; Pavlů *et al.* 2005). Above the treeline, these potential effects are less thoroughly studied (Austrheim & Eriksson 2001; Erschbamer, Virtanen & Nagy 2003), and aerial imagery is not useful to detect fine-grained vegetation changes. Some authors have anticipated plant diversity losses in alpine grasslands due to land-use changes (Tasser & Tappeiner 2002; Niedrist *et al.* 2008), whereas experimental evidences have shown an increase in SR (Speed, Austrheim & Mysterud 2013), or just a rapid change in community composition in the short-term (Mayer *et al.* 2009). However, we do not know yet whether these effects will diminish or increase in the long-term, and how they will interact with the simultaneous effects of climate warming in alpine areas (but see Speed *et al.* 2012). These gaps in the knowledge clearly confine our ability to develop successful management actions to mitigate the impact of global change in alpine grasslands of high conservation value.

The study area

Our study area locates in the Central Pyrenees, and particularly in the Ordesa-Monte Perdido National Park (OMPNP). The Pyrenees extends East-West along ~435 km, from the Biscay bay in the Atlantic ocean to the Mediterranean sea, separating the Iberian Peninsula from continental Europe. This physical barrier of ~150 km wide (10 km at the eastern end) also divides two watersheds and two floristic regions (Eurosiberian and Mediterranean). We carried out most of the research in the OMPNP (42º42' N, 0º03' W), which is located in the Inner Ranges of the Central Pyrenees, in Aragón (Spain), bordering with the Pyrenees National Park in the North face (Fig. 1). Although the present thesis is mainly focused in this National Park, some of our studies were replicated in a treeline located in the Aigüestortes-Estany de Saint Maurici National Park



LVONP OMPNP AESMNP

Fig. 1 Study sites (from left to right): Los Valles Occidentales Natural Park (LVONP), the Ordesa-Monte Perdido National Park (OMPNP), and the Aigüestortes-Estany de Saint Maurici National Park (AESMNP).

(42º28'N 1º03' E; Fig. 1), and several grasslands across the Los Valles Occidentales Natural Park (42º44' N, 0º35' W; Fig. 1). Characteristics of these sites are given in chapters 3 and 4, respectively.

The National Park was set up in the Ordesa valley in 1918, originally restricted to the range of the Pyrenean ibex (*Capra pyrenaica pyrenaica*), which went extinct by the beginning of the XXI century. In 1982, the National Park was extended to encompass the four other valleys radiating from the highest calcareous peak in Europe (Monte Perdido, 3355 m a.s.l.; Fig. 2): Bujaruelo, Añisclo, Escuaín and Pineta. Nowadays the park covers 15.608 ha, plus 19.679 ha in the peripheral zone, distributed across four main municipalities (with less than 2000 inhabitants in total): Torla, Fanlo, Puértolas, Tella-Sin and Bielsa. The OMPNP hosts the category of Biosphere Reserve and World Heritage Site since 1977 and 1997 respectively, and from 1998 onwards it is also included in the Natura 2000 network. There are no populated villages within the National Park, but a large part of its alpine area has traditionally been used as summer pasturing by local shepherds. According to the current law (Plan Rector de Uso y Gestión – PRUG), both biotic and abiotic elements of the OMPNP are under strict protection regimes, and traditional practices such as summer grazing are the only economic activities allowed under regulation.

Geomorphology and geology

The OMPNP shows a high complex topography, with 34 summits above the 3000 m a.s.l. and an elevational gradient ranging from 700 m to 3355 m. U-shaped valleys, glacial cirques, and several hanging valleys (e.g. Cotatuero and Carriata) witness the Quaternary glaciation-deglaciation events. River erosion has been important in shaping the depth and narrow canyons of Añisclo and Escuaín.

Main bedrock type is limestone, whose karstification has created sinkholes, dolines and caves throughout water runs out. Sandstone outcrops are abundant in cliffs, whilst flysch and loam are mostly found in mountain passes, and smooth peaks. The unequal resistance of main rock types to glacier erosion led to idiosyncratic steep cliffs of the OMPNP.

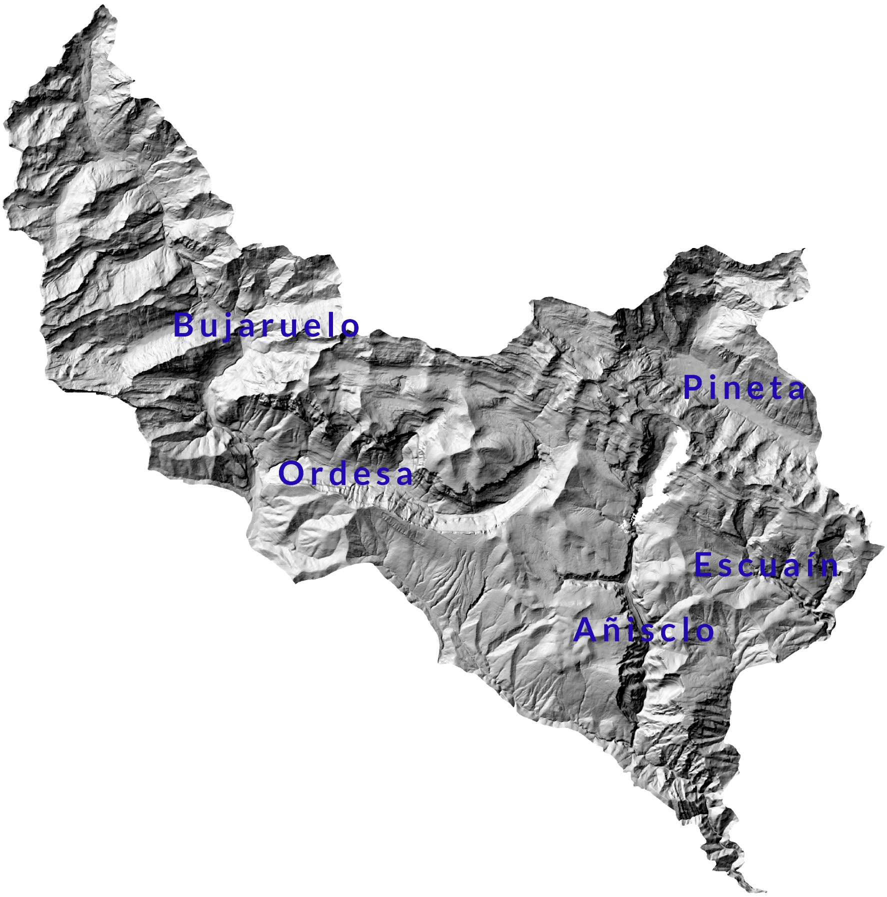


Fig. 2 Hillshade map of the Ordesa-Monte Perdido National Park (including the buffer area) constructed from a 5-m resolution digital elevation model.

Climate

The OMPNP has a mountain climate with a continental influence, as it is evidenced by the marked seasonality in both temperature and precipitation (Fig. 3). According to data from the Góriz Metereological Station (2215 m a.s.l.), annual mean temperature is ~5ºC (1982-2012 period), and daily mean temperature ranges from -0.5ºC in January and 13ºC in August (1991-2012 period). Annual mean precipitation amounts 1676 mm, and it is concentrated in the early fall and spring (Fig. 3). Although year-to-year precipitation variability is very high (from 1103 mm in 2004 to 2506 mm in 1997), February and March are on average the driest months (Fig. 3). In contrast, storm events are concentrated in summer (Benito 2006). At 2200 m, precipitation falls as snow since November to May. On average, snow lasts for 197 days, with a mean maximum accumulation of 152 cm. The vegetation growing period, defined as the number of snow free days with air temperature equal to or above 7ºC, lasts for 128 days at 2215 m (Del Barrio, Creus & Puigdefabregas 1990).

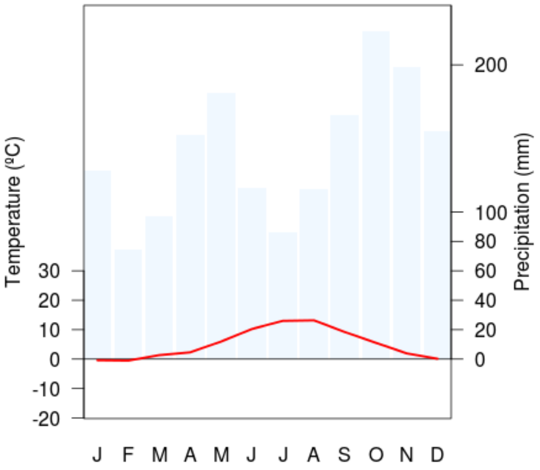


Fig. 3 Climate diagram of the Ordesa-Monte Perdido National Park (Góriz Meteorological Station, 2215 m a.s.l.).

Figure 4 illustrates how estimated climatic variables vary along the elevational gradient. Temperature decreases between 0.45ºC and 0.55ºC every 100 m (Benito 2006). In contrast, precipitation increases on average 41.3 mm every 100 m from the valley bottom to intermediate elevations (Benito 2006). Elevation interacts with the complex geomorphology of the National Park, generating many microclimates. North and south facing slopes show also contrasting characteristics (milder winters and cooler summers, and more even precipitation throughout the year *vs.* higher insolation, drier summers and strong variation in temperature and precipitation throughout the year, respectively). At more local scale, thermal inversion is also remarkable (e.g. +0.62°C annual mean temperature from 1350 to 1700 m; Benito 2006).

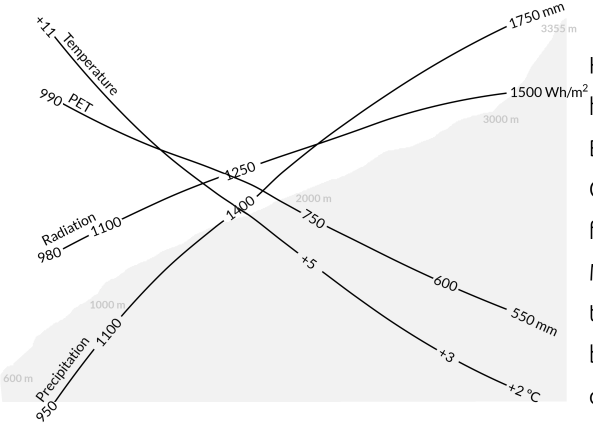


Fig. 4 Extrapolated values of climate variables along the elevational gradient in the Ordesa-Monte Perdido National Park (OMPNP). Mean annual annual temperature and precipitation were gathered from the Góriz Meteorological Station (2215 m a.s.l.) and extrapolated by using a digital elevation model (DEM, 5-m resolution). Evapotranspiration (PET) was obtained from the Atlas Climático de Aragón (Vicente Serrano, 2006, 0.1x0.1 km resolution). Other variables were calculated with a GIS based on the DEM. The area of the OMPNP across elevation is represented in gray.

Vegetation

Here, we present a brief overview of habitats and plant communities based on Benito (2006). Lower elevations of the OMPNP are covered by evergreen mixed forest of *Quercus ilex* subsp. *ilex* and Mediterranean shrublands. Different types of forest are found in the montane belt (800-1700 m, 65% of the total area), depending on topography, altitude and human activity. In the lowest part, marcescent forests of *Quercus cerrioides* predominate in south slopes. Above 1000 m, mixed forest of *Fagus sylvatica*, *Abies alba*, *Pinus sylvestris*, *Coryllus avellana, Fraxinus excelsior, Tilia platiphyllos, Betula pendula,* and *Sorbus aria* are frequent. *Buxus sempervirens* becomes frequent in the understory of all types of forests between 800 and 2000m, as well as in open areas, where it co-occurs with dense thorny cushions of *Echinospartum horridum*. This shrubland extends upwards in stands where the subalpine forest was historically depressed by fire and logging.

In the subalpine belt (1700-2300 m, 6% of the total area), *Pinus sylvestris* is gradually replaced along the elevational gradient by *P. uncinata*, which is the only treeline-forming species in the Pyrenees. Although the potential limit of *P. uncinata* treeline is at ~2100 m a.s.l., treeline position has historically been lowered to increase summer grazing areas. As a result, there are no dense forests above 1900 m a.s.l. in the Park. Nevertheless, the treeline ecotone located in the Sierra de las Cutas is considered to have been hardly affected by local logging and grazing (Gil-Pelegrín & Villar Pérez 1988), and for such reason, it has been studied to seek long-term dendrochronological evidences of climate warming (Camarero & Gutiérrez 2004). The understory of *P. uncinata* forest in sandstone outcrops is dominated by acidophillous shrubs such as *Rhododendron ferrugineum, Vaccinium myrtillus* and *Calluna vulgaris* in north faces with long lasting snowbeds in spring, and *Juniperus communis* and *Arctostaphylos uva-ursi* in south facing slopes and crests.

Grasslands have been expanded at the expense of subalpine forests long ago (Fillat *et al.* 2008). Traditional pastoral management, along with topographic complexity, has led to a complex mosaic of dozens of different grassland communities between the subalpine belt and the limit of the alpine belt (2700 m, 0.25% of the area). Most of them are dominated by hemicriptophytes (grasses and sedges), although at higher elevation (2400-2700m) chamaephytes gain predominance.

In the subnival belt (2700-3555 m, 0.01% of the area), vegetation cover decreases substantially, and rocky habitats, including cliffs and screes, become dominant. Different plant communities are found in this belt depending of main bedrock type, exposure, and site stability. Species of the genus *Silene, Saxifraga*, *Androsace, Artemisia, Draba, Minuartia, Veronic*a, *Cerastium,* and *Linaria* dominate plant communities at this belt.

Simplification of main vegetation types

The OMPNP harbors 122 plant communities, which are the most vegetation types present in calcareous mountains of the Central Pyrenees. For analyses, we have clumped the habitats defined in different vegetation maps into eight main categories (Aldezabal 1997; Villar & Benito 2001 and the Spanish National Forest Inventory 1997-2007; percentage of total areas is given in brackets; see also Fig. 5):

- Nitrophilous communities related to human activity, associated to **anthropogenic areas** (0.4%), consist of vegetation occurring along pathways, around small villages, or in abandoned crops (several alliances of Class *Ruderali-Secalietea*) located at low-elevation in the periphery of the OMPNP, besides shepherd's huts and livestock resting areas in subalpine and alpine belts (Alliance *Rumicion pseudoalpinae*).

- **Wetlands** (0.4%). Communities mainly associated to streams and springs, including dense grasslands with abundant grasses, sedges (*Carex* spp.) and rushes (*Juncus* spp.) that are mainly included in the following phyosociological alliances: *Calthion palustris, Caricion nigrae, Caricion davallianae* and *Molinio-Holoschoenion*. Wetlands can be found from the montane to the lower alpine.

- **Mediterranean forests** (2%), including marcescent forests of *Quercus cerrioides* (mainly at Escuaín valley, and evergreen oak (*Quercus ilex* and *Q. ballota*) forests located in warmer areas of the OMPNP (i.e. the lowest part of Añisclo and Ordesa). These forests have been historically depleted for creating crop areas, and nowadays they are transformed into successional stages after abandonment, mainly shrublands of *B. sempervirens, Amelanchier ovalis, Juniperus* spp. and *Genista* spp.

- **Shrublands** (4%), mostly dominated by *B. sempervirens* and *E. horridum*, are distributed along the montane and subalpine belts. This habitat often locates in south facing slopes across the peripheral area of the Park, mostly in ridges and steep slopes, as well as abandoned areas.

- **Deciduous forests** (7%) occur mostly in valleys and shadow hillsides. Beech forests (often mixed with firs), splattered by elms (*Ulmus montana*), maples (*Acer* spp.) and sorbs (*Sorbus* spp.) widely extend along the Ordesa and Pineta valleys. Localized patches of montane poplar (*Populus tremula*) and ash (*Fraxinus excelsior*) point out avalanche corridors and steep slopes with sliding soils. Gaps of deciduous forests, produced by natural processes or logging, are colonized in a first step by characteristics herbaceous communities with tall herbs such as *Epilobium angustifolium*, *Atropa belladonna*, *Valeriana pyrenaica*, *Aconitum napellus, Heracleum sphondylium*), and some shrubs (*Salix caprea*, *Sambucus ebulus*, *Rubus idaeus*). These communities, named "megaforbs" (*Atropion belladonnae, Adenostylio alliariae* and *Sambuco-Salicion capreae*), show high plant diversity and contain several rare species.

- **Coniferous forests** (16%), i.e. pine and fir forests, are well-represented in the five valleys of the OMPNP, from the montane to the uppermost subalpine treeline. The south facing slopes are dominated by *P. sylvestris* forests (Al. *Deschampsio-Pinion* and), whereas upper subalpine areas are dominanted by *P. uncinata* (Al. *Rhododendro-Vaccinion* and Al. *Seslerio-Pinion*), the only treeline-forming species in the Pyrenees.

- **Rocky habitats** (32%) represent low cover vegetation with small plants, that dominates in cliffs, ridges and screes, across the whole territory, but particularly above the 2700 m. This habitat shows the most singular, rare and specialized flora, including many endemisms. Aside from some ferns (*Asplenium*, *Cystopteris*), genus such as *Saxifraga*, *Androsace*, *Hieracium*, *Potentilla* and *Galium* are frequent. Cliff vegetation is represented by several communities (depending on the geology, elevation and aspect) that are included in Al. *Saxifragion mediae* and Al. *Androsacion vandellii* on calcareous and siliceous substrata respectively. In screes, Al. *Androsacion ciliatae* (on sandstone) and *Iberidion spathulatae* (on limestone) include the most abundant plant communities. Finally, plant communities of the Al. *Saponarion caespitosae*, with many chamaephytes that are endemics of the central Pyrenees are located in ridges.

- **Grasslands** (38%) is the most extended and complex (both in structure and dynamic) vegetation class. Along with mountain grasslands, meadows that support traditional livestock economy and pastures located above the current treeline (hereafter named as alpine grasslands) are included within this category. Due to the complex microtopography (and, as a consequence, variety of microclimates and stocking rates) patches of different grasslands shape an heterogeneous mosaic. Al. *Mesobromion erecti* (on basic soils) and Al. *Nardion strictae* (in acidic ones) include the most abundant dense grasslands (>75% of cover) in flat areas and low steep slopes. Al. *Festucion eskiae* and Al. *Festucion gautierii* grasslands cover medium steep slopes (in acid and basic soils, respectively). Above 2000 m, *Mesobromion* communities gives rise to communities of Al. *Primulion intricatae* that are an important source of food for wild herbivores (chamois, marmots) in summer and autumn. Snowbed communities (*Arabidion coerulae* and *Salicion herbaceae*) with dwarf willows (*Salix pyrenaica, S. retusa, S. herbacea, S. reticulata* and other boreal species in their south distribution limit) increase at the expense of grasslands. The upper limit of grasslands, represented by Al. *Elynion myosuroidis,* is at 2700m.

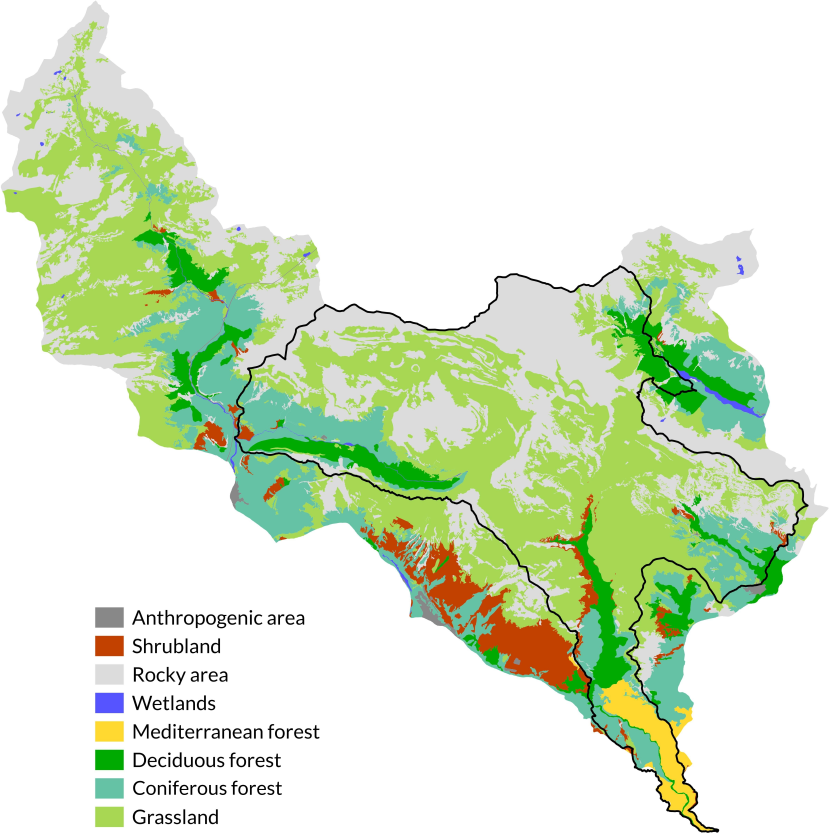


Fig. 5 Simplified vegetation types of the Ordesa-Monte Perdido National Park. This map was created by combining the vegetation map of the National Park (Villar & Benito 2001), the third National Forest Inventory, and the cartography of grasslands of Aldezabal (1997). Black line separates the National Park and the buffer area.

Plant diversity

The Pyrenees harbor more than 3300 autochthonous vascular plants, which constitutes about 25% of the European flora (http://atlasflorapyrenaea.org/florapyrenaea/index.jsp), and an extraordinary sample of the Pyrenean plant species. According to ~44000 plant records compiled from the JACA Herbarium and field surveys since the 1950s, there are more than 1379 plant taxa in the OMPNP. However, the shape of the rarefaction curve of species suggests that the inventory of species is still far from being complete (Fig. 6).

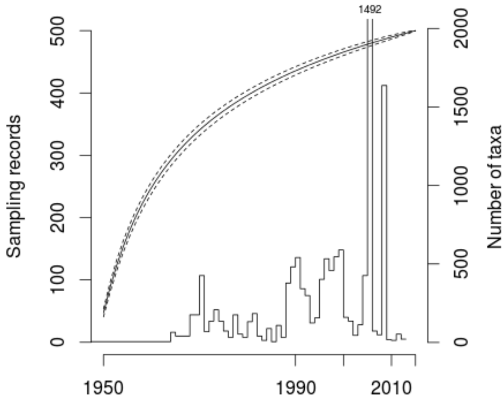


Fig. 6 Number of vegetation sampling records compiled in the JACA Herbarium since the 1950s, and species rarefaction curve (solid line) and 95% confidence intervales (dashed lines).

Out of the 104 families present in the OMPNP, Asteraceae, Poaceae, Rosaceae and Brassicaceae are the most abundant ones. At genus level, *Carex*, *Hieracium, Rosa* and *Festuca* are particularly abundant. The number of endemisms is large (45% of the Pyrenean ones occur in the OMPNP), including some tertiary relict species (*Ramonda myconii*, *Borderea pyrenaica*) that witness previous tropical conditions in the area. Several rare taxa (e.g. *Silene borderei, Carduus carpetanus, Veronica aragonensis, Pinguicula longifolia* subsp. *longifolia*) are locally abundant, and many others (17% of the flora) reach their range limit in the Pyrenees. Almost 100 species occur above 3000 m, including *Silene ciliata, S. acaulis, Saxifraga oppositifolia, S. pubescens, Cerastium alpinum, Veronica alpina, V. numularia, Minuartia cerastifolia* and *Pritzelago alpina*.

Given the biogeographic setting of the OMPNP, almost half of the species are Eurosiberian and about one fifth Mediterranean, whereas boreoalpine species are close to 10% of the total species pool. Twenty-five species are listed in the regional Catalogue of Endangered Species (*Corallorhiza trifida* and *Cypripedium calceoulus* are considered to be under extinction risk), and nine are listed as Species of Community Interest in the Habitats Directive (1992).

Land-use change and climate warming

The landscape of the Pyrenees (and hence that of the OMPNP) has largely been shaped by human activities (e.g. fire, logging, grazing), which dates back to at least the mid-Paleolithic. Nonetheless, the agro-pastoral system was not generalized until the 5300-4500 BP (Fillat 2008).

During the Middle Ages, sheep were introduced in the Pyrenees, and shrublands and forests were removed for increasing grazing areas (Montserrat 1992). The transhumance system (seasonal movement of livestock from high-elevation grasslands to lowlands following the seasonal resources) began in this period, and it has been central for sustaining local populations until recently (Fillat *et al.* 2008).

After centuries of demographic fluctuations, the number of inhabitants and domestic animals rose to its maximum in the beginning of the XX century (Chocarro *et al.* 1990). However, by the end of the civil war, socio-economic conditions provoked a massive migration from rural areas to cities, which led to a dramatic recession of traditional land-use, including grazing practices. Nowadays, the population is 70% less than in the 1900s, many areas are abandoned, and the transhumance is not longer practiced (García-Ruiz *et al.* 1996; Lasanta-Martínez *et al.* 2005). In some cases, sheep have been replaced by less demanding cattle and horses (García-Ruiz *et al.* 1996), but the numeric increase of these animals has not offset the losses of sheep (Lasanta-Martínez *et al.* 2005). Land-use changes have resulted in regrowth and densification of forests as well as shrub expansion at the expense of mountain grasslands (Lasanta-Martínez *et al.* 2005; Komac 2010; Gartzia *et al.* 2014). In contrast, tourism has increased during the last decades: since the 1990s the annual number of visitors is approximately 600.000 (http://www.magrama.gob.es), mostly concentrated in the Ordesa and Pineta valleys during summer.

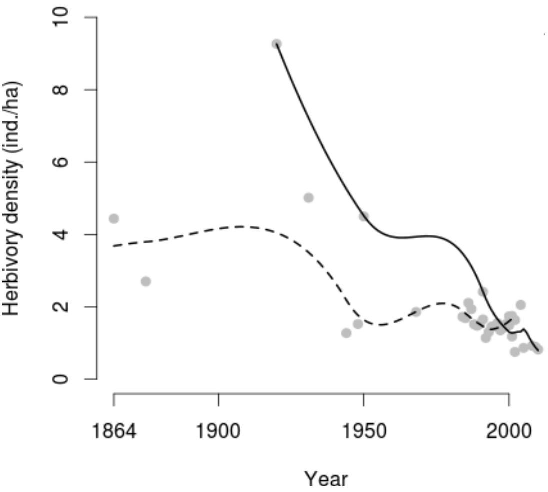


Fig. 7 LOESS lines of the temporal trend in the density of grazing animals in the Ordesa-Monte Perdido National Park (solid line) and the Los Valles Occidentales Natural Park (dashed line). Data (gray dots) was gathered by F. Fillat, R. García-González and T. Lasanta (see also García-Ruiz *et al.* 1996).

Temperatures have increased in the Pyrenees throughout the XX century (Dessens & Bücher 1997). López-Moreno *et al.* (2010b) estimated the increase in the Spanish Pyrenees in +0.3ºC per decades between 1950 and 2006. At regional scale, a general trend towards decreasing annual precipitation, and increasing extremely high temperatures have also been observed since the 1950s (López-Moreno *et al*. 2010a). In addition, regional climatic models forecast that most of these climatic trends will exacerbate in next decades (Nogués-Bravo *et al*. 2007), which may affect the pattern of snowfall frequency and intensity along elevation (López-Moreno *et al*. 2011). Evidences of the impact of ongoing climate warming are multiple and diverse in the OMPNP, from the melting of the glacier of the Monte Perdido (López-Moreno *et al*. 2015), to the enhanced tree recruitment and tree-growth in treelines in response to increasing temperatures (Camarero *et al*. 2015), and the short-term thermophilization of plant communities observed in some summits (Gottfried *et al*. 2012).

Objectives

Objectives

The general aim of this thesis is to contribute to better understand the distribution of different components of plant diversity in high mountains, and their long-term response to global change. The specific objectives associated to each of the five chapters presented in this thesis are the following:

- To assess the sampling effort in the database information of the plant distribution in the Ordesa and Monte Perdido National Park (OMPNP), and its effect on the analysis of diversity patterns (*Chapter 1*).
- To examine the distribution of taxonomic (species and endemism, separately), phylogenetic and functional diversity of vascular plants across such heterogeneous mountain area, and to assess the utility of hotspots for the representation of multiple diversity components (*Chapter 2*).
- To evaluate the dynamics of the tree cover at the treeline in the last decade, and its consequences on underlying alpine plant communities (*Chapter 3*).
- To assess the dynamics of alpine grasslands over the last two decades in response to past grazing reduction and climate warming (*Chapter 4*).
- To disentangle the long-term effects of grazing cessation and climate variability in two alpine grasslands (*Chapter 5*).

To achieve these objectives, we combined information from biodiversity databases (*Chapter 1 and 2*), with observational and experimental data gathered in the field (*Chapter 3, 4 and 5*). First, we explored the data quality of distribution databases, and developed a novel method to quantify the sampling effort (*Chapter 1*). This method was used then in the assessment of hotspots of multiple diversity components in the OMPNP (*Chapter 2*). Next chapters were devoted to explore and quantify the response of alpine plant community to climate warming and grazing reduction, which is one of the main global change drivers in European mountains. Firstly, we analyzed the impacts of the treeline dynamics on field layer vegetation by comparing data taken along several transects in 1998 and 11 years later (*Chapter 3*). Secondly, we assessed changes of alpine grasslands at community, species and trait levels, by resurveying semi-permanent transects established 20 earlier, and taking into account the effect of the sampling error and natural fluctuations of communities (*Chapter 4*). Finally, we tested the long-term effect of herbivore exclusion and temperature on alpine plant communities with data from a 19-yr monitoring (*Chapter 5*).

Chapter 1

A Novel Method to Handle the Effect of Uneven Sampling Effort in Biodiversity Databases

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Abstract

How reliable are results on spatial distribution of biodiversity based on databases? Many studies have evidenced the uncertainty related to this kind of analysis due to sampling effort bias and the need for its quantification. Despite that a number of methods are available for that, little is known about their statistical limitations and discrimination capability, which could seriously constrain their use. We assess for the first time the discrimination capacity of two widely used methods and a proposed new one (FIDEGAM), all based on species accumulation curves, under different scenarios of sampling exhaustiveness using Receiver Operating Characteristic (ROC) analyses. Additionally, we examine to what extent the output of each method represents the sampling completeness in a simulated scenario where the true species richness is known. Finally, we apply FIDEGAM to a real situation and explore the spatial patterns of plant diversity in a National Park. FIDEGAM showed an excellent discrimination capability to distinguish between well and poorly sampled areas regardless of sampling exhaustiveness, whereas the other methods failed. Accordingly, FIDEGAM values were strongly correlated with the true percentage of species detected in a simulated scenario, whereas sampling completeness estimated with other methods showed no relationship due to null discrimination capability. Quantifying sampling effort is necessary to account for the uncertainty in biodiversity analyses, however, not all proposed methods are equally reliable. Our comparative analysis demonstrated that FIDEGAM was the most accurate discriminator method in all scenarios of sampling exhaustiveness, and therefore, it can be efficiently applied to most databases in order to enhance the reliability of biodiversity analyses.

Introduction

Decisions on biodiversity conservation are typically dependent on the degree of knowledge of species distribution [1], therefore, they ideally require the best available spatially explicit information of species distribution [2]. Given that field work necessary to get a database representative of the real biodiversity in large areas is highly resource-consuming, and current funding for this task is scarce [3–4], historical data stored in herbaria, museums, atlas and unpublished material emerge as an outstanding alternative [5]. In fact, biodiversity databases compiling information from these sources have proliferated worldwide in the last decade [6], as it is exemplified by initiatives such as the Global Biodiversity Information Facility (GBIF) [http://www.gbif.org]. Scientists and managers can now take advantage of the enormous effort done during decades of biodiversity inventories [7] and raise new ecological questions [6,8]. In particular, biodiversity databases are being intensively used in relevant conservation issues, such as the predictive distributions of plants and animals under global change scenarios [9–10], the identification of biological hotspots (e.g. [11– 13]), or the design of protected areas [1,14]. The generation of new analytical tools is promoting advances in the study of these fields, however, their reliability remains challenging due to the contingencies of the baseline data [15–22]. For instances, biodiversity database usually contain incomplete distribution data, because the information was collected according to different aims [15]. Evidences of how bias in database information can compromise biodiversity analyses and conservation planning are reported in a large number of studies [19,20,23,24,25,26,27]. Hence, an adequate control of data-quality is needed [15].

Quality control process should regard both database configuration and the evaluation of data suitability for analyses. According to the scheme proposed in Hortal et al. [15], the control routine has two main levels: (i) data-compilation and digitalization, and (ii) sampling effort assessment. The former is related to the reliability of the sources of information, taxonomic bias and the geographical accuracy of the data [8,28,29]. The latter refers to the uneven sampling effort present in non-systematic biodiversity databases [30]. As the first control level has already been discussed elsewhere, in this paper we will focus only on the assessment of the sampling effort bias.

Sampling effort is likely to be temporally, spatially and environmentally biased [20,27,31,32,33]. Temporal bias can be minimized by limiting database information to a time period short enough as to ensure that information remains the same throughout this period.

Regarding spatial and environmental bias, it is well known that some territorial units accumulate more sampling records than others due to diverse factors such as accessibility, habitat singularity, abundance of rare taxa, or differences in funding [24,25,34,35,36,37,38]. Given that most aspects of biodiversity (e.g. species richness) correlate well with sampling effort (e.g. [15]), diversity distribution inferred from raw database information may reflect the spatial distribution of sampling effort rather than the real distribution of diversity [8,15,27]. Hence, biodiversity distribution analyses based on spatially explicit data should account for sampling effort.

Among the range of methods that have been proposed to reduce the bias of sampling effort, those based on species accumulation curves (SAC) [34,39,40] are commonly used. According to SAC's properties, the total number of species recorded rises towards a ceiling as sampling effort increases [39,41]. Once the SAC is constructed, a model is fitted to describe the accumulative-sampling effort relationship (e.g. [42]). The selection of the model should be done with statistical rigor [39], but also according to the discrimination capability, i.e. the probability of correctly identifying well (or poorly) sampled units [43]. The discrimination is a *sine qua non* criterion in scientific fields with important social responsibility such as clinical diagnostic [44–45], whereas it has been hardly applied in ecological classification analysis, including the evaluation of sampling effort. If the methods to assess sampling effort fail to discriminate well from poorly sampled units, the resultant classification would be seriously affected. Likewise, if the discrimination capability differ among methods, then, the reliability of the classification would depend to a great extent on the selected method, and so will do the uncertainty of any analysis based on such information.

In this study, we analyze for the first time the discrimination capability of commonly used SAC based methods to quantify sampling completeness, and present a novel approach. We first compare methods according to their discrimination capability in two contrasting scenarios of sampling exhaustiveness and in an ideal situation, where the true species richness is known. Finally, we define an objective and generalizable procedure to account for sampling effort bias in biodiversity databases using the novel method and discuss its practical benefits for conservation management.

Methods

Review of methods to assess sampling effort bias

The SAC are constructed by plotting the expected (mean) cumulative number of species *S*(*n*), at a given number of samples (*n*) [40,46]. Samples order is randomized by repeatedly re-sampling (without replacement) to rule out its effect on the SAC [40,41,46]. Two main procedures based on SAC have been proposed to assess the sampling completeness: (i) the proportion of species richness out of the total predicted by the richness estimators [47–49], and (ii) the slope of the accumulation curve [30,50].

For the former procedure, the predicted richness should be calculated first, which can be done in several ways. Extrapolation of SAC based on asymptotic functions is one of them. The predicted richness is estimated as the total number of species that would be achieved with a hypothetical infinite sampling effort. The most usual models used to describe the SAC are the negative exponential, the Clench, and the Weibull models [39,50,51,52,53]. The other common way of predicting species richness is by non-parametric estimators based on the number of rare species observed within samples, either from incidence or abundance data [54–55]. The most common estimators in this case are Chao [56], Jacknife (onwards NPE) and Bootstrap [57] estimators, as well as incidence-based and abundance-based coverage estimators, called ICE and ACE respectively [58]. For a complete review of all these methods see [40], [59] and [60].

The second procedure for measuring sampling completeness is the slope of the SAC along the sampling effort gradient, which is minimum when all species have been found [30,50]. There are several alternatives to compute the slope of the curve. One is the geometric definition of the slope as the secant line to the curve:

$$m = \frac{\Delta Y}{\Delta X} \qquad [1]$$

where *Y* is the species richness and *X* the measure of effort. An appropriate procedure for calculating *Y* is the unbiased estimator of true species richness, the so called Mao Tau estimator (hereafter STE) [41,61]. Another way of estimating the slope of the SAC is to calculate the species accumulation rate at a given sampling level, by fitting a function to the curve. To do that, it is necessary to previously examine the level of homogeneity of sample units by comparing the empirical mean randomized SAC, with the expected curve if all individuals had been randomly assigned to the samples. The expected curve may be constructed either by computing a rarefaction curve or a Coleman curve (for details see [40,46]). The slope of the SAC is then calculated with the first derivative of the fitted curve. The final slope of the Clench function (as well as the slope of other asymptotic functions) is the most common method for assessing the accumulation rate [30,39,60]. Two main problems are associated with these asymptotic functions: their limited use at low sampling levels of sampling [15], and the violation of statistical assumptions inherent to non-linear regression models (i.e., correct mean structure, variance homogeneity, and independent and normally distributed errors [62]).

FIDEGAM: a new method to quantify sampling completeness

As an alternative to the methods reviewed above, we have developed the FIDEGAM method, which fits a Generalized Additive Model (GAM) [63–64] with Poisson response, or the negative binomial if data presents overdispersion [65], to each randomized SACs. GAM is an extension of Generalized Linear Models (GLM) [66], which allows flexible modeling of the influence of the response variable [64]. In a GAM framework, statistical assumptions are met because the function is adjusted to non-normal distributed data instead of forcing data to fit an arbitrary known function. Besides, contrary to asymptotic methods the model fits even at low levels of sampling effort. Once GAMs are fitted, the first derivatives and their 95% corresponding confidence intervals along the species accumulation process are calculated. This output describes the whole pattern of species accumulation, being the first derivative at the maximum number of sampling records the measure of sampling completeness (onwards FIDEGAM value). FIDEGAM values range from 0 to 1, corresponding to high and low sampling completeness for a given area, respectively.

Testing and comparing the discrimination capability of the sampling completeness measures

(i) Classification rules to assess discrimination capability. The discrimination capability between different methods should be evaluated under different levels of sampling completeness and according to an objective classification rule [43]. From a statistical point of view, the discrimination capability of a given Y (e.g. a measure of sampling completeness in our case) to distinguish between two alternative states $S1$ (e.g. well sampled unit) and $S2$ (e.g. poorly sampled unit), should be based on a Receiver Operating Characteristics (ROC) curve analysis [43,67,68]. A binary response is needed for ROC analyses, so that Y classifier should be dichotomized according to a cut-point value. Values of the sampling unit above that cut-point would refer to one of the two possible states (i.e. S1), and values below to the alternative state (i.e. S2). The classification criterion used in ROC analysis is related to the probabilities of belonging to one of the states as a function of the values of Y, $P[S1|Y]$. These probabilities are

estimated using a GLM in a binary regression framework [43]. Once the ROC curve for each completeness measure is fitted, their discrimination performance is evaluated using the area under the ROC curve (AUC)

$$AUC = \int_{0}^{1} ROC(t)\,dx \qquad [2]$$

The AUC takes values between 0.5 (uninformative classifier) and 1 (perfect classifier) [44,69,70].

(ii) Discrimination capability of methods in different scenarios. We tested the discrimination capability of the completeness measures based on the observed proportion of species richness out of the total predicted by a non-parametric estimator (NPE), the slope of the Mao Tau estimator (STE) and FIDEGAM using ROC in two contrasting scenarios of sampling completeness and in a ideal situation, where the true species richness is known.

The scenario of high sampling exhaustiveness is derived from a database that contains information of the vascular flora of the Ordesa-Monte Perdido National Park (Spanish Pyrenees; ORDESA thereafter). The National Park is one of the most exhaustively prospected areas in the Iberian Peninsula [71], however, due to the high topographic complexity, the large altitudinal range (from 700 to 3354 m a.s.l.) and severe access difficulties to some points, the sampling effort is expected to be unevenly distributed along the *ca*. 30000 ha of the Park. The ORDESA database comprises more than 44000 spatially explicit records of 1379 vascular plant species along the 321 UTM cells (1 km^2; sampling units) of the Park (excluding Bujaruelo valley), compiled from herbarium samples, phytosociological *relevés*, and *visu* records collected over the last 50 years in the JACA Herbarium (http://proyectos.ipe.csic.es/floragon/index.php). To homogenize the different sources of information we defined ‘’sampling record’’ as each input of information of plants occurrence (from one to multiple species) that differs in date, site, method and/or author.

The second scenario was created emulating the structure (sampling units/sampling records/species per record) of the ORDESA database and using a random procedure, which involves the following steps (see Fig. S1 for further details):

1. For each sampling unit (n = 180), assign the number of sampling records according to three levels of sampling intensity (20–50, 51–80 and 81–110 sampling records) at random.

2. For each sampling record, randomly determine how many (between 1 and 30) and which species are recorded from a virtual pool (400 species).

The resultant information was compiled in a database named SIMULAU. We assumed that the sampling effort has been enough to detect the true richness in all the sampling unit of this database. We then subsampled from SIMULAU to achieve an scenario of low sampling exhaustiveness (SIMULAU$_{sub}$). To do so, we repeated Steps 1 and 2, but in this case the number of sampling records and species was randomly assigned according to the information gathered in SIMULAU. To ensure low levels of sampling exhaustiveness in SIMULAU$_{sub}$, we limited the maximum number of sampling record per sampling unit, and the maximum number of detected species per sampling record to 25 and 20, respectively.

The next step was to produce the smoothed SAC for each sampling unit in the ORDESA and SIMULAU$_{sub}$ database using specaccum function (1000 permutations) in the VEGAN package [72] in R [73]. Then, the three completeness measures were estimated for each SAC. The NPE was calculated as the proportion of species richness out of the total predicted by the Jacknife estimator using the poolaccum function (1000 permutations) in the VEGAN package. The slope for Mao Tau estimator (STE) was computed from the SACs as

$$\frac{y_i - y_{i-3}}{x_i - x_{i-3}} \qquad [3]$$

being i the last position of both species richness (*y*) and number of records (*x*). We finally used FIDEGAM method by fitting GAM models with Poisson response to the each accumulation curve (obtained at random) using penalized splines [64,74]. Optimum effective degrees of freedom (equivalents to degrees of smoothness) were automatically selected using the unbiased risk estimator criterion (UBRE) [75]. The first derivative of the resultant curve and its 95% confidence intervals were computed for each sampling unit (Fig. 1), being the first derivative at the maximum number of sampling records the FIDEGAM measure of sampling completeness (Fig. 1).

The final step consisted in examining the discrimination performance of the three completeness measures calculated, to correctly classify well and poorly sampled sampling units. In most real situations, the true species richness is unknown, therefore, a surrogate of the sampling effort is needed to categorize sampling units. Here, we used the number of sampling records as a surrogate in the ORDESA and SIMULAU$_{sub}$ [30,47,76]. We set the cut-point value according to the preliminary analysis [77] at the 50th percentile (i.e. the median) of the number of records per sampling unit [78] (see further details on Appendix S1). Thereby, units with higher number of sampling records than the median were classified as well sampled and those below as poorly sampled. To evaluate the role that the surrogate could play on the results, we categorize the sampling units of SIMULAU according to an ideal scenario where the degree of sampling completeness is known. Given that all species were detected in the sampling units of SIMULAU, we calculated the true sampling completeness for each unit as the

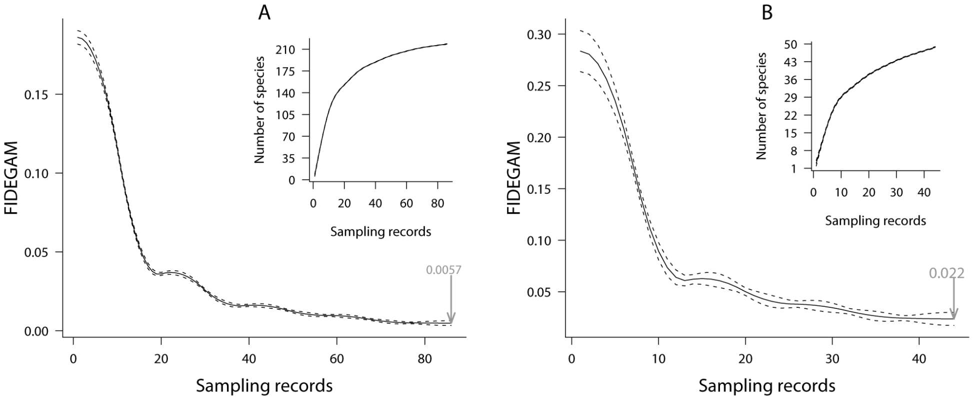

Fig. 1 The sampling completeness measured from two smoothed species accumulation curves (1000 randomization each) using the FIDEGAM method in the Ordesa-Monte Perdido National Park. FIDEGAM values (in grey) recorded at the maximum number of sampling records indicates higher sampling completeness in the sampling unit A than in B. Dashed lines correspond to confidence intervals of FIDEGAM values.

$$\frac{SR_{sub}-SR_{true}}{SR_{true}}\times 100 \qquad [4]$$

where SR_{sub} is the species richness in SIMULAU$_{sub}$ and SR_{true} the true richness obtained from the SIMULAU database [79–80]. The inventory of sampling unit exceeding the 70% of completeness are usually considered as nearly completed [81–82], therefore, we categorized sampling units according to such value.

Once the binary response variable was created for all scenarios, we proceed to estimate the capability of each completeness measure (NPE, STE and FIDEGAM) for discriminating between classes using ROC-GLM regression for binary responses with logit link. The probability of belonging to each state was calculated as a function of the value of completeness measure

$$P[Y=well\ sampled \mid completeness\ measure] \qquad [5]$$

whereas AUC values were computed using the *roccurve* function in the *pcvsuite* package [83] and their 95% confidence intervals estimated by bootstrap regression techniques [84].

Results

According to our logistic model (equation 5), the predicted probabilities of a method for correct discrimination should reach 1 and 0 for well and poorly sampled units, respectively. Therefore, when representing this ideal discrimination in a kernel density plot, maximum densities of predicted probabilities of well and poorly sampled areas should clump at 0 and 1 values of the x-axis. On the contrary, higher densities of predicted probability values would lie between 0 and 1 if the method fails in discriminating. Figure 2 shows strong differences in the predicted probabilities for well and poorly sampled units among methods, evidencing the higher discrimination capacity of FIDEGAM. This pattern was consistent in the three examined scenarios despite that different surrogates for categorizing the sampling units were used if true richness was known or unknown (Fig. 2). In all cases, FIDEGAM showed an excellent performance for discrimination according to the observed AUC values (Table 1). On the contrary, NPE failed to correctly discriminate sampling units in the ORDESA database, whereas, STE only classified correctly poorly sampled units (Fig. 2A), being good the discrimination quality (Table 1).

At lower levels of sampling exhaustiveness (i.e., using data from SIMULAU$_{sub}$), the probability of NPE and STE for correct discrimination decreased (Fig. 2B), reaching undesirable AUC values (Table 1). The same results were obtained in the simulated scenario when sampling units were categorized according to the true sampling completeness (Fig. 2C, Table 1).

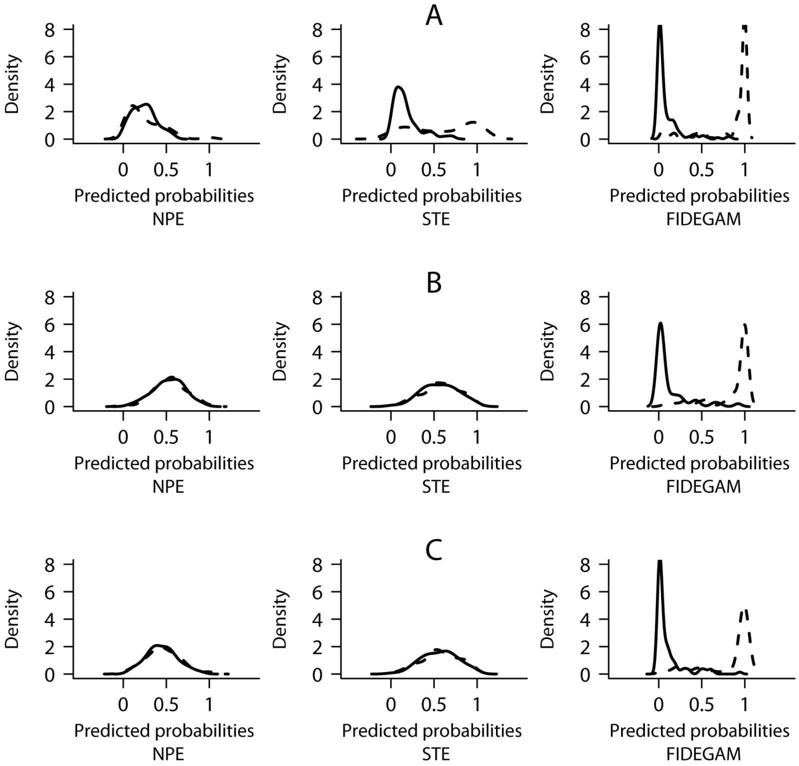

Fig. 2 Kernel density plots of predicted probabilities of discrimination between well (dashed line) and poorly sampled units (continuous line) for NPE, STE and FIDEGAM methods. In the scenarios of high (A) and low (B) levels of sampling exhaustiveness, the sampling units were categorized as well and poorly sampled according to the number of records (see Appendix S1), whereas, when the true richness was known (C), the true sampling completeness (see equation 4 on text) was used as a categorization criterion. Probabilities were calculated according to ROC-GLM regression models.

To better interpret the results obtained in the discrimination analysis, we plotted the relationship between the true percentage of species detected (defined as the ratio between the richness observed in SIMULAU$_{sub}$ and SIMULAU) and the completeness values of NPE, STE and FIDEGAM in SIMULAU$_{sub}$ (Fig. 3). By fitting a Poisson-GLM to this relationship, we found that values of both NPE and STE did not correlate with that (R^2 = 0.28 and R^2 = 0.14, respectively), whereas FIDEGAM values did (R^2 = 84.19) (Fig. 3).

Table 1. AUC values and 95% bootstrap confidence intervals (in brackets) obtained in the discriminatory analysis of methods for sampling completeness quantification. The analyses were repeated in two scenarios of high and low levels of sampling exhaustiveness (from the ORDESA and SIMULAU$_{sub}$ databases, respectively), and in an ideal situation where the true species richness was known (SR$_{true}$). Grading guidelines for AUC values indicate fail (0.50-0.60), poor (0.60-0.70), fair (0.70-0.80), good (0.80-0.90) and excellent (0.90-1.00) discrimination.

Method	Level of sampling exhaustiveness		SR$_{true}$
	high	low	
NPE	0.64 (0.59, 0.71)	0.49 (0,41, 0.59)	0.52 (0.40, 0.59)
STE	0.81 (0.75, 0.86)	0.49 (0.40, 0.57)	0.48 (0.40, 0.57)
FIDEGAM	0.92 (0.88, 0.95)	0.98 (0.97, 1.00)	0.97 (0.95, 0.98)

NPE is the proportion of species richness out of the total predicted by a non-parametric estimator (Jacknife)[57]; STE is Mao Tau estimator [41,61]; FIDEGAM is the first derivate of a GAM with Poisson response fitted to species accumulation curves.

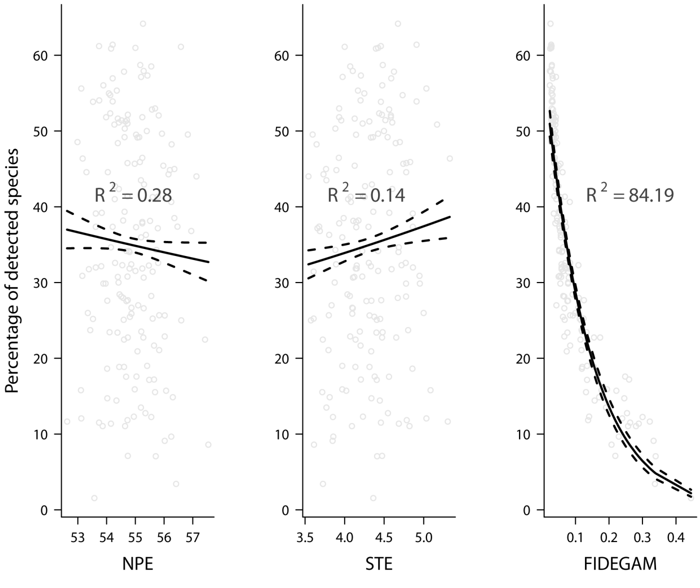

Figure 3. Relationship between the sampling completeness calculated using the FIDEGAM method and the percentage of detected species in a simulated scenario, where the true richness is known. Dashed lines state the 95% confidence intervals.

Handling with sampling effort bias in biodiversity analyses: a case study

To illustrate how the measure of sampling completeness can be used to enhance the reliability of biodiversity analysis, we analyzed the patterns of distinctiveness along the Ordesa-Monte Perdido National Park (excluding Bujaruelo valley) using the ORDESA database. The distinctiveness indicates to what extent one area is distinct from other areas in terms of taxonomic, functional or/and genetic diversity [85–86]. The identification of most distinctive areas constitutes a basis for establishing priority conservation areas at different scales. We calculated here an easy-to-use index based on taxonomic distinctiveness according to the formula detailed in Jennings et al. [81], but it is also possible to use other metrics of distinctiveness and beta diversity.

Figure 4A represents distinctiveness in the National Park from the raw information in ORDESA, and suggests that most areas of the Park would be highly distinctive. To what extent is this pattern reliable? We quantified the sampling completeness of each sampling unit with FIDEGAM and found that most of the poorly sampled ones were those of highest distinctiveness values (Figs. 4 and 5). Hence, the distinctiveness pattern obtained from raw information is highly uncertain. To minimize such uncertainty, we excluded poorly sampled areas from analysis according to an objective criterion based on the maximization of the discrimination capability using the Youden index (*J*) [87]. The *J* value in the ROC curve is

$$J = P_{S2(c0)} + P_{S1(c0)} - 1 \qquad [6]$$

being *P* the probability of correctly classifying, *S*1 and *S*2 well and poorly sampled units respectively, and c*0* the optimum cut-point, and the corresponding value of FIDEGAM the optimum threshold to separate well sampled units from poorly. In the ORDESA database the *J* index was 0.85 (confidence interval: 0.75–0.93) and the corresponding threshold 0.029. After excluding sampling units with FIDEGAM values above such threshold (i.e., poorly sampled areas), we recalculated the distinctiveness values and found that the resulting pattern of distinctiveness totally differed from the previous one (Fig. 4B). This result evidences how the inclusion of uncertain information in biodiversity analysis (poorly sampled units in this case) distorts the overall picture of the spatial pattern of distinctiveness.

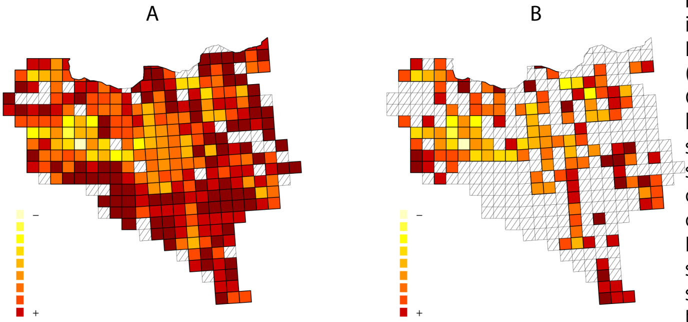

Fig. 4 Taxonomic distinctiveness in the Ordesa-MontePerdido National Park calculated using all (A) and selected (B) sampling units. Grid cells correspond to UTMs of 1 km2. Striped cells indicates sampling units with less than three sampling records,where the quantification of sampling completeness is impossible using FIDEGAM method, in A, and poorly sampled units in B. Well and poorly sampled units were defined using their completeness value of FIDEGAM and according to a threshold value that maximize the discrimination capability between sampling units (see text for details).

Discussion

Many biodiversity databases have been constructed from heterogeneous sources of information because of the large spatio-temporal ranges they usually cover. The information that they contain, therefore, does not always represent the reality due to large differences in sampling effort across time and space. This fact constitutes one of the main limiting factors for the reliability of the results provided by analyses based on them. Different methods have been proposed to account for spatial sampling effort bias, but not all of them perform equally. Here, we have demonstrated that SAC based methods differ in terms of statistical robustness, but also in their capability to discriminate between well and poorly sampled units.

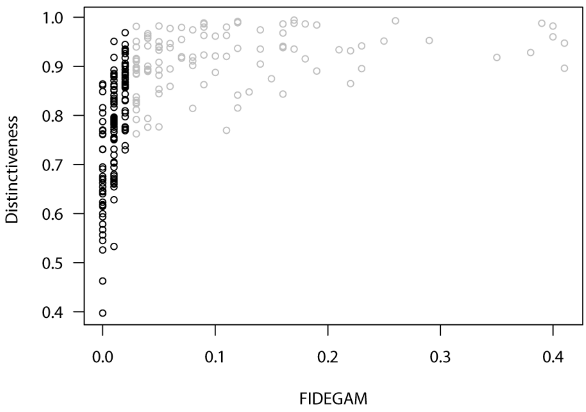

Fig. 5 FIDEGAM values and taxonomic distinctiveness in the Ordesa-Monte Perdido National Park. Low values of FIDEGAM correspond to high sampling completeness. Black and grey dots indicate well and poorly sampled units ($1\ km^2$) respectively, according to an optimum threshold value of FIDEGAM that maximizes discrimination capability.

Statistical assumptions cannot be disregarded even in the most-up-date statistical methods [88]. Some methods for quantifying sampling completeness do not fulfill such assumptions (see [89]), whereas others (e.g. the classic asymptotic function [39]) present severe limitations. Even when the statistical assumptions are not violated, not all methods are equally reliable because there are strong differences in their discrimination capability, as we have shown here. The novel method we have proposed in this study, FIDEGAM, outperforms others regardless of the sampling exhaustiveness, and both when true richness was known and unknown, evidencing its robustness. The most striking feature of the method is its excellent performance at low levels of exhaustiveness, because most regions and living groups worldwide are not exhaustively sampled [15]. In turn, other methods based on NPE and STE estimators, often misclassified well and poorly ed units, which may constitute another source of bias to the original problem of sampling effort bias. As a result of this low discrimination capacity, NPE and STE also failed to represent the true proportion of detected species in a simulated scenario.

The sampling exhaustiveness of the database is an important constrain for the use of both non-parametric estimators and asymptotic methods [90]. Beyond discrimination capability, even the computation of sampling completeness values is limited by using asymptotic methods (e.g. Michaelis-Menten) in scarcely prospected areas. These areas ought to be ruled from the sampling effort assessment, and as a result, a large amount of information is susceptible to be lost. This situation is less dramatic using the FIDEGAM method, because it requires a lower number of sampling records (i.e. three) than the asymptotic ones.

The assessment of sampling completeness can be easily incorporated into biodiversity analyses to reduce the uncertainty of results. A promising procedure is to incorporate sampling completeness values as a covariate (or offset) in the analysis of biodiversity patterns (Pata et al., unpublished data), although the most frequent alternative is to only consider the areas that are well surveyed (i.e. those with a sampling effort above some threshold) [15,91,92,93]. If sampling effort is similarly distributed across space (regardless of the level of sampling exhaustiveness), the selection should be done according to comparable values of sampling effort rather than to high values [35,94]. The full interest of this procedure relies on how to define a threshold value in order to classify the suitability of different areas [30,95]. An arbitrary value may be justified when the knowledge of the studied system is robust, otherwise the subjectiveness should be avoided. In the example presented, the threshold value was defined according to the maximization of discrimination capability, thereby, minimizing in this way the potential bias intrinsic to method. The straightforward advantage of proceeding objectively is that the method can be equally used in other databases, regardless of the nature and spatial resolution of the information.

Correctly identifying well and poorly sampled areas is also of paramount importance for the interpretation of biodiversity distribution [27,92], and FIDEGAM has been proved to provide an accurate layer of uncertainty over results obtained from raw data. This would allow us to know at which locations results of biodiversity analysis is reliable, and where the prospective biological exploration is necessary if we want to extend results of standard analysis of biodiversity [53,92].

To summarize, our results have highlighted that an adequate selection of the assessment method is as important as the decision itself of taking into account the sampling effort for enhancing the reliability of database analyses. FIDEGAM provides the best discrimination capability and minor dependence on exhaustiveness. Therefore, we recommend this method to overcome sampling effort bias when analyzing the information gathered in biodiversity databases. By no means, a method for sampling completeness quantification will replace the advantages provided by further biological prospections. However, given the urgencies of biodiversity conservation and the limitations for intensive data gathering, we consider the quantification of sampling completeness the best alternative to enhance the reliability of biodiversity analyses based on non-exhaustive database.

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Author Contributions

Conceived and designed the experiments: IP MPP. Analyzed the data: IP MPP. Contributed reagents/materials/analysis tools: DG. Wrote the paper: IP MPP DG MBG.

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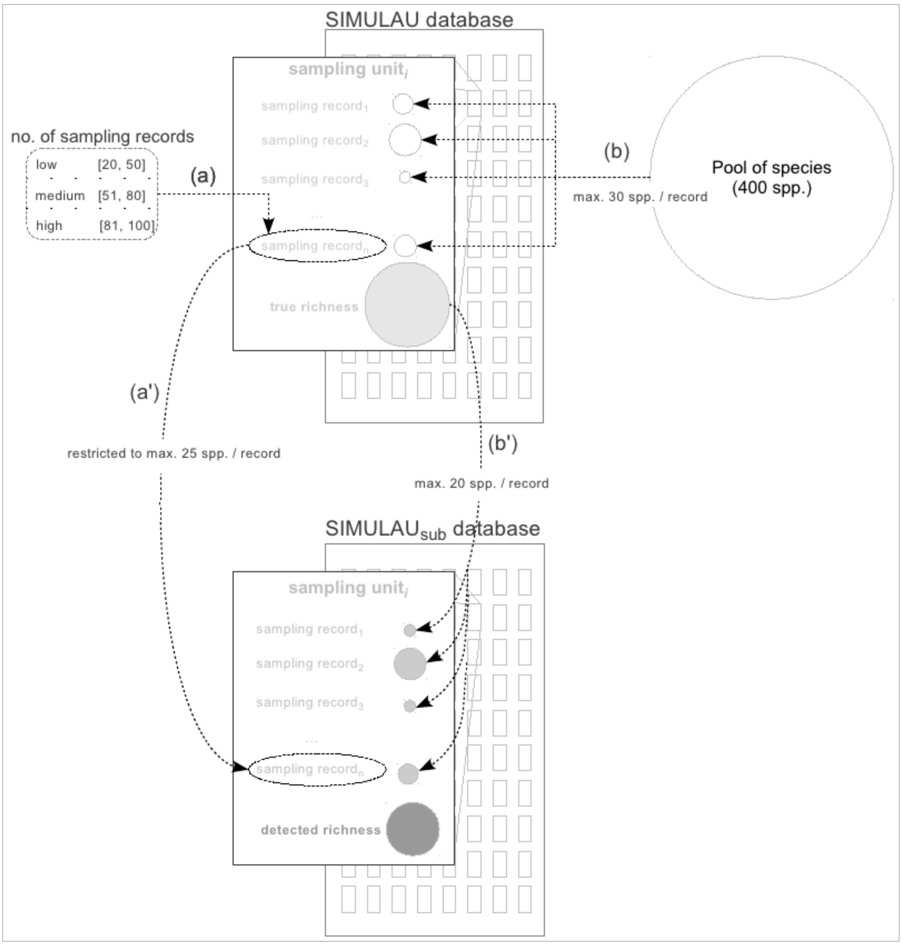
Supporting Information

Fig. S1 Diagram of the simulation procedure to create the ideal scenario, where the true richness is known (SIMULAU), and the scenario of low levels of sampling exhaustiveness ($SIMULAU_{sub}$). Dashed lines state a random assignation of the number of sampling records to a sampling unit (a; a'), the number of species detected in a given sampling records (b; b'). In the SIMULAU database the total number of species observed at each of the sampling units was defined as the true richness. We generated the scenario of low sampling exhaustiveness ($SIMULAU_{sub}$) by subsampling at random the SIMULAU database. Note that we restricted the number of record per sampling unit and the maximum number of species observed per record, to ensure low levels of sampling completeness.



Appendix S1. Preliminary studies to establish an adequate threshold based on the number of sampling records for the discrimination analysis.

The establishment of a threshold to separate two states (e.g. well and poorly sampled) was done using graphical diagnostic and model-based plots, and, in the case of the SIMULAU database, using the maximum chi-square method [1- 3].

In the simulated scenario where the true richness was known (SIMULAU), we could easily transform the richness into a binary variable according to a common accuracy measure [4-6] as

$$\frac{SR_{sub} - SR_{true}}{SR_{true}}$$

where SR_{sub} is the species richness subsampled from the simulated scenario ($\mathrm{SIMULAU}_{\mathrm{sub}}$) and SR_{true} the richness observed in each of the sampling unit in SIMULAU. The inventory is considered nearly complete above 70% of such measure [7-8 but see 9], therefore, we defined well sampled units as those exceeding such values. Once the binary variable was obtained, we calculated the proportion of poorly sampled units in SIMULAU to the mean number of records by quantile (Fig. A1).

The Fig. A1 confirms the existence of a threshold: sampling units with more sampling records than the median were well sampled, whereas, those with less number were poorly sampled. The difference between well and poorly sampled units is ca. 50% as separated by median (Fig. A1). Additionally, we modeled the probability of units for being poorly sampled as a function of number of sampling records using a binomial GLM, and found consistent results (Fig. A1). Please, note that the same conclusion (but inverse results) would be achieved in these analyses using the well sampling units.

Finally, we tested for the best threshold value, using the maximum chi-square method. In this method the quantiles of the number of sampling records are evaluated as a cut-points (i.e.threshold). A two-sampled test with the corresponding chi-square statistic and p-value was computed for each quantile, and that with a maximum chi-square statistic (or equivalently the minimum p-value) was selected as the cut-point which best separates well and poorly sampled units. We corrected the p-values to avoid type I errors due to multiple testing [1]. We found that the median of the number of sampling records yields the highest statistic and the lowest p-value (Table A1 and Fig. A2).

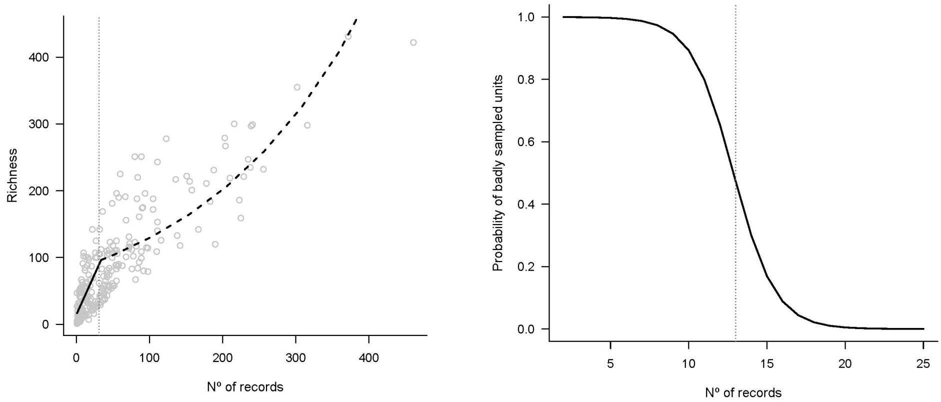


Fig. A1 Percentage of poorly sampled units to the mean number of sampling records by quantile (left), and binomial GLM-based plot of the probabilities of poorly sampling units as a function of the number of sampling records (right) in a simulated scenario. Dotted line states the median of the number of sampling records.

Table A1 Results of the maximum chi-square test to find the best threshold to separate sampling units. The test was repeated for each quantile, and the maximum and minimum chi-square statistic and p-value (in bold), respectively, indicates the number of sampling records which best separate well and poorly sampling units .

Quantile	Cut-point	Chi-square	p-value
5	2,95	7,49	6,22E-003
10	3,90	17,84	2,40E-005
15	5,00	33,64	6,63E-009
20	6,00	44,09	3,14E-011
25	7,75	57,36	3,62E-014
30	8,00	76,35	2,38E-018
35	10,00	89,41	3,21E-021
40	11,00	103,79	2,25E-024
45	11,55	103,79	2,25E-024
50	**13,00**	**129,14**	**6,33E-030**
55	14,00	120,57	4,76E-028
60	15,00	108,67	1,91E-025
65	17,00	91,55	1,09E-021
70	18,30	74,31	6,67E-018
75	19,00	53,90	2,11E-013
80	20,00	38,00	7,09E-010
85	21,00	28,10	1,15E-007
90	23,00	11,94	5,50E-004
95	24,00	6,41	1,13E-002

Given that the true richness is unknown in the real scenario built from the ORDESA database, we cannot applied the previous procedure. As an alternative, we modeled the relationship between the number of sampling records and observed richness using two approaches:

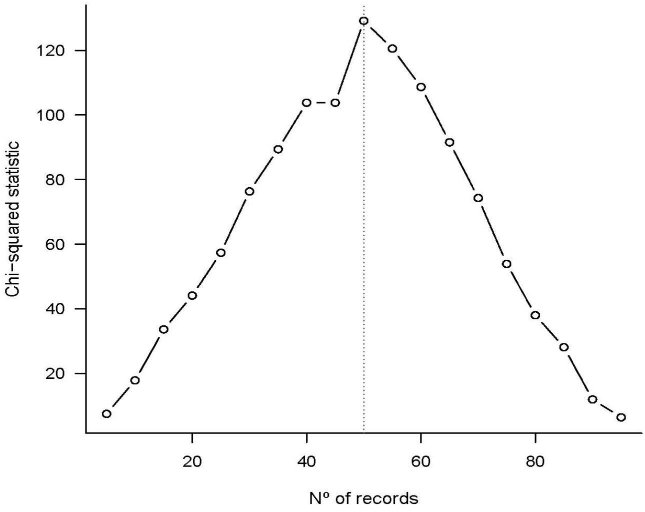


Fig. A2 The relationship between the number of sampling records and statistics obtained using the maximum chi-square test. Dotted line states the median of the number of sampling records.

(a) Piecewise regression, which is based on regression models that joins two or more lines at some unknown points (breakpoints) [10-11]. The resulting breakpoints can be considered as the thresholds for the variable in study (sampling records in our case).

(b) Quantile regression, which estimates the relationships between richness (response variable) and number of sampling records (covariate) for all quantiles of the distribution [12-13].

The unique breakpoint resulting from the piecewise regression was 32.6 (± 0.618 standard error), which corresponds to the 55th quantile. Figure A3 shows the fitted lines obtained with the piecewise regression model. In line with this result, we found different estimated slopes (i.e. different relation between species richness and number of sampling records) above and below the median using the quantile regression (Fig. A4).

Altogether, the results obtained in the simulated and the ORDESA databases support that the median of the number of sampling records was the most adequate threshold value for the discrimination analysis.

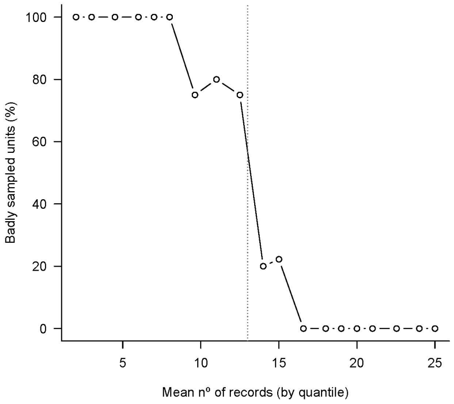


Fig. A3 Piecewise regression of the sampling records and the species richness. Lines represent the fitted models above (dashed line) and below (black line) the obtained breakpoint, which matchs with the median of the number of sampling records (dotted grey line).

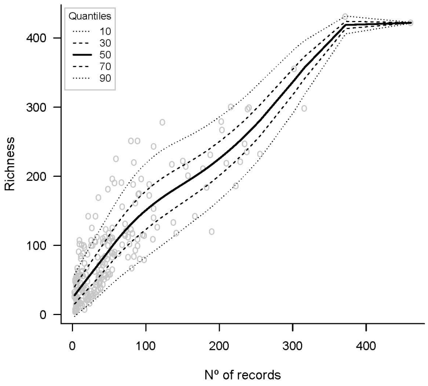


Fig. A4 Scatter plot of number of sampling records and species richness. Black lines represent the estimated slopes obtained in a quantile regression.

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Chapter 2

On the limits of hotspots for the representation of multifaceted diversity at small-scale

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Abstract

Recent studies have challenged the use of conventional procedures based on species richness (SR) or endemism richness (ER) for area prioritization at large scale, because they underrepresent other levels of diversity. However, we do not know if this conflict persists at small spatial scales at which most management decisions are taken. Here, we assessed the spatial congruence of hotspots of plant SR, ER, phylogenetic diversity (PD) and functional diversity (FD) across a mountain National Park in the Central Pyrenees, their performance for the representation of multifaceted diversity, and the sensitivity of results to spatial resolution and sampling completeness of data. Analyses were carried out with on the basis of two information sources: plant database (grid-cell occurrences) *vs* standardized community surveys. The four diversity components were spatially congruent across grid cells, and as a result, multiple diversity components were effectively represented in hotspots. However, we demonstrated that hotspots identification was biased by sampling differences among grid cells, their congruence was caused by the influence of SR on metrics of PD and FD, and representation of diversity in hotspots was inflated due to the relative coarse spatial resolution of data. On the basis of community surveys data, and with metrics of PD and FD not affected by SR, diversity hotspots were no longer congruent and presented large gaps in the representation of multifaceted diversity. Taken together, our results demonstrate that accounting for sampling bias and the spatial resolution of data may be crucial for implementing realistic and ecologically meaningful priority areas for conservation. But they also confirmed the limitations of conventional hotspots as priority

areas of multifaceted diversity at small scale. When different diversity components are not spatially congruent, the selection of different types of hotspots in a complementary fashion could be a more efficient strategy for the representation of multifaceted diversity.

Introduction

'Agony', 'crisis', 'urgency', and 'emergency' are terms repeatedly used in the conservation literature to depict the status of biodiversity. However, resources allocated to conservation are usually limited, and therefore conservation science is largely about establishing priorities (species, populations, habitats or areas) in a way that conservation returns are maximized (Bottrill et al. 2008). Priorities are often centered around areas that best complement their biological composition (complementarity areas; Kirkpatrick 1983; Vane-Wright et al. 1991), whereas in many other cases the priority is given to hotspots, defined as exceptionally diverse areas in terms of number of species (sometimes endemisms), and in some cases with high rates of habitat loss (Myers 1988; Prendergast et al. 1993; Reid 1998; Myers et al. 2000). A feature common to both procedures is that priorities are identified at species level. However, differences in evolutionary history and functional characteristics of species are relevant from a conservation perspective (Vane-Wright et al. 1991; Faith 1992; Petchey & Gaston 2002).

Phylogenetic diversity (PD) represents the amount of evolutionary history of species (Faith 1992). Areas with high PD are essential for preserving the evolutionary potential of diversity (Mace et al. 2003; Forest et al. 2007), and the future uses and benefits that it would deliver to humans (Vane-Wright et al. 1991; Faith 1992). The relevance of functional diversity (FD) in conservation science revolves around the key role that some traits play in the maintenance of diversity (Petchey & Gaston 2002) and ecosystem functioning (Díaz & Cabido 2001; Cadotte et al. 2011). While PD describes functional complementarity under trait conservatism (Flynn et al. 2011), it fails to replace FD in many others (Prinzing et al. 2008; Pavoine et al. 2013). Therefore, measures of both phylogenetic and functional components of diversity may be used in a complementary way to achieve a more comprehensive view of biodiversity (Cadotte, Albert & Walker 2013).

Whether species-based priority areas present large gaps in the representation of other diversity components is related to the spatial congruence between components (e.g. Orme et al. 2005). From global-to-regional scale, some studies have supported the use of species richness (SR) as a surrogate for endemism richness (ER), PD and FD (Kerr 1997; Rodrigues & Gaston 2002; Sechrest et al. 2002; López-Osorio & Miranda-Esquivel 2010), whereas many others have not (e.g. Orme et al. 2005; Ceballos & Ehrlich 2006; Forest et al. 2007; Devictor et al. 2010; Mouillot et al. 2011). Accordingly, conservationists have been encouraged to embrace more integrative procedures of areas prioritization than those based on SR (Fleishmann et al. 2006; Redding & Mooers 2006; Devictor et al. 2010; Winter et al. 2013). Yet, it is uncertain whether this conflict persists at more fine-grained scales, at which most management decisions (including monitoring) are undertaken (Nantel et al. 1998; Schwartz 1999 and references herein), and micro-reserves are delineated (Gjerde et al. 2004; Laguna et al. 2004).

The spatial mismatch among diversity components is expected to reflect ecological and historical causes (Orme et al. 2005; Davies & Buckley 2011; Fritz & Rahbek 2012). However, the correlation between the metrics used to measure each diversity component may confound these causes (Pavoine et al. 2013). Likewise, the spatial resolution (i.e. the grain size) at which distributional data are captured may be different from the one at which ecological factors operate (Rahbek 2005), which may lead to the misidentification of diversity hotspots (Hurlbert & Jetz 2007). To complicate matters further, the sampling effort of the baseline information may distort diversity maps, and hence, the identification of priority areas (Freitag & Jaarsveld 1998; Guilhaumon et al. 2008). While the effects of the spatial resolution of data and the sampling effort on SR maps are well documented (Hortal et al. 2007), it is still unclear the extent to what other diversity components, and hence the spatial congruence among multiple diversity components, might be affected.

In this study we explore the spatial pattern of taxonomic, phylogenetic and functional diversity of vascular plants in the Ordesa-Monte Perdido National Park (OMPNP; Central Pyrenees). The OMPNP harbors 1379 plant taxa, nearly 20% of the Iberian Peninsula in only 0.07% of the territory, and provides an excellent representation of most abundant habitats found in the calcareous Pyrenees. Maps of SR, ER, PD and FD were inferred from database records (1x1 km grid-based occurrences) and community survey data to assess (i) the spatial congruence between hotspots of these diversity components; (ii) the representation of multiple diversity components (i.e. multifaceted diversity) at each type of hotspot; and (iii) sensitivity of results to the spatial resolution of data and sampling completeness.

Material and Methods

Study site

The Ordesa-Monte Perdido National Park (42°N, 0°E) extends over an area of 34000 ha (including the buffer area) in the Central Pyrenees. It presents a complex geomorphology, with an elevational range from 700 m to 3354 m (the highest calcareous summit in Europe). The main bedrock type is limestone, but flysch and sandstone outcrops are relatively abundant. Main habitats are, in order of decreasing abundance (see also Supporting Information): grasslands, most of which have traditionally used for summer pasturing; rocky areas, including alpine rocky grasslands, screes and cliffs; coniferous forests dominated by *Abies alba*, *Pinus sylvestris* or *P. uncinata;* deciduous forests, including those dominated by *Fagus sylvatica,* and mixed ones; mediterranean forests, mainly dominated by *Quercus ilex*; and shrublands. Other habitats such as wetlands and anthropogenic areas (vegetation occurring along pathways) covered less than 1% of the OMPNP.

Distributional data

All analyses were conducted on the basis of two information sources: plant database and community surveys. The former consists of 44000 records corresponding to 1379 taxa distributed across 323 grid-cells of 1x1 km. This information was obtained from the 'Floragon' database (http://proyectos.ipe.csic.es/floragon/index.php), which includes georeferenced plant samples and visual records from herbarium collections, as well as distributional data digitalized from published and unpublished studies. Despite that the OMPNP is the most exhaustively prospected area in the Iberian Peninsula (Font et al. 2010), distributional information of plants is still incomplete and there are large differences in the sampling completeness between grid cells (Pardo et al. 2013).

Community survey data were obtained from 1218 selected phytosociological surveys downloaded from the SIVIM website (http://www.sivim.info/sivi/). This survey method has recurrently been used for the standardized description of vegetation communities throughout the 20th century in Europe. For analyses, each community survey was classified into one of the aforementioned habitats. While compiled surveys did not cover every single vegetation patch in the National Park, they did represent quantitatively well most of the habitats (Supporting Information), and provide a virtually complete sampling of communities.

Multiple components of diversity

Maps of SR and ER (endemisms of the Pyrenees) were built at two spatial resolution from species' occurrences in grid cells and community surveys data. For PD estimation, we first generated a molecular phylogeny of the whole flora resolved to the genus level, following Roquet et al. (2013). DNA sequences for 10 regions were downloaded from Genbank: three conserved regions (*matK*, *ndhF* and *rbcL*), plus 7 regions less conserved that were clustered to the family or order level for the alignment (*atpB*, *ITS*, *psbA-trnH*, *rpl16*, *rps16*, *rps4-trnS* intergenic spacer, *trnL-F*). Alignment for each region was performed with 3 methods: Kaling (Lassmann & Sonnhammer 2005), MAFFT (Katoh et al. 2005) and MUSCLE (Edgar 2004). The best alignment was determined with MUMSA (Lassmann & Sonnhammer 2006), checked visually with Seaview (Gouy et al. 2010) and depurated later on with TRIMAL software (Capella-Gutierrez et al. 2009). All regions were concatenated with FASconCAT (Kück & Meusemann, 2010). For phylogenetic inference, we conducted a maximum likelihood (ML) by using RAxML (Stamatakis et al., 2008) with the model GTR+Gamma, applying a supertree constraint at the family-level on the basis of Davies et al. (2004) and Moore et al. (2010), and setting one partition for each DNA region. Node support was estimated using bootstrap values. Once the topology of the best ML tree was obtained, we dated the tree with penalized-likelihood as implemented in r8s (Sanderson 2003) and used a wide range of fossil data to calibrate the tree (25 fossils extracted from Smith et al. 2010 and Bell et al. 2010). Finally, we transformed the polytomies at the genus level into dichotomies of branches of length zero at random with the *multi2di* function in picante (Kembel et al. 2010).

On the basis of this phylogeny and SR maps, we calculated Faith's PD (1992) as the sum of the branch lengths of the co-occurring taxa for each geographic unit. Among existing PD metrics, we selected the one by Faith (1992) because it has been successfully used in similar studies (e.g. Sechrest et al. 2002; Forest et al. 2007; Fritz & Rahbek 2012), it provides a more robust basis for conservation than other measures (Pio et al. 2011), and it is probably the most intuitive one. All phylogenetic analyses were done in R 2.14.1 (R Development Core Team 2011) by using PICANTE (Kembel et al. 2010), APE (Paradis et al. 2004) and GEIGER (Harmon et al. 2008) R-packages.

Functional diversity was estimated on the basis of eight traits related to life-history (Raunkiaer's life form, life span), plant propagation, dispersal syndrome, pollination system, sexual expression, inflorescence architecture and floral color (Table 1). We additionally included mean population size of adults (a few individuals; <25 individuals; <100 individuals; <1000 individuals; and >1000 individuals). Trait information was compiled from the literature and on line databases (Table 1), except mean population size which was determined according to our botanical expertise. Species with no trait information (< 10%) were excluded for estimation of FD. In preliminary analyses we tested the phylogenetic correlation of traits along the phylogeny with '*phylo.signal.disc*' (999 randomizations), a randomization procedure developed in R by E. Rezende, and found that all ecological and biological traits as well as mean population size were significantly correlated. Following Petchey and Gaston (2002), we calculated functional pairwise distance between species based on Gower's metric (1971), and performed a hierarchical clustering analysis to produce a functional dendrogram by using *daisy* (Maechler et al. 2013) and *hclust* R-functions, respectively. Next, we used *treedive* function in the VEGAN R-package (Oksanen et al. 2013) to calculate FD as the sum of the total branch lengths connecting recorded taxa in geographic unit along the dendrogram (Petchey & Gaston 2002).

Table 1 Description of biological and ecological traits used for the calculation of functional diversity.

Trait	Description	Categories	Source
Raunkier's life-form	Position of renewal buds during unfavorable seasons for growing	Terophythes; Geophytes; Hemicryptophytes; Chamaephytes; Phanerophytes	1
Life-span		Annual; short lived (< 5 yr); long lived (≥ 5 yr)	1
Plant propagation	Main process of creating new individuals	Sexual; Vegetative; Mixed	1, 2
Dispersal syndrom	Seed dispersal agent	Autochory; Endochory; Exochory; Anemochory assumed due to small seed size (less than 1mm, and without special morphological characters); None	1, 3, 4
Pollination system	Based on flower shape, as a proxy of insect accessibility	Insect and wind pollination; insect pollination (flowers can only be pollinated by specialized insects); No insect pollination	1, 5
Sexual expression	Spatial pattern of male and female organs	Complex; Dioecious; Hermaphroditic; Monoecious	1, 5

(*continue next page*)

Inflorescence architecture	Abundance and arrangement of flower in the inflorescence	Dense; Specialized; Incospicuous; Lax; Solitary	1, 5
Floral color		Colorless; White; Yellow; Blue; Pink; Red; Multiple	1, 5

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These metrics of PD and FD provide an integrative view of diversity, and hence they are necessarily affected by the number of species (Schweiger et al., 2008). To tease apart the taxonomic, phylogenetic and functional components of diversity, we modeled PD and FD as a function of SR, and worked on residuals (onwards PD_R and FD_R; Davies et al. 2008; Devictor et al. 2010; Fritz & Rahbek 2012). The relationship between SR and PD, as well as FD, was quadratic, and diversity values were not apparently autocorrelated according to the distributional pattern of residuals (Supporting Information). Indeed, the fit and normality of residuals did not improve when models with autocorrelation structures (i.e. generalized least squared models) were considered.

Spatial congruence of hotspots

Hotspots were defined as the top 5% geographic units (n=16 grid cells, and n=64 community surveys) in terms of SR, ER, PD, PD_R, FD, and FD_R. The spatial congruence between different types of hotspots was measured as

$$\frac{A \cap B \cap C \cap D}{A+B+C+D} \qquad [1]$$

where A, B, C and D are the set of hotspots of each diversity component. Dividend was substituted by (A∩B∩C), and (A∩B) to calculate the overlap between all possible combinations of three and two types of hotspots, respectively.

Diversity representation in hotspots

We quantified the percentage of each diversity component represented in each type of hotspots, and observed values were compared with those resulting from a random selection of 5% geographic units to assess the effectiveness of hotspots in diversity representation. Differences between observed diversity values and those expected at random were tested with a two-sided permutation test (1000 random permutations) at 0.05 significance level.

The effect of sampling effort

We evaluated the relationship between the sampling effort of grid cells with observed diversity values by means of quantile regression (Koenker & Bassett 1978). This method allows to disentangle the effect of the independent variable (sampling effort) on the dependent variable (diversity metric) across the quantiles of interest (the highest ones in this study; Cade & Noon 2003). Sampling effort of each grid cell was calculated in a previous study (Pardo et al. 2013), from the first derivative of a Generalized Additive Model fitted to species accumulation curves at each grid cell (FIDEGAM). For the sake of interpretation, FIDEGAM values were subtracted from one, so that values close to zero indicate incomplete sampling of species, and those close to one complete sampling. Finally, conditional quantiles of taxonomic, phylogenetic and functional metrics were regressed on sampling completeness values with *qr* function in QUANTREG R-package (Koenker 2013), and bootstrapped standard errors.

Results

The influence of SR on PD and FD values in grid cells was very high (r^2= 0.95, 320 d.f., p-value < 0.001; r^2=0.98, 320 d.f., p-value < 0.001, respectively). Accordingly, different types of grid-cell hotspots were spatially congruent (~80%; Fig. 1). Half of them were also spatially congruent with ER hotspots (Fig. 1). The relationship between grid-cell values of the four diversity metrics and their sampling completeness was significantly positive, being the effect stronger for the highest quantile, as indicated the slopes of quantile regressions (Fig. 2 and see Supporting information the for full results). Hotspots SR, ER, PD and FD were found in exhaustively sampled grid cells (FIDEGAM values above 0.9) that included at least five types of habitats (Fig. 3). The area of these habitats was relatively similar in different types of hotspots (Fig. 3). On average, 78% of total diversity of each diversity components was represented in hotspots of SR, ER, PD and FD, and in all cases it was significantly higher than that expected at random (Table 2). When the effect of species number was removed from phylogenetic and functional metrics, hotspots of PD_R decoupled from those of FD_R , and they were no longer congruent with those of SR and ER (Fig.. 1). The effect of sampling completeness on PD_R observed in grid cells was still strong and significant, whereas it was not significant on FD_R. In this case, hotspots of PD_R did not represent other diversity components more than expected at random (Table 2), whereas FD_R provided a poor representation of ER.

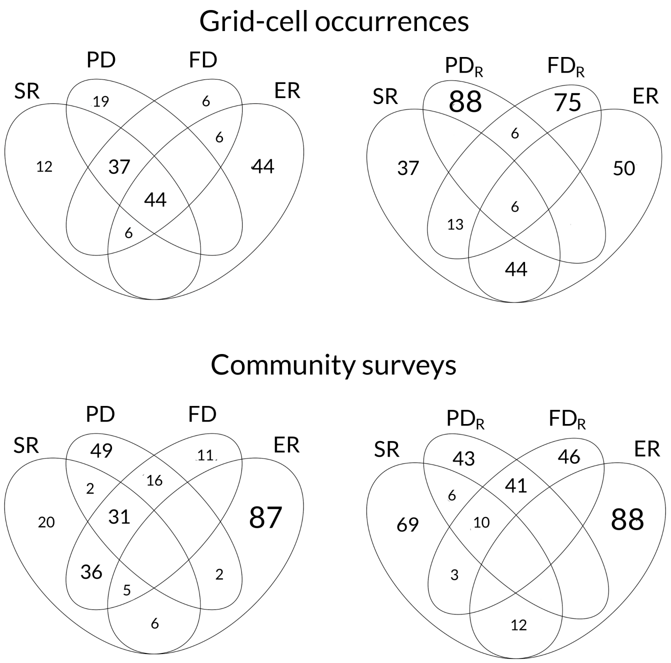

Fig. 1 Percentage of overlap among hotspots of species richness (SR), endemism richness (ER), phylogenetic diversity (PD) and functional diversity (FD) across the Ordesa-Monte Perdido National Park, calculated from two sources of information. Quadratic regressions between species richness and phylogenetic diversity and functional diversity were used to identify phylogenetic and functional hotspots without the influence of species richness (PD_R and FD_R, respectively; right panel).

Results obtained from community survey data were substantially different from those of grid-cells occurrences. The relative influence of SR on PD values was lower than observed in grid cells (r^2=0.71, 1215 d.f., $p < 0.001$), and accordingly, the overlap between their hotspots decreased to 33% (Fig. 1). In contrast, the effect of SR on FD was almost as high as in grid cells (FD: r^2= 0.93, 1215 d.f., p-value < 0.001), and hotspots congruence was also high (72%; Fig. 1). Hotspots of both PD$_R$ and FD$_R$ were relatively congruent, and spatially decoupled from those of SR. The spatial mismatch of hotspots of ER with any other type of hotspots was high (88%; Fig. 1). Hotspot of SR, ER and FD were mostly concentrated in grasslands, whereas both types of phylogenetic and FD$_R$ hotspots were more frequent in forests, and in particular in deciduous ones (Fig. 4).

On average, hotspots inferred from community survey data included 25% less diversity of any component than did grid-cells hotspots. In this case, diversity representation in hotspots of SR and ER was not significantly higher than expected at random (Table 2), whereas hotspots of PD, FD and FD$_R$ did effectively represent, at least, another diversity component (Table 2).

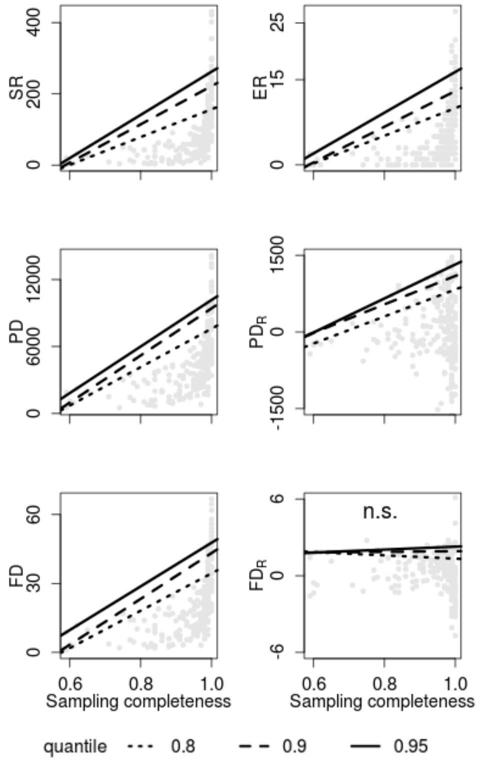

Fig. 2 Quantile regression between sampling completeness and species richness (SR), endemism richness (ER), phylogenetic diversity (PD), functional diversity (FD), and the residuals from the quadratic regression between SR and PD and FD (PD$_R$ and FD$_R$, respectively). The effect of sampling completeness was significant ($p < 0.05$) in all quantiles of diversity components, except in FD$_R$. Gray dots show diversity records from grid-cell occurrences.

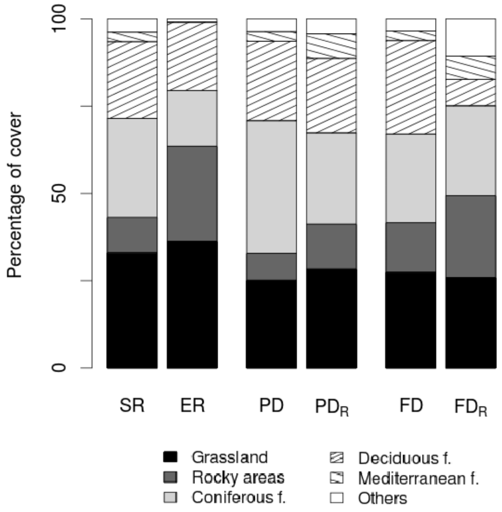

Fig. 3 Percentage of cover of habitats in grid-cell hotspots of species richness (SR), endemism abundance (ER), phylogenetic diversity (PD), functional diversity (FD), and the residuals from the quadratic regression between SR and PD and FD (PD_R and FD_R, respectively) in the Ordesa-Monte Perdido National Park.

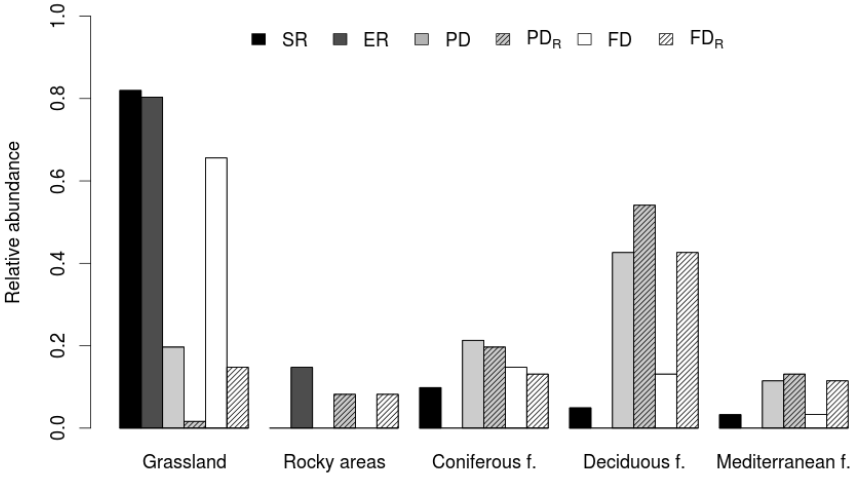

Fig. 4 Relative abundance of community hotspots of species richness (SR), endemism abundance (ER), phylogenetic diversity (PD), functional diversity (FD), and the residuals from the quadratic regression between SR and PD and FD (PD_R and FD_R, respectively) in the Ordesa-Monte Perdido National Park. Habitats are ranked in order of decreasing cover from left to right, and non-abundant habitats are omitted for clarity.

Table 2 Percentage of individual diversity components represented in different types of hotspots of species, and those expected at random.

		Diversity component			
Source of information	Hostpots type	SR	ER	PD	FD
Grid-cell occurrences					
	Random (Q_5–Q_{95})	[30, 56]	[32, 59]	[51, 75]	[50, 73]
	SR	**76**	**74**	**88**	**85**
	ER	**60**	**74**	75	**74**
	PD / PD_R	**75** / 51	**67** / 41	**88 / 77**	**85** / 68
	FD / FD_R	**76 / 65**	**73** / 48	**88 / 80**	**85 / 79**
Community surveys					
	Random (Q_5–Q_{95})	[38, 48]	[4, 60]	[57 , 68]	[53, 63]
	SR	**50**	32	64	64
	ER	29	**62**	43	40
	PD / PD_R	**50** / 36	30 / 12	**72** / 63	**65** / 57
	FD / FD_R	**50** / 47	32 / 32	67 / **70**	**68 / 68**

*Random expectations were calculated by selecting 5% of geographic units at random and by iteratively (n=1000) calculating their diversity values. Random values (5th and 95th quantile of the distribution; Q_5 and Q_{95}) were used as a reference to statistically assess the the effectiveness of each type hotspot for the representation of diversity components. Observed diversity values higher than the 95 quantile of the random distribution are shown in bold.

Abbreviations: Hotspots of species richness (SR), endemism richness (ER), phylogenetic diversity (PD), functional diversity (FD), and the residuals of the quadratic regression between species richness and phylogenetic diversity (PD_R) and functional diversity (FD_R)

Discussion

We found that the assessment of taxonomic, phylogenetic and functional diversity hotspots was strongly affected by the metrics used for phylogenetic and functional diversity, and the spatial resolution of baseline data and associated sampling completeness. Integrative measures of diversity such as Faith's PD (and the analogous FD) resulted to be uninformative for the identification of hotspots in grid cells, because the effect of SR on PD (and FD) values overrode almost completely the contribution of the phylogeny (and functional characterisitics; Pio et al. 2011; Pavoine et al. 2013). This large effect explains the high congruence and surrogacy observed between SR and PD (and FD), which is generally expected when phylogenies are balanced (i.e. similar ramification across branches; Rodrigues & Gaston 2002). However, the strength of the significant effect of SR on PD (but not on FD), and the spatial congruence between their hotspots, decreased at community scale. Thus, suggesting that the spatial resolution of data may determine when PD (and FD to a lesser extent) really provides an integrative view of diversity beyond the topology of the phylogeny.

Once the effect of SR was removed from PD and FD values, hotspots of these diversity components were not congruent anymore regardless of the baseline data used. This result was consistent with the overall mismatch between diversity component reported at large scale (Forest et al. 2007; Devictor et al. 2010; Mouillot et al. 2011). However, does this mismatch really indicate that different ecological mechanism have shaped each diversity component (Orme et al. 2005), or is it just the product of a methodological issue? We quantitatively demonstrated that the high quantiles of diversity components were tightly related to the sampling completeness of grid cells, which means that distributional pattern of diversity components were distorted, and their hotspots biased towards most intensively sampled grid cells. While the effect of sampling sampling effort in the distribution of SR is well-known (e.g Hortal et al. 2007), our results demonstrated for the first time that other diversity components can also be affected. Although the strength of the sampling bias may vary from one diversity database to another, the effect of sampling bias should not be neglected in future assessments of multiple diversity components, and previous ones may need some revision.

There were not sampling effort differences between community surveys, therefore mismatch between diversity hotspots inferred from these data was reliable. Hotspots of SR and ER were mostly found in phylogenetically poor grasslands, which suggest the relative abundance of recent and species-rich lineages in this habitat (Forest et al. 2007; Davies & Buckley 2011). We speculate that such lineages may result from vicariance and allopatric speciation processes associated to glacial-interglacial episodes throughout the Pleistocene in the Alpine arc (Tribsch 2004). In contrast, most of the hotspots of PD_R were found in forests, which were not particularly rich in terms of species but included species from both ancient and modern lineages. As some Tertiary taxa evolved under a more humid climate than today (Barrón et al. 2010), it is plausible that they had found more suitable conditions for persistence in certain forests than in more open habitats (De Frenne et al. 2013). Hotspots of FD_R were also frequent in forest which may be consistent with their less severe environmental filtering they suffer, compared to open grasslands at higher elevation, where the harsh environmental conditions and the long grazing history might have exerted a strong selection in some traits (e.g. long lifespan, vegetative reproduction; de Bello et al. 2013).

The surrogacy between diversity components is often interpreted according to the degree of overlap between measures (Prendergast et al. 2003; Brooks et al. 2006). However, our results from grid-cell occurrences show that the mismatch between different hotspots does not necessarily translate into a poor representation of diversity components (see also Rodrigues & Gaston 2002). This may be explained by coarse spatial resolution of data (1x1 km) relative to the extend of the study area and its topographic complexity. As a result, a large number of phylogenetically and functionally different species (up to 431) from multiple habitats were included in each grid cell, which inflated the representation of diversity in hotspots. However, these hotspots lack of ecological significance, and we would risk leaving out from protection ecologically meaningful areas of plant diversity is priority is given to them.

Consistent with the overall mismatch among diversity components, hotspots of a single component resulted to be a poor strategy for representation of multifaceted diversity. Hotspots of integrative measures of diversity (PD and FD) performed better than those based on species, but they still underrepresented endemic plants. We are aware that the configuration of priority areas is much more complex than it has been addressed in this study, as other aspects than biological ones (e.g. socioeconomic ones or land-use conflicts) should be considered (Cardawine et al. 2009). Still, under the strong regime of protection of a National Park, representation of biodiversity is central for the identification of small reserves (Gjerde et al 2004; Laguna et al. 2004), and for this task, the use of hotspots of a single diversity component seems to be a limited strategy. In absence of further small-scale evidences the generalization of this result is constrained, but we expect similar results in mountain ranges with similar ecological characteristics and evolutionary histories, such as those of the Alpine arc.

Taken together, our findings demonstrated that the correlation between diversity metrics, a coarse spatial resolution of data, and sampling biases associated to sources of information can mask true spatial mismatch among diversity components, its ecological significance, and inflate the performance of diversity hotspots for representation of multifaceted diversity. Therefore, these aspects should not longer be ignored in the assessment of priority areas of multifaceted diversity. When different ecological and historical factors have shaped the distribution of each diversity component, such as in temperate mountains, prioritization strategies should not be focused on a single diversity component (Devictor et al. 2010; Winter et al. 2013). We suggest that priority areas of different diversity components could be selected in a complementary fashion to have a higher impact on conservation of multifaceted diversity at small-scale.

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Supporting Information

Vegetation map of the OMPNP (Appendix S1), the assessment of habitat representativity in community survey data (Appendix S2), the model-based approach used for estimating PD_R and FD_R (Appendix S3), and full results of the quantile regression between sampling completeness and diversity components (Appendix S4). The authors are solely responsible for the content and functionality of these materials. Queries (others than absence of the material) should be directed to the corresponding author.

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Appendix S1. Vegetation map of the Ordesa-Monte Perdido National Park.

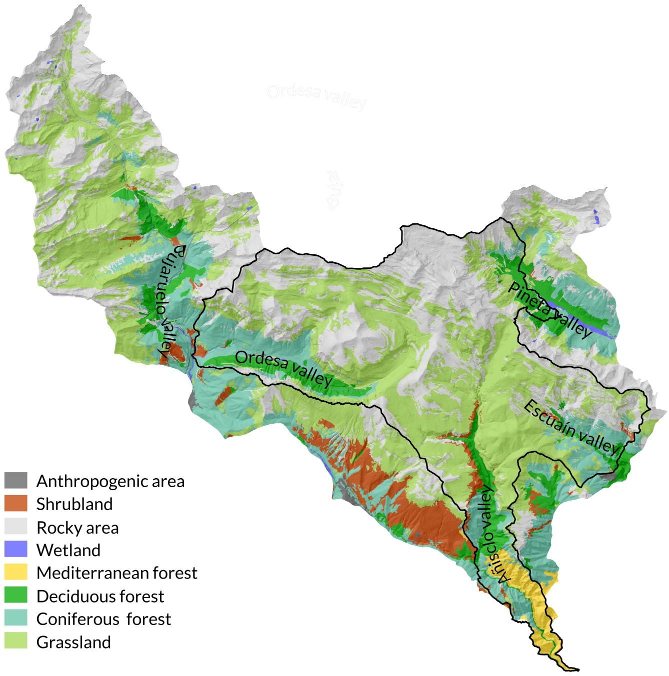

Fig. S1 Vegetation map the Ordesa-Monte Perdido National Park (1:150.000). This map was created by combining the vegetation map of the National Park (Villar & Benito 2001), the third National Forest Inventory*, and the grassland cartography of Aldezabal (1997). Black line separates the National Park and the buffer area.

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*http://www.magrama.gob.es/es/biodiversidad/servicios/banco-datos-naturaleza/informacion-disponible/ifn3_bbdd_descargas.htm.aspx

Appendix S2. Habitat representativeness in community surveys.

In order to evaluate the representativeness of data from community surveys, we calculated the number of surveys carried out in each habitat type of the Ordesa-Monte Perdido National Park (including the buffer area). Each surveys was classified into one of the eight habitats according to their syntaxonomy, and the botanical expertise of one of the co-authors (D.G.). Fig. S2 shows that all the habitats, except coniferous forest were relatively well covered in community surveys. The poor quantitative representation of pine forest may be attributed to their low taxonomic diversity, which probably deviated past descriptive efforts to more heterogeneous habitats of the National Park instead.

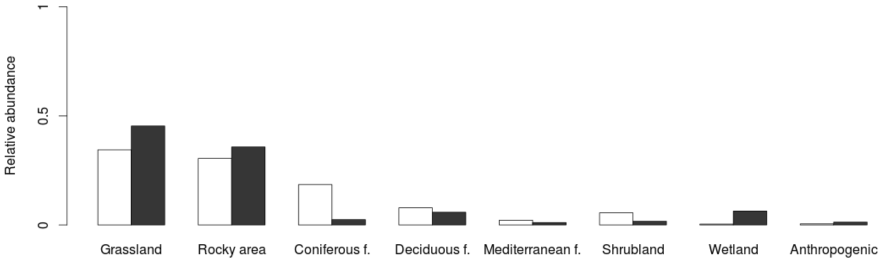

Fig. S2 Relative abundance of the different types of habitats across the Ordesa-Monte Perdido National Park in terms of areas extent (bars in white), and that of community surveys of each habitat (bars in black) out of the total surveys (n = 1218). Habitats are ranked in order of decreasing abundance from left to right along the x-axis.

Appendix S3. A model based approach to remove the effect of species richness in the estimation of phylogenetic and functional diversity.

As in previous studies (e.g. Devictor et al. 2010; Davies & Buckley 2011; Fritz & Rahbek 2012; Zupan et al. 2014), we modeled the two diversity measures as a function of species richness (SR), and used model residuals as surrogate of net phylogenetic and functional diversity (PD_R and FD_R, respectively). We initially fitted linear and quadratic models, and evaluated the models according to their fit (Fig. S3) and AIC values (Akaike 1974; Table S3). Other aspects such as the normality of residuals were visually inspected. Almost without exception, inclusion of the quadratic term in the model led to higher proportion of variance explained (Fig. S3), and did not penalize as indicated the lower AIC values irrespective of the source of information (Table S3).

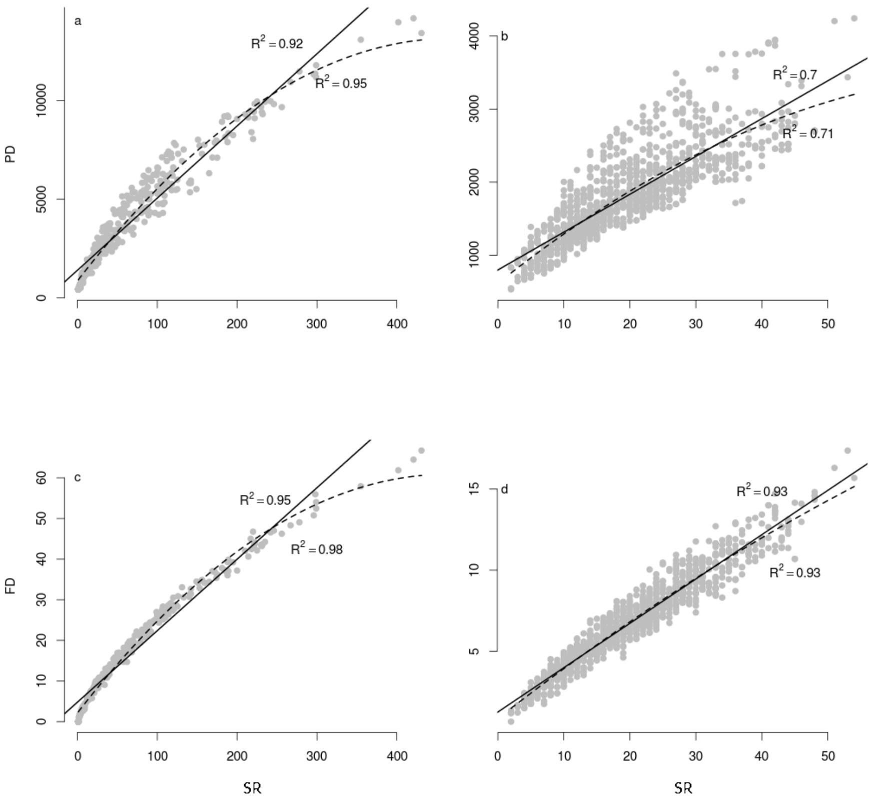

Fig. S3 Significant ($P < 0.001$) positive relationship between the values of species richness (SR) and phylogenetic diversity (PD) and functional diversity (FD) in grid-cell occurrences (a, c) and community surveys (b, d). Linear and quadratic regressions are shown by solid and dashed lines (respectively). Grey dots represent observed values.

Table S3 Fit of linear and quadratic models between species richness and phylogenetic diversity and functional diversity.

Model variables	Information source	Model	df	AIC
PD ~ SR	Grid-based occurrences	linear	3	5267.04
		quadratic	4	5098.63
	Community surveys	linear	3	17573.47
		quadratic	4	17543.62
FD ~ SR	Grid-based occurrences	linear	3	1636.14
		quadratic	4	1189.46
	Community surveys	linear	3	2675.85
		quadratic	4	2646.86

Abbreviations: SR, species richness; PD, phylogenetic diversity; FD, functional diversity; AIC, Akaike Information Criteria.

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Appendix S4. Results of quantile regression between sampling completeness and diversity components

Table 1 Estimates of the coefficients of regression quantiles between sampling completeness and multiple diversity aspects from grid-based occurrences of taxa.

Response variable	Quantile	Intercept (SE)	*t* statistic	Slope (SE)	*t* statistic
SR	80th	-232.58 (90.47)	-2.57*	389.47 (100.84)	3.86***
	90th	-314.58 (56.16)	-5.60***	536.58 (62.36)	8.60***
	95th	-343.35 (50.10)	-6.85***	605.40 (68.74)	8.80***
ER	80th	-14.39 (4.23)	-3.40***	24.39 (4.50)	5.41***
	90th	-18.43 (3.38)	-5.45***	31.43 (4.10)	7.67***
	95th	-19.54 (3.30)	-5.91***	35.90 (4.34)	8.26***
PD	80th	-9611.34 (3159.29)	-3.36***	17206.90 (3001.64)	5.73***
	90th	-11680.07 (1610.80)	-7.25***	21096.32 (1776.23)	11.87***
	95th	-10683.43 (1555.53)	-6.86***	20847.99 (2021.78)	10.31***
FD	80th	-46.85 (14.84)	-3.15***	81.2 (16.05)	5.06***
	90th	-56.40 (8.78)	-6.42***	99.62 (9.79)	10.17***
	95th	-47.23 (9.26)	-5.09***	94.95 (10.58)	8.97***
PD_R	80th	-1813.39 (306.12)	-5.92***	2645.80 (382.8)	6.91***
	90th	-1686.69 (397.08)	-4.24***	2783.46 (420.43)	6.62***
	95th	-2008.56 (506.42)	-3.96***	3329.01 (556.96)	5.97***
FD_R	80th	2.62 (1.31)	1.99*	-1.28 (-1.28)	-0.89
	90th	1.67 (1.22)	1.37	0.24 (1.33)	0.18
	95th	1.09 (1.59)	0.68	1.17 (1.67)	0.70

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Abbreviations: SR, species richness; ER, endemism richness; PD, phylogenetic diversity; FD, functional diversity; PD_R, phylogenetic diversity measured as the residuals of the quadratic regression between species richness and Faith's (1992) phylogenetic metric; FD_R, functional diversity measured as the residuals of the quadratic regression between spcies richness and functional metric of Petchey & Gaston (2002).

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Chapter 3

Uncoupled changes in tree cover and field layer vegetation at two Pyrenean treeline ecotones over 11 years

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Abstract

Background: The alpine treeline ecotone is regarded as a sensor of the effects of global change on alpine plant communities. However, little is known about how treeline dynamics influence the diversity and composition of alpine plant communities. Such information is necessary to forecast how ascending montane forests may affect the composition of alpine flora.

Aims: We analysed the temporal variations in tree cover, plant diversity and composition, and the effect of tree cover dynamics on field layer vegetation over a period of 11 years, at two alpine treeline ecotones in the central Pyrenees, Spain.

Methods: Tree and field layer vegetation was sampled in permanent transects in 1998 and 2009, using the point-intercept method. Temporal changes in tree cover, plant species richness and abundance were characterised along the ecotone by using a randomisation approach, rarefaction curves, and a non-parametric multivariate test, respectively.

Results: Tree cover increased significantly at one of the sites, whereas plant species richness only increased at the other site where tree cover had not changed. Vegetation composition changed significantly at both sites, but it was not spatially coupled with changes in tree cover along the ecotone.

Conclusions: A change of tree cover does not necessarily trigger changes in the ground flora at the treeline over relatively short periods (decade scale). The results challenge our ability to infer short-term biodiversity impacts from upslope advance of forests. Integrated tree and field layer monitoring approaches are necessary to produce a better understanding of the impact of

ongoing global change on treeline ecotones.

Introduction

Climate warming is causing the extension of plant species' ranges towards higher altitudes and shifts in community composition in mountain regions during the last decades (Grabherr et al. 1994; Peñuelas and Boada 2003; Walther 2003; Walther et al. 2005; Cannone et al. 2007; Lenoir et al. 2008, Kullman 2010, Pauli et al. 2012). The elevational treeline has been regarded as a reliable sensor of such climate effects worldwide (e.g. Hessl and Baker 1997; Kullman 2002; Daniels and Veblen 2003; Camarero and Gutiérrez 2004; Baker and Moseley 2007; Gehrig-Fasel et al. 2007; Mathisen et al. 2013) because its limit is expected to be determined by a thermal threshold (Jobbagy and Jackson 2000; Körner and Paulsen 2004; Holtmeier and Broll 2005; Körner 2012). Therefore, treelines should respond to ongoing climate warming by shifting upwards (Grace et al. 2002). However, some studies have reported tree cover increases within the ecotone while minor or no upward advances occurred, especially during the second half of the twentieth century (Camarero and Gutiérrez 2004). The reduction in grazing, especially since the 1960s, has promoted forest re-growth (Motta and Nola 2001; Holtmeier 2003; Ninot et al. 2008; Ameztegui et al. 2010), and it might override the effects of climate warming in treeline ecotones that have historically been below their natural threshold due to grazing (e.g. Gehrig-Fasel et al. 2007; Batllori and Gutiérrez 2008; Palombo et al. 2013).

Regardless of the main drivers of treeline responses, if the uppermost limits of montane forests ascend, adjacent alpine communities are expected to undergo changes. An overall assumption is that a treeline advance and an increase of tree density should lower species diversity and modify microenvironmental conditions in adjacent grasslands and in the field layer of the ecotone, respectively (Dirnböck et al. 2003; Walther 2003; Tinner and Kaltenrieder 2005, Illerbrun and Roland 2011). An increase of tree cover would modify light availability, moisture regime, soil properties and nutrient availability (Holtmeier and Broll 2005), creating forest-like microclimatic conditions in the treeline ecotone. Vegetation composition would therefore shift towards a greater dominance of shade-tolerant species, and the original shade-intolerant alpine flora would be gradually replaced by taxa typical of forest (Hofgaard and

Wilmann 2002; Grytnes 2003; Sundqvist et al. 2008). However, the consequences of canopy closure are often based on studies of forest succession (e.g. Dahlgren et al. 2006; Laughlin et al. 2011), rather than on direct evidence from treeline ecotones (but see Illerbrun and Roland 2011).

Despite the large amount of literature on treeline dynamics, relatively little attention has been paid to the consequences of treeline changes on plant diversity in the field layer (Camarero and Gutiérrez 2002; Hofgaard and Wilmann 2002; Camarero et al. 2006; Sundqvist et al. 2008; Batllori et al. 2009a). The scarce available information is from interpretations of chronosequences that use a space-for-time approach for assessing temporal dynamics, which has recently been criticised (Johnson and Miyanishi 2008). Therefore, empirical data are required for a reliable evaluation of the consequences of treeline dynamics on ground layer flora. Here, we report a detailed study on the dynamics of tree cover and associated field layer vegetation in the Pinus uncinata treeline ecotone in two national parks in the central Pyrenees (Spain) between 1998 and in 2009. The aim of this study was to assess the short-term impact of tree cover dynamics on field layer vegetation in the treeline ecotones. Specifically, we addressed the following questions: (i) did tree cover in two undisturbed treeline ecotones increase between 1998 and 2009? (ii) did field layer plant diversity and composition change significantly? and if so, (iii) were changes in tree and field vegetation layers coupled in space?

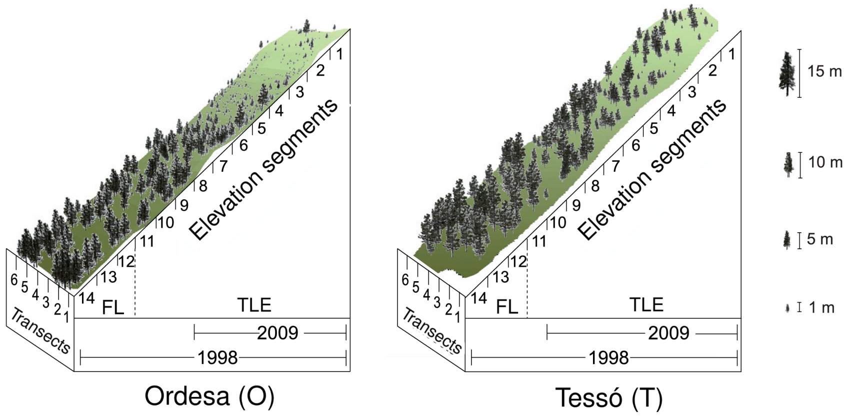
Materials and methods

Study area

We defined the treeline ecotone as the transition zone between the closed upper montane forest (hereafter forest limit) to the uppermost scattered and stunted trees (following Holtmeier 2003). The study sites were located in two protected areas: the Ordesa site (hereafter abbreviated as O) in the buffer zone of Ordesa-Monte Perdido National Park; the Tessó site (T) in the buffer zone of Aigüestortes-Estany de Saint Maurici National Park, central Pyrenees, Spain. On the basis of available historical data and comparison of aerial photographs taken in 1946 and 1988, these ecotones have been considered as undisturbed during the past century (Camarero and Gutiérrez 2002, 2004; Camarero et al. 2006). We examined orthophotographs taken in 1998 and 2009 to confirm that no wildfires, insect outbreaks, snow avalanches or logging occurred during this period. Repeated historical photographs also evidenced null elevational ascent of both treelines during the twentieth century (Camarero et al. 2000; Camarero and Gutiérrez

2004).

The sites were selected to represent the main contrasting structures of *Pinus uncinata* treeline ecotones (Camarero and Gutiérrez 2002). Site O was defined as a krummholz abrupt treeline, where the size and growth form of *P. uncinata* individuals changed abruptly upslope along the ecotone, from tall trees near the forest limit to a dense belt of krummholz individuals near the treeline (Camarero and Gutiérrez 1999) (Fig. 1). In contrast, the density and height of *P. uncinata* individuals decreased gradually with increasing elevation at site T (Camarero and Gutiérrez 1999), a diffuse treeline ecotone (sensu Harsch et al. 2009). The two sites also differed in terms of climate, geology and vegetation composition. Site O (42° 37' N, 0° 02' W, 2110 m a.s.l.) was located on a south-facing gentle slope (5–10°), under continental and Mediterranean influence (1660 mm annual precipitation and 5 °C mean temperature), with a yearly maximum snow-pack of 1.0–2.0 m (Góriz Meteorological Station, 42° 39' N, 00° 01' E, 2215 m a.s.l.; 1981–1989 and 1992–1995 data [Camarero and Gutiérrez 2002]). The bedrock was mainly limestone, although it contained many sandstone patches (Camarero and Gutiérrez 2002). Rock outcrops were abundant in the uppermost part of the ecotone. Field layer was dominated by hemicryptophytes, such as *Calluna vulgaris*, *Carex sempervirens* and *Festuca rubra*. Site T (42° 28'



Ordesa (O)

Tessó (T)

Fig. 1 Schematic representation of the sampling design and tree layer structure at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain. *Pinus uncinata* individuals were located and their height was measured in 1995 (modified from Camarero and Gutiérrez, 1999). Vegetation transects were set from the treeline to the upper part of the forest in 1998, whereas in 2009 the study was restricted to the treeline ecotone (TLE), excluding the forest. The position of the forest limit (FL) was established according to Camarero et al. (2006)

N, 01º 03' E, 2330 m) was located on a north-east-facing steep slope (20–30º), under continental local climate (ca. 1600 mm of annual precipitation; 3 ºC of mean temperature), and with a yearly maximum snowpack of 1.5–3.0 m (Esterri Meteorological Station, 42º 37' N, 01º 07' E, 1054 m a.s.l., 1970–1997 data [Camarero and Gutiérrez 2002]). The bedrock was shale, and the soil contained abundant organic matter (Camarero and Gutiérrez 2002). At this site, *Rhododendron ferrugineum* and *Vaccinium myrtillus* dwarf shrubs were dominant near the forest limit, whereas patchily distributed species, such as *Dryas octopetala* were more abundant in gaps near the treeline.

The mean temperature has increased at a rate of +0.3 ºC per decade on average between 1950 and 2006 in the Pyrenees (López-Moreno et al. 2010), causing a decrease of snow depth (López-Moreno 2005). The same increasing rate of temperature has been recorded during the last decade at a Meteorological Station near to site O (R. García-González, unpublished data). This rate constitutes an accelerated warming in comparison with historical available temperature records before 1950 (Bücher and Dessens 1991). On the other hand, grazing activities have diminished along the central Pyrenees during the twentieth century, due to the phasing out of transhumance and the depopulation of rural areas (García-Ruiz et al. 1996; Lasanta-Martínez et al. 2005). For instance, the estimated number of sheep in the Ordesa-Monte Perdido National Park was reduced by 75% since 1960 to recent decades (Komac 2010). The mean stocking rate in 1990s was below 1.5 and 1 LAU (1 Large Animal Unit = eight sheep or one head of cattle) per month ha^{-1} in the Ordesa-Monte Perdido and Aigüestortes-Estany de Saint Maurici National Parks, respectively (Aldezábal et al. 1992; Bas et al. 1994). However, we assumed that grazing pressure was much lower at the study sites, based on their distant location away from the main summer pastures and livestock routes. Only a few goats were observed in the surroundings of site O (Aldezábal et al. 1992), their impact on P. uncinata individuals being negligible (Camarero and Gutiérrez 1999).

Sampling methods

In summer 1998, Camarero and Gutiérrez (2002) laid six line transects (each 140 m long) along the maximum slope from alpine grassland to the closed upper montane forest at both study sites. Transects were separated from each other by 6 m (Fig. 1), and their position was permanently marked by using anchors, metal sticks and milestones. The abundance of *P. uncinata* and vascular plant taxa was estimated by using the point-intercept method (Barbour et al. 1987). A metal rod (diameter: 2 mm) was placed every 1 m along each transect, and all taxa contacted by the rod and its vertical projection were recorded (Camarero and Gutiérrez 2002). In 2009 transects were relocated and the tree and field layer vegetation was sampled in the same way as 11 years earlier (see Camarero and Gutiérrez 2002). As we were only interested on the dynamics of the treeline ecotone, we shortened transect length to the distance between the treeline and forest limit (i.e. the uppermost 80 and 100 m at sites O and T, respectively), to exclude the sampling points located within the forest (see Fig. 1). More than 600 and 1000 plant records were gathered each year at sites O (486 sampling points) and T (606 sampling points), respectively. The botanical nomenclature of field layer species follows de Bolòs et al. (2005).

Statistical analyses

Changes over time in tree and field layer vegetation were first examined by considering each transect as a statistical unit after confirming the lack of spatial autocorrelation in preliminary analyses (Mantel test; $R = 0.41$ and 0.25 for Ordesa and Tessó sites, respectively; $P > 0.05$). In addition, we explored temporal changes along elevation by splitting transects into 10 m-long segments (see Fig. 1).

Temporal changes in tree cover

We estimated the cover of *P. uncinata* individuals (hereafter tree cover) per transect ($n = 6$) by dividing the number of hits by the total number of sampling points in each survey (1998 and 2009). According to our previous knowledge of the treeline ecotones, we would expect an increase of tree cover rather than a decrease over 11 years. Therefore, we tested only for the increase in tree cover both at each treeline ecotone and along elevation segments, using one-tailed Wilcoxon tests.

To assess whether forest limit significantly shifted upwards over the 11-year study period, we tested whether the tree cover values along the elevational gradient in 2009 were the same as those observed below the forest limit in 1998. Different subjective criteria have been used to determine a threshold that separates the treeline ecotone from the forest below (e.g. Jobbagy and Jackson 2000; Holtmeier 2003). To overcome such limitations, we computed a site-specific threshold value on the basis of the tree cover values recorded below each forest limit in 1998 (Camarero et al. 2006) (Fig. 1), and tested if the forest limit had shifted by using a randomisation procedure proposed by Harper and MacDonald (2001). First, we randomly selected one elevation segment below the forest limit (segments 12 to 14 in 1998, see Fig. 1) in each transect and calculated the mean tree cover (*n* = 6) below the forest limit at each treeline ecotone. These steps were repeated 10,000 times for each ecotone, and the 2.5th percentile (two-tailed test, *α* = 0.5) of the resulting distribution of mean cover values was selected as the threshold value. Finally, the tree cover values observed in 2009 were compared with such threshold values. We considered that the forest limit had shifted upwards when a set of consecutive segments of 2009 presented tree cover values that did not significantly differ ($P > 0.05$) from those observed in forest in 1998.

Temporal changes in plant diversity

Changes in plant diversity were analysed by considering the variation of species richness and evenness. We compared species richness between 1998 and 2009 by using sample-based rarefaction curves with 95% confidence intervals (Gotelli and Colwell 2001; Colwell et al. 2004). Both curves and confidence intervals were calculated by using the EstimateS software (Colwell 2005), and following the analytical formulas detailed in Mao et al. (2005) (see also Colwell et al. 2004). The differences between sample-based rarefaction curves could be due to differences in species richness, but also to differences in the number of individuals recorded (Gotelli and Colwell 2001; Colwell et al. 2004). As we were only interested in the former and given that we recorded a different number of plant individuals in 1998 and 2009, we re-scaled the rarefaction curve to the number of plant individuals in the 2 years (Gotelli and Colwell 2001). Once rarefaction curves were plotted, significant differences ($P < 0.05$) between 1998 and 2009 were established when confidence intervals did not overlap (Colwell et al. 2004). In addition, we tested the changes of species richness between years at each elevation segments by using a two-tailed Wilcoxon test.

As an estimation of evenness we chose the Simpson index (1-D) (Legendre and Legendre 1998) which is preferable over other indices because it stabilises at small sample sizes (Magurran 2004). Differences of evenness between surveys were tested by using a two-tailed *t*-test with the Welch correction for groups with unequal variances (onwards Welch test), once normal distribution of the data was confirmed. We applied the same procedure for testing the changes in evenness along the elevation gradient.

Temporal changes in vegetation composition

We tested the change in the composition and abundance of field layer species (henceforth vegetation composition) in the treeline ecotone by using a non-parametric multivariate analysis of variance based on distance matrices (Anderson 2001; McArdle and Anderson 2001). This multivariate analysis was carried out by using the *adonis* function (Oksanen et al. 2010) in R (version 2.10.1; 2009), after excluding all unidentified species (see Table 1). This test is analogous to a MANOVA (Anderson 2001) and compares the variability in composition dissimilarity among transects from the same observation year to the variability in composition dissimilarity among transects from 1998 and 2009. We first calculated the dissimilarity among transects within and between observation years (the distance matrix), based on the Chao distance. This distance minimises the contribution of rare species (defined as those with only one or two hits in one of the sampling years) to the estimated dissimilarity (Chao et al. 2005, 2006); even so, the vegetation turnover might still be biased by our sampling limitation to track the changes of species occurring at low densities. To further explore this, we additionally calculated distance matrices by excluding less abundant species (defined as those species with abundance values lower than the observed third quartile; see further details in Appendix S1, online supplemental material). Next, the pseudo *F*-ratio statistic was computed for each distance matrix. A large value of this test statistic means that transects sampled in 1998 and 2009 differ in vegetation composition. The significance of this statistic was tested by using a permutation test (n = 10,000) carried out under the true null hypothesis of no difference between observation years. Each permutation transect was reassigned to one of the two possible observation years at random, and a new pseudo *F*-ratio was calculated. Finally, the significance of the test (α = 0.05) was obtained by comparing the pseudo *F*-ratios obtained under permutation and those pseudo F-ratios corresponding to collected data (Anderson 2001; McArdle and Anderson 2001). As in similar analyses of variance, the *adonis* test is sensitive to data heteroscedasticity (Anderson 2001, 2006). To confirm that significant differences between years were not caused by data heteroscedasticity across sampling years, we carried out a permutation test (10,000 randomisations), based on a multivariate dispersion analysis (Anderson 2006) by using the *permutest.betadisper* function (Oksanen et al. 2010).

The temporal turnover of vegetation was examined along the elevation gradient by using a dissimilarity index. We merged species abundances recorded at each elevation segment and computed the Chao index by using the *vegdist* function (Oksanen et al. 2010). High values indicate large changes in the composition of the assemblage between years. We also re-calculated Chao index by excluding the less abundant species (see Appendix S1) to determine whether the turnover pattern was affected by sampling limitations (i.e. insufficient capture of species occurring at low densities). Finally, temporal changes in the abundance of each taxon were tested by using a x^2 test.

Table 1. List of species recorded at the Ordesa (O) and Tessó (T) treeline ecotones, central Pyrenees, Spain, in 1998 and 2009. Nomenclature follows de Bolòs et al. (2005).

Site	Species
O	Both in 1998 and 2009: *Agrostis capillaris, Alchemilla* gr. *alpina, Allium senescens* subsp. *montanum, Antennaria dioica, Anthyllis montana, Calluna vulgaris, Campanula rotundifolia, Carex sempervirens, Deschampsia flexuosa, Festuca rubra, Galium pumilum* subsp. *marchandii, Geranium cinereum, Globularia cordifolia* subsp. *repens, Hieracium pilosella, Hieracium* sp., *Hippocrepis comosa, Jasione crispa, Juniperus communis* subsp. *communis, Nardus stricta, Pimpinella saxifraga, Plantago maritima* subsp. *alpina, Plantago monosperma, Potentilla alchemilloides, Potentilla crantzii, Sideritis hyssopifolia, Thymelaea tinctoria* subsp. *nivalis, Trifolium alpinum, Viola rupestris* subsp. *rupestris.* Only in 1998: *Arenaria purpurascens, Asperula pyrenaica, Astragalus monspessulanus, Phyteuma hemisphaericum.* Only in 2009: *Androsace villosa, Anthyllis vulneraria, Danthonia decumbens, Dianthus benearnensis, Erigeron uniflorus, Euphrasia salisburgensis* subsp. *salisburgensis, Hypochoeris radicata, Saponaria caespitosa, Sedum album, Thymus serpyllum* subsp. *nervosus.*
T	Both in 1998 and 2009: *Anemone narcissiflora, Botrychium lunaria, Carlina acaulis, Carex sempervirens, Dianthus hyssopifolius, Dryas octopetala, Festuca gautieri, Galium pumilum, Helianthemum nummularium, Hieracium lactucella, Juniperus communis* subsp. *communis, Lotus corniculatus* subsp. *alpinus, Pyrola chlorantha, Rhododendron ferrugineum, Saxifraga moschata, Vaccinium myrtillus, Veronica bellidioides.* Only in 1998: *Anemone nemorosa, Anemone vernalis, Daphne cneorum, Galium verum, Helictotrichon sedenense.* Only in 2009: *Alchemilla flabellata, Androsace villosa, Anemone hepatica, Antennaria dioica, Bupleurum ranunculoides* subsp. *gramineum, Campanula cochlearifolia, Cruciata glabra, Gentiana verna, Iberis sempervirens, Luzula nutans, Plantago monosperma, Polygonum viviparum, Primula elatior* subsp. *intrincata, Sempervivum montanum, Soldanella alpina, Vaccinium uliginosum* subsp. *microphyllum.*

Results

Tree cover significantly increased from 20% to 35% at site O (one-tailed Wilcoxon test; $W = 4$, $P = 0.013$) between 1998 and 2009. Near the forest limit, tree cover in 2009 was not statistically different from the threshold values (randomisation test; $P > 0.05$), indicating that tree cover in these elevation segments of the treeline ecotone was similar to that observed within the forest (Fig. 2). We also detected significant increases of tree cover in the third and fifth elevation segments (one-tailed Wilcoxon test, W statistic ranges from 5.5–7, $P < 0.05$; Fig. 2). Tree cover at site T was below 21% in 2009, and unlike at O, statistically the same as 11 years earlier (one-tailed Wilcoxon test; $W = 7.5$, $P = 0.054$). Tree cover did not significantly increase in any of the elevation segments either ($P > 0.05$ in all segments; Fig. 2).

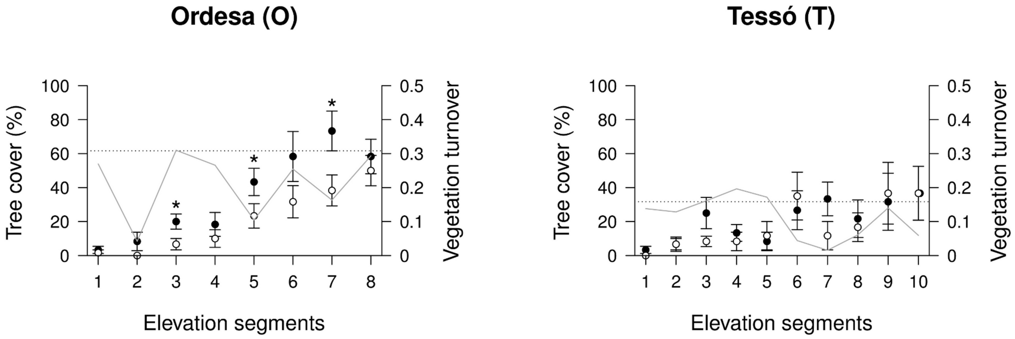


Fig. 2 *Pinus uncinata* cover in 1998 (empty circles) and 2009 (filled circles) (means and standard errors), and vegetation turnover estimated as Chao index (grey line), at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain. Elevation segments are numbered from the treeline to the forest limit. Asterisks indicate significant (one-tailed Wilcoxon test, $P < 0.05$) increase of tree cover after 11 years. The dotted line corresponds to the threshold value of tree cover that separates the treeline ecotone from the forest limit.

Plant richness of the field layer vegetation did not significantly differ between 1998 and 2009 at site O (Fig. 3), but evenness significantly increased from 0.82 to 0.86 through time (two-tailed Welch test; $t = 3.60$, d.f. = 8.18, $P = 0.005$). At site T, plant richness increased significantly from 23 to 36 recorded taxa ($P < 0.05$; Fig. 3), whereas evenness in 2009 was identical to that in 1998 (0.78; two-tailed Welch test; $t = 0.63$, d.f. = 9.8, $P = 0.54$). Neither richness nor evenness changed significantly (two-tailed Wilcoxon test and two-tailed Welch test, respectively, $P > 0.05$) along the elevation gradient at site T (Appendix S2, online supplemental

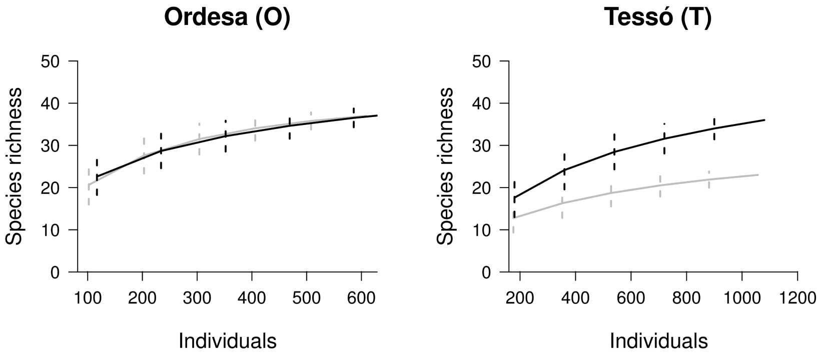


Fig. 3 Individual-based rarefaction curves of the Mao-Tau expected species richness and 95% confidence intervals in 1998 (grey line) and 2009 (black line) at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain.

material).

Vegetation composition changed significantly between 1998 and 2009 at both sites (O adonis; F = 9.03, R^2 = 0.44, P = 0.003; T adonis; F = 17.01, R^2 = 0.63, P < 0.001). None of these results were biased due to data heteroscedasticity (permutest.betadisper; F = 1.31, P = 0.283 in O and F = 0.53, P = 0.487 in T). At the two treeline ecotones, the species only detected in 2009 were typical of grasslands (Table 1). These shifts in composition were mostly driven by species with cover < 5% at site O (Fig. 4). In particular, we only found significant increases (x^2 test; P < 0.05) in the abundance of alpine grasses, such as *Nardus stricta*, *Danthonia decumbens* and *Agrostis capillaris* at this treeline ecotone (Fig. 5), whereas the newly detected species at site T contributed the most to the observed change (Fig. 4). However, when we repeated analyses excluding less abundant species (n = 14 and 12, O and T, respectively), we also found a significant shift in vegetation composition between years (O adonis; F = 15.18, R^2 = 0.60, P = 0.002; T adonis; F = 36.05, R^2 = 0.78, P < 0.001). Four species accounted for more than two- thirds of the hits both in 1998 and 2009 in the field layer at O: *Calluna vulgaris*, *Festuca rubra*, *Carex sempervirens* and *Hippocrepis comosa*, and only the abundance of the latter shifted significantly between 1998 and 2009 (Fig. 5). At site T *Rhododendron ferrugineum*, *Vaccinium myrtillus*, *Dryas octopetala*, *Festuca gautieri* and *Carex sempervirens* accounted for 90% of the cover hits in 1998 and 89% in 2009, and only the abundance of two of the species changed significantly (Fig. 5).

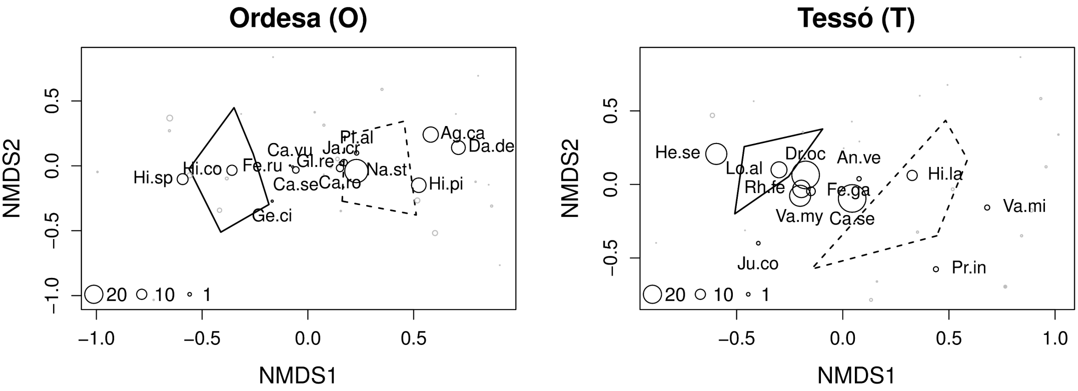


Fig. 4 Non-metric multidimensional scaling ordination of field layer vegetation composition at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain. Convex hulls join vegetation samples from 1998 (solid lines) and 2009 (dashed line). Circles indicate the position of the less abundant (grey) and most abundant (black) plant species in the multivariate space. The size of the circles represents the absolute change in abundance of the species from 1998 to 2009. For clarity only the names of most abundant species are shown, names are abbreviated by writing the first two letter of the genus name and the first two letter of the (sub)species names (e.g. *Dryas octopelata* = Dr.oc). See Table 1 for a list of species names.

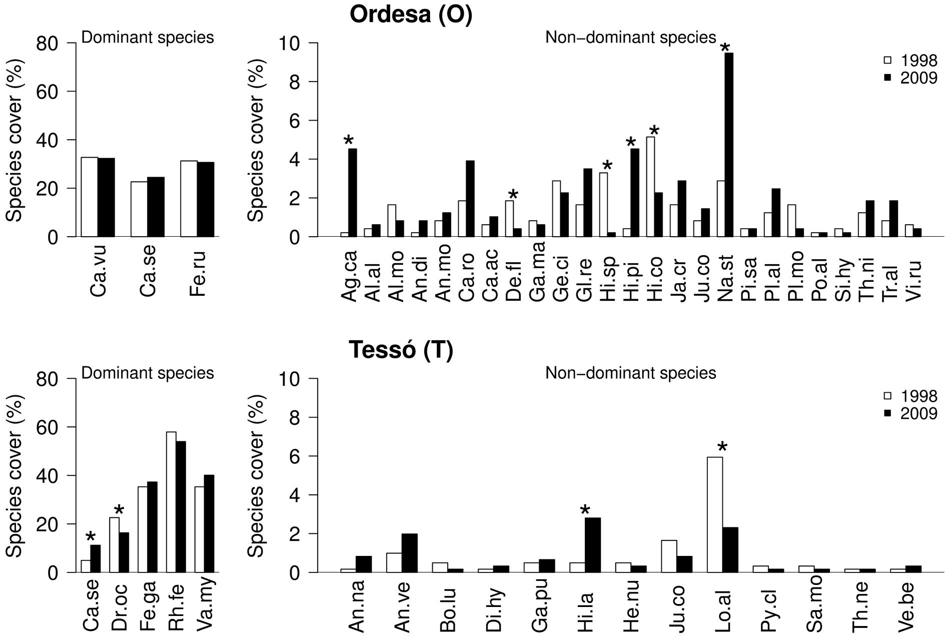


Fig. 5 Changes in the cover of shared dominant (on the left) and non-dominant species (on the right) at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain, between 1998 and 2009. Asterisks indicate significant temporal changes in abundance (x 2 test, P < 0.05, n = 6 transects). Codes for species correspond to the first two letter of the genus name and the first two letter of the (sub)species names (e.g. *Globularia cordifolia* subsp. *repens* = Gl.re). See Table 1 for a list of species names.

The highest values of vegetation turnover were patchily distributed along the elevation gradient at site O, whereas at site T, the highest turnover was restricted to the upper elevation segments (Fig. 2). The same elevation pattern, though with lower turnover values, was found at both sites when the less abundant species were excluded, except for the uppermost segment in T (see Appendix S1). At both treeline ecotones, the patterns of highest vegetation turnover and significant increases in tree cover were spatially uncoupled along the elevation gradient (Fig. 2).

Discussion

We found that changes in *Pinus uncinata* canopy cover and field layer plant communities were spatially uncoupled in Pyrenean treeline ecotones at a decadal scale. Even if the upwards shift of the forest limit at site O might have drastically modified field layer microsite conditions (e.g. by reducing the light reaching the field layer) (Smith et al. 2003), vegetation turnover was not higher there than in other parts of the treeline ecotone. Moreover, contrary to overall expectation, the species involved in the turnover were not those of shade-adapted, but grasses. Taken together, these results suggest that canopy closure did not trigger short-term changes in the composition of the field layer vegetation in the treeline ecotone.

The main driver of vegetation dynamics remains unclear after removing the effect of tree cover. Unlike some subarctic forest tundra ecotones (Pajunen et al. 2012), the shift in field layer vegetation cannot be attributed to denser shrub canopies, because none of the shrubs species (*Calluna vulgaris*, *Rhododendron ferrugineum* and *Vaccinium myrtillus*) increased in cover over the 11 years. At site O, *Nardus stricta*, *Agrostis capillaris* and *Danthonia decumbens* significantly increased at the expense of other grasses and non-dominant forbs. These species are abundant in grassland in the Pyrenees (Fillat et al. 2008), and their increase in abundance may be linked to the past decline in grazing intensity. Indeed, increasing abundance of *N. stricta* and *A. capillaris* has also been observed after two decades of grazing exclusion in nearby grasslands (D. Gómez and R. García-González, pers. comm.). However, climate-mediated responses cannot be discarded either, because these graminoids might have responded rapidly to the rising temperatures due to their modular organisation and high tissue turnover (Shaver et al. 1997). Whatever the driver was, the higher values of vegetation turnover was associated with the distribution of graminoids, whereas the lower turnover corresponded to localities where biotic conditions were little altered, such as rock outcrops or locations where shrubs and tussock-forming sedges (*Carex sempervirens*) were already established in 1998 (Camarero and Gutiérrez 2002). By contrast, a higher vegetation turnover was found in the upper half of the treeline ecotone at site T. In the uppermost elevation segment, we found a discordance between the turnover based on all species and that based on a subset of most abundant ones (Appendix S1), which indicates a higher contribution of newly detected species to vegetation turnover there. The fact that higher turnover values matched the distribution of the dwarf shrub that decreased the most, i.e. *Dryas octopetala* (Camarero and Gutiérrez 2002), led us to suggest that a competition release allowed the increase in abundance of less competitive species (*Plantago monosperma*, *Primula elatior* subsp. *intrincata*, *Soldanella alpina*) near the treeline (Choler et al. 2001; Virtanen et al. 2010). We cannot directly infer causality from our descriptive study; however, the low grazing since 1990s, and the decrease by *D. octopetala* similarly to that found in response to experimental warming (Klanderud and Totland 2005) suggests a potential role of rising temperature on the reduction of this dwarf shrub. In contrast, the stable dense layer of ericoid shrubs (*R. ferrugineum* and *V. myrtillus*) may have prevented the entrance of new species in the lower half of the elevation gradient at site T.

It is important to note that the reported changes in vegetation composition and species richness could also reflect stochastic processes that were not identified by our study, such as year-to-year fluctuations in the abundance of each species (Magurran et al. 2010). However, given that all recorded species were long-lived that buffer stochastic fluctuations to a great extent (García et al. 2008), the contribution of temporal variability to the observed vegetation changes may be regarded as minor. Another important caveat in our study was the number of sampling points per transect established in 1998 (Camarero and Gutiérrez 2002), which could be insufficient to capture species occurring at very low densities. To overcome such potential bias, we reduced the weight of species with low cover on vegetation analysis by using the dissimilarity distance that yielded the most conservative results (see Materials and methods). More importantly, we demonstrated that the observed results were robust to the removal of species with lower cover from analyses (except the aforementioned case in the uppermost elevation segment at site T; see Appendix S1). Finally, the detection of species not recorded in 1998 should be interpreted as an increase of their abundance in the treeline ecotone, rather than as a migration from adjacent belts. Taken together, the results indicate moderate changes in field layer plant diversity, supporting previous conclusions from high-latitude treelines (Virtanen et al. 2010).

Regarding the dynamics of *P. uncinata*, we only found a change in tree cover at site O. This result deviates from general predictions of lower sensitivity to rising temperature of abrupt treeline ecotones as compared with diffuse ones, which are less subjected to additional constrains (e.g. wind, snow) (Harsch et al. 2009; Harsch and Bader 2011). Wind has limited pine recruitment and growth through mechanical damage during the last decades (Camarero et al. 2000). Therefore, the negative impacts of the wind (winter desiccation, summer cooling, needle abrasion by snow particles or mechanical damage to exposed stems [Scott et al. 1993; Smith et al. 2003; Holtmeier and Broll 2010]) should be locally lessened to allow the increase in tree cover increase. In this context, a positive interaction between *P. uncinata* adults (Smith et al. 2003; Batllori et al. 2009b) may explain the upwards extension of the forest limit, whereas in the krummholz band (see Fig. 1), the increased tree cover may be associated to more favourable microsite conditions under krummholz individuals (Germino et al. 2002; Smith et al. 2009; Holtmeier and Broll 2010), some field layer species (e.g. Calluna vulgaris) (Bartolomé et al. 2008), or/and the shelter of surface geomorphologic features (Resler 2006). By contrast, the dense patches of R. ferrugineum might have prevented the recruitment of *P. uncinata* at site T (Pornon and Doche 1996; Ninot et al. 2008; Batllori et al. 2009b).

Conclusions

Our study evidences an unexpected uncoupling between the dynamics of tree cover and field layer vegetation at the upper montane treeline ecotone over a decade. This result suggests that an upwards extension of the upper montane forest does not necessarily mean a loss of plant diversity in adjacent grasslands in the short term. So far, such local uncoupling between tree and field layer vegetation has not been evidenced from other sites. Thus, there is still little basis for robust generalisations based on comparative approaches. Further long-term studies that include field layer vegetation are required for assessing the full impact of ongoing global change on treeline dynamics and related plant diversity. Meanwhile, alpine plant diversity losses inferred from gross dynamics of treeline ecotones at large or regional scales should be cautiously interpreted.

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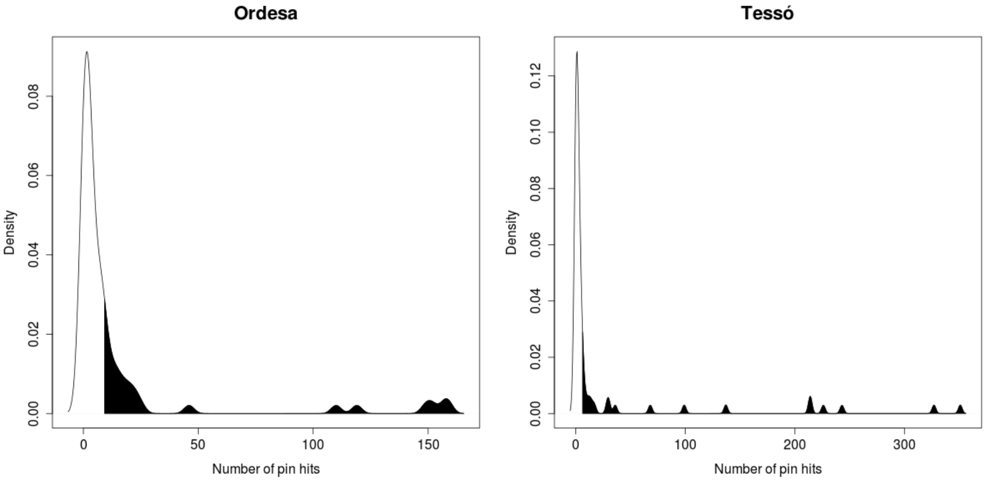
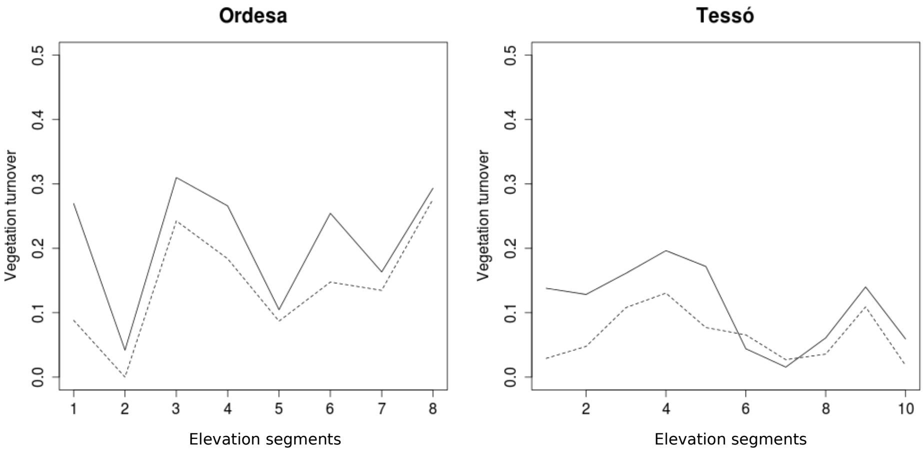
Supporting Information

Appendix S1. Evaluation of the effect of less abundant species on temporal vegetation composition changes along the elevational gradient. The number of sampling points established in 1998 (Camarero & Gutiérrez 2002) could be limited for estimating accurately the abundance of field layer species occurring at very low densities. If this was the case, this sampling bias could cause an overestimation of the vegetation turnover along the elevational gradient in the present study. In order to assess to what extent our results were biased, we compared the turnover values based on all identified species to those obtained after excluding less abundant species. We first defined less abundant species as those with lower abundance values (pin hits) than the observed third quartile. In contrast, the abundant ones were those above the third quartile in any of the two sampling years. The kernel density plots below show the distribution of the abundance of species (measured as number of pin hits) at the Ordesa and Tessó treeline ecotones, respectively. The range of data corresponding to the abundant species is shown in black.

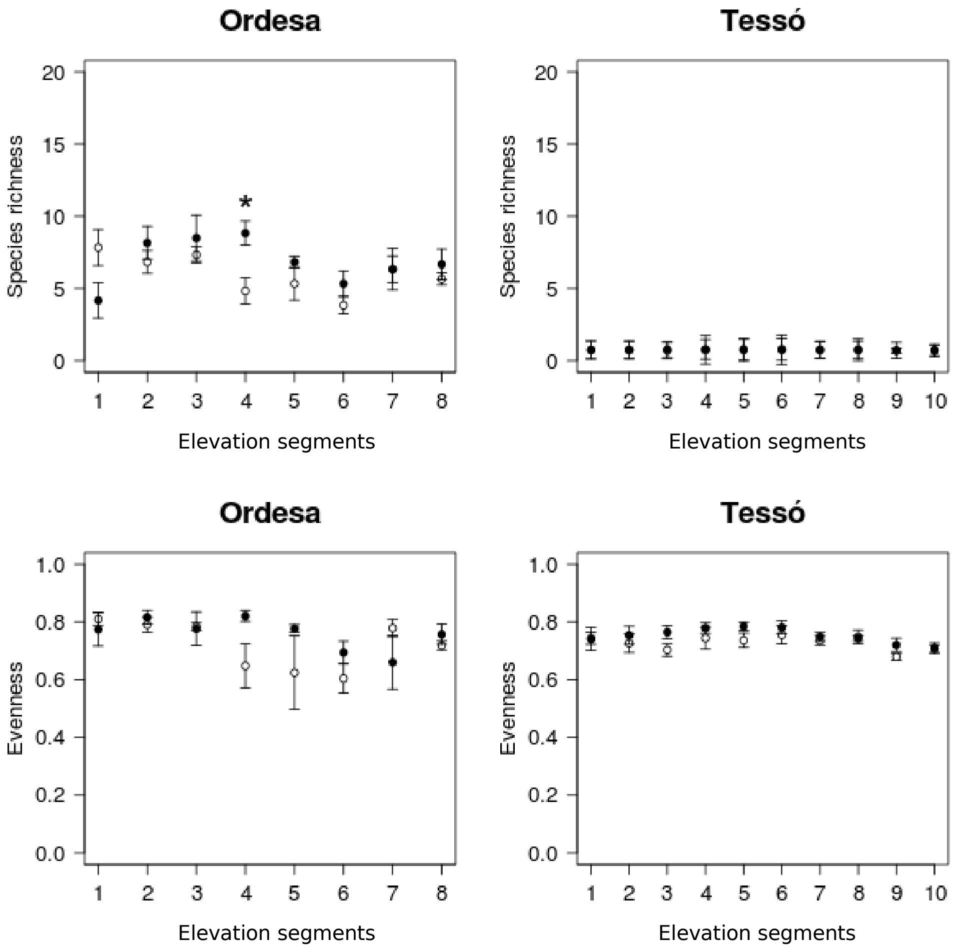


As a result of this classification, 14 species (*Agrostis capillaris, Calluna vulgaris, Campanula rotundifolia, Carex sempervirens, Danthonia decumbens, Festuca rubra, Geranium cinereum, Globularia cordifolia* subsp. *repens, Hieracium* sp., *Hieracium pilosella, Hippocrepis comosa, Jasione crispa, Nardus stricta, Plantago maritima* subsp. *alpina*) were classified as abundant from a total of 44 at site Ordesa. Whereas at site Tessó, the abundant species were less than one third of the total (12 abundant species: *Anemone vernalis, Carex sempervirens, Dryas* *octopetala, Festuca gautieri, Helictotrichon sendenense, Hieracium lactucella, Junniperus communis* subsp. *communis, Lotus corniculatus* subsp. *alpinus, Primula elatior* subsp. *intrincata, Rhododendron ferrugineum*, *Vaccinium myrtillus, Vaccinium uliginosum* subsp. *microphyllum*).

Finally, we re-calculated the change in the vegetation composition between 1998 and 2009 along the elevational gradient (dashed line), and compared the resultant pattern to that obtained with all species (solid line). Note that the elevational pattern was the same, although obviously turnover values were lower, except for the uppermost segment in the Tessó treeline ecotone.



Appendix S2. Species richness and evenness (measured as Simpson index) along the altitudinal segments at the Ordesa and Tessó treeline ecotones in 1998 (empty circles) and 2009 (filled circles). Asterisks indicate significant differences (*P* < 0.05) in species richness (two-tailed Wilcoxon test) and evenness (two-tailed Welch test) between years.



Chapter 4

Little evidence of the impact of global change on alpine grasslands over the last two decades in the Pyrenees

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Abstract

1. Reduction of traditional grazing activities is a major conservation issue in many European mountains. At highest elevation, however, the effects of climate warming on plant communities are predominant. While the impact of these drivers on vegetation are well-documented in mountain grasslands and mountain summits, respectively, only a few studies have examined the response of alpine grasslands, where both drivers concur to a greater extent.

2. We investigated the long-term dynamics of 12 alpine grasslands in two valleys of the Central Pyrenees (Spain), where grazing has declined by 70%, and annual temperature has increased in 1.8ºC since the 1950s. Grasslands were surveyed in the 1990s and two decades later. Three of these grasslands were also annually monitored to measure their year-to-year fluctuations. We also carried out a survey to estimate sampling error. Temporal trajectories of grasslands were analyzed by means of ordination analyses, and the significance of compositional shift was tested with multivariate analyses of variance based on distance matrices. Species were classified according to their shift in abundance as increasers and decreasers, and by accounting for the predicted sampling error. Differences between response groups were assessed in terms of species' distribution, abundance, and ecological and biological traits, and by accounting for their phylogenetic correlation.

3. We found minor changes in species composition and abundance of alpine grasslands between the 1990s and 2010s. In fact, compositional shifts of these grasslands over two decades were smaller than year-to-year fluctuations. Less than the half of total species (n=171) shifted in abundance between surveys more than expected by sampling error alone, and there were no significant differences in traits between species with opposing dynamics.

4. Our results revealed that alpine grasslands can show a larger ecological inertia than generally expected. The evidences of global change impact were subtle in comparison with those observed in mountain grasslands and summits of the study area and other European mountains. We suggest that grazing, though residual from a historical perspective, might has been sufficient to prevent some of the drastic structural changes (i.e. shrubs expansion) observed elsewhere. Finally, our results also highlight the importance for accounting for sampling error and natural fluctuations of communities, which can be higher than generally assumed for long-lived species and at high-elevation. Otherwise, the uncertainty associated to resurvey data will be high.

Introduction

Global change is transforming mountain ecosystems, threatening the high biodiversity they harbor (Grabherr et al., 1994; Walther et al., 2002; Gottfried et al., 2012), and the numerous ecosystem services they deliver (Schroter, 2005; Fontana et al., 2014). Since these impacts are expected to intensify throughout the 21st century (Sala et al., 2000; Dirnböck et al., 2003), understanding the way global change is affecting mountain ecosystems has become an urgent target of ecological research. Despite the many generalities about the effects of global change on mountain biodiversity (including distributional shift of species or community changes; Theurillat & Guisan, 2001; Walther, 2003), it renders difficult to downscale them to particular regions (Harrison et al., 2010). The impact of global change (land-use change and climate warming) will vary from one mountain to another (Cernusca et al., 1996; Engler et al., 2011), but also along elevational and/or topographic gradients within a mountain range (Fagre et al., 2003; (Speed et al., 2013). Testing general expectations under different situations is therefore central for refining our knowledge about the biotic consequences of global change in mountains.

Land-use change is the main driver of change in most European mountains (Tasser & Tappeiner, 2002; Dirnböck et al., 2003). Human activities such as forest clear-cut and burning or seasonal livestock grazing have been central for shaping the highly valuable landscape of these mountains (Chocarro et al., 1990; Cernusca et al., 1996; 2008; Colombaroli et al., 2013; Schwoerer et al., 2015). However, the generalized shift in socio-economic conditions of rural communities over the 20th century has led to a decline of traditional land-use, causing a drastic reduction of livestock grazing, and in some cases, a complete land-abandonment (MacDonald et al., 2000; Lasanta-Martínez et al., 2005; Cocca et al., 2012). Land-use changes have translate into forest re-growth and shrub expansion into grasslands below the treeline (Chauchard et al., 2007; Gehrig-Fasel et al., 2007; Gellrich et al., 2007; Améztegui et al., 2010; Gartzia et al., 2014). As a results, changes in plant composition, diversity reduction, and a steady impoverishment of foraging quality are expected in grasslands (Milchunas et al., 1988; Huntly, 1991; Vesk & Westoby, 2001; Tasser & Tappeiner, 2002; Borer et al., 2014). However, biological consequences of land-use changes are still poorly understood in alpine grasslands (Austrheim & Eriksson, 2001; Erschbamer et al., 2003), because only a few studies have examined in detail the temporal dynamics of these communities so far, and results are not consistent (Dullinger et al., 2003; Niedrist et al., 2008; Vittoz et al., 2008; Evju et al., 2009; Mayer et al., 2009; Speed et al., 2013). Since biological responses might be non-linear and lagged at high elevation (Bullock et al., 2001; Theurillat & Guisan, 2001), the dynamics of alpine grasslands should be assessed with a long-term perspective.

Alpine plant communities are also affected by climate warming. Increasing temperatures have been coupled with upwards shift of species (Kullman, 2002; Walther et al., 2005; Lenoir et al., 2008; but see Grytnes et al., 2014), specially in treeline ecotones (Körner & Paulsen, 2004; Harsch et al., 2009) and summits (i.e. subnival belt), where the treeline-forming species and those with thermal affinities have enhanced at the expense of alpine ones, respectively (Keller et al., 2000; Walther et al., 2005; Erschbamer et al., 2008; Parolo & Rossi, 2008; Gottfried et al., 2012). Fewer studies have empirically examined the impacts of climate warming on alpine grasslands communities, and results are inconsistent (Cannone et al., 2007; Vittoz et al., 2008, 2009; Virtanen et al., 2010). In addition, little is now about the synergistic effects of climate warming and grazing reduction in alpine areas under natural conditions (Theurillat & Guisan, 2001; Hoiss et al., 2012).

Revisitation studies are increasingly used to examine the long-term dynamics of plant communities (e.g. Lavergne et al., 2006; Vittoz et al., 2008; Wilson & Nilsson, 2009; Harrison et al., 2010; Kapfer et al., 2011). However, they are not exempt of limitations. For instance, if original sampling units are not accurately relocated, then obtained data will produce a biased picture of temporal changes in plant communities (Conway-Cranos & Doak, 2011; Chytrý et al., 2014). In addition, with only two censuses in time, we cannot detect natural fluctuations in plant communities, which may confound the interpretation of community trajectories (Magurran et al., 2010).

Here, we report on the long-term community dynamics of several alpine grasslands located across two valleys of the Spanish Pyrenees, where the number of livestock (mainly sheep) has declined in more than 70% throughout the 20th century (Aldezabal et al., 1992; García-Ruiz et al., 1996), and annual mean temperature has increased at rate of +0.3ºC per decade since the 1950s (López-Moreno et al., 2010). We analyzed the response of alpine grasslands to these drivers of change by using data from vegetation resurveys after two decades and a specific surveys carried out to estimate the sampling error between survey. In addition, included information about the year-to-year fluctuations of some of these grasslands. Combination of the three datasets provides a rather unique opportunity to distinguish the temporal response of alpine communities to external drivers of change from their natural dynamics in a realistic way. Finally, we investigated the role of grazing reduction and climate warming on the dynamics of plant communities by analyzing ecological and biological traits of species from different response groups (Noy-Meir et al., 1989; Bullock et al., 2001; Wiegmann & Waller, 2006; Naaf & Wulf, 2011; McCune & Vellend, 2013).

Material and methods

Study site

We carried out fieldwork in two protected areas of the Central Pyrenees: the Ordesa-Monte Perdido National Park and the Valles Occidentales Natural Park. These areas are representative of the semi-cultural landscape of the Pyrenees, and harbor a great diversity of grassland communities, most of which have traditionally been used for summer pasturing (from June to late September). The main bedrock type is limestone, occasionally mixed with outcrops of flysch and sandstone, and both areas share a mountain climate with Mediterranean influence in south facing slopes, which is characterized by mild summers and a marked seasonal precipitation. At 2200 m a.s.l., annual temperature is below 5ºC, precipitation ranges from 1600 to 2000 mm (data from Góriz Metereological Station; Remón, 2004), and the length of vegetative growing period is ~130 days (Del Barrio et al., 1990). In both protected areas, the number of livestock heads (mainly sheep) reached historical minimums in the early 1970s (García-Ruiz et al., 1996; Aldezabal, 1997), and occasionally, sheep flocks have been replaced by cattle. In contrast, populations of wild herbivores have fluctuated (Pyrenean chamois) or probably increased (wild-board) over the last decades (García-González, unpublished)

Vegetation sampling

In the early 1990s several vegetation surveys were conducted to evaluate the vegetation-herbivore interaction in both protected areas (Aldezabal, 1997). Vegetation was sampled following the point intercept method (pin diameter: < 1 mm; 20 cm intervals) along four transects oriented to N, S, E, W, radiating from a central point. Plant identity, height (measured as the distance from the top of the plant to the ground), and predation status (bitten or not) was recorded at each sampling point. This sampling procedure have been annually repeated over the last two decades in three of the grasslands (Table 1). We used this information to assess the year-to-year fluctuation of alpine grasslands. Other grasslands were permanently marked for exact relocation in successive years.

Table 1. Main characteristics of resurveyed and annually monitored grasslands.

Code	Location	Elevation (m a.s.l.)	Aspect	Vegetation cover (%)	Most abundant species	SR[1]	Grazing intesity	Sampling years
a	42°45'37'' N 0°35'26'' W	1835	-	67	*Festuca nigrescens, Nardus stricta, Agrostis capillaris, Carex caryophyllea*	44	medium	1990, 2011
nar	42°36'28'' N 0°1'45'' E	1930	N	99	*Festuca nigrescens, Trifolium alpinum, Nardus stricta, Agrostis capillaris*	14	medium	1993–2013*
bro	42°36'30'' N 0°1'40'' E	1930	N	100	*Festuca nigrescens, Briza media, Carex caryophyllea, Agrostis capillaris*	24	medium	1993–2013*
d	42°44'35'' N 0°33'59'' W	2053	S	95	*Festuca eskia, Festuca nigrescens, Trifolium alpinum, Nardus stricta*	19	high	1990, 2011
b	42°45'60'' N 0°35'51'' W	2046	-	97	*Festuca nigrescens, Trifolium alpinum, Nardus stricta, Carex rupestris*	51	medium	1990, 2011
q	42°38'22'' N 0°3'58'' W	2100	W	97	*Festuca eskia, Nardus stricta, Trifolium alpinum, Festuca nigrescens*	19	low	1992, 2010[R]
r	42°37'57'' N 0°2'27'' W	2100	SSE	64	*Carex rupestris, Thymelaea tinctoria nivalis, Geranium cinereum, Festuca nigrescens*	61	low	1992, 2010[R]
s	42°38'17'' N 0°3'35'' W	2150	S	61	*Festuca gautieri, Hippocrepis comosa, Koeleria vallesiana, Carex rupestris*	47	low	1992, 2010[R]
e	42°44'5'' N 0°34'5'' W	2233	?	54	*Festuca gautieri, Festuca eskia, Koeleria vallesiana, Helictrochicon sedenense*	33	low	1990, 2011
rua	42°46'23'' N 0°36'5'' W	2244	N	90	*Festuca nigrescens, Trifolium alpinum, Alopecurus gerardii, Poa alpina*	37	low	1991–2012
i	42°40'1'' N 0°0'55'' E	2380	S	98	*Festuca nigrescens, Lotus corniculatus, Agrostis capillaris, Poa alpina*	33	medium	1993, 2010
c	42°40'21'' N 0°1'20'' E	2675	NNE	35	*Festuca glacialis, Poa alpina, Oxytropis neglecta, Galium pyrenaicum*	30	low	1993, 2010

[1] Species richness in the 1990s.

*Data from 1999 is missing.

[R] Sampling was repeated three times in a day for estimating the sampling error.

In 2010 and 2011, we successfully relocated transects in 12 (including the three annually monitored) of the initially surveyed grasslands. Main characteristics of these grasslands are given in Table 1. Vegetation transects (more than 8,400 sampling points) were resurveyed following the original sampling protocol, and the relative abundance of each species was then estimated as the number of hits out of the total sampling points per grassland and year. A few conflictive taxa for identification (some *Hieracium* and *Alchemilla* species) were clumped in higher taxonomic groups for analyses. For the sake of simplicity, these groups (and subspecies) were treated as species throughout the text.

Sampling error

To estimate the sampling error performed during the resurvey, we sampled three grasslands with contrasting characteristics successively (three times) along a single day in 2011 (Table 1). Each time, we completely removed the ruler to reproduce the alignment process. We then modeled the difference in the relative abundance of species between surveys (henceforth sampling error) as a function of their initial abundance with a generalized additive model (GAM, p-splines and Gaussian distribution; Hastie & Tibshirani; Wood, 2006).

Trait data

For each species, we compiled information about its distribution, regional and local abundance, and biological and ecological traits from the literature, on line databases and *in situ* measures (Table 2). Hereinafter, and for the sake of simplicity, we will use the term trait loosely to refer to all these characteristics of species.

Community-level analyses

Long-term changes in species composition and abundance of grasslands were examined by mean of a non-metric multidimensional scaling based on Bray-Curtis metric (NMDS). We included annually gathered data from the three grasslands in the NMDS ordination, to compare the magnitude of vegetation shifts between decades to year-to-year fluctuations. The temporal trajectories of grasslands were assessed in relation to with elevation by projecting smooth surfaces of elevation onto the NMDS ordination. To do so, we modeled the composition of grasslands as a non-linear function of elevation with a GAM (thin plate splines; Gaussian distribution; Wood, 2003).

Table 2. Description of distributional attributes and biological and ecological traits of species.

Trait	Description	Values
Maximum elevation[1]	Highest occurrence of species	Between 1400 m and 3500 m
Elevation range[1]	Difference between lowermost and uppermost records of the species	Between 300 m and 2832 m
Species uppermost belt[1]	The uppermost elevation belt where a species has been recorded	Montane; Subalpine; Alpine; Subnival
Chorology[1]	According to the spatial distribution of species	Widespread; Mediterranean; Eurosiberian; Boreoalpine; Alpine; Pyrenean endemism
Range limit[1]	Whether species' range limit is in the Pyrenees	At range limit; Not at range limit
Regional abundance[2]	Number of occurrences in *relevées* (n=1218) conducted in the study area	Counts between 1 and 218
Local abundance[3]	Relative abundance in samples of this study	Continuous values between 0 and 15.40
Ecological guild[1]		Graminoid; Forb (non-legume); Legume; Shrub; Tree
Life-form[1,4]	Based on the position of renewal buds during unfavourable seasons for growing	Terophythes; Geophytes; Hemicryptophytes; Chamaephytes; Phanerophytes
Life-span[1]		Annual; short(< 5 yr); large (≥ 5 yr)
Plant propagation[1,4]	Main process of creating new individuals	Sexual; Mixed; Vegetative
Pollination system[1,6]		Insect and wind pollination; insect pollination (flowers can only be pollinated by specialized insects); No insect pollination
Dispersal syndrome[1,5,7]	Main agent for long-distance dispersal of seeds	Zoochory, Anemochory, No long-distance dispersal (NLDD)
Plaint height[3]	Distance from the top of the plant to the ground averaged from multiple field surveys	From 1 cm to 67 cm
Jacob's selectivity index[3]	Number of plants with bits as a proportion of the relative abundance of the species in samples	Between -1 (avoidance) to 1 (preference)

[1] On line database http://atlasflorapyrenaea.org/florapyrenaea/index.jsp and http://proyectos.ipe.csic.es/floragon/index.php

[2] http://www.sivim.info/sivi/

[3] Field.measures

[4] Klimeš L., Klimešová J., Hendriks R. & Groenendael J. van (1997) Clonal plant architectures: a comparative analysis of form and function. Pages 1-29. In: Kroon H. de, & Groenendael J. van (1997) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, the Netherlands.

[5] Poschlod, P., Kleyer, M., Jackel A., Dannemann A., & Tackenberg O. (2003) BIOPOP—a database of plant traits and internet application for nature conservation. *Folia Geobotanica* 38:263–271;

[6] Kuhn, I., & Klotz W. D. A. (2004) BiolFlor—a new plant-trait database as a tool for plant invasion ecology. *Diversity and Distributions* 10:363–365

[7] Kleyer M., et al. (2008) The LEDA Traitbase: a database of life-history traits of the Northwest European flora. Journal of Ecology 96:1266–1274.

The significance of the shift in plant community composition of grasslands between decades was tested with a multivariate analysis of variance based on distance matrix (Bray-Curtis, 9,999 iterations; Anderson et al., 2006). In preliminary analyses, we found no significant differences in the composition of grasslands between the two studied areas, but within grasslands below and above the natural treeline (henceforth lower alpine and upper alpine grasslands, respectively). We therefore restricted randomizations of survey years within each group of grasslands. To assess whether the temporal dynamics of grasslands was influenced by past grazing regime (low, medium and high) of grasslands, the multivariate analysis was repeated by including the interaction between this factor and survey year. All these analyses were done in R (R Development Core Team, 2011) by using 'metaMDS', 'vegdist', 'ordisurf' and 'adonis' functions from the VEGAN package (Oksanen et al., 2013).

For each trait and survey year, we calculated the community weighted mean value (CWM; Garnier et al., 2004) as:

$$CWM = \sum_{i=1}^{S} p_i x_i \qquad [1]$$

where *S is* the total number of species within a given grassland p_i ,is the relative abundance of species i , and x_i is its trait value. Differences in the CWM of traits between the 1990s and 2010s were tested with paired Wilcoxon signed-rank tests. In the case of categorical traits, we performed tests for each category, separately.

Species-level analyses

Plant species were classified as 'increaser', 'decreaser' and 'no change' according to their change in the median abundance (across sites) between decades. Predicted values of sampling error were used as confidence intervals, so that only those species whose change in abundance between surveys was higher than the predicted error were considered as true increasers or decreasers.

Difference among response groups regarding categorical traits were tested by means of contingency tables, and chi-square tests, with Bonferroni correction for *post hoc* comparison between response groups. Trait values with a low number (<5) of counts were ignored for analyses, and *P*-values were estimated by Monte-Carlo simulations (999 iterations) to avoid incorrect approximations. The chi-square test assumes the independence between data points, however, closely related species are prone to share more biological traits than do distant ones due to their common evolutionary history (Felsenstein, 1985). The correlation of trait values along the phylogeny (henceforth phylogenetic signal) was tested with '*phylo.signal.disc*' (999 randomizations), a function developed in R by E. Rezende. This function reconstructs the ancestral state of traits by parsimony to calculate the number of transition from one state to another across the phylogeny. The number of evolutionary transitions is then compared with a null-model obtained by shuffling trait values across species. This analysis was conducted on the basis of a phylogenetic tree resolved at genus level obtained in a previous study (Pardo et al. unpublished, see Appendix S1). All functions for phylogenetic analyses were obtained from APE (Paradis et al., 2004), GEIGER (Harmon et al., 2008), PICANTE (Kembel et al., 2010) and PHYTOOLS (Revell 2012) R-packages.

For traits with significant phylogenetic signal, original counts in contingency tables were substituted by values (the nearest integer) of the taxonomic distinctness index (TD; Clarke & Warwick, 1998) to account for the phylogenetic relationship among species (Pyšek et al., 2010; Hempel et al., 2013). Values of TD were computed with 'taxondive' from VEGAN package.

Differences in quantitative traits among response groups were tested with ANOVA, and *post hoc* Tukey's tests for pairwise comparison when original or transformed data met assumption of normality and homocedasticity. Otherwise, trait differences were assessed by means of Kruskal-Wallis tests, and *post hoc* Dunn's tests. In this case, the strength of the phylogenetic signal was evaluated according to Pagel's λ (1999), as calculated with 'phylosig' function.

Results

Community-level change

Changes in species composition and abundance of alpine grasslands were relatively small between the 1990s and 2010s (Fig. 1), and not statistically significant (multivariate analysis of variance; d.f. = 1, $F = 0.16$, $R^2 = 0.1$, $P > 0.05$). In the case of grasslands annually monitored, the magnitude of the compositional shift between surveys was year-to-year fluctuations. Some of these natural fluctuations were also larger than the compositional shift observed in most other grasslands (Fig. 1).

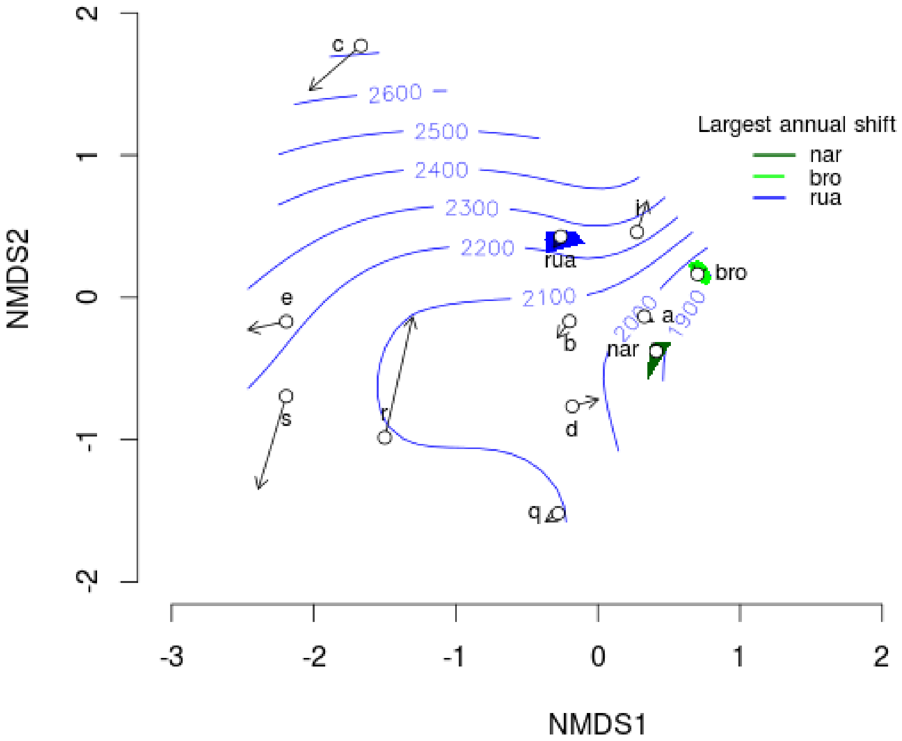

Fig. 1 Non-metric multidimensional scaling (NMDS; stress = 0.01) ordination of grasslands communities between the 1990s (white dots) and 2010s (arrow tips). The length of the arrows indicate the magnitude of the compositional shift of grasslands. Three of the grasslands (nar, bro, rua) were also annually monitored, and their trajectories over the two decades are represented with convex hulls. Their largest compositional shift observed between successive years is drawn as a reference for interpreting the compositional shift between decades. Blue lines state the elevation (m a.s.l) of grasslands fitted onto the NMDS. See Table 1 for code and description of grasslands.

Vegetation composition of grasslands in the 1990s was significantly determined by elevation (df = 8.8, F = 200.10, deviance explained = 96%, *P*-value < 0.001), and the elevational pattern of distribution of grasslands was similar two decades after. Figure 1 shows that only the uppermost grasslands “migrate” downwards in the NMDS ordination. Despite the marked differences in the composition of grasslands along elevation, there were no significant differences in the long-term trajectories of lower and upper alpine grasslands (multivariate analyses of variance; only the interaction effect is reported: df = 1, $F = 0.13$, $R^2 < 0.1$, P-value > 0.05). Temporal trajectories of grasslands were not significantly related to their grazing regimes either (multivariate analyses of variance; only the interaction effect is reported: df = 1, $F= 0.15$, $R^2 < 0.1$, $P > 0.05$).

There were no significant differences in CWM of any trait between the 1990s and 2010s (see Appendix S2 for results).

Species-level change

The number of species recorded in grasslands decreased from 153 in the 1990s to 140 in the 2010s. Several species (n=31) were only recorded in the 1990s, but their presence was visually confirmed in the surroundings of vegetation transects during the resurvey. In contrast, 18 species were only recorded in the 2010s.

The sampling error performed with the point intercept method was high, and significantly distorted the temporal change in abundance of species (52.3% explained deviance, df= 7.8, F= 16.9, $P < 0.001$). Figure 2 shows that this error was higher for the less abundant species, including those only recorded in one of the surveys. After accounting for the sampling error, we found that 27% of total recorded species (n=171) increased in abundance and 17% decreased between the 1990s and 2010s, whereas 56% did not change in abundance more than

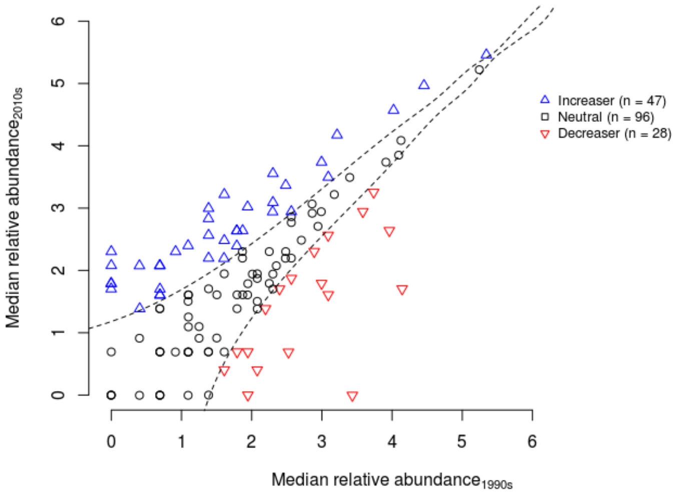

Fig. 2 Species abundance change in grasslands between the 1990s and 2010s. Axis values are multiplied by 1000 and log-transformed for illustration purposes. Dashed lines delimit the predicted change in abundance of species between decades due to sampling error.

predicted by sampling error alone (Fig. 2). These percentages were substantially different when the sampling error was ignored (44% increasers, 53% decreasers and 3% no change species).

Only three true increasers (*Festuca nigrescens, Festuca gautieri* and *Nardus stricta*) and two decreasers (*Briza media* and *Hippocrepis comosa*) were relatively abundant in the early 1990s. The change in abundance was greater for decreaser species (Fig. 1): *Ranunculus gouanii, Briza media, Calluna vulgaris, Silene acaulis, Astragalus sempervirens* and *Hippocrepis comosa*. In contrast, the change in increaser species (specially abundant ones) was smaller. No shrubs or trees species were increasers.

Overall, species from different response groups were similar regarding their distributional and biogeographic affinities (Table 3). The only apparent difference, though not statistically significant, was the higher frequency of subalpine species in increasers (Fig. 3, Table 3). Differences between species groups regarding ecological and biological traits were also non-significants (Table 3). Five of these traits (life-form, ecological guild, plant propagation, long-distance dispersal syndrome and pollination system) were significantly correlated across the phylogeny ($P < 0.05$, see result in Appendix S3). We therefore repeated analyses with phylogenetically corrected tests. Accounting for the phylogeny produced different results only in the case of dispersal syndrome (Table 3). Figure 3 shows that almost half of the increasers have structures for the long-distance dispersal, whereas wind dispersal was relatively infrequent in this group. In contrast, the frequency of anemochory was higher in the other response groups. However, *post hoc* comparison between groups revealed that only the difference between increaser and stable species was significant (chi-square with Bonferroni correction; increaser vs. no change $P < 0.01$; increaser vs. decreaser $P = 0.09$).

Finally, we detected that plant height and Jacob's selectivity index was higher in increasers and decreasers than in stable species (Fig. 3). However, this trend was not significant (Table 3).

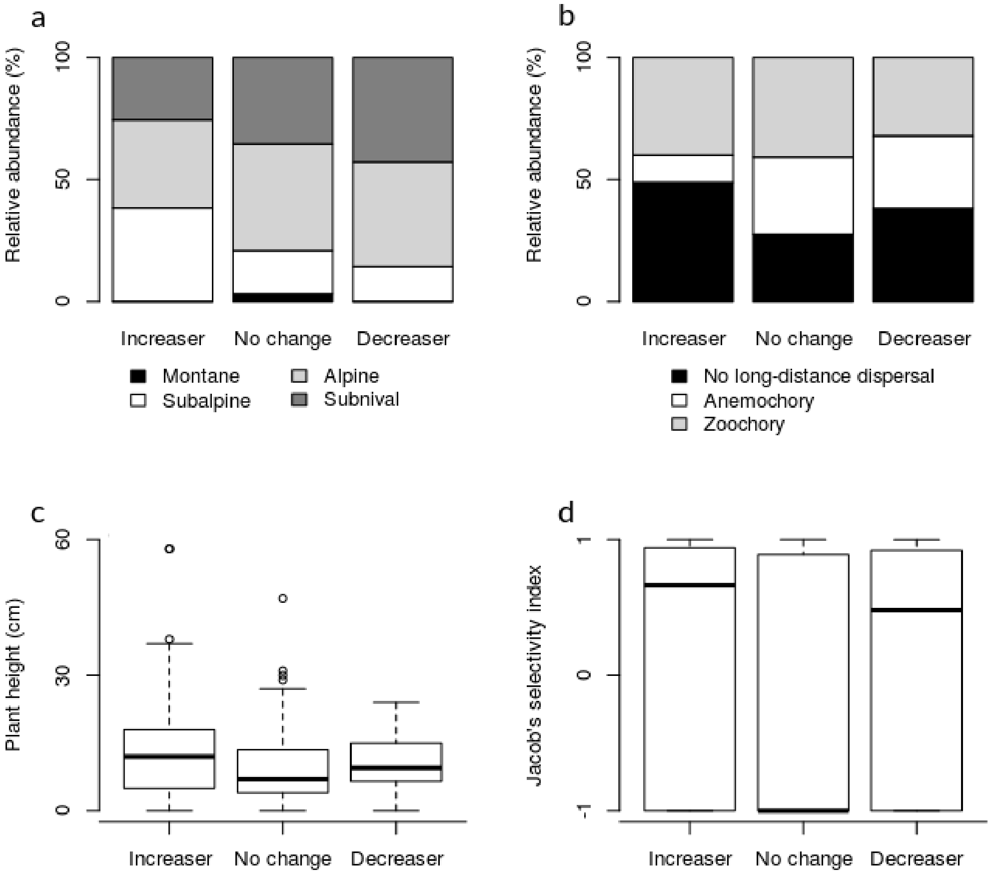

Fig. 3 The percentage of species of each response group within categories of species uppermost elevation belt (a), and long-dispersal syndrome (b). In the lower panel, boxplots of plant height (c) and the Jacob's selectivity index (d) are shown for each response group. See Table 3 for result of the test for the difference among response groups regarding these traits.

Table 3. Trait differences between response groups of species, defined according to their change in median relative abundance between the 1990s and 2010s. Test statistics are indicated in the footnote. See Table 1 for description of traits.

Trait	Trait mean value / dominant trait category			Differences between species groups		
	Increaser (n=47)	No change (n=96)	Decreaser (n=28)	Test	Statistic	*P*
Maximum elevation (m)	2495	2613	2619	1	1.85	ns
Elevation range (m)	1645	1436	1646	2	5.52	ns
Species uppermost belt	Subalpine/ alpine	Alpine	Subnival	3	8.92	ns
Chorology	Eurosiberian	Alpine	Eurosiberian	3	7.83	ns
Range limit	Not at limit	Not at limit	Not at limit	3	2.05	ns
Regional abundance	35	34	40	2	0.60	ns
Local abundance	0.65	0.33	0.20	2	4.00	ns
Ecological guild	Forb	Forb	Forb	3/4	7.85/1.12	ns/ns
Life-form	Hemicrypto.	Hemicrypto.	Hemicrypto.	3/4	0.28/0.19	ns/ns
Plant propagation	Mixed	Mixed	Mixed	3/4	0.53/2.22	ns/ns
Pollination system	No insect pollination	No insect pollination	Insect and wind pollination	3/4	4.32/3.00	ns/ns
Dispersal syndrome	No long-dist.	No long-dist.	No long-dist.	3/4	4.67/11.03	ns/*
Plant height (cm)	17	11	10	2	4.74	ns
Jacob's selectivity index	0.13	-0.20	0.14	2	4.66	ns

*$P < 0.05$; non-significant ($P > 0.05$; ns)

[1]ANOVA (2 df; F-value); [2]Kruskal-Wallis (2 df; K); [3]Chi-square test (999 iterations); [4]Phylogenetically corrected Chi-square test (999 iterations, see Hempel et al. 2013). [5]Wilcoxon signed-rank test (paired samples; V)

Discussion

We found little evidence of change over the last two decades in 12 alpine grasslands of the Central Pyrenees, an area that has undergone a drastic reduction of grazing and concomitant climate warming. Changes in vegetation composition of most grasslands were subtle and smaller than can be expected due to natural fluctuations, and more than 50% of species remained stable after two decades. In addition, and given that the sampling error was particularly high for less abundant species, it is unlike that missing species in one of the surveys were true local extinctions or colonizations. Altogether, results at community and species level point to a relative stability. Therefore, our results does not match general expectations of a rapid compositional shift in response to grazing reduction (Milchunas et al., 1988; Westoby et al., 1989; Olff & Ritchie, 1998), and they contrast with the short-term response of other alpine and tundra grasslands to herbivory exclusion or simulated warming (Klanderud & Totland, 2005; Walker et al., 2006; Mayer et al., 2009; Borer et al., 2014).

The slow dynamics of plant communities is often attributed to the longevity of species and short-growing periods (Eriksson et al., 2002; Wilson & Nilsson, 2009; Kullman, 2010), that help to keep stable trends in plant populations (Morris et al., 2008). However, we suggest that these mechanisms alone may be insufficient for explaining the relative stability of studied grasslands. In fact, other grasslands at similar elevations have undergone drastic changes such as a rapid expansion of shrubs and/or tree re-growth under similar environmental conditions (Cannone et al., 2007; Vittoz et al., 2009 Komac et al., 2011; Gartzia et al., 2014). However, we did not detect a single seedling of *Pinus uncinata* (the only treeline-forming species) or dominant shrubs in the 2010s. This result is somehow surprising given the absence of topographic factors limiting the arrival of tree and shrub propagules, at least, in the grasslands located close to the subalpine treeline (Gartzia et al., 2014).

Grazing reduction is usually coupled with an effective increase in competition for light (Olff & Ritchie, 1998; Adler et al., 2004; Pavlů et al., 2005; Borer et al., 2014). However, we found no indirect evidences supporting such increase. In fact, the CWM of plant height was not statistically different between the 1990s and 2010s. None of the traits with stronger association to grazing (Jacob's index; life form, plant height; see De Bello et al., 2005; Evju et al., 2009) shifted in abundance (i.e. CWM) since the 1990s, or presented statistically different frequencies between response groups. Taken together, these results, and the lack of shrub expansion (Speed et al., 2012; Kaarlejärvi et al., 2013), may suggest the persistence of grazing. At first glance, this may seem contradictory given the drastic reduction in the number of domestic herbivores in the study area. However, we suggest that present-day grazing, though residual from a historical perspective, might has been enough to prevent larger changes in vegetation of grasslands between the 1990s and 2010s.

Grazing might has also buffered the impacts of climate warming (Speed et al., 2012, Pardo et al. 2015), which may help to explain why most traits indirectly related to climatic niche of species (i.e. elevation range, biogeography and geographic distribution) were stable over two decades. This finding contrasts with the short-term change reported in many European summit floras, including some of the Ordesa-Monte Perdido National Park (Erschbamer et al., 2008; Gottfried et al., 2012). Grazing provides one potential explanation for explaining the contrasting sensitivity to climate warming between studied alpine grasslands and summits (which are only grazed occasionally) in the Ordesa-Monte Perdido National Park.

The methodological approach used in this study provides another possible explanation for our conservative results. In fact, we found that natural fluctuations in communities dominated by long-lived species and at high elevation were higher than it is often assumed. Likewise, the sampling error was unexpectedly high even if our transects were semi-permanent. If we have not accounted for these two sources of uncertainty our interpretation of data had definitely been different, and most probably exaggerated (for instance the number of increaser and decreaser species were two and three times higher, respectively, when sampling error was not considered). The same may hold for other resurvey studies, which may need some reinterpretation. While it would be necessary exhaustive basic research for better understanding the baseline change of biodiversity (Magurran et al. 2010), the sampling error would easily be measured in existing monitoring initiatives (e.g. GLORIA) with a little effort (Conway-Cranos & Doak, 2011).

Conclusion

Taken together, our results suggest that two decades were insufficient for reverting the ecological inertia of the historical land-use in alpine grasslands, but also that the effects of environmental drivers of change were unexpectedly weak at community, species and trait level during this period. In particular, we have shown that response of alpine grasslands to grazing reduction can deviates from general expectations, thus challenging our ability to make accurate general predictions about the fate of these ecosystems. Although the background of global change in the study area, the Central Pyrenees, is similar to that in many other European mountains, the number of grasslands resurveyed for this study clearly limits our ability to generalize results. However, it is unlike that the mechanism underlaying the long-term stability of grasslands were exclusive of our study area. Future research on the factors limiting tree and shrub expansion (including herbivory) in alpine areas would provide an avenue to anticipate contrasting dynamics in alpine grasslands.

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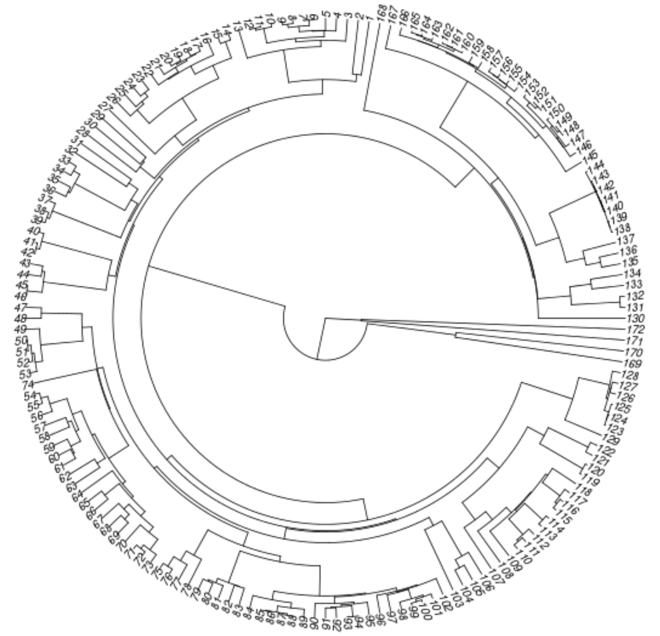
Appendix S1. List of species and phylogenetic relationship of species recorded in grasslands during field surveys in the 1990s and 2010s. The phylogenetic tree was pruned from a megaphylogeny of the Pyrenean flora obtained for a previous study (Parde et al. unpublished), where it build following the procedure detailed in Roquet et al. 2013 *Ecography*.



1. Urtica dioica
2. Rhamnus pumila
3. Sanguisorba minor
4. Potentilla alchemilloides
5. Potentilla aureaaurea
6. Potentilla crantzii
7. Potentilla erecta
8. Potentilla neumanniana
9. Potentilla nivalis nivalis
10. Alchemilla alpigena
11. Alchemilla flabellata
12. Alchemilla glabra
13. Geum montanum
14. Oxytropis neglecta
15. Astragalus sempervirens
16. Vicia pyrenaica
17. Trifolium alpinum
18. Trifolium montanum
19. Trifolium pratense pratense
20. Trifolium repens
21. Trifolium thalii
22. Medicago suffruticosa
23. Anthyllis montana
24. Anthyllis vulneraria
25. Lotus corniculatus
26. Hippocrepis comosa
27. Polygala alpina
28. Salix reticulata
29. Viola biflora
30. Viola rupestris rupestris
31. Helianthemum nummularium
32. Thymelaea tinctoria nivalis
33. Draba aizoides aizoides
34. Arabis ciliata
35. Pritzelago alpina
36. Murbeckiella pinnatifida
37. Geranium cinereum cinereum
38. Geranium pyrenaicum
39. Geranium sylvaticum sylvaticum
40. Saxifraga moschata
41. Saxifraga oppositifolia
42. Saxifraga praetermissa
43. Sedum album
44. Sedum atratum atratum
45. Sempervivum arachnoideum
46. Sempervivum montanum montanum
47. Gentiana alpina
48. Gentiana verna verna
49. Asperula hirta
50. Galium verum verum
51. Galium cespitosum
52. Galium pumilum
53. Galium pyrenaicum
54. Acinos alpinus
55. Thymus praecox polytrichus
56. Horminum pyrenaicum
57. Scutellaria alpina
58. Sideritis hyssopifolia
59. Ajuga pyramidalis
60. Teucrium chamaedrys
61. Teucrium pyrenaicum
62. Euphrasia alpina
63. Pedicularis pyrenaica
64. Linaria alpina
65. Globularia cordifolia
66. Globularia repens
67. Veronica alpina
68. Veronica arvensis
69. Veronica nummularia
70. Plantago alpina
71. Plantago lanceolata
72. Plantago media
73. Plantago monosperma monosperma
74. Myosotis alpina
75. Seseli montanum nanum
76. Meumathamanticum
77. Conopodiummajusmajus
78. Bupleurum ranunculoides
79. Campanula cochlearifolia
80. Campanula scheuchzeri
81. Phyteuma orbiculare
82. Jasione crispa
83. Jasione laevis laevis
84. Carlina acaulis caulescens
85. Cirsium acaule acaule
86. Cirsium eriophorum
87. Carduus carlinifolius
88. Carduus carlinoides carlinoides
89. Serratula tinctoria
90. Tragopogon lamottei
91. Crepis pygmaea pygmaea
92. Taraxacum dissectum
93. Leontodon hispidus hispidus
94. Leontodon pyrenaicus pyrenaicus

95. *Pilosella lactucella*
96. *Doronicum grandiflorum*
97. *Achillea millefolium*
98. *Aster alpinus*
99. *Erigeron alpinus*
100. *Erigeron uniflorus*
101. *Solidago virgaurea*
102. *Antennaria dioica*
103. *Calluna vulgaris*
104. *Androsace villosa*
105. *Androsace vitaliana vitaliana*
106. *Primula integrifolia*
107. *Chenopodium bonus-henricus*
108. *Paronychiakapela*
109. *Minuartia verna*
110. *Arenaria grandiflora grandiflora*
111. *Arenariamoehringioides*
112. *Arenaria purpurascens*
113. *Cerastium arvense*
114. *Cerastium fontanum vulgare*
115. *Silenea caulis*
116. *Silene ciliata*
117. *Silene vulgaris*
118. *Saponaria caespitosa*
119. *Armeria alpina*
120. *Armeria pubinervis*
121. *Polygonum aviculare*
122. *Polygonum viviparum*
123. *Ranunculus acris*
124. *Ranunculus amplexicaulis*
125. *Ranunculus bulbosus*
126. *Ranunculus carinthiacus*
127. *Ranunculus gouanii*
128. *Ranunculus parnassiifolius*
129. *Thalictrum alpinum*
130. *Merendera montana*
131. *Allium senescens montanum*
132. *Allium sphaerocephalon sphaerocephalon*
133. *Iris latifolia*
134. *Crocus nudiflorus*
135. *Luzula campestris*
136. *Luzula nutans*
137. *Luzula spicata*
138. *Carex caryophyllea*
139. *Carex curvula*
140. *Carex macrostyla*
141. *Carex ornithopoda*
142. *Carex parviflora*
143. *Carex rupestris*
144. *Kobresia myosuroides*
145. *Bromus erectus erectus*
146. *Anthoxanthum odoratum*
147. *Helictotrichon sedenense*
148. *Koeleria pyramidata*
149. *Koeleria vallesiana vallesiana*
150. *Trisetum flavescens baregense*
151. *Agrostis capillaris capillaris*
152. *Agrostis rupestris*
153. *Briza media media*
154. *Alopecurus gerardii*
155. *Phleum alpinum*
156. *Poa alpina*
157. *Poa supina*
158. *Dactylis glomerata*
159. *Deschampsia flexuosa*
160. *Festuca eskia*
161. *Festuca gautieri*
162. *Festuca glacialis*
163. *Festuca nigrescens*
164. *Festuca pyrenaica*
165. *Festuca rubra*
166. *Sesleriacoerulea*
167. *Nardus stricta*
168. *Borderea pyrenaica*
169. *Pinus uncinata*
170. *Juniperus communis*
171. *Botrychium lunaria*
172. *Cystopteris fragilis*

Appendix S2. Temporal changes in mean community-weighted mean of traits between

Table S2. Results of paired Wilcoxon signed-rank tests for the difference in community-weighted mean value of traits observed in the 1990s and 2010s.

Trait		V	*P*	Trait		V	*P*
Maximum elevation (m)		47	0.57	Life-form	Phanerophytes	3	0.37
Elevation range (m)		41	0.91		Chamaephytes	37	0.36
Species uppermost belt	Montane	10	0.56		Hemicryptophytes	30	0.52
	Subalpine	18	0.11		Geophytes	27	0.65
	Alpine	57	0.18		Terophythes	10	0.55
	Subnival	35	0.79	Plant propagation	Sexual	47	0.57
					Mixed	32	0.62
Chorology	Widespread	56	0.20		Vegetative	25	0.81
	Boreoalpine	17	0.09	Pollination system	Insect and wind pollination	32	0.62
	Eurosiberian	48	0.15		insect pollination	58	0.15
	Mediterranean	31	0.90		No insect pollination	29	0.50
	Alpine	46	0.62	Dispersal syndrome	Zoochory	39	1
	Pyrenean endemism	32	0.29		Anemochory	35	0.8
Range limit	At range limit	47	0.57		No long-distance dispersal	49	0.47
	Not at range limit	30	0.84	Plant height (cm)		31	0.57
Regional abundance		41	0.91	Jacob's selectivity index		28	0.42
Local abundance		43	0.79				
Ecological guild	Tree	–	–				
	Shrub	3	0.37				
	Forb	46	0.62				
	Legume	56	0.20				
	Graminoid	27	0.38				

Appendix S3. Test for the correlation of traits along the phylogeny.

Correlation of categorical traits was tested with '*phylo.signal.disc*' (999 randomizations), a function developed in R by E. Rezende. Phylogenetic signal of continuous traits was evaluated according to Pagel's λ (1999), as calculated with 'phylosig' in the PHYTOOLS R-package (Revell 2012).

Table S3. Results of test for phylogenetic signal. Min and Max, are the maximum evolutionary transition observed in a null-model (999 iterations) obtained by shuffling trait values at random.

Trait	Transitions observed [min, max]	*λ*	*P*
Maximum elevation (m)		0	1
Elevation range (m)		0	1
Species uppermost belt	79 [69,88]		0.51
Chorology	89 [85,195]		0.02
Range limit	41 [33,49]		0.26
Regional abundance		0	1
Local abundance			
Ecological guild	7 [39,48]		0
Life-form	40 [46,59]		0
Plant propagation	47 [46,66]		0.001
Pollination system	23 [70,92]		0
Dispersal syndrome	31 [47,58]		0
Plant height (cm)		0	1
Jacob's selectivity index		0	1

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Chapter 5

Long-term response of plant communities to herbivore exclusion at high elevation grasslands

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Abstract

Understanding the effects of herbivores on plant communities is needed for anticipating how variation in grazing regimes will impact natural and semi-natural ecosystems. Prominent ecological hypotheses predict that drastic reductions of herbivory, as have occurred in many European mountains, will trigger fast diversity loss and structural changes in grasslands, because grazing tolerant species are expected to be rapidly replaced by taller and more competitive species. The aims of this study were to test generalizations of herbivory effects (mostly from lowlands) in high elevation grasslands over ecological relevant times scales, and to unravel the contribution of climatic conditions to observed changes. Species richness, canopy height, plant community structure, and transition probabilities among ecological groups were monitored throughout 19 years in herbivore exclusions and control plots in the Central Pyrenees. We used ordination analyses to track the long-term community response to herbivore exclusion, and generalized additive models to assess the non-linear effects of herbivore exclusion and climatic conditions on measured variables. Contrary to expectations, herbivore exclusion did not significantly affect species richness, and although canopy height increased, it was not drastic enough to suppress shade intolerant species. The strongest shifts in plant community structure and transition probabilities between ecological groups occurred during a sequence of warm and dry growing seasons, whereas in control plots, these changes were smaller, and largely reversed after cooler and wetter climatic conditions returned. Our results suggest that long-term effects of grazing cessation in high elevation grasslands can be weaker and slower than predicted. However, these effects can act synergistically with dry and warm events. Therefore, the maintenance of past grazing activities can be key in the face of ongoing climatic warming.

Introduction

Understanding the community effects of herbivory is a pivotal issue for the management of healthy plant communities. Many human activities shift the pattern of herbivore effects in both natural and semi-natural ecosystems worldwide. Therefore, these effects are of particular concern for understanding the optimal management of domestic herbivores as well as for defining conservation policies (Noy-Meir et al. 1989; Westoby et al. 1989; Hobbs and Huenneke 1992). In some areas, the main management question is how to lessen grazing pressure (e.g. Zhao and Zhou 1999), while in others, economic shifts have resulted in rapid reduction of herbivory that may have equally negative effects on plant diversity and foraging value of grasslands (e.g. Wells 1969; Stohlgren 1999; Tasser and Tappeiner 2002).

While herbivory has been one of the most intensively studied ecological interactions (McNaughton 1985; Milchunas et al. 1988; Huntly 1991), ecologists have crafted a wide range of sometimes opposing generalities about herbivore effects on plant communities and when and why these effects will be strong or weak (Milchunas et al. 1988; Westoby et al. 1989; Olff and Ritchie 1998; Proulx and Mazumder 1998). During the last decades, the Generalized Grazing Model (GGM) has provided the most prominent theoretical framework for understanding the variable impact of herbivory across different plant communities (Oesterheld and Semmartin 2011). The GGM is largely based on the hump shaped response of plant richness to disturbance (i.e. grazing) predicted by the 'Intermediate Disturbance Hypothesis' (Grime 1973). However, the GGM suggests that the strength of the response of plant communities to grazing will be different across gradients of precipitation and evolutionarily history of grasslands. In particular, the GGM predicts that competition between plant species shifts from soil resources to light along gradients of humidity (Milchunas et al. 1988), and primary productivity (Cingolani et al. 2005). Therefore, largest effects of herbivory on plant richness are expected in sub-humid and/or productive areas (Milchunas et al. 1988; Milchunas and Lauenroth 1993). The model also predicts that plant communities with long grazing histories will rapidly shift in response to large changes in grazing intensity, because they have evolved under divergent selection for grazing and canopy dominance, and developed pools of native species adapted to different levels of herbivory. Given that non-resilient species were eliminated from the initial species pool long ago, the predicted structural changes are likely to be more reversible than those occurring in communities with a short grazing history (Cingolani et al. 2005).

Empirical support for the GGM is mostly from low elevation grasslands and shrublands

(Milchunas and Lauenroth 1993), whereas the herbivore impact on high elevation grasslands remains relatively less thoroughly studied (Erschbamer et al. 2003). This fact contrasts with the key role that wild and domestic herbivores play on the maintenance of highly valuable ecological and cultural landscapes at high elevations (Austrheim and Eriksson 2001; Chemini and Rizzoli 2014; Fillat et al. 2008), which in some cases are alarmingly endangered (Habel et al. 2013). In European mountains, the shift in socio-economic conditions of rural societies has weakened seasonal grazing to historical minimums, including the complete abandonment of many pastures (García-Ruiz et al. 1996; Luick 1998; MacDonald et al. 2000, Zervas 1998). As a result, forest and shrubs have encroached into semi-natural grasslands (Welch and Scott 1995; Gehrig-Fasel et al. 2007; Améztegui et al. 2010; Pajunen et al. 2012), and even more drastic changes to these ecosystems have been predicted (Sala et al. 2000).

Several studies conducted to address the specific impact of cessation of grazing on mountains have reported a decline in species richness (Rawes 1981; Tasser and Tappeiner 2002; Niedrist et al. 2008; Speed et al. 2013), whereas a few others have shown no changes in species richness, but rapid changes in the community composition and structure (Hill et al. 1992; Hope et al. 1996; Krahulec et al. 2001; Mayer et al. 2009). The lack of consistent results along with the particularly slow response of alpine plant species (Evju et al. 2009) limit our current understanding of the long-term effects of grazing reductions. To further complicate matters, the land-use change is coincident with climate warming in mountains (Dessens and Bücher 1997; Beniston 2006). As disentangling their separate effects is not easy under natural conditions, the interaction between these two drivers of change is still understudied at high elevation. However, this gap needs to be addressed to forecast the biological consequences of grazing reduction and to guide successful management of high elevation grasslands, particularly in the face of ongoing climate change.

In this study, we experimentally assess the long-term impacts of herbivore exclusion on plant species richness, canopy height, and relative abundance of species in some of the most common grassland communities of the Pyrenees (Fillat et al. 2008). The studied grasslands can be defined as sub-humid (annual precipitation *1700) and long grazing history systems (Chocarro et al. 1990) following the classification criteria used in Milchunas and Lauenroth (1993). For this sort of grasslands, the GGM predicts a rapid shift in community structure towards taller and more competitive species in response to grazing cessation, and in some cases, a decline in species richness via competitive exclusion (Milchunas et al. 1988). If a few dominant grasses increase at the expense of forbs and shade intolerant grasses, we would also expect a decline in species richness (Milchunas et al. 1988; Hobbs and Huenneke 1992) and pasture value for livestock (Crawley 1983). We also expect that structural and diversity impacts of herbivore exclusion will be faster and more dramatic if climate-induced migration of high competitive lowland species (e.g. shrubs) alters the pool of available species (Olofsson et al. 2009; Speed et al. 2012). In contrast, whether climate-driven impacts would alter the equilibrium composition of grazed grasslands is more uncertain (Vittoz et al. 2009), and will depend on whether climate can override the effects of herbivory (Post and Pedersen 2008).

Methods

Study site

The study sites were located in the Ordesa-Monte Perdido National Park, Central Pyrenees, Spain (42°40'18'' N, 0°3'20'' E). This mountain park extends along five valleys radiating from the highest calcareous summit in Europe (3355 m a.s.l.), covering an elevational gradient of ~2400 m. Subalpine and alpine grasslands cover more than one third of the total area of the National Park (15608 Ha.), and have been used as summer pastures for livestock since at least the fifteenth century (Fillat et al. 2008), and probably into ancient pre-history. However, this traditional land-use has been dramatically modified owing to socio-economic shifts in the Pyrenean region during the last century, with the total number of domestic herbivores (mainly sheep) drastically diminished since the 1950s (García-Ruiz et al. 1996; Aldezabal 1997).

In 1992, two exclosures of 144 m^2 each were established to assess the effect of grazing abandonment on plant diversity and community composition and structure in high elevation grasslands. They were selected to represent the two most abundant types of grasslands along the Pyrenees (Fillat et al. 2008): *Bromion erecti* (henceforth Bromion) and *Nardion strictae* (henceforth Nardion). The former harbors high species richness (>40 sps) and plants of high fodder value (e.g. *Lotus corniculatus*, *Medicago suffruticosa* and *Trifolium* spp.) whereas the latter has lower species richness (<20), is usually dominated by the tussock-forming grass *Nardus stricta*, and poorer in terms of nutrient quality (Gómez 2008). In both communities *Festuca nigrescens* was the most abundant species at the beginning of the study. For Bromion communities, the mean aboveground net primary production was 483 g of dry matter per m^{-2} year^{-1}, and 378 for Nardion (García-González et al. 2002). Such values correspond to productive grasslands following the classification criteria of the GGM in Milchunas and Lauenroth (1993).

Exclosures are located in a flat area (1930 m a.s.l.) 600 and 450 m distant from the nearest subalpine forest limit and shrubland, respectively, and separated each other by 125 m. The area was frequently visited by domestic herbivores (mostly cattle) for summer pasturing (from July to October) and a very few wild ungulates. Activity of other small herbivores (e.g., marmots and moles) was negligible in study area. The stocking rate was relative high throughout the study: 0.79 LU ha^{-1} (1LU = 1.25 cow = eight sheep) (Aldezabal et al. 1992).

The monitored grasslands share most topographic and environmental features, such as N aspect, length of vegetative period (Del Barrio et al. 1990), mean annual temperature (5 °C) and precipitation (*1700 mm). However, edaphic conditions were different: the soil of Bromion was basic, high permeable and with moderate levels of organic matter, whereas that of Nardion was acidic (pH 4), humic, with high levels of organic matter and water retaining capacity (Badía et al. 2002).

Climatic data

We gathered daily temperature, precipitation, and snow depth records between 1982 and 2012 from the nearby Góriz Meteorological Station (42º39’49''N, 00º00'50''E; 2215 m a.s.l.). In 2006, we also set up a Station at the Nardion site, which was used to validate the temperature records from Góriz, and to fill the gaps in the temperature series. We calculated the mean, maximum and minimum temperature, and the mean thermal sum (measured in degree days) for each year, summer (from June to September), snow free period and growing season, defined as the span between the snow melt-out and the exact date of vegetation sampling (between July 28th and August 1st). For each of these periods, we also calculated the accumulated precipitation.

Vegetation sampling

Between 1993 and 2012 (with the exception of 1999), we annually monitored the vegetation community within the exclosures by using the point intercept method along two perpendicular transects (10 m long each) at each site. At each semi-permanent sample point (n = 200, spaced every 10 cm along the transects), we recorded the height and species of the tallest plant. When the point was not covered (e.g. bare soil, dung, or litter), it was registered as a ‘non-plant’. Control transects were also established outside the exclosure, where the same sampling procedure was applied. This yielded a total of 400 sample points per site. The abundance of each species was calculated as the number of points covered by the species out of the total number of points per treatment (exclosure *vs* control). The sum of the heights of all recorded plant individuals was used as a surrogate of canopy height. Community structure was defined according to the composition and abundance of species.

Although the beginning and end of transects were permanently marked with sticks, we could not assume that each sample point was exactly relocated every year. Even if we did, it is unlike that the same plant individuals were always recorded, due to the small size of plants, their architecture, microtopography, and simple inaccuracy. While inaccuracy does not influence the relative abundance estimates, it might result in serious sampling error for analyses that track transitions between plants at each point (Conway-Cranos and Doak 2011). To account for such potential bias, we estimated the sampling error in point relocation, following Conway-Cranos and Doak (2011). One of the two transects at each control site was successively resampled three times in a single day in 2011. Each time, we removed and replaced the transects to reproduce the alignment process in annual resampling. Using the resulting data in combination with the annual sampling allowed us to correct transition estimates for sampling uncertainty (Conway-Cranos and Doak 2011).

Data analyses

Climate trends in annual mean temperature and precipitation were tested with the Mann–Kendall test (Mann 1945; Kendall 1975). For vegetation analyses, we first analyzed the effect of grazing exclusion on species richness, canopy height and abundance of dominant species over the 19 years by using the following Generalized Additive Model (GAM; Wood 2006; Hastie and Tibshirani 1990):

$$y_i = \alpha + \beta \, treatment + f(x_i) \, treatment + \varepsilon_i \qquad [1]$$

where y is the response variable in year i, α the intercept, β the factor indicating the treatment (control *vs* herbivore exclusion), $f(x_i)$ the smooth function of time, and ε_i the random error. Species richness and the abundance of dominant species were initially modeled using Poisson distribution, whereas data on canopy height was log transformed to fit into a Gaussian distribution. The effect of the grazing exclusion was evaluated as a smooth function of time by using cubic splines (Wood 2006) and the number of knots were selected from three to a maximum of five, iteratively. When we observed that an increase in the number of knots did not lead to a better explanation of the residuals, we selected the lowest number to minimize model overfitting (Wood 2006). Given that annual data within each plot is used as an independent sample, clearly, our sampling design is pseudo-replicated (with one single plot per treatment in each site), so *P* values may be inaccurate. For such reason, *P* values higher than 0.01 were not considered as significant. We used the same approach for testing the interactive effect of climate and herbivore exclusion by substituting the 'time' variable in Eq. (1) with temperature and/or precipitation variables. Inclusion of interaction effects between climatic variables was infeasible because of limited sample size. From the set of candidate climatic variables (see above), we selected thermal sum and accumulated precipitation during the growing season based upon their explanatory power (i.e. the proportion of deviance explained by the model).

To assess the effect of herbivory on the community structure over our 19 years of data, we performed a non-multidimensional scaling ordination based on the species abundances and the Bray-Curtis dissimilarity measure (Bray and Curtis 1957), comparing the trajectories of community structure in exclusion and control plots. Although the Bray-Curtis is a robust measure of plant community response (Faith et al. 1987), the use of any other dissimilarity measures would lead to different ordination results (Anderson et al. 2006), and thus, to different interpretations. To assess the robustness of the observed trajectories of communities, we repeated ordination analyses with (i) two other dissimilarity measures (Euclidean and Horn-Morisita; Horn 1966), (ii) only common species, and (iii) incidence data (see Online Resource 1).

To further characterize the dynamics of grazed and ungrazed communities we used community transition models (Horn 1975; Hill et al. 2004). Because of the high number of species and specific functional groups (geophytes, rosette forbs) with low values at both sites, model construction with individual species or specific functional groups was problematic. Therefore, we decided to parameterize models using four ecological classes: (1) the dominant grass *F. nigrescens*, which was the only tall species (>35 cm based on field averaged field records); (2) other non-dominant grasses; (3) forbs; and (4) non-plants (i.e. bare soil, dung and litter). Non-dominant grasses were long-lived hemicriptophytes with similar leaf morphology, plant propagation and dispersal syndromes, whereas forbs were mostly dominated by highly palatable and small size hemicriptophytes. Nevertheless, and given the existent heterogeneity mostly within forbs (e.g. legumes, rosette forbs, thistles, all of which appeared in very low abundance), transition analyses were only used to identify broad effects of herbivory exclusion, such as gap creation or colonization (Non-plants ↔ other plants), competition relaxation (Dominant grass →

other plants), stability (transition within groups) and ecological changes (Forbs ↔ Grasses). Transition probabilities between each set of groups were estimated using the approach of Conway-Cranos and Doak (2011). Estimation of error-corrected transition rates were conducted using Matlab programs modified from Conway-Cranos and Doak (2011). All other analyses were conducted using R 2.14.1 (R Development Core Team 2011), and 'kendall' (http://www.stats.uwo.ca/faculty/aim/), 'mgcv' (Wood 2006) and 'vegan' (Oksanen et al. 2013) R-packages.

Results

Meteorological records from Góriz Station indicated a significant increasing trend in annual mean temperature since 1981 ($\tau = 0.32$; $P < 0.05$) (Fig. 1). The period between 2001 and 2012 was on average warmer (+0.3 °C) than the previous decade. Most notably, the average (3-years fixed interval) of the thermal sum during the growing season evidenced a warm period between 2002 and 2006 (Fig. 1), whereas the prior and subsequent periods were markedly cooler. The pattern in annual mean precipitation over the last decades was less clear, with no linear trend in precipitation ($\tau = 0.06$; $P = 0{,}67$). However the driest growing seasons were concentrated in early 2000s (Fig. 1).

We did not find any significant ($P < 0.01$) effect of herbivore exclusion on species richness at any of the grasslands between 1993 and 2012 (Table 1). Temporal fluctuations in species richness were also unrelated to temperature and precipitation regimes in both cases. In contrast, canopy height increased significantly in response to herbivore exclusion (Table 1). In the Bromion exclusion, a significant decrease in canopy height was observed during the warm and dry period, which was reversed at the end of the study (Fig. 2). In the Nardion exclusion, on the contrary, canopy height gradually increased throughout the study (Table 1; Fig. 2), although in this case, the effect was not statistically significant ($P > 0.01$). The separate effects of thermal sum and accumulated precipitations on canopy height were significant ($P < 0.001$) in both grasslands, but only in the case of Bromion explained a reasonable amount of the observed variability (Table 1). In this case, the highest temperatures decreased canopy height only in the control plot.

Exclusion of herbivores affected the community structure at both sites, but the differences between control and exclusion plots after 19 years were stronger at Nardion (Fig. 3) The community structure of Bromion diverged considerably from the control in 2001, but it was partially reversed after 2006 (Fig. 3). In Nardion, changes in the community structure after herbivore exclusion were only noticeable after the 8th year (Fig. 3). At the end of the study, control plots approached initial community structures in both cases. Interestingly, interpretation of ordination analyses was consistent regardless of the dissimilarity measure used, and consideration or not of rare species, but not when incidence data was used (see Online Resource 1).

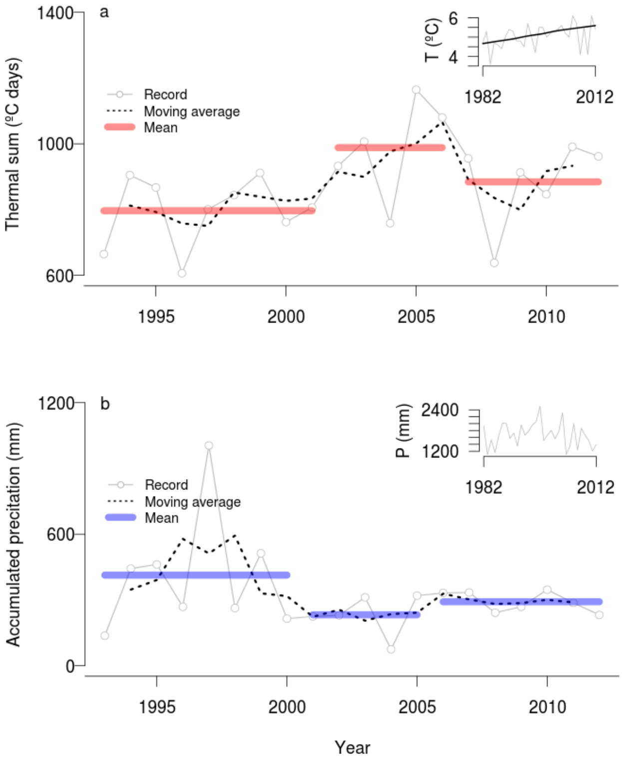

Fig. 1 Trends of thermal sum (a) and accumulated precipitation (b) in the growing season in the Ordesa-Monte Perdido National Park for the last decades. Moving averages (3-years fixed interval) of these variables indicate a warmer period between 2002 and 2006, and a dry period between 2001 and 2005. On average, corresponding bracketing time periods were cooler and more humid, respectively (horizontal lines). Embedded subplots show records of mean annual temperature (a) and precipitation (b) between 1983 and 2012. The black line indicates a significantly increasing trend in annual temperature ($P < 0.05$) according to the Mann–Kendall trend test

The dominant grass *Festuca nigrescens* underwent a markedly different shift in abundance in the different sites and treatments (Fig. 4). At the Bromion site, herbivore exclusion led to declining *F. nigrescens* during the warm and dry period, but that trend was reversed afterwards (Fig. 4). At the Nardion site, the decreasing trend of dominant *F. nigrescens* was gradual and significant since the first years of herbivore exclusion, and mirrored the increase of *Nardus stricta*. As a result, *N. stricta* became the most dominant species at the end of the study. In control plots, temporal fluctuations of the dominant species were less intense that in exclusion plots, and neither of these grasses showed substantial changes in abundance after 19-years.

Table 1 Summary of generalized additive models used to test the effect of herbivore exclusion and climate on species richness and log-transformed canopy height across 19-years

Response variable	Site	Predictor variable	Treatment parameter	Smooth term				Deviance explained (%)
				Control		Exclusion		
				edf	statistic	edf	statistic	
Species richness	Bromion	year	-0.12	1	0.02	1	1.28*	40.7
		T_{sum}	-0.12	1	0.21	1	0.88	38.5
		P_{acc}	-0.12	1	0.06	1	0.63	35.0
	Nardion	year	-0.14	1	1.07	1	0.72	48.6
		T_{sum}	-0.14	1	0.01	1	1.05	40.3
		P_{acc}	-0.14	1	0.98	1	0.10	41.2
Canopy height	Bromion	year	0.69***	1.95	1.63	2.85	5.65**	78.6
		T_{sum}	0.69***	1.70	6.92**	1.77	1.23	75.7
		P_{acc}	0.69***	1	0.77	2.69	0.88	66.3
	Nardion	year	0.32***	2.47	1.73	1	6.78*	50.4
		T_{sum}	0.32***	1.86	3.07	1.82	0.43	45.2
		P_{acc}	0.32***	1	2.06	1	1.50	35.7

Models were formulated using herbivore exclusion as a treatment, and time, growing thermal sum (T_{sum}) and accumulated precipitation (P_{acc}) as predictor variables. Cubic splines were used to account for potential non-linear relationships, with a maximum degrees of freedom of the model (edf) set to five to avoid overfitting. Note that test statistics of the smooth terms are F and Chi squared statistics for species richness' and canopy height's models, respectively

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

Despite the permanent location of transects, our spatial sampling error rates were estimated as 0.64 and 0.33 at the Bromion and Nardion sites, respectively. For the sake of simplicity, and given the importance of the warm and dry period between 2002 and 2006 for community changes, transition probabilities were calculated separately for the warm period and the bracketing cooler time periods (1992–2001 and 2007–2012, respectively), and for each of the two treatments. The main difference in the transition probabilities of the two grasslands was related to grasses, whose probability to be replaced by forbs was higher than that of any other ecological groups in Bromion. In contrast, grasses were the most persistent group in Nardion. Regardless of the climatic period, we found more stable transitions (i.e. less transitions between groups) in presence of herbivores at both sites (Fig. 5). At the Bromion site, largest changes in transition probabilities in response to herbivore exclusion took place between 2002 and 2006, when the transition from the dominant grass and other grasses to forbs increased, as did the persistence of forbs relative to preceding time period. Conversely, there was a decrease

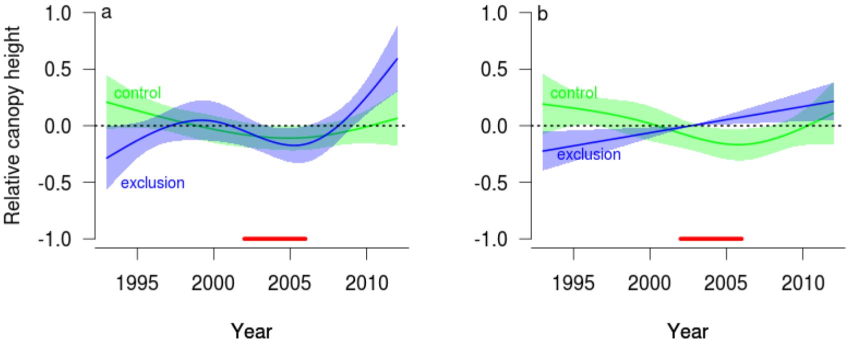

Fig. 2 Effect of herbivore exclusion on log-transformed canopy height in the Bromion (a) and Nardion (b) grasslands across 19-years. Shaded area represents 95 % confidence intervals for control and exclusion plots. Canopy height values are centered on 0 to facilitate comparison between sites and plots. Red line highlights an exceptionally warm and dry period detected between 2002 and 2006

in the transition from all groups towards *F. nigrescens* (Fig. 5). These changes were detectable at both control and exclusion plots, being higher in the latter case (Fig. 5). After 19-years of herbivore exclusion, however, only the persistence of forbs remained higher than initially seen. In Nardion, the clearest changes during the 2002–2006 period were the reduction of transition probabilities from all groups to the dominant grass, and the increasing persistence of grasses and forbs relative to that observed in previous time period. After 2006, the transition between ecological groups approached initial values in presence of herbivory (Fig. 5). Unlike in Bromion, the shifts in transition probabilities observed (except that between dominant and non-plants)

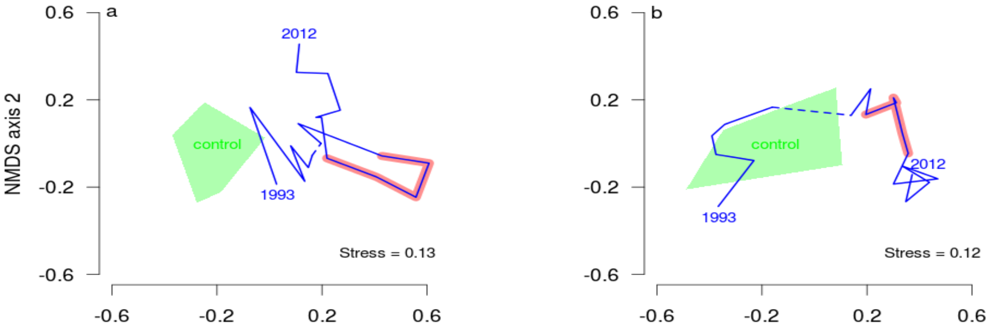

Fig. 3 Long-term trajectory of plant community structure in response to herbivore exclusion in the Bromion (a) and Nardion (b) grasslands. Ordination is based on non-metric multidimensional scaling (NMDS), and shows plant community dynamics in controls (convex hull) and exclusion plots (solid line) between 1993 and 2012 (no data was recorded in 1999; dashed lines). Shade line stresses the exceptionally warm and dry period between 2002 and 2006

during the warm and dry period continued afterwards in the exclusion plot (Fig. 5).

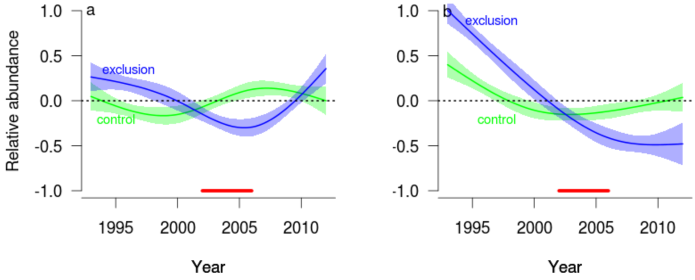

Fig. 4 Long-term dynamics of the most dominant species in response to herbivore exclusion. (a) Relative abundance of Festuca nigrescens at Bromion site. (b) Relative abundance of F. nigrescens at Nardion site. (c) Relative abundance of *Nardus stricta* at Nardion site. Shaded areas represent 95 % confidence intervals. Abundance values are re-scaled to 0 to ease visual comparison between sites and plots. Red line highlights an exceptionally warm and dry period detected between 2002 and 2006

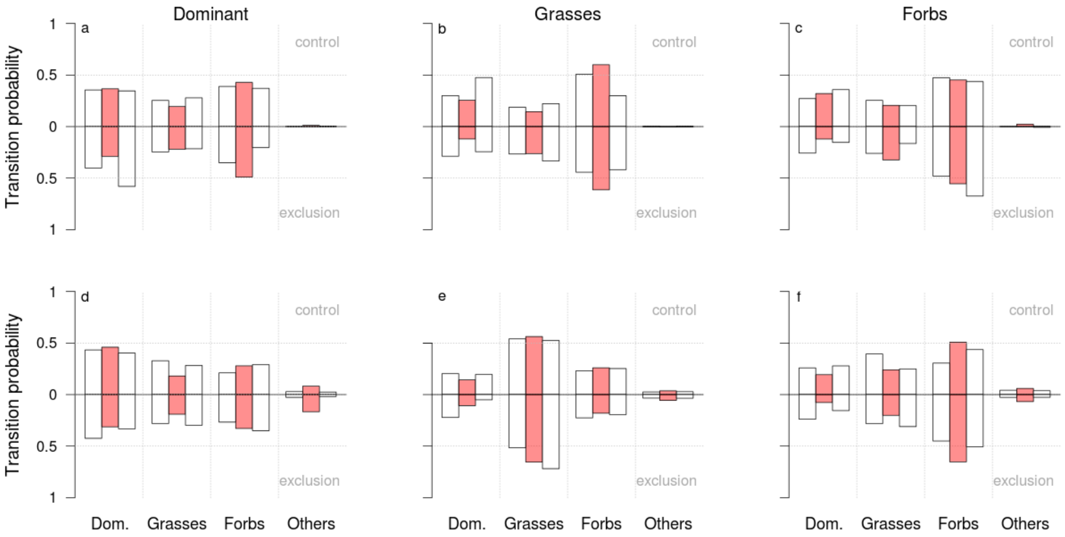

Fig. 5 Corrected mean transition probabilities between main ecological groups (dominant species, non-dominant grasses, forbs and other non-plants) in Bromion (a, b, c) and Nardion (d, e, f) grasslands. Each plot shows the averaged transition probabilities between a given group and the others for an exceptionally warm period (2002–2006; bar in red) and bracketing cooler time periods (1993–2000 and 2007–2012, respectively; bars in white)

Discussion

Contrary to expectations, we found that two decades of herbivore exclusion did not trigger large changes in community structure and diversity losses. Although the canopy height increased significantly within exclosures over 19 years as predicted by the GGM, this change was not drastic enough to modify competition for light and thereby, to suppress shade intolerant species. The lack of effect of herbivore exclusion on species richness may also be related to stronger effects of rates of local colonization and extinction on grassland structure (Glenn and Collins 1992; Olff and Ritchie 1998), or to the fact that herbivores were not selecting the most competitive species (Crawley 1983), and hence that their relative importance in altering interspecific competition was minor.

For productive subhumid grasslands, the GGM predicts rapid community shifts in response to changes in grazing regimes (Milchunas and Lauenroth 1993). However, we found that high-elevation grasslands responded slowly to the most extreme reduction of grazing possible. In fact, the response was mostly related to the shift in abundance of species, and marked divergences in community structure between control and exclusion plots did not happen before the ninth year of exclusion. This result is in disagreement with theoretical expectations, but also contrasts with previous findings from exclusion experiments in other mountain regions, where community changes (including those in composition) were stronger soon after excluding herbivores (Rawes 1981; Hill et al. 1992; Krahulec et al. 2001; Tasser and Tappeiner 2002; Mayer et al. 2009; Speed et al. 2013). Importantly, our results were irrespective to the dissimilarity measure used to characterize grasslands trajectories, and potential bias related to the detection of rare species.

Why was the community response to the exclusion of large herbivores so weak in this study? We speculate about four different possibilities, not mutually exclusive. First, the lack of response might be due to the absence of life forms such as dwarf-shrubs, which have been shown to fast modify community physiognomy and structure (e.g. De Bello et al. 2005; Olofsson et al. 2009). Second, the reduced growing period and predominance of long-lived species at high elevations might have slowed down the response of communities to herbivore exclusion (Tasser and Tappeiner 2002; Mayer et al. 2009). Third, given that the studied plots were surrounded by large grassland areas, immigration of species from lower elevations (including shrubs) might be reduced compared to grasslands close to the forest limit and/or shrublands (e.g. Speed et al. 2013). Likewise, the colonization of more competitive lowlands species (including shrubs) may also be limited in the absence of grazing (Austrheim and Eriksson 2003), because above ground biomass and species colonization and seedling emergence are negatively related (Eskelinen and Virtanen 2005). The fact that all the new species detected within the exclosure across the 19-years were also present in the surrounding grasslands support this explanation. Finally, continued intensive grazing throughout the last centuries might have depleted the pool of strictly non-tolerant species from the system, thus limiting the resilience mechanisms proposed by the GGM that allow for reversible floristic changes in response to grazing intensity (Cingolani et al. 2005).

A striking finding of this study was that hervibory played an important role in modulating the effect of climate in community structure and turnover between ecological groups. In both grasslands, plant assemblages become more vulnerable to a period of unusually warm and dry conditions in absence of herbivory. This was evidenced by higher changes in both the plant community structure and transition probabilities of ecological groups within exclosures between 2002 and 2006. The exceptionally warm and dry growing seasons affected negatively the dominant *Festuca nigrescens* in both sites, although it trigger different plant community responses. *Festuca nigrescens* was mostly replaced by forbs in Bromion, which explains the temporal shift in the community structure found in this grassland. Importantly, the initial community structure and group replacements were partially recovered once the exceptional climatic conditions reverted at this site, indicating a high resilience of this grasslands.

In Nardion site, *F. nigrescens* was mostly replaced by the *Nardus stricta*, which became the most dominant since the 2002. The replacement of one dominant species from another suggests a lower competitive release, and may explain why transition from grasses to forbs between 2002 and 2006 was lower than in Bromion. In this case the dominance of N. stricta continued after the end of the warm and dry event. This trend may constitute a potential risk from a management perspective, because *N. stricta* can spread in absence of herbivores up to form dense tussocks (Chadwick 1960; Hulme 1996; Tasser and Tappeiner 2002), which can be difficult to control even by restoring past grazing regimes (Rosef et al. 2004). As the low foraging value *N. stricta* spreads at the expense of more nutritive grasses and legumes, it can also result in an impoverishment of the quality of grassland for livestock production, and in some cases, in a diversity loss (Cernusca et al. 1996; Fillat et al. 2008). Our result do not corroborate such extremes, but they do warn about the potential influence of exceptional climatic conditions in favouring the spread of *N. stricta*.

As the community structure in all control plots was relatively stable across the 19-yr, we suggest that herbivory buffered changes of plant community in response to exceptional climatic events (Post and Pedersen 2008; Olofsson et al. 2009; Speed et al. 2012; Kaarlejärvi et al. 2013). A straightforward implication of this result is that the maintenance of past grazing stocking rates (or restoration in case of recent loss) may not only be valuable for the conservation of current biological diversity (Austrheim and Eriksson 2001), and productive communities (e.g. in the case of Nardion grasslands), but an efficient strategy to slow down some of the detrimental effects expected under ongoing climate warming (Theurillat and Guisan 2001).

Taken together, our results do not support the application of the GGM for guiding successful management of high elevation grasslands. As the GGM was mainly based on studies from low-elevation grasslands and shrubslands (Milchunas et al. 1988; Milchunas and Lauenroth 1993) it does not contemplate some particular aspects of high elevation grasslands (i.e. predominance of long-lived species, short growing periods) that may be critical in modulating the response of grasslands to grazing. At first glance, the weak long-term impact of the herbivory exclusion on grasslands may be perceived as good news for managers. However, caution is recommended when interpreting these results, because as we have shown, the relative slow response of plant communities to exclusion treatment would accelerate under climatic conditions, similar to those predicted for many European mountain (Engler et al. 2011).

Summarizing, we have shown that the long-term effect of herbivore exclusion on high elevation plant communities can be more moderate than expected, but also that they are modulated by the climatic conditions. Importantly, our results demonstrate that the strength of the response of grasslands to herbivory exclusion is likely to be enhanced under exceptional warm and dry periods, such as those expected under ongoing climate warming. Unfortunately, as in many other long-term experiments, the lack of replication limits our ability to generalize these results. However, we must be aware that the huge variability of grassland communities will make it difficult to establish accurate generalizations even if we had triplicated the number of grasslands. In absence of further detailed studies, our study provides novel insights into the long-term response of particular ecologica communities under climate and land-use change. Grassland management should consider not only the direct effect of herbivore activity or climate warming, but also their combined effect across a variety of communities over ecological relevant times scales.

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Supporting Information

Online Resource 1. Sensitivity of ordination analyses to the choice of the dissimilarity measure, type of vegetation data, and consideration of rare species.

The choice of the dissimilarity measure, the type of vegetation data (qualitative or quantitative), and in the case of quantitative data, the abundance of rare (or most abundant) species would influence the ordination of communities. The dissimilarity measure used in this study (i.e. Bray-Curtis) was more suitable than others in the context of this study, given the characteristics of our data (quantitative) and the goals of the investigation, and because it has been shown to perform better than alternative measures in similar studies conducted elsewhere (see Magurran 2004 and references herein). However, it is not immediately clear, to what extent some our findings were sensitive to this choice. Therefore, we repeated analyses with different metrics to assess the consistency of our ordination outputs. Figure S1 shows that trajectories of control and experiment were relatively robust to the dissimilarity measure used. In all cases, except when rare species were not included in the analysis, the effect of the warm and dry period was similar to that initially observed. When ordination was solely focus upon common species (Fig. S1 g-h) the difference between control and treatment blurred at the Nardion grassland. This result supported that, besides the significant increase in dominance of *Nardus stricta* (see Fig. 4), the change in abundance of less common species was also behind the divergent trajectory of this community after several years of herbivore exclusion.

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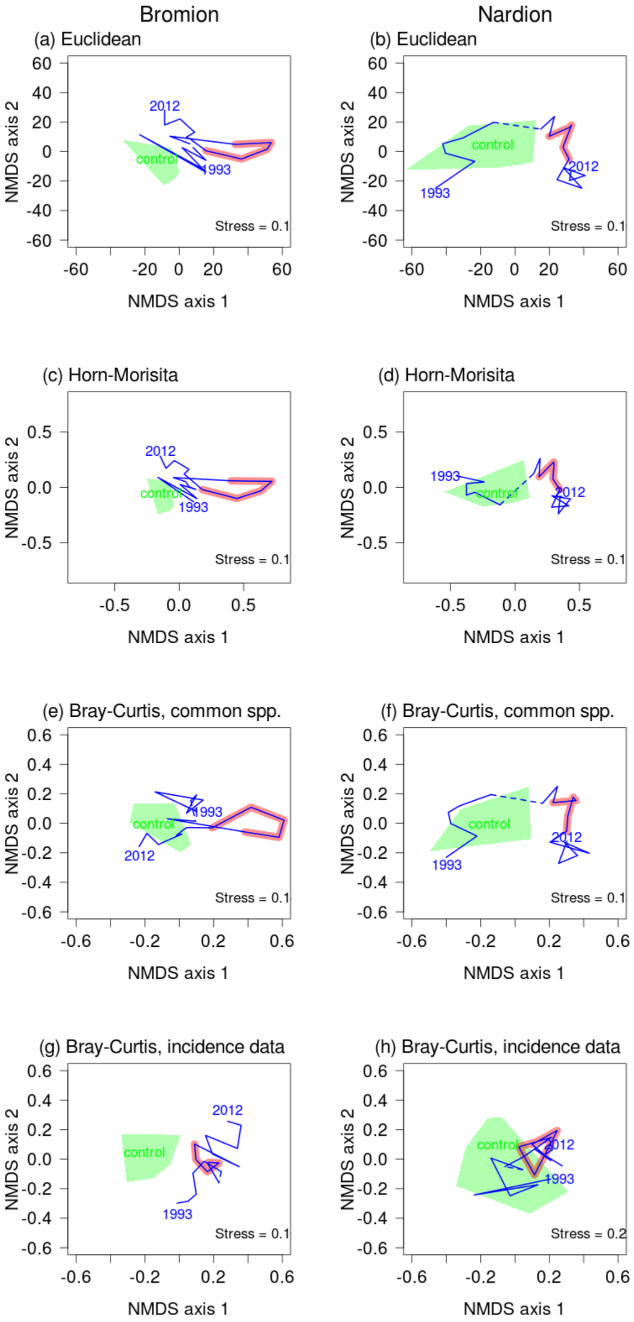

Fig. S1 Non-multidimensional scaling (NMDS) ordinations on the trajectory of plant communities in response to herbivore exclusion (solid line) between 1993 and 2012 (with the exception of 1999; dashed line). Ordination was repeated with three dissimilarity measures, (a-d) and with Bray-Curtis measure on the basis of quantitative data of only common species (those whose abundance was higher than the third quartile of the distribution; e and f) and incidence data of all species (g and h), respectively. Convex-hull represent multivariate dispersion of control communities. Shade line indicates the sequence of exceptionally warm and dry growing seasons between 2002 and 2006.

General Discussion

This thesis analyses the distribution of different components of plant diversity in a mountain National Park, and assesses how the structure and composition of the richest habitats of the Pyrenees have changed over the last decades. We have used a multiple approach to address these questions, paying particular attention to the uncertainty derived from the data used. Our intention was to provide readers with a realistic view of what kind of diversity the National Park harbors, where, and how it is responding to land-use changes and climatic warming in alpine grasslands.

Spatial distribution of plant diversity

The effect of sampling effort on biodiversity databases

Despite the Ordesa-Monte Perdido National Park (OMPNP) is the most exhaustively prospected area of the Iberian Peninsula (Font *et al.* 2010), our knowledge about the spatial distribution of plants across the park is still incomplete and geographically biased (*Chapter 1*). Such bias resulted from spatial differences in the sampling effort due to non-systematic prospections, and from adding together data on species occurrences from heterogeneous sources of information (Reddy & Dávalos 2003; Soberon & Peterson 2004; Hortal & Lobo 2005). In chapters 1 and 2, we empirically demonstrated that the uneven distribution of sampling effort strongly distorted diversity patterns observed in the OMPNP.

Not surprisingly, we found that the richest areas of the OMPNP were those with highest sampling effort. While this is a well documented pattern (Dennis, Sparks & Hardy 1999; Hortal, Lobo & Jiménez-Valverde 2007; Boakes *et al.* 2010; Robertson, Cumming & Erasmus 2010), our study adds to existing literature that other diversity components such as phylogenetic diversity (PD) and functional diversity (FD) can also be affected by the sampling effort differences. This may be due to correlation between phylogenetic and functional diversity metrics with species richness (SR; Pavoine *et al.* 2013). However, the effect of sampling effort persisted on PD (but not on FD) after removing the influence of SR. In addition, such effect was stronger in the highest quantiles of these diversity metrics, which implies that the identification of diversity hotspots was also affected (*Chapter 2*). Such possibility should not longer be ignored in analyses of the spatial congruence between multiple diversity components, and findings from previous studies may need some revision (Orme *et al.* 2005; Mouillot *et al.* 2011). Furthermore, the assessment of sampling effort bias in databases should be systematic to reduce the uncertainty of any subsequent basic or applied analysis (Dennis *et al.* 1999; Hortal *et al.* 2007).

Although several methods have been used to quantify sampling effort in databases (Colwell & Coddington 1994; Fagan & Kareiva 1997), not all of them perform well under all situations (*Chapter 1*). Most conventional methods have been used with little consideration of their statistical assumptions (Soberón & Llorente 1993), and as shown in *Chapter 1*, this can result in an inaccurate estimation of the sampling effort. Our results also warn about the limitations of some of these methods for discriminating between well and poorly sampled units. A more statistically robust alternative is provided by our FIDEGAM method (*Chapter 1*), which outperformed others in terms of discrimination capability (the probability of correctly identifying well and poorly sampled units; Pepe 2003), even when the sampling exhaustiveness was very low. Thereby, FIDEGAM can be applied to a wider range of situations than other conventional methods (Soberón & Llorente 1993), providing a more reliable estimate of the sampling effort in databases.

A major challenge is, however, how to account for sampling effort bias in diversity analyses. There is not a 'silver bullet' to solve the problem, and all existing alternatives present drawbacks. For comparative analyses, the sampling effort should be balanced between geographic units (Freitag & Jaarsveld 1998). A common procedure is to remove poorly surveyed areas (Gaston & Rodrigues 2003; Soberon & Peterson 2004; Santos *et al.* 2010). Nevertheless, new limitations may arise if the reduction of area suitable for analyses leads to a substantial reduction in the total amount of diversity examined. This was not the case of our study, because the removal of poorly sampled units (40%) only reduced 2% total species richness. However, we should recall that the effect of the sampling effort was not exclusive of poorly sampled areas (see *Chapter 2*), so by no means the removal of these areas would reduce the noise completely.

Another alternative to overcome the effect of sampling effort is to extrapolate biodiversity data to poorly prospected areas by using species distribution modeling (Cabeza & Moilanen 2001; Soberon & Peterson 2004). While this is a widely accepted approach, we believe that interpolation of present-day species occurrences would have been problematic given the fine-grain resolution of our study and the topographic complexity of the OMPNP, but also the low-resolution of available predictor variables (e.g. temperature, which was only gathered from a single Meteorological Station) relative to the many existing micro-habitats (Scherrer & Körner 2010).

Instead, model predictions of diversity components might have been more suitable (Ferrier 2002; Lobo & Martín-Piera 2002). Calibration of these kind of models should be done on the basis of the information of well surveyed areas (Hortal & Lobo 2006), which in our case did not sufficiently represent the existing environmental and topographic variability within the studied area. Therefore, a more powerful strategy may be to include sampling effort values as a variable in the explanatory models. Thus, the fit between environmental variables and SR can be improved substantially, increasing the accuracy of projected diversity values (Pardo *et al.* unpublished).

All aforementioned alternatives should be regarded as the best-so-far solutions to the actual problem: the incomplete, and spatially and taxonomically biased inventory of biodiversity. It is widely acknowledge that the systematic collection of data is invaluable for testing a number of ecological questions (Robertson *et al.* 2010), and conservation planning (Margules & Pressey 2000), scientists cannot undertake such task without help. Involving citizens in data collection is one successful way to tackle the problem (Bonney *et al.* 2009). We suggest that producing maps of poorly sampled areas would help to guide people in this task, increasing survey efforts in localities that contribute the most to fill the gaps in biodiversity maps (Sastre & Lobo 2009).

Distribution of multiple diversity components and its implication for area prioritization

Procedures for the identification of priority areas of SR are sensitive to the quality of the data (Freitag & Jaarsveld 1998; Guilhaumon *et al.* 2008). In *Chapter 2*, we quantitatively demonstrated that spatial distribution of other diversity components than SR can be similarly biased, but also that the spatial resolution of the baseline data can mask the ecological significance of the mismatch among diversity components (*Chapter 2*). When analyses were carried out with data gathered in a standardized way (phytosociological *relevés*) and at ecologically meaningful spatial resolution (plant communities), we found an overall mismatch between different types of hotspots, a result that is consistent with findings at larger spatial scales (Prendergast *et al.* 1993; Devictor *et al.* 2010; Mouillot *et al.* 2011). In our study, however, PD and FD hotspots showed a higher congruence than in previous ones, most probably due to phylogenetic conservatism of traits used in the calculation of FD (see appendices in *Chapter 2*).

The spatial mismatch among diversity components presented here undermines the utility of hotspots as a conservation tool. In fact, the representation of multiple diversity components in any type of hotspot was lower than if we had chosen a set of areas at random.

For example, many areas with abundant endemics would be left out if priority areas for conservation were defined according to any of the other diversity components. We are fully aware that many other aspects (e.g cost, threats) than biodiversity should be considered to judge the conservation utility of a prioritization procedure (Brooks 2010). However, achieving a good representation of diversity is critical in conservation planning (Margules & Pressey 2000), and from this perspective, the use of hotspot of a single diversity component was a poor strategy for representation of multifaceted diversity in high mountain areas. An intriguing question raised then is whether other prioritization procedures would do a better job. According to some preliminary tests, we also found large gaps in the representation of multifaceted diversity in complementary areas selected with a greedy algorithm (Pardo *et al*. 2013b). While these two evidences alone are not conclusive, they did underpin the limitations of conventional procedures for identification of small priority areas for conservation of multifaceted diversity in topographically complex areas.

The spatial mismatch among different hotspots suggest that the distribution of each diversity components was shaped by different mechanisms (Davies & Cadotte 2011; Pavoine *et al*. 2013 and references herein). We can only speculate about the causes of the spatial mismatch between diversity components from our study. However, the distribution of different diversity components across different habitats and elevations (Fig. 1) suggests that ecological factors (e.g. environmental filtering) and historical factors (glaciations, grazing) were important in explaining the mismatch. In particular, the low amount of evolutionary history and the high endemicity found at high elevation grasslands evoke the importance of vicariance and allopatric speciation during Quaternary glaciations (Tribsch 2004). The concentration of PD in low elevation forests reveals the co-occurrence of relatively modern species with those from old lineages (including several pteridophytes). The highest values of FD also were also concentrated in some forests at low elevation, most probably due to the less severe environmental filtering than in high-elevation grasslands, where grazing and climatic conditions exert a strong selection pressure on species attributes (de Bello et al. 2013).

Beyond the causes of the spatial mismatch between diversity components, our results highlight the complementary contribution of different types of habitats to biodiversity. While the high SR component of grasslands observed in this thesis was previously demonstrated across the central Pyrenees (García & Gómez 2007), our findings reveal a hidden conservation value of some particular forests (mostly beech and mixed forests with ash and hazel) as historical reservoirs of PD.

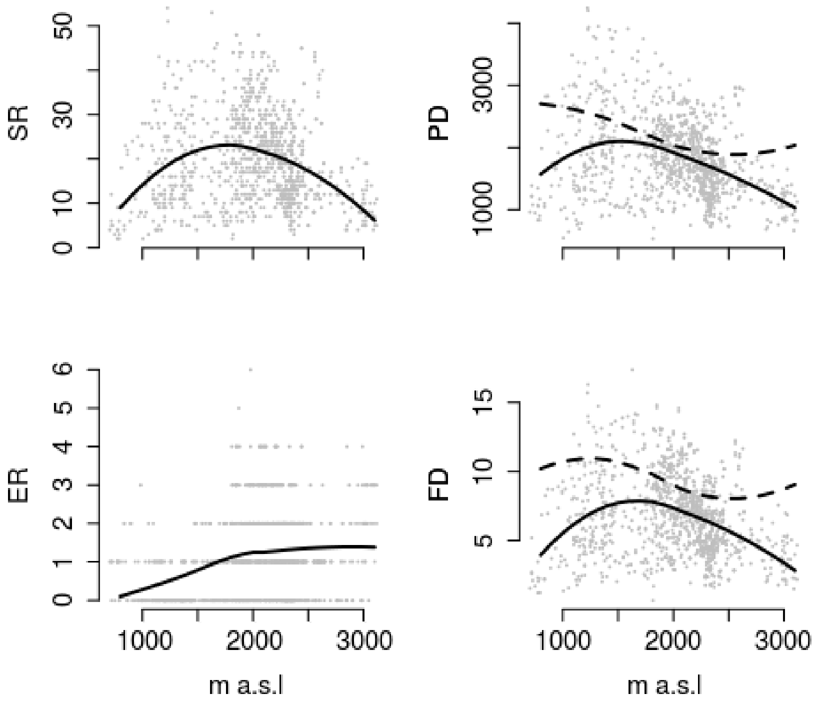

Fig. 1 Distribution of species richness (SR), endemism richness (ER), phylogenetic diversity (PD) and functional diversity (FD) along the elevational gradient (100 m intervals). Trends are depicted by fitting local non-parametric regressions (LOESS). Dashed lines indicate the LOESS smooth curve of the residuals from the quadratic regression between SR and PD and FD, respectively.

Temporal response of alpine plant communities to global change

Grasslands are the richest communities of the Pyrenees, but they are also ones of the most exposed to the impact of global changes. Our research evidenced, however, very few noticeable changes in alpine grasslands over the last two decades. Furthermore, our results from both observational and experimental studies pointed out a relative long-term stability of alpine grasslands during the studied period. This finding contrasts with both expected and predicted changes in mountain vegetation due to global drivers, and some of the evidences already reported below the treeline and in summits. In the following lines we attempt to connect results from *Chapters 3, 4* and *5* to existing literature, and to discuss their implication in the context of global change.

The treeline ecotone

Climate warming is expected to enhance tree recruitment and growth rates in treeline ecotones, which may translate into a loss of suitable habitat for alpine plants and associated animals (Illerbrun & Roland 2011). In *Chapter 3*, we reported an increase in tree cover in an undisturbed treeline of the OMPNP between 1998 and 2009, which is consistent with the expectations of climate warming (Camarero & Gutiérrez 2004; Körner & Paulsen 2004; Batllori & Gutiérrez 2008; Harsch *et al.* 2009), and the trend observed in another treeline nearby (Escuaín valley, Camarero *et al.* 2015). Surprisingly, this structural change did not affect the diversity and composition of the field layer vegetation over a decade. We invoke two possible reasons, not mutually exclusive: (i) the response of field-layer vegetation was slow (i.e. a lagged response), and (ii) our method was not powerful enough to detect subtle changes in vegetation.

The tree cover increased unevenly across the treeline ecotone, most probably because tree recruitment and/or growth were restricted to most suitable microsite conditions near adult trees or boulders (Batllori *et al.* 2009; Holtmeier & Broll 2010). Since alpine species might have found safe sites within short distance, the observed increase of the tree layer might not actually entailed habitat loss for these species. Nevertheless, the impact of the expansion of tree cover expansion on the field layer vegetation was also negligible when data were parsed into elevational segments, even where forest-like conditions were reached (and thus, the habitat loss was effective for alpine specie). Interestingly, species with clear forest affinities did not enhance either. As colonization of this kind of species seems inevitable under forest-like conditions, our results may be interpreted as a colonization credit (Piqueray *et al.* 2011). To check that the lagged response was not the product of our sampling method, we started monitoring species' abundance changes in 20x20 cm permanent plots located under 10 randomly selected krummholz pines, and in open areas (N=100 in total). Although the increasing trend in tree cover was visually evident after four years, preliminary analyses suggest that vegetation changes underneath the trees were subtle (Tejero, Pardo & García 2015), which may support the existence of a lag between the increase in tree cover and the changes in field-layer vegetation in the treeline ecotone.

Alpine grasslands

Wild and domestic herbivores have historically shaped plant communities mainly through selective foraging (Augustine & McNaughton 1998), affecting local plant extinctions and colonizations, and patterns of spatial heterogeneity through the continuous creation of small disturbances (Olff & Ritchie 1998). Due to the long grazing history of Pyrenean grasslands, herbivory plays a central role in the dynamics of these systems (Fillat *et al.* 2008). Therefore, grazing reduction is considered a major perturbation for grasslands. Prevalent grazing models anticipate rapid and deep changes in the diversity, structure and composition of grasslands in response to grazing reduction (Milchunas, Sala & Lauenroth 1988; Cingolani, Noy-Meir & Díaz 2005). However, our results from both observational and experimental studies provide little support for these general expectations (*Chapters 4 and 5*).

Revisitation of several grasslands located in two different valleys after 20 years revealed that plant community changes were, with few exceptions, smaller than expected by natural fluctuations alone. Evidences of competition for light (i.e. increase in dominance of tall species) or structural changes (increase of shrubs) were not apparent either (*Chapter 4 and 5*). Indeed, only some low abundant species underwent significant changes in abundance, and species that significantly increased or decreased did not differ in terms of traits related to herbivory.

Such weak and slow effect of grazing reduction was confirmed in grasslands under experimental exclusion of herbivores (*Chapter 5*), where the impact of the treatment on SR of grasslands was non-significant after two decades. In addition, it was only after the 9th year that divergent trajectories were observed between control and fenced areas. These results contrast with the stronger and faster plant community response observed in other exclusion experiments and abandoned grasslands at lower elevation elsewhere (Rawes 1981; Hill et al. 1992; Krahulec et al. 2001; Tasser and Tappeiner 2002; Mayer et al. 2009; Speed et al. 2013), evidencing the difficulty of crafting general rules on the effect of herbivores on plant communities.

Fingerprints of climate warming were not detected in resurveyed grasslands either. Contrary to predicted, compositional shifts in plant communities were not associated to the elevation affinities of species (Fig 1 of *Chapter* 4). Likewise, none of the species increasing in abundance was exclusively from lowlands and/or presented higher warmth affinities than those that decreased over two decades. These results do contrast with the general trend towards 'thermophilization' of European summits recorded in less than a decade, some of them located in the OMPNP (Pauli *et al.* 2007; Erschbamer *et al.* 2011; Gottfried *et al.* 2012). Nevertheless, the community effects of an exceptionally warm and dry period observed in the experimental exclusion was suggestive of the potential impact of climate warming on alpine grasslands. Interestingly, these effects were buffered in presence of herbivores, which may stress the importance of herbivory in the face of climate warming (see also Post & Pedersen 2008; Speed *et al.* 2012; Kaarlejärvi 2014).

Taken together, our results from both observational and experimental studies stress the ecological inertia of alpine plant communities in the OMPNP (Table 1). Several studies have linked the slow dynamics of plant communities to the longevity of alpine species and the short growing periods at high elevation (Mayer *et al.* 2009; Nagy & Grabherr 2009; Wilson & Nilsson 2009). The contribution of long lifespans to stability is well documented in demographic studies (Morris *et al.* 2008; García, Picó & Ehrlén 2008). Indeed, several studies have demonstrated that alpine species can buffer detrimental effects of climate warming through compensatory changes in their demographic rates (e.g. Doak & Morris 2010; García-Camacho, Albert & Escudero 2012). The presence of species with clonal propagation in studied grasslands might also point to the same direction, because these species have higher ability to persist when the environmental conditions are unfavorable (e.g. Klimesova & Klimes 2007). Nevertheless, we hold that these aspects alone cannot explain some of our results such as the lack of increase in abundance of shrubs (Cannone, Sgorbati & Guglielmin 2007).

Table 1 Summary of the commonly expected impacts of grazing reduction and climate warming on alpine grasslands tested in the chapters (Ch.) of this thesis. Plus and minus symbols state whether a given expectation was supported or not, respectively, in our studies.

	Grazing reduction				Climate warming			
Scale	Expected change	Ch.3	Ch.4	Ch.5	Expected change	Ch.3	Ch.4	Ch.5
Regional	Landscape biotic homogenization			–	Homogenization along the elevational gradient		–	
Community	Structural changes	+	–	–	Upwards shift of communities/species	–	–	
	Transitory changes		+	+	Transitory changes		+	+
	Increasing competition of light		–	–	Shrubs colonization	–	–	–
	Decrease in species richness			–	Increase in species richness			
Species	Change in the dominant species	–	–	+	Decrease in abundance of cold adapted species	–	–	
	Increasing abundance of dominants	+	+	+	Increase in abundance of low elevation species	–	–	–
	Increase in abundance of palatable species	–	+	+				

The probability of shrub colonization at the OMPNP decreases as a function of the distance to woody communities (Gartzia, Alados & Pérez-Cabello 2014). However, some of the alpine grasslands of our study are located close to the treeline and shrublands, and no biophysical barriers are apparent between them. Possible explanations for the absence of shrubs is that our grasslands are located in non suitable areas for the most invasive shrubs (e.g. *Echinospartum horridum*), and/or that current grazing, though residual from a historical perspective, is enough to control shrub expansion (Olofsson *et al.* 2009). In fact, we cannot discard that the generalized reduction in grazing had resulted in a concentration of remaining flocks (and locally cow herds) in more accessible and favorable areas for summer grazing. If this hypothesis was correct, then grazing reduction would have not been locally relevant in some of the revisited grasslands and our results would not be so unexpected there. The buffering effect of grazing (*Chapter 5*) may also help to explain why alpine areas were less sensitive to climate warming than in high summits, where herbivory was occasional or inexistent (Villar & Benito 2007). However, this tentative hypothesis (the local maintenance of stocking rates) does not explain the observed stability of grasslands under experimental exclusion of herbivores (*Chapter 5*), and why the dynamics of grasslands in less favorable areas for grazing was also stable (*Chapter 4*).

Whatever the reason, the message drawn from our findings is that, overall, alpine grasslands may have a larger ecological inertia than expected. Our results complement others carried out in montane areas and summits, and provide insight for understanding the interaction between both drivers of global change at an ecologically relevant scale. This was only possible due to the long datasets used in this thesis. However, our studies were poorly replicated and constrained by the initial sampling design, which may not be ideal for characterizing and monitoring diversity structures. We are fully aware that the number of grasslands surveyed in this thesis represents only a small sample of existing types of alpine grasslands, which clearly limits the generalization of our findings. Moreover, given the disproportionate heterogeneity of plant communities and existing micro-environmental variability in the alpine belt (Scherrer & Körner 2010; Scherrer, Schmid & Körner 2011), it would be unwise to assume homogeneous community responses to global change. However, our results come from grasslands under different grazing regimens because they are located in different valleys and altitudes (including extreme ones such as herbivore exclusion) and yielded rather consistent results. We therefore conclude that the long-term stability of alpine grasslands was not idiosyncratic, and that many other alpine grasslands in the region could show similar responses to the ongoing global changes.

In the particular context of the OMPNP, the long-term stability of alpine grasslands contrasts with some evident changes in land cover (Landsat imagery between 1984-2007, Gartzia, Alados & Pérez-Cabello 2014), and the rapid colonization of shrubs into grasslands observed below the potential treeline (~2100 m a.s.l.; aerial photographs from 1981 and 2003, Komac, Alados & Camarero 2011). The community effects of climate warming in the studied grasslands were also subtle compared with those observed in the GLORIA project in four summits of the National Park in less than a decade (Gottfried *et al.* 2012; Pauli *et al.* 2012). Although the comparison among these different studies is merely qualitative, and having acknowledged that the response of plant communities to global change can differ even within elevation belts, the strength of changes in plant diversity of the OMPNP could be represented as a 'reverted glass' (Fig. 2). Nevertheless, the narrow stem of the glass should be hold with extreme caution, because the current state of apparent stability of alpine grasslands may suddenly revert due to lagged responses to grazing reduction and/or as climate tipping points are exceeded (Doak & Morris 2010). The good news is that the slow dynamics of alpine grasslands offers a window of opportunity for implementing timely management before reaching an irreversible state (Hughes et al. 2013). As it has been stressed elsewhere, the conservation of this semi-cultural alpine landscape, and their high diverse communities, will depend on our ability to maintain and, in some cases, to restore traditional grazing activities (Olsson, Austrheim & Grenne 2000; Spiegelberger *et al.* 2006; Chemini & Rizzoli 2014).

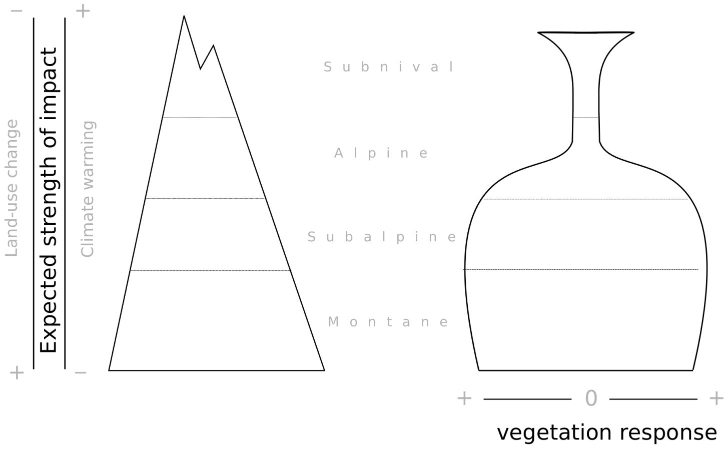

Fig. 2 Expected impact of land-use change and climate warming along the elevational gradient of the OMPNP.

Getting the most out of long-term data

We have stressed in the introduction of this thesis the importance of the long-term data for understanding ecological processes and the consequences of global change, especially where remote sensing methods fail to detect changes in the structure and composition of heterogeneous habitats. Our results highlight their value, but also that they should be complemented with analyses considering sampling uncertainty and natural stochasticity of communities. Sampling error and temporal fluctuations may confound the interpretation of results, and accounting for such aspects when analyzing long-term data is therefore crucial to obtain solid conclusions.

Annual monitoring data showed that natural fluctuations of grasslands were higher than it is often assumed for communities dominated by long-lived species and at high-elevation. Several mechanisms have been postulated to explain these natural fluctuations, including the stochastic emergence of hidden populations of dormant seeds and meristems (Klimešová & Klimeš 2007). Nevertheless, year-to-year fluctuations of plant communities are not only due to natural stochasticity, they may also be the product of the observer's accuracy and the sampling method. The effect of sampling error is widely acknowledged, but rarely measured in resurvey studies. In *Chapters 3* and *4* we carried out specific research for measuring this sampling error, and found that it was surprisingly high due to the inexact relocation of sampling points along semi-permanent transects. While this error did not substantially affect temporal differences in community composition of grasslands (pers. observ.), it did result in inaccurate estimates of species' abundance (*Chapter 4*), and transition probabilities between ecological groups (*Chapter 5*). So, if we had not accounted for the sampling error, we would have obtained different, and definitely more uncertain results. It is obvious that the magnitude of the sampling error may vary from one sampling method and natural system to another. Yet, uncertainties around results will not dissipate until they are quantitatively evaluated (Magurran *et al.* 2010). Fortunately, accounting for such error requires much less effort than obtaining the long-term data, and it could be estimated even only once over the whole monitoring period, which would considerably simplify the process.

Future research and gaps to be solved

This thesis aimed to contribute to better understand the distribution of multifaceted diversity in mountains, and its response to global change. Many interesting questions arose during the development of this study, that could not be addressed because of lack of time. We would like to mention some of the most relevant ones.

An interesting area of future research would be to investigate what causes the uncoupled distribution of diversity components observed in *Chapter 2*. By jointly investigating the pattern of phylogenetic structure and trait combinations it would be possible to gain insight into the relative importance of the ecological processes (environmental filtering) and historical factors (Cavender-Bares *et al.* 2009; Kraft et al. 2011; de Bello *et al.* 2012). This information would be useful to improve our ability to anticipate the effects of global change on biodiversity (e.g. Suding et al. 2005 in Adler 2013).

Continued long-term monitoring of studied grassland seems necessary to measure the lag in the response of alpine communities to drivers of global change, as well as to evaluate what tipping points would revert the relative state of stability observed in our studies. However, our monitoring of grasslands should be improved to enhance the statistical explanatory power of the analyses on the effect of grazing reduction and climate warming. On the one side, an accurate estimation of past and current spatial distribution of stocking rates are necessary to better understand the impact of grazing reduction in the region. On the other side, climatic records from meteorological stations are insufficient to characterize the local climatic conditions experienced by plants (Scherrer & Körner 2010). Therefore, devices to record microclimatic conditions at each of the monitoring sites will considerably increase our ability to unravel the impact of climate change on vegetation dynamics.

Finally, further observational and experimental studies need to be undertaken in alpine plant communities to test the generality of our findings. This is a challenge in the alpine belt because the large heterogeneity of plant communities in those areas, the difficult accessibility and harsh conditions to set enough replicates, and the difficulty to get funding for long-term monitoring (Strayer et al. 1986; Lindenmayer et al. 2012). Our Institute managed to keep herbivory exclusions for 25 years now, and additional exclosures along the elevational gradient were established throughout the course of this thesis. This is however clearly insufficient to obtain general conclusions, considering the huge variability and extension of grasslands. The rationale progression of this work seems to be the integration of regional data into multi-site networks that put together data from other mountain ranges. We believe that meta-analyses including different studies will help to clarify the limitations of the prevalent grazing hypothesis (e.g. the Generalized Grazing Model), and to better anticipate the dynamics of alpine grasslands.

Conclusions

Conclusions

1. In this thesis we have focused on the spatial patterns of multiple diversity components of vascular plants and the long-term dynamics of the richest habitat in the Central Pyrenees. Most studies were conducted in the Ordesa-Monte Perdido National Park (OMPNP), an outstanding European area in terms of plant diversity and monitoring of community changes over the last decades.

2. Quality assessments of databases is needed to conduct reliable biodiversity analyses. We developed a novel analytical method to quantify the sampling effort in databases constructed from multiple sources (museum samples, field surveys, etc.). This method, tested with the plant database of the OMPNP and several simulated datasets, performed better than others regardless of the level of sampling exhaustiveness. It can thus be suitable for a wide spectrum of databases, and will help to account for uncertainty in biodiversity analyses.

3. The sampling effort distorts the distribution of taxonomic, phylogenetic and functional diversity inferred from biodiversity databases, which in turn has a strong effect on the selection of priority areas for conservation. In addition, the spatial resolution at which data are gathered might blur the ecological significance of the distribution of diversity components, and mask the poor utility of hotspots of a single diversity component for representation of multifaceted diversity.

4. On the basis of unbiased distributional data at fine-spatial resolution, we demonstrated that taxonomic, phylogenetic and functional components of diversity are not spatially congruent in the OMPNP. The highest concentrations of species and endemism were observed in alpine grasslands, whereas some forests, specially mixed and deciduous ones, harbored the highest values of phylogenetic and functional diversity.

5. Despite the past drastic reduction in grazing and concomitant temperature increase in the Central Pyrenees, we found little evidences of change in plant species composition and abundance in several alpine grasslands over the last two decades. Our species traits analysis did not reveal any indirect evidence of the effect of grazing reduction or/and climate warming either.

6. Observational and experimental studies also demonstrated that the vegetation response of alpine communities to drastic environmental changes such as grazing cessation or tree

encroachment, might take decades. Alpine communities, however, could be particularly sensitive to the effects of exceptionally warm and dry periods in absence of herbivores. Maintenance or restoration of traditional grazing seems to be a good management strategy to conserve the diversity of the richest habitats in Central Pyrenees, and perhaps the only possible at local scale to slow down its response to climate warming.

7. Taken together, our results do not support the widespread expectations of global change for alpine plant communities. They also contrasts with the strong and rapid changes in land cover observed in some grasslands below the treeline, and in several European summit floras (including in the OMPNP). Such discrepancies evidence our limitations to craft general predictions about the impact of global change in mountain plant diversity.

8. Our studies have also demonstrated that the effect of sampling error and natural fluctuations may confound the interpretation of results obtained from long-term data. Accounting for data uncertainty and temporal stochasticity of plant communities will definitely improve our conclusions about the real biological consequences of global change.

Resumen

INTRODUCCIÓN

Los diferentes componentes de la biodiversidad

La biodiversidad fue definida en la Convención de Biodiversidad de 1992 como "la variación genética (diversidad intra-específica), taxonómica (inter-específica) y de funciones ecosistémicas". Es intuitivo que dos comunidades (por ejemplo, una formada por un haya y un roble, y otra formada por un haya y un helecho), aun compartiendo el mismo número de especies, no albergan la misma diversidad. Sin embargo, la medición de la biodiversidad se restringe a menudo a un número de especies, ignorando las diferencias interespecíficas (Magurran & McGill 2011). La diferencia entre las especies viene determinada por la semejanza de rasgos que presentan (Cadotte, Carscadden & Mirotchnick 2011). Bajo la asunción de evolución browniana de los rasgos (Felsenstein 1985; Harvey & Purvis 1991), y por tanto, de una diferencia fenotípica entre las especies proporcional a la distancia con respecto al ancestro común, la diversidad filogenética (PD) constituye una medición de la diferencia general entre las especies. Si bien ciertos rasgos no cumplen el patrón evolutivo anteriormente mencionado (un claro ejemplo de evolución convergencia son las plantas crasas que provienen de dos familias distantes; Gravel *et al.* 2012; Kelly, Grenyer & Scotland 2014), en términos generales, la diferencia fenotípica (del conjunto de rasgos) parece estar bien representada por la historia evolutiva (Cavender-Bares *et al.* 2009; Vellend *et al.* 2011).

En el ámbito de la biología de la conservación, donde la limitación de recursos obliga a establecer prioridades a diferentes niveles (especies, poblaciones, hábitats, áreas), la diferencia entre las especies juega un papel relevante (Vane-Wright, Humphries & Williams 1991). A mayor historia evolutiva (PD), mayor potencial evolutivo y diferenciación fenotípica (Vázquez & Gittleman 1998; Mace, Gittleman & Purvis 2003; Forest *et al.* 2007). En consecuencia, mayores serán también las opciones de futuro de la biodiversidad, así como el número de usos y beneficios potenciales que podamos obtener de ella (Vane-Wright *et al.* 1991; Faith 1992, 1994).

El componente funcional de la diversidad, definido como el valor y rango de los rasgos que influencian el funcionamiento ecosistémico (Tilman *et al.* 2001), media en el mantenimiento de los procesos claves de los ecosistemas (p. ej. resiliencia o resistencia frente a la perturbación, o producción primaria; Chapin III *et al.* 2000; Hooper *et al.* 2005; Cadotte *et al.* 2011; Isbell *et al.* 2011). Al igual que la PD, la diversidad funcional (FD) es una medida de la diferencia entre las especies, pero solo respecto a los rasgos que median en la(s) función(es) de interés (Lavorel &

Garnier 2002; Petchey & Gaston 2006). Si los rasgos involucrados en una determinada función ecosistémica aparecen conservados en la filogenia, entonces la PD puede ser también una buena medida predictora de dicha función (e.g. Maherali & Klironomos 2007; Flynn *et al.* 2011; Srivastava *et al.* 2012), y quizás la única posible cuando los rasgos se desconocen o no se pueden medir fácilmente (Crozier 1997; Cadotte *et al.* 2009; Mouquet *et al.* 2012). En cualquier caso, ambas medidas usadas de manera complementaria aportan información valiosa sobre la diferencia entre las especies, y su importancia para el mantenimiento y funcionamiento de la biodiversidad (Cadotte, Albert & Walker 2013).

Las distribución espacial de la biodiversidad y los retos para su conservación

Buena parte de la información obtenida durante décadas de prospección e inventariado de la biodiversidad ha sido recopilada en bases de datos. Esta información, aun siendo incompleta, nos permite estudiar la distribución de la biodiversidad a diferentes escalas (Kier *et al.* 2005; Robertson, Cumming & Erasmus 2010; Pimm *et al.* 2014), así como los factores ecológicos e históricos subyacentes. Esta información es también fundamental en el diseño de espacios protegidos, ya que nos permite identificar las áreas con mayor interés para su conservación, bien sea porque presentan una concentración excepcional de especies (*hotspots*; Myers 1988; Prendergast *et al.* 1993; Reid 1998; Myers *et al.* 2000), una mayor complementariedad de especies entre sí (Kirkpatrick 1983; Margules, Nicholls & Pressey 1988) o un mayor número de especies singulares (Pressey *et al.* 1993). Existe un gran debate en torno a estos tres criterios para identificar áreas prioritarias, y cuándo es preferible aplicar uno u otro (Williams *et al.* 1996; Jepson & Canney 2001; Brummitt & Lughadha 2003; Kareiva & Marvier 2003). Sin embargo, un aspecto común a todos ellos es que están formulados a nivel taxonómico, y por lo tanto, independientemente del criterio aplicado, la representación de otros componentes de la diversidad podría ser reducida en las áreas prioritarias. Existen múltiples estudios que apuntan en esta dirección (Possingham & Wilson 2005; Orme *et al.* 2005; Ceballos & Ehrlich 2006), aunque solo unos pocos han considerado los componentes filogenéticos y funcionales de la diversidad (Devictor *et al.* 2010; Prado *et al.* 2010; Mouillot *et al.* 2011; Taberlet *et al.* 2012). Además, prácticamente la totalidad de estos estudios se han realizado a gran escala, y por tanto, se desconoce si el problema existe a escalas más reducidas, donde se implementan la mayoría de las medidas de gestión de la biodiversidad (e.g. Gjerde et al., 2004; Harris et al., 2005; Laguna et al., 2004; Nantel et al., 1998; Schwartz, 1999).

La biodiversidad a lo largo del tiempo

Los mapas de biodiversidad están trazados a partir de datos recopilada en diferentes años, asumiendo que la información (p. ej. la presencia de una especie en un determinado lugar) no está desfasada. Sin embargo, la biodiversidad en todos sus nivel es cambiante, ya sea por razones naturales, o por el impacto directo e indirecto de la actividad humana. De hecho, este último motor de cambio ha dado lugar a una tasa de pérdida de biodiversidad sin precedentes en la historia reciente (Pimm *et al.* 2014), que podría ser equiparable a las cinco extinciones masivas de nuestro planeta (Barnosky *et al.* 2011). El cambio de uso de suelo y el cambio climático son los principales motores de cambio global en los ecosistemas terrestres (Vitousek *et al.* 1997; Sala *et al.* 2000), y las evidencias de sus impactos sobre la biodiversidad son relativamente frecuentes (Chapin III *et al.* 2000; Walther *et al.* 2002; Parmesan 2006; Walther 2010). Dado que todo apunta a que el escenario de cambio global será aun peor en las próximas décadas (Millennium Ecosystem Assessment 2005; IPCC 2014), urge conocer dónde, cómo y con qué intensidad se verán afectados los ecosistemas naturales. Solo alcanzado un alto grado de conocimiento de estas cuestiones seremos capaces de desarrollar medidas de gestión para paliar los posibles impactos del cambio global (Chapin III *et al.* 2000).

La predicción de los efectos del cambio global sobre los ecosistemas ha acaparado una gran atención en la comunidad científica, dando lugar al desarrollo de potentes modelos predictivos para determinar la distribución futura de las especies. A pesar de estar sometidos a una mejora continua, los modelos de distribución de especies actuales presentan serias limitaciones para anticipar la respuesta de la biodiversidad frente al cambio global (Elith & Leathwick 2009; Wisz *et al.* 2013). No menos importante es la ausencia de datos empíricos con los que realizar una validación realista de estos modelos (Guisan & Theurillat 2005). Por consiguiente, resulta evidente la necesidad de conocer primero cómo están respondiendo los ecosistemas de manera precisa, para poder después realizar predicciones fiables.

El estudio de las respuestas de la biodiversidad al cambio global debe abordarse con una perspectiva temporal amplia, dado que estas no tienen por qué ser lineales y/o inmediatas (Harrison, Damschen & Grace 2010; Bertrand *et al.* 2011). Este aspecto resulta particularmente relevante en el caso de las comunidades dominadas por especies longevas o con distribución vegetativa, cuya respuesta frente al cambio global podría ser lenta, y enmascarar las consecuencias reales (e.g. extinción, o colonización de especies; Tilman *et al.*, 1994; Piqueray *et al.* 2011; Dullinger *et al.* 2012). Una razón no menos importante para el estudio a largo plazo de los impactos del cambio global es que los ecosistemas experimentan cambios naturales, de carácter estocástico, que podrían dar lugar a interpretaciones erróneas. Desafortunadamente, los estudios detallados a largo plazo iniciados en las décadas pasadas son escasos.

Una alternativa recurrente para el estudio de la dinámica temporal de las comunidades vegetales a largo plazo es revisitar localidades que fueron muestreadas en el pasado. Sin embargo, el análisis de datos históricos y actuales está sujeto a incertidumbre. Factores tales como la precisión de la relocalización de las unidades de muestreo pueden dar lugar a una sobre-estimación de los cambios biológicos observados. Si bien en algunos casos excepcionales es posible minimizar el efecto de estas fuentes de incertidumbre (p. ej. muestreos en parcelas permanentes, protocolos detallados), los resultados entre dos observaciones en el tiempo deberían interpretarse con extrema cautela, ya que podrían simplemente reflejar la dinámica natural (cambios estocásticos) del sistema de estudio. Además, para poder discernir la causa exacta del cambio temporal de las comunidades, puede ser necesario realizar manipulaciones experimentales. Si las monitorizaciones a largo plazo son escasas, los experimentos a largo plazo lo son aún más. Además, en la mayoría de los casos no están adecuadamente replicados, y por lo tanto, la valiosa información que se puede extraer de ellos es difícilmente generalizable.

Las montañas como laboratorio para el estudio de la distribución de la biodiversidad y su respuesta al cambio climático

Las montañas albergan mayor diversidad que las zonas colindantes, y en muchos casos constituyen auténticos puntos calientes de riqueza de especies y endemismos. A pesar de las diferencias existentes entre las distintas cadenas montañosas del mundo, todas ellas comparten ciertas características generales (gradiente altitudinal, gran heterogeneidad espacial, y fragilidad) que hacen de ellas excepcionales laboratorios naturales para el estudio de la distribución de la biodiversidad, y su respuesta frente al cambio global (Körner 2007a; Sanders & Rahbek 2012). Además, las montañas juegan un papel importante en el abastecimiento de recursos e innumerables servicios ecosistémicos para las poblaciones humanas (Beniston 2006), lo que pone de manifiesto la transcendencia de los estudios científicos en montaña.

La variación climática a lo largo del gradiente altitudinal juega un papel fundamental para la diversidad de las montañas. Este gradiente climático, en realidad representa dos gradientes opuestos: la temperatura y variables asociadas disminuyen con la elevación, mientras que la precipitación y la humedad aumentan (Körner 2007a). Por esa razón no es de extrañar que la mayor acumulación de especies vegetales ocurra en zonas intermedias del gradiente altitudinal (Rahbek 1995). El efecto de la altitud sobre otros componentes de la diversidad vegetal ha sido mucho menos estudiado (ver Bryant *et al.* 2008, Ndribe *et al.* 2014), y existe un menor consenso a la hora de explicar su distribución. En cualquier caso, sea en mayor o menor medida, el efecto del gradiente climático sobre los diferentes componentes de la diversidad es patente. Por esta razón, las montañas, y en consecuencia su diversidad vegetal, son particularmente vulnerables al impacto del cambio climático. Esto coincide con el notorio aumento de temperaturas experimentado en muchas zonas montañosas, que en muchos casos es superior al observado en las zonas bajas.

Las montañas están experimentado también un importante cambio de uso del suelo. El paisaje de la mayoría de las montañas europeas ha sido moldeado por la actividad humana durante siglos (Fillat 2008). Las zonas montanas y colinas fueron deforestadas para la extracción de combustible, mientras que el bosque subalpino fue mermado mediante tala y quema para ampliar las zonas de pastoreo estival. Estas actividades agro-pastorales centenarias dieron lugar a un paisaje de gran heterogeneidad y valor cultural en la alta montaña, y fueron claves para el desarrollo de las ricas comunidades alpinas (Olff & Ritchie 1998; Adler, Raff & Lauenroth 2001; Deléglise, Loucougaray & Alard 2011). Sin embargo, estas actividades experimentaron un drástico retroceso durante el siglo pasado, debido al fuerte flujo migratorio de la población rural hacia las ciudades y el auge de otros sectores económicos. Como resultado, ha habido un descenso generalizado de las actividades ganaderas, y de manera más puntual, una concentración de estas en las zonas más accesibles (fondo de valle).

Los impactos del cambio global sobre la diversidad de la alta montaña: predicciones vs. observaciones

Son muchas la generalidades sobre los posible impactos del calentamiento climático en montañas (Theurillat & Guisan 2001; Crawford 2008). Una de las más recurrentes y que goza de mayor respaldo empírico es el ascenso altitudinal de las especies siguiendo el aumento de temperaturas (Grabherr, Gottfried & Pauli 1994; Lenoir *et al.* 2008; Chen *et al.* 2011; Pauli *et al.* 2012; Morueta-Holme *et al.* 2015). En este aspecto, las especies más sensibles son las que aparecen en las cumbres, dado que no pueden escapar hacia arriba, y su hábitat ocupa un área infinitamente inferior al de las especies de zonas más bajas. La termofiliación de las montañas, es decir, el aumento de especies con mayor afinidad térmica a costa de las especies más adaptadas a las condiciones de cumbre, ha sido observada en varias montañas (Keller, Kienast & Beniston

2000; Parolo & Rossi 2008; Erschbamer *et al.* 2008; Gottfried *et al.* 2012). Sin embargo, Grytness y colaboradores (2014) han presentado evidencias que cuestionan el papel principal de la temperatura sobre los cambios en las comunidades vegetales de cimas.

Otra expectativa generalizada es el desplazamiento de algunos límites bioclimáticos. Uno de los más frecuentemente estudiados es el límite superior del bosque subalpino, donde el crecimiento arbóreo está limitado por las frías temperaturas de la alta montaña. El incremento de la cobertura arbórea es concebido como uno de los cambios más drásticos posibles para las comunidades alpinas. Sin embargo, no existen apenas estudios que hayan cuantificado las consecuencias reales del aumento de la cobertura arbórea y/o avance del límite del bosque superior sobre las ricas comunidades alpinas adyacentes de modo detallado (Illerbrun & Roland 2011).

Respecto al impacto de los cambios de uso del suelo, las evidencias de la sucesión ecológica debido al cese del pastoreo (reforestación natural y matorralización) son muy evidentes en los pastos montanos y subalpinos (Gehrig-Fasel *et al.* 2007; Gellrich *et al.* 2007; Améztegui, Brotons & Coll 2010; Komac, Alados & Camarero 2011; Gartzia, Alados & Pérez-Cabello 2014). Por el contrario, el impacto del descenso de pastoreo ha sido menos explorado en las comunidades de pastos alpinos (Austrheim & Eriksson 2001; Erschbamer, Virtanen & Nagy 2003), si bien son esperables cambios en su diversidad, composición y calidad nutritiva para el ganado (Milchunas, Sala & Lauenroth 1988; Olff & Ritchie 1998; Krahulec *et al.* 2001; Vesk & Westoby 2001; Tasser & Tappeiner 2002; Dullinger *et al.* 2003; Pavlů *et al.* 2005). Algunos estudios han mostrado que la riqueza específica disminuye con el descenso de pastoreo (Tasser & Tappeiner 2002; Niedrist *et al.* 2008), mientras que otros han observado un aumento (Speed, Austrheim & Mysterud 2013), o un rápido cambio en la composición de la comunidad a corto plazo (Mayer *et al.* 2009). No obstante, nuestro conocimiento actual sobre la respuesta a largo plazo de las comunidades alpinas al descenso de pastoreo es todavía limitado, y desconocemos cómo esta respuesta podría interactuar con los efectos del calentamiento climático (ver Speed *et al.* 2012). Por lo tanto, es necesario abundar en el conocimiento de estas cuestiones, puesto que solo así será posible desarrollar medidas de gestión eficientes para la conservación de estas comunidades de gran valor ecológico y cultural.

El área de estudio: el Parque Nacional de Ordesa y Monte Perdido

Todos los estudios de esta tesis fueron realizados en el Parque Nacional de Ordesa y Monte Perdido (PNOMP; 42º42' N, 0º03' W), y de manera más puntual en el límite del bosque localizado en la zona periférica del Parque Nacional de Aigüestortes i Estany de Saint Maurici National Park (42º28'N 1º03' E), así como en varios pastos alpinos del Parque Natural Los Valles Occidentales (42º44' N, 0º35' W). La descripción de estas áreas de estudio pirenaicas se presenta en los capítulos 3 y 4 respectivamente.

El PNOMP se extiende a lo largo de cinco valles que convergen en el pico calcáreo más alto de Europa. Se trata de un parque de alta montaña, de tamaño reducido (15.608 ha, más 19.679 ha de zona periférica) y una geomorfología extraordinariamente compleja. La geología predominantemente es calcárea, si bien la arenisca es abundante en los acantilados, y el flysch y la marga aparecen en los pasos de montaña y picos inferiores. El clima por encima de los 1600 m.s.n.m es típico de montaña con una influencia continental notable, y una temperatura media de 5ºC, siendo enero el mes más frío (-0.5ºC) y junio el mes más cálido (13ºC). Las precipitaciones anuales oscilan entre 1103 mm (en 2004) y 2506 mm (en 1997), y se concentran en primavera y otoño. Cabe destacar que no existen épocas de sequía marcada a lo largo del verano. La nieve ocupa vastas áreas del parque de noviembre (octubre) a mayo (junio). Dada la topografía del parque, existen un gran número de microclimas, y grandes diferencias entre las laderas norte y sur que dejan su impronta en la vegetación. A una escala menor, los estrechos cañones dan lugar a eventos de inversión térmica.

A pesar de que no hay núcleos poblados dentro del parque, y que el Sobrarbe es una de las zonas menos pobladas de la Península Ibérica, su explotación ganadera (principalmente pastoreo estival) ha sido intensa durante siglos. En la actualidad el parque goza de un estricto régimen de protección, y tanto el pastoreo como la tala de árboles son actividades reguladas por el PRUG (Plan Rector de Uso y Gestión; DECRETO 49/2015).

Respecto a la vegetación, en las zonas bajas podemos diferenciar los bosques de *Quercus ilex* subsp. *ilex* y comunidades seriales de matorral mediterráneo, así como bosques submediterráneos de *Quercus cerrioides*. Por encima de los 1000 m se encuentran los bosques mixtos, a menudo dominados por el haya y el abeto. En estos ambientes el boj es relativamente abundante. Las bujedas y los matorrales de erizón (*Echinospartum horridum*) se extienden hasta los 2000 m. A medida que vamos ganando altitud, el bosque de *Pinus sylvestris* es sustituido por el del *Pinus uncinata.* Ambos tipos de bosque han sido explotados para aumentar las áreas destinadas al pastoreo. Como resultado, el bosque de *Pinus uncinata* se encuentra por debajo de su límite potencial (2100 m), si bien todavía quedan en el parque zonas localizadas que no han sido sustancialmente modificados por la actividad humana. Los pastos ocupan zonas del bosque subalpino. Algunos de estos pastos supraforestales presentan una estructura y composición similar a los estrictamente alpinos, habiéndose acuñado en castellano el término subalpino alpinizado para denominar la zona donde se encuentran estos pastos. Por motivos prácticos, hemos considerado los pastos del subalpino alpinizado como alpinos. Los pastos alpinos presenta una gran riqueza de especies, en su mayoría hemicriptófitas, pero al ganar en altitud abundan las caméfitas. En la zona subnival, la cobertura de la vegetación es muy reducida, y aparecen diferentes comunidades de plantas dependiendo del tipo de roca, exposición y estabilidad, entre las que se encuentran las plantas de los géneros *Silene, Saxifraga*, *Androsace, Artemisia, Draba, Minuartia, Veronica*, *Cerastium* y *Linaria.*

A pesar de la enorme diversidad de comunidades vegetales, para los análisis de diversidad hemos diferenciado ocho tipos de vegetación (entre paréntesis su abundancia): comunidades nitrófilas asociadas a la actividad humana (0.4%); humedales (0.4%); bosques Mediterráneos (2%); matorrales (4%); bosques de hoja caduca (7%); pinares (16%); ambientes rocosos (32%); pastos (38%).

De acuerdo con los registros del herbario de Jaca, el número total de taxones se estima en 1379 (si bien el número real de especies es a buen seguro mayor) pertenecientes a 104 familias, y 483 géneros. Más del 45% de los endemismos Pirenaicos están presentes en el PNOMP, incluyendo algunos elementos del terciario tales como *Ramonda myconii*, *Borderea pyrenaica.* Además, un notable número de especies raras (p. ej. *Silene borderei, Carduus carpetanus, Veronica aragonensis, Pinguicula longifolia* subsp. *longifolia*) están presentes en el parque. Veinticinco especies están listadas en el Catálogo de Aragón de Especies Amenazas, entre ellas cabe destacar *Corallorhiza trifida* y *Cypripedium calceoulus* que están consideradas en peligro de extinción. Están presentes también nueve taxones en la Directiva Hábitats. Por grupos biogeográficos, la flora se constituye principalmente por especies euro-siberianas y mediterráneas, mientras que un 8% son boreoalpinas.

Al igual que en el resto del Pirineo, el paisaje del PNOMP es en buena medida el resultado de la actividad milenaria del ser humano. El sistema agro-pastoral se generalizó durante la Edad Media, y fue entonces cuando se dio inicio a la trashumancia. Este modelo de pastoreo ha jugado un papel central para la economía de las áreas rurales desde entonces. Si bien a inicios del siglo XX se registraron los máximos históricos del número de cabezas de ganado, a mediados de los años 50 este modelo de explotación extensivo entró en crisis debido al despoblamiento generalizado de las áreas rurales. En la actualidad, la población es un 70% inferior a la existente a comienzos del siglo XX. Como resultado, el número de cabezas de ganado se ha visto reducido sustancialmente, y en algunos casos, el ganado ovino ha sido sustituido por el bovino y/o equino, sin llegar a compensar el descenso. Como contrapartida, el turismo ha emergido durante las últimas décadas. Más de 600.000 personas visitan anualmente el PNOMP, principalmente los valles de Ordesa y Pineta durante la época estival. El descenso del pastoreo ha dado lugar a una reforestación natural y matorralización de los pastos montanos y del subalpino inferior.

Por otra parte, los registro climáticos han puesto de manifiesto una tendencia al alza de las temperaturas (+0.3ºC por década) desde los años 50. Las predicciones climáticas señalan que la tasa de calentamiento será aún mayor a lo largo del siglo XXI. A escala regional se ha registrado también un incremento de los eventos de calor extremo, así como una tendencia general de descenso de precipitaciones. Como consecuencia del calentamiento climático, el glaciar del Monte Perdido se está fundiendo y también se ha observado un incremento en el reclutamiento de árboles y de su tasa de crecimiento en el límite superior del bosque (Camarero et al. 2015), así como un aumento de especies termófilas en algunas cumbres del parque (Gottfried et al. 2012).

OBJETIVOS

Los objetivos generales de esta tesis son ampliar nuestro conocimiento sobre la distribución de los diferentes componentes de la diversidad vegetal en la alta montaña, y evaluar su respuesta frente al efecto del descenso del pastoreo y aumento de temperaturas durante las últimas dos décadas. A continuación se presentan los objetivos asociados a cada uno de los cinco capítulos recopilados en esta tesis:

- Cuantificar el efecto del esfuerzo de muestreo en nuestra base de datos, y evaluar su impacto sobre los análisis de los patrones de diversidad (*Capítulo 1*).

- Determinar la distribución de los componentes taxonómico (especies y endemismos por separado), filogenético y funcional de la diversidad vegetal en la alta montaña, y su representación en diferentes tipos de hotspots (*Capítulo 2*).

- Evaluar los efectos del aumento de la cobertura arbórea sobre las comunidad vegetal del límite superior del bosque a lo largo de 11 años (*Capítulo 3*).
- Analizar la dinámica a largo plazo (las dos últimas décadas) de los pastos alpinos en un contexto de descenso de pastoreo y aumento de temperaturas (*Capítulo 4*).
- Separar el efecto a largo plazo del cese del pastoreo y la variabilidad climática sobre las comunidades de pastos alpinas (*Capítulo 5*).

Para abordar estos objetivos combinamos el análisis de información disponible en bases de datos (*Capítulo 1* y *2*), con aproximaciones descriptivas y experimentales a partir de datos de campo (*Capítulo 3, 4* y 5). En primer lugar examinamos el efecto del esfuerzo de muestreo en la información de la bases de datos a partir de una nueva técnica que desarrollamos (*Capítulo 1*). A continuación utilizamos los valores del esfuerzo de muestreo para determinar su efecto sobre la distribución espacial de los diferentes componentes de la diversidad vegetal en el PNOMP (*Capítulo* 2). En este mismo capítulo, y utilizando una base de datos alternativa, examinamos el grado de congruencia espacial entre los hotspots de los diferentes componentes de la diversidad. En los siguientes capítulos nos centramos en el estudio de la respuesta de las comunidades alpinas al descenso del pastoreo y aumento de las temperaturas. En el *Capítulo 3*, analizamos el efecto de la dinámica del límite superior del bosque alpino sobre las comunidades alpinas, comparando datos de vegetación históricos y actuales obtenidos en transectos semi-permanentes. En el *Capítulo 4*, revisitamos una docena de parcelas de vegetación muestreadas hace más de dos décadas, y tomamos mediciones del error de muestreo y de la fluctuación temporal de las comunidades. A partir de esta información analizamos la dinámica a largo plazo de los pastos alpinos a escala de comunidad, especies y rasgos funcionales (*Capítulo 4*). Finalmente, testamos el efecto a largo plazo (19 años) del cese del pastoreo y su interacción con el clima en un experimento de exclusión (*Capítulo 5*).

PUBLICACIONES

Capítulo 1 - *A novel method to handle the effect of uneven sampling effort in biodiversity databases*

Muchos análisis de diversidad están basados en bases de datos que contienen información espacial de la distribución de las especies. Esta información es a menudo incompleta y contiene sesgos espaciales debido a las diferencias en el esfuerzo de muestreo realizado para su obtención. Varios estudios han puesto de manifiesto la importancia de tener en cuenta el esfuerzo de muestreo en las bases de datos a la hora de elaborar mapas de biodiversidad precisos o identificar áreas prioritarias para la conservación, y se han desarrollado varios métodos para ello. En el capítulo 1 evaluamos las propiedades estadísticas de dos de los métodos más frecuentemente para la medición del esfuerzo de muestreo, y proponemos uno nuevo que cumple todas las asunciones estadísticas. Mediante curvas ROC testamos la capacidad de estos tres métodos (todos ellos basados en curvas de acumulación) para discriminar unidades espaciales bien e insuficientemente muestreadas, tanto en escenarios reales como simulados, donde la riqueza real es conocida. Nuestro método presentó una mayor correlación con la diversidad real debido a su excelente capacidad de discriminación, en todos los escenarios. Por contra, los otros dos métodos mostraron una peor capacidad para la discriminación entre las unidades bien y poco muestreadas, siendo su utilidad particularmente limitada en escenarios de baja intensidad de muestreo (pocos registros). Nuestros resultados ponen de manifiesto que no solo es importante medir el esfuerzo de muestreo en las bases datos, sino cómo hace, ya que su estimación podría resultar inexacta con algunos de los métodos existentes. De acuerdo a los criterios testados (cumplimiento de las asunciones estadísticas y capacidad discriminatoria), nuestro método resultó ser la alternativa más robusta para cuantificar el esfuerzo de muestreo. Dado que su aplicación es posible en un amplio espectro de situaciones, concluimos que esta nueva herramienta puede contribuir a controlar la incertidumbre de los análisis de biodiversidad basados en bases de datos.

Capítulo 2 *- On the limits of hotspots for the representation of multifaceted diversity at small-scale*

El efecto del esfuerzo de muestreo sobre los patrones de riqueza de especies (SR) es intuitivo, a mayor esfuerzo, mayor número de especies. También es conocido que este efecto puede distorsionar la selección de áreas prioritaria para la conservación de especies. Sin embargo, se desconoce en qué medida el esfuerzo de muestreo puede distorsionar los patrones de distribución de otros componentes de la diversidad tales como la diversidad filogenética (PD) y la diversidad funcional (FD). Por otra parte, resulta necesario estudiar el grado de agregación espacial entre los diferentes componentes de la diversidad, con el fin de evaluar si los diferentes componentes están suficientemente representados en los espacios protegidos Varios estudios han dado cuenta del escaso solapamiento espacial entre los diferentes componentes a escala global o regional, pero desconocemos si este problema persiste a escala más reducidas donde se toman la mayoría de decisiones de gestión para la biodiversidad. A partir de dos fuentes de información, base de datos a partir de presencias de especies en UTMs de 1x1 km *vs.* inventarios estandarizados de comunidades, y la construcción de una filogenia y un dendrograma funcional para todas las especies del PNOMP, hemos evaluado las siguientes cuestiones: (i) el grado de congruencia espacial entre los hotspots locales de los diferentes componentes (SR, PD, FD y riqueza de endemismo; ER), (ii) la representación de estos componentes en los hotspots, y (iii) el efecto del esfuerzo de muestreo y la resolución espacial de los datos sobre los resultados. A partir de ambas fuentes de información, observamos una distribución segregada de los diferentes componentes de diversidad, excepto entre la PD y FD, que puede ser atribuible a la señal filogenética de los rasgos incluidos en este estudio. La ausencia de solapamiento entre SR, ER y PD-FD es consistente con los resultados obtenidos previamente a gran escala. Sin embargo, nuestros resultados indican que la falta de congruencia espacial entre los diferentes componentes de la diversidad puede no en todos los casos atiende a cuestiones ecológicas. De hecho, la distribución de los diferentes tipos de hotspots deducida a partir de la base de datos resultó estar severamente sesgada por las diferencias en el esfuerzo de muestreo entre las UTMs. Además, la representación de la diversidad en los hotspots estuvo exagerada como resultado de una resolución espacial inapropiada dada la compleja topografía del área de estudio. Los hotspots a partir de inventarios de comunidades (sin diferencias en el esfuerzo de muestreo) también mostraron una escasa coincidencia espacial. Los hotspots de SR y ER se concentraron en los pastos, mientras que los valores máximos de PD y FD fueron observados en hábitats forestales (principalmente bosques mixtos), con valores de diversidad relativamente bajos. En este caso, ningún tipo de hotspot resultó ser representativo de los múltiples componentes de la diversidad. En conjunto, nuestros resultados ponen de manifiesto la importancia de evaluar la calidad de la información biológica disponible a la hora de identificar los hotspots de manera verosímil, pero también la escasa utilidad de los diferentes tipos de hotspots por separado para la protección de la diversidad a escala local.

Capítulo 3 **-** Uncoupled changes in tree cover and field layer vegetation at two *Pyrenean treeline ecotones over 11 years*

Tal y como demostramos en el capítulo anterior, las comunidades más ricas en términos de especies y endemismos se encuentra en los pastos de alta montaña. Sin embargo, estos pastos están altamente expuestos al impacto del cambios de uso de suelo (descenso de pastoreo) y cambio climático (aumento de temperaturas). El límite del bosque superior es un indicador recurrente del aumento de las temperaturas, ya que su límite superior natural está determinado por las condiciones climáticas. El incremento de la cobertura arbórea, constituye sin duda uno de los cambios estructurales más importantes para las comunidades alpinas. Sin embargo, existen muy pocas evidencias empíricas que nos permitan conocer en detalle el efecto de la dinámica del límite del bosque sobre las comunidades alpinas adyacentes. Esta información es necesaria para poder predecir el impacto real del ascenso del límite del bosque sobre la diversidad alpina. En aras de dar respuesta a este cuestión sin resolver, revisitamos varios transectos semi-permanentes de vegetación situados en dos ecotonos con características opuestas. En cada transecto anotamos la cobertura arbórea y composición de especies, y a partir de los datos obtenidos en 1998 y 2009 evaluamos la evolución temporal de estas variables. Tras 11 años, la cobertura arbórea incrementó en uno de los límites del bosque, mientras que la diversidad vegetal solo cambió en el otro. Dentro de cada ecotono los cambios en la diversidad y el aumento de la cobertura arbórea no estuvieron espacialmente ligados, y no se detectó un incremento en la abundancia de las especies con afinidades forestales. Estos resultados sugieren que el efecto del incremento de la cobertura arbórea sobre las comunidades alpinas puede no ser inmediato, y por lo tanto, que nuestra habilidad para inferir el impacto a corto plazo del avance del límite del bosque sobre las comunidades alpinas es limitada.

Capítulo 4 - *Little evidence of the impact of global change on alpine grasslands over the last two decades in the Pyrenees*

El descenso generalizado del pastoreo constituye una gran amenaza para la conservación de los pastos de montaña. Si bien la respuesta de la vegetación a la reducción de pastoreo está relativamente bien documentada por debajo del límite del bosque, esta es más incierta en los pastos alpinos, debido a que los estudios a largo plazo son escasos, y poco se sabe sobre su posible interacción con los efectos del calentamiento climático. En este capítulo estudiamos la dinámica de las comunidades alpinas durante las dos últimas décadas a lo largo de un gradiente altitudinal en dos valles del Pirineo central. Para ello, combinamos información obtenida tras la revisitas de pastos en transectos semi-permanentes (1989/1990-2010/2011), información sobre la variación natural de las comunidades observada en monitorizaciones a largo plazo, y mediciones del error de muestreo tomadas en campo. La combinación de esta información nos permitió evaluar la magnitud del cambio temporal (revisitas), teniendo en cuenta la fluctuación natural de los pastos (monitorización anual), y descontando el error de muestreo. Los resultados de nuestros análisis revelaron una relativa estabilidad de los pastos alpino. Entre 1989/1990 y 2010/2011 la mayoría de las comunidades no varió entre décadas más de lo que es esperable debido a cambios naturales (y reversibles). Ninguna de la comunidades experimentó un incremento de especies provenientes de zonas más bajas, y no se observó tampoco una homogeneización, ni signos de matorralización en las comunidades. De las 171 especies detectadas, menos de la mitad experimentaron cambios significativamente superiores a los esperados por error de muestreo, y todas ellas presentaron una abundancia baja. Las diferencias a escala de rasgos funcionales entre las especies con dinámicas temporales opuestas fue escasa tras 20 años, e inexistente cuando se tuvo en cuenta la autocorrelación evolutiva de estos rasgos, y su abundancia. Nuestros resultado señalan que los pastos alpinos pueden tener un inercia ecológica mucho más grande de lo generalmente esperado, y mayor que la observada en pastos subalpinos y montanos, así como en las comunidades de cimas de montana.

Capítulo 5 - Long-term response of plant communities to herbivore exclusion at high elevation grasslands

Los modelos teóricos de pastoreo predicen un efecto drástico del descenso del pastoreo sobre la diversidad y composición de los pastos de alta montaña. Mediante un experimento de exclusión a largo plazo (19 años) testamos esta predicción en dos pastos alpinos. Tras dos décadas, observamos un incremento en la biomasa, pero no efectos significativos sobre la riqueza de especies. Además, las trayectorias temporales a nivel de comunidad, y las transiciones entre grupos ecológicos (principalmente gramíneas dominantes, gramíneas no dominantes y dicotiledóneas) no fueron sustancialmente divergentes entre los controles hasta el 9º año y coincidiendo un periodo excepcionalmente cálido y seco. Nuestros resultados indicaron una alta inercia ecológica de las comunidades frente al cese del pastoreo, dejando en evidencia las limitaciones existentes para anticipar la dinámica de los pastos alpinos a partir de los modelos existentes. No obstante, la mayor vulnerabilidad de los pastos a los efecto climáticos en ausencia de pastoreo llama la atención sobre los posibles efectos aditivos que podrían tener en el futuro los principales motores de cambio global si no se revierten las tendencias actuales de abandono del territorio, y la actividad ganadera de alta montaña.

DISCUSIÓN GENERAL

Distribución espacial de la diversidad vegetal

El sesgo del esfuerzo de muestreo en la bases de datos

A pesar de que el PNOMP es el área con mayor número de registro de plantas de la Península Ibérica (Font *et al.* 2010), nuestro conocimiento de cómo está distribuida la diversidad vegetal es todavía incompleto y está espacialmente sesgado. Este sesgo es común a las bases de datos que aglutinan información de diferentes fuentes, y se debe a la diferencia de esfuerzo de muestreo realizado para la obtención de la información florística en las diferentes unidades geográficas. Tal y como han documentado otros estudios con anterioridad, los lugares mejor prospectados resultaron ser los más ricos (Dennis, Sparks & Hardy 1999; Hortal, Lobo & Jiménez-Valverde 2007; Boakes *et al.* 2010; Robertson, Cumming & Erasmus 2010). Sin embargo, nuestros resultados demuestran por primera vez que la distribución de otros componentes de la diversidad también pueden verse afectada. En el caso de las medidas integrales de diversidad, tales como la PD de Faith (1992) y su medida análoga de FD, el sesgo del esfuerzo de muestreo fue probablemente debido a su correlación con SR. Sin embargo, obtuvimos un resultado similar al repetir los análisis con medidas de independientes de PD y FD (no afectadas por el SR), lo que sugiere que la probabilidad de muestrear especies evolutivamente lejanas incrementa en función del esfuerzo de muestreo. En cualquier caso, el sesgo de muestreo hacía las zonas mejor prospectadas claramente distorsionó nuestra selección de áreas prioritarias para la conservación (UTMs de 1x1 km).

Si bien es importante medir el esfuerzo de muestreo, no menos importante es la manera de hacerlo. En el capítulo 1, demostramos que no todas las metodologías para cuantificar el esfuerzo de muestreo resultaron ser igualmente precisas. En consecuencia, la fiabilidad de la discriminación entre las unidades espaciales bien y mal muestreadas dependió del método utilizado. Para apreciar la importancia de este resultado basta hacer un paralelismo con un caso clínico, y pensar en falsos positivos. Los valores obtenidos con algunos de los métodos indicaron que el paciente estaba enfermo, sin estarlo. Por el contrario, nuestra nueva metodología, FIDEGAM, generó muy pocos falsos positivos. Cabe destacar que el uso de esta metodología es posible incluso en escenarios de esfuerzo de muestreo muy bajo, lo que permite su aplicación en zonas de estudio muy poco prospectadas.

Una vez medido el esfuerzo de muestreo, el siguiente reto es incorporar esta información en los análisis de diversidad para corregir posibles sesgos. No existe una única alternativa, y todas ellas presentan limitaciones. La aproximación más intuitiva, y que fue usada en el ejemplo presentado en el capítulo 1, es considerar exclusivamente las unidades espaciales bien muestreadas. Sin embargo, nuestros resultados de la regresión cuantil entre el esfuerzo de muestreo y los valores de los diferentes componentes de la diversidad mostraron que el sesgo fue precisamente más notorio entre las zonas mejor muestreadas (Capítulo 2). Otra posibilidad es incluir la medida del esfuerzo de muestreo como variable independiente (o peso) en la modelización espacial de la diversidad, para así aumentar su ajuste y poder predictivo (Pardo *et al.* inédito).

En cualquier caso, todas estas alternativas constituyen una solución temporal, mientras se va recopilando más información sobre la distribución de la diversidad. Esta tareas es, no obstante, inabordable a corto plazo por la comunidad científica, y cada vez son más las voces que abogan por involucrar a diferentes colectivos (p. ej. voluntarios; Bonney *et al.* 2009). La generación de mapas de esfuerzo de muestreo podría ayudar a identificar las áreas donde el inventariado florístico es más incompleto, y por lo tanto, que más podrían contribuir a completar los mapas reales de diversidad (Sastre & Lobo 2009).

La distribución de los múltiples componentes de la diversidad vegetal y su implicación para la selección de áreas prioritarias

En el capítulo 2 hemos visto que aspectos metodológicos (esfuerzo de muestreo, resolución espacial de los datos, selección de métricas de diversidad) pueden dificultar la identificación de las áreas prioritarias locales para la conservación de los múltiples componentes de la diversidad (Capítulo 2). Cuando los análisis fueron repetidos a partir de información recogida de manera estandarizada (inventarios fitosociológicos), encontramos un escaso solapamiento espacial entre los diferentes tipos hotspots. La única excepción observada fue entre los hotspots de PD y FD, muy probablemente debido a la alta señal filogenética de los rasgos utilizado en este estudio. Nuestros análisis no nos permiten determinar de modo preciso las causas de distribución dispar entre los diferentes componentes de la diversidad, sin embargo, parece claro que los factores tanto ecológicos (p. ej. filtrado ambiental) como históricos jugaron un papel clave. Por ejemplo, la baja acumulación de historia evolutiva y notable abundancia de endemismos en las comunidades de la alta montaña podrían reflejar los procesos de vicarianza y especiación alopátrica ocurridos durante las glaciaciones cuaternarias (Tribsch 2004). Por el contrario, la concentración de PD en bosques mixtos a altitudes relativamente bajas, podría ser debida a la mayor frecuencia de especies terciarias en zonas con mayor estabilidad ambiental.

En cualquier caso, estos resultados ponen de manifiesto el valor de diferentes tipos de hábitats. Tal y como es conocido los pastos resultaron ser los hábitats más ricos (García & Gómez 2007), pero también con mayor concentración de endemismos. Un resultado más sorprende fue el valor, hasta ahora oculto, de algunos tipos de bosques (bosques mixtos de avellano y fresnos) como reservorios de historia evolutiva.

La respuesta temporal las comunidades alpinas al cambio global

Los pastos constituyen las comunidades más ricas de la alta montaña, pero también unas las más expuestas a los efectos del descenso de pastoreo y del calentamiento climático. En contra de lo esperado, los indicios de cambio en las comunidades alpinas durante las dos últimas décadas fueron escasos.

El límite superior del bosque subalpino

El reclutamiento árboles y el incremento del cobertura arbórea son dos de los cambios estructurales más grandes posibles en las comunidades alpinas. En el capítulo 3, observamos un aumento de la cobertura arbórea en un límite del bosque de PNOMP entre 1998 y 2009. Este resultado es consistente con la dinámica observada en otros límites del bosque (Camarero & Gutiérrez 2004; Körner & Paulsen 2004; Batllori & Gutiérrez 2008; Harsch *et al.* 2009), incluido el del valle de Escuaín (Camarero *et al.* 2015). Sin embargo, estos cambios estructurales no tuvieron efecto aparente sobre la diversidad y composición de la vegetación. Este sorprendente resultado podría atribuirse a que la respuesta de la vegetación alpina al incremento de la cobertura arbórea fue muy lenta, y/o a que nuestro método no fue suficientemente adecuado para capturar cambios menores en la vegetación.

El incremento de la cobertura arbórea no fue homogéneo a lo largo del ecotono, probablemente porque el reclutamiento y/o crecimiento de los individuos estuvo asociado a condiciones microclimáticas favorables (Batllori *et al.* 2009; Holtmeier & Broll 2010), y pudo no constituir una pérdida de hábitat para las especies alpinas. Sin embargo, tampoco se detectaron evidencias de cambios en la vegetación a escala más detallada (segmentos altitudinales), ni siquiera donde la cobertura arbórea alcanzó niveles similares a los observados en el bosque subalpino. Dado que bajo esa situación la colonización de especies con afinidades forestales parece inevitable, nuestros resultados podrían reflejar un desfase de la vegetación, es decir, un "deuda de colonización" (Piqueray *et al.* 2011). Para verificar si los cambios en la vegetación ocurrieron a una escala más fina de la que pudo detectar con nuestro método, iniciamos una monitorización en un centenar de parcelas permanentes de 20x20 cm bajo 10 pinos seleccionados al azar, y en zonas sin cobertura arbórea. Tras cuatro años, los resultados preliminares mostraron un claro incremento de la cobertura arbórea, mientras que solo se detectó una ligera tendencia al alza de las especies acidófilas en las parcelas con cobertura arbórea (Tejero, Pardo & García 2015). Por consiguiente, podemos descartar que la falta de respuesta de la vegetación al aumento de la cobertura arbórea en el ecotono fuera producto del método de muestreo utilizado.

Pastos alpinos

La herbivoría juega un papel principal en la configuración la diversidad vegetal de los pastos (Augustine & McNaughton 1998). En consecuencia, los modelos generalizados de herbivoría predicen cambios importantes en la diversidad y composición de especies de las comunidades de pastos frente a un hipotético descenso de pastoreo de magnitud similar al observado en el Pirineo (Milchunas, Sala & Lauenroth 1988; Olff & Ritchie 1998; Cingolani, Noy-Meir & Díaz 2005). Sin embargo, los resultados de nuestros estudios descriptivos y experimentales muestran una escasa respuesta de los pastos alpinos frente al drástico descenso de pastoreo observado en el Pirineo (Capítulos 4 y 5). Resulta particularmente llamativa la ausencia de matorralización en los pastos estudiados, así como el hecho de que la mayoría de las especies no experimentaran cambios en su abundancia. Tampoco se encontraron diferencias notables entre los rasgos funcionales de las especies con dinámicas temporales opuestas. Estos resultaron son consistentes con la lenta dinámica de las comunidades de pastos observada en respuesta a la exclusión experimental de ganado (Capítulo 5), donde el tratamiento no tuvo un efecto significativo sobre la riqueza de especies a lo largo de 19 años. Nuestros resultados contrastan con los obtenidos en otros experimentos de exclusión, y en pastos montanos y subalpinos abandonados (Rawes 1981; Hill et al. 1992; Krahulec et al. 2001; Tasser and Tappeiner 2002; Mayer et al. 2009; Speed et al. 2013), dejando en evidencia las limitaciones de los modelos generalizados de herbivoría para anticipar la respuesta de los pastos alpinos en un contexto de descenso de pastoreo y abandono.

Las evidencias indirectas del efecto del calentamiento climático fueron también prácticamente nulas en los pastos revisitados, resultado que contrasta con la rápida respuesta observada en varias cimas de montañas Europeas, inclusive en el PNOMP. Sin embargo, sí que observamos que un periodo excepcionalmente cálido y seco tuvo impacto inmediato sobre la dinámica de los pastos dentro de la exclusión. Este resultado nos lleva a sugerir que el cese del pastoreo y el aumento de temperaturas podrían tener un efecto aditivo sobre los pastos alpinos.

En conjunto, los resultados obtenidos en la revisita de los pastos y el experimento de exclusión, señalan una gran inercia ecológica de los pastos alpinos. Otros estudios realizados en la tundra y pastos de alta montaña han atribuido la relativa estabilidad de estas comunidades a la longevidad y reproducción vegetativa de las especies (Mayer *et al.* 2009; Nagy & Grabherr 2009; Wilson & Nilsson 2009), así como a los cortos periodos vegetativos existentes en la alta montaña, que pueden, en buena medida, amortiguar los cambios ambientales (Klimesova & Klimes 2007; Morris *et al.* 2008; García, Picó & Ehrlén 2008). No obstante, no creemos que estos aspectos fueran los únicos subyacentes a la estabilidad de los pastos. Por ejemplo, ¿podrían explicar estas características de los pastos alpinos la ausencia de matorralización? No lo creemos posible. Es más, dado que algunos de los pastos estudiados están situados en la proximidad de comunidades, es posible que el pastoreo actual, aun siendo residual desde un punto de vista histórico, fuera suficiente para controlar la expansión del matorral. De hecho, no es descartable que la reducción general en número de cabezas de ganado hubiera dado lugar a una concentración del ganado remanente en las zonas más favorables para el pastoreo. Si esta hipótesis fuera cierta, el descenso en la presión ganadera no habría sido sustancial y nuestros resultados no serían tan sorprendentes. Sin embargo, esta hipótesis no permite explicar la inercia ecológica observada en las parcelas situadas en zonas más desfavorables para el pastoreo, y en las exclusiones de ganado.

En cualquier caso, somos conscientes de que la escasa replicación espacial de nuestros estudios limita claramente la generalización de los resultados, más aún en un ambiente como el alpino donde la alta variabilidad micro-climatica da lugar a un gran número de comunidades (y dinámicas). Por eso cabe esperar encontrar diferentes respuestas a los cambios globales en el área alpino. Sin embargo, el hecho de que la dinámica temporal de pastos alpinos con diferentes características fuera similar bajo condiciones natural y experimentales sugiere que los resultados aquí mostrados sean relativamente frecuentas.

En el contexto particular del PNOMP, la gran estabilidad de los pastos alpinos estudiados en esta tesis contrasta con la rápida reforestación y matorralización de los pastos montanos y subalpinos observada durante las últimas tres décadas (Komac, Alados & Camarero 2011; Gartzia, Alados & Pérez-Cabello 2014). Las evidencias del efecto del calentamiento climático fueron también insignificantes en los pastos revisitados comparado con las obtenidas en las cuatro cimas del PNOMP monitorizadas dentro del marco del proyecto GLORIA (Gottfried *et al.* 2012; Pauli *et al.* 2012), donde se observó un rápido incremento en la abundancia de las especies termófilas. La ausencia de impacto del cambio global sobre los pastos alpinos mostrada en esta tesis debería interpretarse con cautela porque detrás del aparente estado de estabilidad podría esconderse una respuesta ralentizada. De hecho, estabilidad observada podría revertir rápidamente a medida que algunos umbrales ecológicos o puntos de inflexión climáticos son excedidos (Doak & Morris 2010). Por lo tanto, nuestros resultados no debieran invitar a bajar la guardia, y se deberían tomar medidas de gestión orientadas a garantizar la conservación de estos pastos excepcionalmente ricos ahora que existe margen. El mantenimiento o restauración de las actividades del pastoreo tradicional se vislumbra como una solución simple y efectiva para paliar los efectos del descenso del pastoreo (Olsson, Austrheim & Grenne 2000; Spiegelberger *et al.* 2006; Chemini & Rizzoli 2014), y quizás la única posible a escala local para reducir el impacto del calentamiento global sobre la diversidad vegetal.

Líneas de investigación futuras

Dado que el estudio de la distribución de la biodiversidad y su evolución temporal se puede abordar desde múltiples perspectivas, hay muchos aspectos que han quedado sin cubrir en esta tesis, y otros emergen de los resultados de esta tesis. A continuación mencionamos brevemente algunos de los más relevantes:

- Estudiar las causas de la distribución de los diferentes componentes de la diversidad vegetal observada en el Capítulo 2. El estudio de la estructura filogenética y de rasgos biológico podría resultar muy útil para determinar la importancia de los factores ecológicos (filtrado ambiental, competencia) e históricos (Kraft *et al.* 2007; Cavender-Bares *et al.* 2009; de Bello *et al.* 2012).

- Continuar la monitorización de los pastos estudiados en esta tesis resulta necesario para poder detectar posible respuesta desfasadas en el tiempo, o puntos críticos en su dinámica. No obstante, la monitorización de las comunidades debería ser complementada con la toma de datos ambientales de manera más detallada y una estimación precisa de la presión ganadera de las parcelas a lo largo del tiempo para poder afinar así nuestra capacidad predictiva.

- Comprobar la generalización de nuestros resultados en otras áreas alpinas. Sin embargo, esto puede no resultar ser una tarea fácil dada la escasez de estudios detallados a largo plazo en la alta montaña. Esto es particularmente evidente en el caso de los experimentos de exclusión, que están frecuentemente poco o nada replicados (véase un excepción en Austrheim *et al.* 2008). En el curso de esta tesis hemos establecido nuevas exclusiones a lo largo del gradiente altitudinal del PNOMP, y el análisis de esta información nos permitirá obtener una visión más general del efecto del cese del pastoreo en el futuro, aunque todavía limitada. El meta-análisis de los resultados obtenidos en exclusiones de diferentes montañas podría constituir la mejor, sino la única, manera de establecer generalidades.

CONCLUSIONES

1. En esta tesis hemos estudiado la distribución espacial de los diferentes componentes de la diversidad vegetal, y la dinámica a largo plazo de las comunidades vegetales más ricas del Pirineo Central, y en concreto del Parque Nacional de Ordesa y Monte Perdido (PNOMP). Este parque constituye un punto caliente en términos de riqueza de especies de plantas a escala continental, así como un referente en el estudio de las comunidades a largo plazo.

2. Los controles de calidad de la información en la bases de datos biológicas son necesarios para asegurar la fiabilidad de los análisis de la biodiversidad en esta información. Hemos desarrollado un nuevo método analítico para cuantificar el efecto del esfuerzo de muestro en las bases de datos que aglutinan información proveniente de múltiples fuentes (pliegos de herbario, muestreos de campo, etc.). Este método, testado con la bases de datos reales (la del PNOMP) y simuladas, presentó mejores cualidades que los métodos existentes, y pudo ser utilizado en un mayor número de situaciones. Por consiguiente, este método constituye una herramienta prometedora para reducir la incertidumbre de los análisis de la biodiversidad a partir de bases de datos.

3. La distribución de los componentes taxonómico, filogenéticos y funcionales de la diversidad puede verse sesgada por las diferencias en el esfuerzo de muestreo empleado para la recopilación de la información de las bases de datos. En consecuencia, nuestra capacidad para identificar áreas prioritarias para la conservación puede verse mermada. Asimismo, la resolución espacial de la información puede diluir el significado ecológico de la distribución de los componentes, y enmascarar la escasa utilidad de los hotspots para la representación

de múltiples componentes de la biodiversidad.

4. A partir de información recogida con similar esfuerzo de muestreo, y a escala detallada, demostramos que los taxonómico, filogenético y funcional de la biodiversidad no siguen una misma distribución espacial en el PNOMP. Los pasto alpinos resultaron ser las comunidades más ricas, y con mayor abundancia de endemismos, mientras que la mayor concentración de diversidad filogenética y funcional fue observada en ciertos bosques mixtos y caducifolios.

5. A pesar del contexto de cambio global de nuestro áreas de estudio, encontramos muy pocas evidencias de cambio en la vegetación de varías comunidades alpinas durante las dos últimas décadas. Tampoco se encontraron evidencias significativas de cambio a nivel de especies y de rasgos ecológicos y biológicos.

6. Nuestros estudios, tanto descriptivos como experimentales, pusieron de manifiesto que la respuesta de las comunidades alpinas frente a cambios ambientales drásticos, tales como el cese de pastoreo, o incremento de la cobertura arbórea, puede llevar décadas. No obstante, las vulnerabilidad de las comunidades alpinas frente a periodos excepcionalmente cálidos y secos podría incrementar en ausencia de herbívoros. El mantenimiento del pastoreo tradicional es posiblemente una de las mejores estrategias posibles para la conservación de las excepcionalmente ricas comunidades de pastos del Pirineo Central, y quizás la única posible a escala local para frenar los impactos del calentamiento climático sobre la diversidad vegetal.

7. En general, nuestro resultados no apoyan las predicciones más extendidas sobre el impacto del cambio global en las comunidades alpinas. Además, la relativa estabilidad de los pastos alpinos a lo largo de las dos últimas décadas contrasta con la rápida transformación de la cubierta vegetal observada en los pasto montanos y subalpinos, así como con las más rápida respuesta de las comunidades de cima de varias montañas Europeas (incluido el PNOMP). La diversidad de respuestas posibles en un mismo contexto ambiental resalta las limitaciones

existentes para generar predicciones generales sobre el impacto del cambio global sobre la diversidad vegetal de alta montaña.

8. Finalmente, nuestros resultados señalan que el error de muestreo y la dinámica natural de los ecosistemas pueden dar lugar a una interpretación errónea de la valiosa información obtenida en estudios a largo plazo. Sin duda, resulta necesario minimizar la incertidumbre asociada a los datos, y abundar en el conocimiento de la dinámica natural de las comunidades para poder afinar nuestras conclusiones sobre el impacto del cambio global.

Report of Supervisor

Report of the supervisor

Dr. María Begoña García, supervisor of the Doctoral Thesis presented by Iker Pardo Guereño (IPG), certifies that the five studies included in this work have been or are ready to be submitted to international journals with peer review, all of them well recognized by the scientific community.

The two first publications appeared in 2013. One in ***Plant Ecology & Diversity*** (Impact Factor: 1.140; ranked as 111/199 in Plant Sciences), which is the Journal of the Botanical Society of Scotland. The second one in ***PLOS ONE*** (IF: 3.534; ranked as 8/55 in the Multidisciplinary area), a peer-reviewed open access scientific journal published by the Public Library of Science (PLOS) since 2006.

The third one is currently available as *early view* in ***Biodiversity and Conservation***, whose IF was 2.365 in 2014, and was ranked as 59/144 in Ecology, and 14/43 in Biodiversity & Conservation disciplines. A fourth one is now under review in ***Conservation Biology***, the Journal of the Society of the Conservation Biology (IF of 4.165 in 2014, ranked as 5/43 in Biodiversity Conservation, 16/221 in Environmental Sciences, and 22/144 in Ecology). A fifth manuscript will be submitted in November to ***Journal of Applied Ecology***.

I certify that all the five studies presented by IPG in this Doctoral thesis have been led by him under my supervision, demonstrating the full capacity of the candidate to develop independent and high quality research in the field of Ecology and Biodiversity and Conservation. In the next paragraphs I detail his contribution to the manuscripts and papers already published, in relation to other authors.

Chapter 1.
Pardo, I, MP Pata, D Gómez & MB García. 2013. A Novel Method to Handle the Effect of Uneven Sampling Effort in Biodiversity Databases. ***PLoS ONE*** 8(1): e52786. doi:10.1371/journal.pone.0052786
Contributions: IPG conceived the study, and together with MPP performed analysis of the data. He also lead the writing of the manuscript, with the assistance of other coauthors. DG and MBG compiled the database and assisted with manuscript writing.

Chapter 2.
Pardo I, Roquet C, Lavergne J, Olesen JM & García MB (under review). On the limits of hotspots for the representation of multifaceted diversity at small-scale. ***Conservation Biology***
Contributions: IPG and MBG designed the study. IPG compiled data information and carried out the analyses of the paper, with the methodological advise of CR of SL. He also wrote the paper, with help of MBG and JMO.

Chapter 3.
Pardo I, Camarero JJ, Gutiérrez E, M.B. García. 2013. Uncoupled changes in tree cover and field layer vegetation at two Pyrenean treeline ecotones over 11 years. **Plant Ecology & Diversity** 6: 355-364
Contributions: IPG resampled transects and carried out all the analyses to compare results with the ones obtained 11 years earlier by JJC and EG. MBG designed the study, and together with JJC helped during the manuscript writing.

Chapter 4.
Pardo I, Aldezabal A, García-González R, Gómez D & García MB (ms to be submitted). Little evidence of the impact of land-use change and climate warming on high elevation grasslands over the last 20 years. To be submitted to **Journal of *Applied Ecology***
Contributions: IPG revisited all the grasslands sampled two decades earlier by AA, RG-G and DG, compiled all the information, carried out comparative analyses, and wrote the manuscript. MBG designed the study together with IPG, and all authors provided assistance throughout the writing of the manuscript.

Chapter 5.
Pardo I, DF Doak, R García-González, D Gómez, MB García (early view). Long-term response of plant communities to herbivore exclusion at high elevation grasslands. ***Biodiversity and Conservation*** 10.1007/s10531-015-0996-3
Contributions: IPG performed analyses with the help of DFD, using the information obtained through time by RGG and DG. He also carried out fieldwork in the last year to estimate the resampling error. All authors contributed at some point in the design of the study, and assisted in the writing of the ms.

Besides these five chapters, IPG is leading another manuscript on the distribution of the main biogeographical plant groups in the same OMPNP, and its sensitivity to climate change, which will be hopefully submitted soon to an international peer-reviewed journal.

Zaragoza, October 2015

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Publications

PLOS ONE

A Novel Method to Handle the Effect of Uneven Sampling Effort in Biodiversity Databases

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Abstract

How reliable are results on spatial distribution of biodiversity based on databases? Many studies have evidenced the uncertainty related to this kind of analysis due to sampling effort bias and the need for its quantification. Despite that a number of methods are available for that, little is known about their statistical limitations and discrimination capability, which could seriously constrain their use. We assess for the first time the discrimination capacity of two widely used methods and a proposed new one (FIDEGAM), all based on species accumulation curves, under different scenarios of sampling exhaustiveness using Receiver Operating Characteristic (ROC) analyses. Additionally, we examine to what extent the output of each method represents the sampling completeness in a simulated scenario where the true species richness is known. Finally, we apply FIDEGAM to a real situation and explore the spatial patterns of plant diversity in a National Park. FIDEGAM showed an excellent discrimination capability to distinguish between well and poorly sampled areas regardless of sampling exhaustiveness, whereas the other methods failed. Accordingly, FIDEGAM values were strongly correlated with the true percentage of species detected in a simulated scenario, whereas sampling completeness estimated with other methods showed no relationship due to null discrimination capability. Quantifying sampling effort is necessary to account for the uncertainty in biodiversity analyses, however, not all proposed methods are equally reliable. Our comparative analysis demonstrated that FIDEGAM was the most accurate discriminator method in all scenarios of sampling exhaustiveness, and therefore, it can be efficiently applied to most databases in order to enhance the reliability of biodiversity analyses.

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Introduction

Decisions on biodiversity conservation are typically dependent on the degree of knowledge of species distribution [1], therefore, they ideally require the best available spatially explicit information of species distribution [2]. Given that field work necessary to get a database representative of the real biodiversity in large areas is highly resource-consuming, and current funding for this task is scarce [3–4], historical data stored in herbaria, museums, atlas and unpublished material emerge as an outstanding alternative [5]. In fact, biodiversity databases compiling information from these sources have proliferated worldwide in the last decade [6], as it is exemplified by initiatives such as the Global Biodiversity Information Facility (GBIF) [http://www.gbif.org]. Scientists and managers can now take advantage of the enormous effort done during decades of biodiversity inventories [7] and raise new ecological questions [6,8]. In particular, biodiversity databases are being intensively used in relevant conservation issues, such as the predictive distributions of plants and animals under global change scenarios [9–10], the identification of biological hotspots (e.g. [11–13]), or the design of protected areas [1,14]. The generation of new analytical tools is promoting advances in the study of these fields, however, their reliability remains challenging due to the contingencies of the baseline data [15–22]. For instances, biodiversity database usually contain incomplete distribution data, because the information was collected according to different aims [15]. Evidences of how bias in database information can compromise biodiversity analyses and conservation planning are reported in a large number of studies [19,20,23,24,25,26,27]. Hence, an adequate control of data-quality is needed [15].

Quality control process should regard both database configuration and the evaluation of data suitability for analyses. According to the scheme proposed in Hortal et al. [15], the control routine has two main levels: (i) data-compilation and digitalization, and (ii) sampling effort assessment. The former is related to the reliability of the sources of information, taxonomic bias and the geographical accuracy of the data [8,28,29]. The latter refers to the uneven sampling effort present in non-systematic biodiversity databases [30]. As the first control level has already been discussed elsewhere, in this paper we will focus only on the assessment of the sampling effort bias.

Sampling effort is likely to be temporally, spatially and environmentally biased [20,27,31,32,33]. Temporal bias can be minimized by limiting database information to a time period short enough as to ensure that information remains the same throughout this period. Regarding spatial and environmental bias, it is well known that some territorial units accumulate more sampling records than others due to diverse factors such as accessibility, habitat singularity, abundance of rare taxa, or differences in funding [24,25,34,35,36,37,38]. Given that most aspects of biodiversity (e.g. species richness) correlate well with sampling effort (e.g. [15]), diversity distribution inferred from raw database information may reflect the spatial distribution of sampling effort rather than the real distribution of diversity [8,15,27]. Hence, biodiversity distribution analyses based on spatially explicit data should account for sampling effort.

Among the range of methods that have been proposed to reduce the bias of sampling effort, those based on species accumulation curves (SAC) [34,39,40] are commonly used. According to SAC's properties, the total number of species recorded rises towards a ceiling as sampling effort increases [39,41]. Once the SAC is constructed, a model is fitted to describe the accumulative-sampling effort relationship (e.g. [42]). The selection of the model should be done with statistical rigor [39], but also according to the discrimination capability, i.e. the probability of correctly identifying well (or poorly) sampled units [43]. The discrimination is a *sine qua non* criterion in scientific fields with important social responsibility such as clinical diagnostic [44–45], whereas it has been hardly applied in ecological classification analysis, including the evaluation of sampling effort. If the methods to assess sampling effort fail to discriminate well from poorly sampled units, the resultant classification would be seriously affected. Likewise, if the discrimination capability differ among methods, then, the reliability of the classification would depend to a great extent on the selected method, and so will do the uncertainty of any analysis based on such information.

In this study, we analyze for the first time the discrimination capability of commonly used SAC based methods to quantify sampling completeness, and present a novel approach. We first compare methods according to their discrimination capability in two contrasting scenarios of sampling exhaustiveness and in an ideal situation, where the true species richness is known. Finally, we define an objective and generalizable procedure to account for sampling effort bias in biodiversity databases using the novel method and discuss its practical benefits for conservation management.

Methods

Review of methods to assess sampling effort bias

The SAC are constructed by plotting the expected (mean) cumulative number of species *S(n)*, at a given number of samples (*n*) [40,46]. Samples order is randomized by repeatedly re-sampling (without replacement) to rule out its effect on the SAC [40,41,46]. Two main procedures based on SAC have been proposed to assess the sampling completeness: (i) the proportion of species richness out of the total predicted by the richness estimators [47–49], and (ii) the slope of the accumulation curve [30,50].

For the former procedure, the predicted richness should be calculated first, which can be done in several ways. Extrapolation of SAC based on asymptotic functions is one of them. The predicted richness is estimated as the total number of species that would be achieved with a hypothetical infinite sampling effort. The most usual models used to describe the SAC are the negative exponential, the Clench, and the Weibull models [39,50,51,52,53]. The other common way of predicting species richness is by non-parametric estimators based on the number of rare species observed within samples, either from incidence or abundance data [54–55]. The most common estimators in this case are Chao [56], Jacknife (onwards NPE) and Bootstrap [57] estimators, as well as incidence-based and abundance-based coverage estimators, called ICE and ACE respectively [58]. For a complete review of all these methods see [40], [59] and [60].

The second procedure for measuring sampling completeness is the slope of the SAC along the sampling effort gradient, which is minimum when all species have been found [30,50]. There are several alternatives to compute the slope of the curve. One is the geometric definition of the slope as the secant line to the curve:

$$m = \frac{\Delta Y}{\Delta X} \qquad (1)$$

where Y is the species richness and X the measure of effort. An appropriate procedure for calculating Y is the unbiased estimator of true species richness, the so called Mao Tau estimator (hereafter STE) [41,61]. Another way of estimating the slope of the SAC is to calculate the species accumulation rate at a given sampling level, by fitting a function to the curve. To do that, it is necessary to previously examine the level of homogeneity of sample units by comparing the empirical mean randomized SAC, with the expected curve if all individuals had been randomly assigned to the samples. The expected curve may be constructed either by computing a rarefaction curve or a Coleman curve (for details see [40,46]). The slope of the SAC is then calculated with the first derivative of the fitted curve. The final slope of the Clench function (as well as the slope of other asymptotic functions) is the most common method for assessing the accumulation rate [30,39,60]. Two main problems are associated with these asymptotic functions: their limited use at low sampling levels of sampling [15], and the violation of statistical assumptions inherent to non-linear regression models (i.e., correct mean structure, variance homogeneity, and independent and normally distributed errors [62]).

FIDEGAM: a new method to quantify sampling completeness

As an alternative to the methods reviewed above, we have developed the FIDEGAM method, which fits a Generalized Additive Model (GAM) [63–64] with Poisson response, or the negative binomial if data presents overdispersion [65], to each randomized SACs. GAM is an extension of Generalized Linear Models (GLM) [66], which allows flexible modeling of the influence of the response variable [64]. In a GAM framework, statistical assumptions are met because the function is adjusted to non-normal distributed data instead of forcing data to fit an arbitrary known function. Besides, contrary to asymptotic methods the model fits even at low levels of sampling effort. Once GAMs are fitted, the first derivatives and their 95% corresponding confidence intervals along the species accumulation process are calculated. This output describes the whole pattern of species accumulation, being the first derivative at the maximum number of sampling records the measure of sampling completeness (onwards FIDEGAM value). FIDEGAM values range from 0 to 1, corresponding to high and low sampling completeness for a given area, respectively.

Testing and comparing the discrimination capability of the sampling completeness measures

(i) Classification rules to assess discrimination capability. The discrimination capability between different methods should be evaluated under different levels of sampling completeness and according to an objective classification rule [43]. From a statistical point of view, the discrimination capability of a given Y (e.g. a measure of sampling completeness in our case) to distinguish between two alternative states $S1$ (e.g. well sampled unit) and $S2$ (e.g. poorly sampled unit), should be based on a Receiver Operating Characteristics (ROC) curve analysis [43,67,68]. A binary response is needed for ROC analyses, so that Y classifier should be dichotomized according to a cut-point value. Values of the sampling unit above that cut-point would refer to one of the two possible states (i.e. $S1$), and values below to the alternative state (i.e. $S2$). The classification criterion used in ROC analysis is related to the probabilities of belonging to one of the states as a function of the values of Y, $P\ [S1|Y\ ]$. These probabilities are estimated using a GLM in a binary regression framework [43]. Once the ROC curve for each completeness measure is fitted, their discrimination performance is evaluated using the area under the ROC curve (AUC)

$$AUC = \int_0^1 ROC(t)dx \tag{2}$$

The AUC takes values between 0.5 (uninformative classifier) and 1 (perfect classifier) [44,69,70].

(ii) Discrimination capability of methods in different scenarios. We tested the discrimination capability of the completeness measures based on the observed proportion of species richness out of the total predicted by a non-parametric estimator (NPE), the slope of the Mao Tau estimator (STE) and FIDEGAM using ROC in two contrasting scenarios of sampling completeness and in a ideal situation, where the true species richness is known.

The scenario of high sampling exhaustiveness is derived from a database that contains information of the vascular flora of the Ordesa-Monte Perdido National Park (Spanish Pyrenees; ORDESA thereafter). The National Park is one of the most exhaustively prospected areas in the Iberian Peninsula [71], however, due to the high topographic complexity, the large altitudinal range (~700 to 3354 m a.s.l.) and severe access difficulties to some points, the sampling effort is expected to be unevenly distributed along the *ca.* 30000 ha of the Park. The ORDESA database comprises more than 44000 spatially explicit records of 1379 vascular plant species along the 321 UTM cells (1 km^2; sampling units) of the Park (excluding Bujaruelo valley), compiled from herbarium samples, phytosociological *relevés*, and *visu* records collected over the last 50 years in the JACA Herbarium (http://proyectos.ipe.csic.es/floragon/index.php). To homogenize the different sources of information we defined "sampling record" as each input of information of plants occurrence (from one to multiple species) that differs in date, site, method and/or author.

The second scenario was created emulating the structure (sampling units/sampling records/species per record) of the ORDESA database and using a random procedure, which involves the following steps (see Figure S1 for further details):

1. For each sampling unit (n = 180), assign the number of sampling records according to three levels of sampling intensity (20–50, 51–80 and 81–110 sampling records) at random.
2. For each sampling record, randomly determine how many (between 1 and 30) and which species are recorded from a virtual pool (400 species).

The resultant information was compiled in a database named SIMULAU. We assumed that the sampling effort has been enough to detect the true richness in all the sampling unit of this database. We then subsampled from SIMULAU to achieve an scenario of low sampling exhaustiveness (SIMULAU$_{\mathrm{sub}}$). To do so, we repeated Steps 1 and 2, but in this case the number of sampling records and species was randomly assigned according to the information gathered in SIMULAU. To ensure low levels of sampling exhaustiveness in SIMULAU$_{\mathrm{sub}}$, we limited the maximum number of sampling record per sampling unit, and the maximum number of detected species per sampling record to 25 and 20, respectively.

The next step was to produce the smoothed SAC for each sampling unit in the ORDESA and SIMULAU$_{\mathrm{sub}}$ database using *specaccum* function (1000 permutations) in the VEGAN package [72] in R [73]. Then, the three completeness measures were estimated for each SAC. The NPE was calculated as the proportion of species richness out of the total predicted by the Jacknife estimator using the *poolaccum* function (1000 permutations) in the VEGAN package. The slope for Mao Tau estimator (STE) was computed from the SACs as

$$\frac{y_i - y_{i-3}}{x_i - x_{i-3}} \tag{3}$$

being i the last position of both species richness (y) and number of records (x). We finally used FIDEGAM method by fitting GAM models with Poisson response to the each accumulation curve (obtained at random) using penalized splines [64,74]. Optimum effective degrees of freedom (equivalents to degrees of smoothness) were automatically selected using the unbiased risk estimator criterion (UBRE) [75]. The first derivative of the resultant curve and its 95% confidence intervals were computed for each sampling unit (Figure 1), being the first derivative at the maximum number of sampling records the FIDEGAM measure of sampling completeness (Figure 1).

The final step consisted in examining the discrimination performance of the three completeness measures calculated, to correctly classify well and poorly sampled sampling units. In most real situations, the true species richness is unknown, therefore, a surrogate of the sampling effort is needed to categorize sampling units. Here, we used the number of sampling records as a surrogate in the ORDESA and SIMULAU$_{\mathrm{sub}}$ [30,47,76]. We set the cut-point value according to the preliminary analysis [77] at the 50th percentile (i.e. the median) of the number of records per sampling unit [78] (see further details on Appendix S1). Thereby, units with higher number of sampling records than the median were classified as well sampled and those below as poorly sampled. To evaluate the role that the surrogate could play on the results, we categorize the sampling units of SIMULAU according to an ideal scenario where the degree of sampling completeness is known. Given that all species were detected in the sampling units of SIMULAU, we calculated the true sampling completeness for each unit as the

$$\frac{SR_{sub} - SR_{true}}{SR_{true}} \times 100 \tag{4}$$

where SR_{sub} is the species richness in SIMULAU$_{\mathrm{sub}}$ and SR_{true} the true richness obtained from the SIMULAU database [79–80]. The inventory of sampling unit exceeding the 70% of completeness are usually considered as nearly completed [81–82], therefore, we categorized sampling units according to such value.

Once the binary response variable was created for all scenarios, we proceed to estimate the capability of each completeness measure (NPE, STE and FIDEGAM) for discriminating between classes using ROC-GLM regression for binary responses with logit link. The probability of belonging to each state was calculated as a

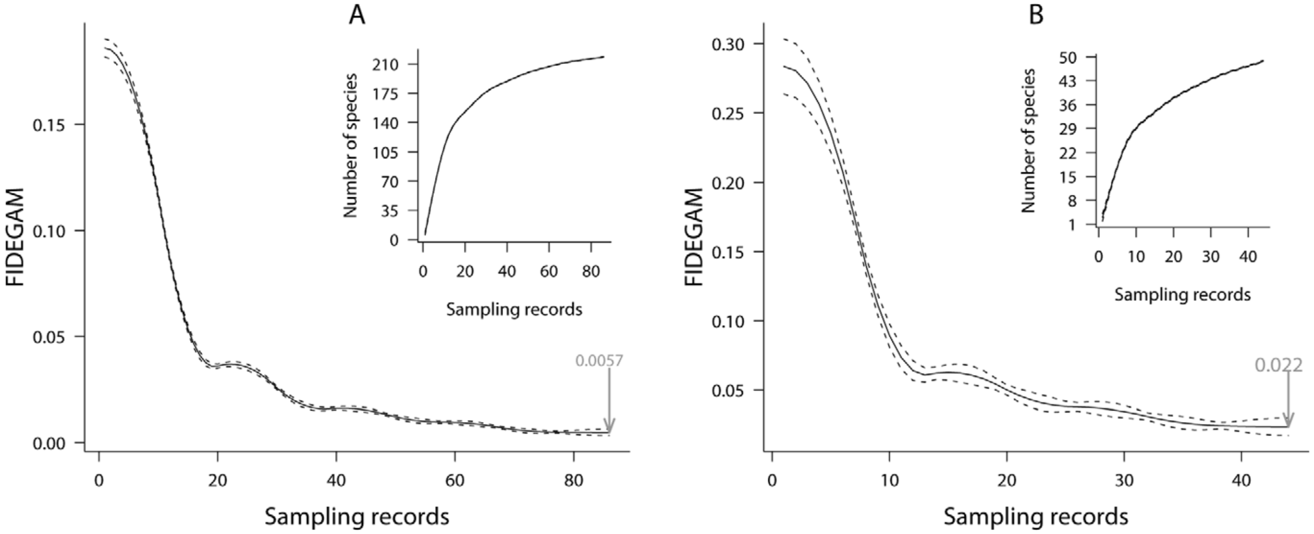

Figure 1. The sampling completeness measured from two smoothed species accumulation curves (1000 randomization each) using the FIDEGAM method in the Ordesa-Monte Perdido National Park. FIDEGAM values (in grey) recorded at the maximum number of sampling records indicates higher sampling completeness in the sampling unit A than in B. Dashed lines correspond to confidence intervals of FIDEGAM values. doi:10.1371/journal.pone.0052786.g001

function of the value of completeness measure

$$P[Y = well\ sampled|completeness\ measure] \quad (5)$$

whereas AUC values were computed using the *roccurve* function in the *pcvsuite* package [83] and their 95% confidence intervals estimated by bootstrap regression techniques [84].

Results

According to our logistic model (equation 5), the predicted probabilities of a method for correct discrimination should reach 1 and 0 for well and poorly sampled units, respectively. Therefore, when representing this ideal discrimination in a kernel density plot, maximum densities of predicted probabilities of well and poorly sampled areas should clump at 0 and 1 values of the x-axis. On the contrary, higher densities of predicted probability values would lie between 0 and 1 if the method fails in discriminating. Figure 2 shows strong differences in the predicted probabilities for well and poorly sampled units among methods, evidencing the higher discrimination capacity of FIDEGAM. This pattern was consistent in the three examined scenarios despite that different surrogates for categorizing the sampling units were used if true richness was known or unknown (Figure 2). In all cases, FIDEGAM showed an excellent performance for discrimination according to the observed AUC values (Table 1). On the contrary, NPE failed to correctly discriminate sampling units in the ORDESA database, whereas, STE only classified correctly poorly sampled units (Figure 2A), being good the discrimination quality (Table 1).

At lower levels of sampling exhaustiveness (i.e., using data from SIMULAU$_{sub}$), the probability of NPE and STE for correct discrimination decreased (Figure 2B), reaching undesirable AUC values (Table 1). The same results were obtained in the simulated scenario when sampling units were categorized according to the true sampling completeness (Figure 2C, Table 1).

To better interpret the results obtained in the discrimination analysis, we plotted the relationship between the true percentage of species detected (defined as the ratio between the richness observed in SIMULAU$_{sub}$ and SIMULAU) and the completeness values of NPE, STE and FIDEGAM in SIMULAU$_{sub}$ (Figure 3). By fitting a Poisson-GLM to this relationship, we found that values of both NPE and STE did not correlate with that ($R^2 = 0.28$ and $R^2 = 0.14$, respectively), whereas FIDEGAM values did ($R^2 = 84.19$) (Figure 3).

Handling with sampling effort bias in biodiversity analyses: a case study

To illustrate how the measure of sampling completeness can be used to enhance the reliability of biodiversity analysis, we analyzed the patterns of distinctiveness along the Ordesa-Monte Perdido National Park (excluding Bujaruelo valley) using the ORDESA database. The distinctiveness indicates to what extent one area is distinct from other areas in terms of taxonomic, functional or/and genetic diversity [85–86]. The identification of most distinctive areas constitutes a basis for establishing priority conservation areas at different scales. We calculated here an easy-to-use index based on taxonomic distinctiveness according to the formula detailed in Jennings et al. [81], but it is also possible to use other metrics of distinctiveness and beta diversity.

Figure 4A represents distinctiveness in the National Park from the raw information in ORDESA, and suggests that most areas of the Park would be highly distinctive. To what extent is this pattern reliable? We quantified the sampling completeness of each sampling unit with FIDEGAM and found that most of the poorly sampled ones were those of highest distinctiveness values (Figures 4 and 5). Hence, the distinctiveness pattern obtained from raw information is highly uncertain. To minimize such uncertainty, we excluded poorly sampled areas from analysis according to an objective criterion based on the maximization of the discrimination capability using the Youden index (J) [87]. The J value in the ROC curve is

$$J = P_{S2(c0)} + P_{S1(c0)} - 1 \quad (6)$$

being P the probability of correctly classifying, $S1$ and $S2$ well and poorly sampled units respectively, and c_0 the optimum cut-point,

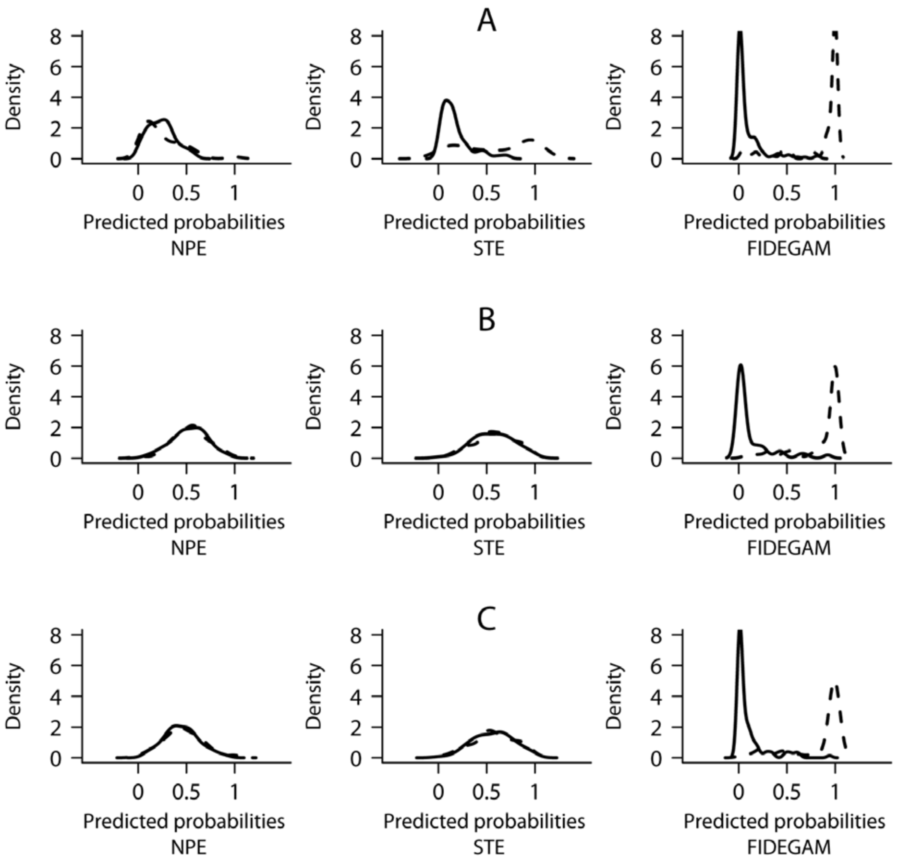

Figure 2. Kernel density plots of predicted probabilities of discrimination between well (dashed line) and poorly sampled units (continuous line) for NPE, STE and FIDEGAM methods. In the scenarios of high (A) and low (B) levels of sampling exhaustiveness, the sampling units were categorized as well and poorly sampled according to the number of records (see Appendix S1), whereas, when the true richness was known (C), the true sampling completeness (see equation 4 on text) was used as a categorization criterion. Probabilities were calculated according to ROC-GLM regression models.
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and the corresponding value of FIDEGAM the optimum threshold to separate well sampled units from poorly. In the ORDESA database the *J* index was 0.85 (confidence interval: 0.75–0.93) and the corresponding threshold 0.029. After excluding sampling units with FIDEGAM values above such threshold (i.e., poorly sampled areas), we recalculated the distinctiveness values and found that the resulting pattern of distinctiveness totally differed from the previous one (Figure 4B). This result evidences how the inclusion of uncertain information in biodiversity analysis (poorly sampled units in this case) distorts the overall picture of the spatial pattern of distinctiveness.

Table 1. AUC values and 95% bootstrap confidence intervals (in brackets) obtained in the discriminatory analysis of methods for sampling completeness quantification.

Method	Level of sampling exhaustiveness		SR_{true}
	high	low	
NPE	0.64 (0.59, 0.71)	0.49 (0,41, 0.59)	0.52 (0.40, 0.59)
STE	0.81 (0.75, 0.86)	0.49 (0.40, 0.57)	0.48 (0.40, 0.57)
FIDEGAM	0.92 (0.88, 0.95)	0.98 (0.97, 1.00)	0.97 (0.95, 0.98)

NPE is the proportion of species richness out of the total predicted by a non-parametric estimator (Jacknife) [57]; STE is Mao Tau estimator [41,61]; FIDEGAM is the first derivate of a GAM with Poisson response fitted to species accumulation curves.
The analyses were repeated in two scenarios of high and low levels of sampling exhaustiveness (from the ORDESA and $SIMULAU_{sub}$ databases, respectively), and in an ideal situation where the true species richness was known (SR_{true}). Grading guidelines for AUC values indicate fail (0.50–0.60), poor (0.60–0.70), fair (0.70–0.80), good (0.80–0.90) and excellent (0.90–1.00) discrimination.
doi:10.1371/journal.pone.0052786.t001

Discussion

Many biodiversity databases have been constructed from heterogeneous sources of information because of the large spatio-temporal ranges they usually cover. The information that they contain, therefore, does not always represent the reality due to large differences in sampling effort across time and space. This fact constitutes one of the main limiting factors for the reliability of the results provided by analyses based on them. Different methods have been proposed to account for spatial sampling effort bias, but not all of them perform equally. Here, we have demonstrated that SAC based methods differ in terms of statistical robustness, but also in their capability to discriminate between well and poorly sampled units.

Statistical assumptions cannot be disregarded even in the most-up-date statistical methods [88]. Some methods for quantifying sampling completeness do not fulfill such assumptions (see [89]), whereas others (e.g. the classic asymptotic function [39]) present

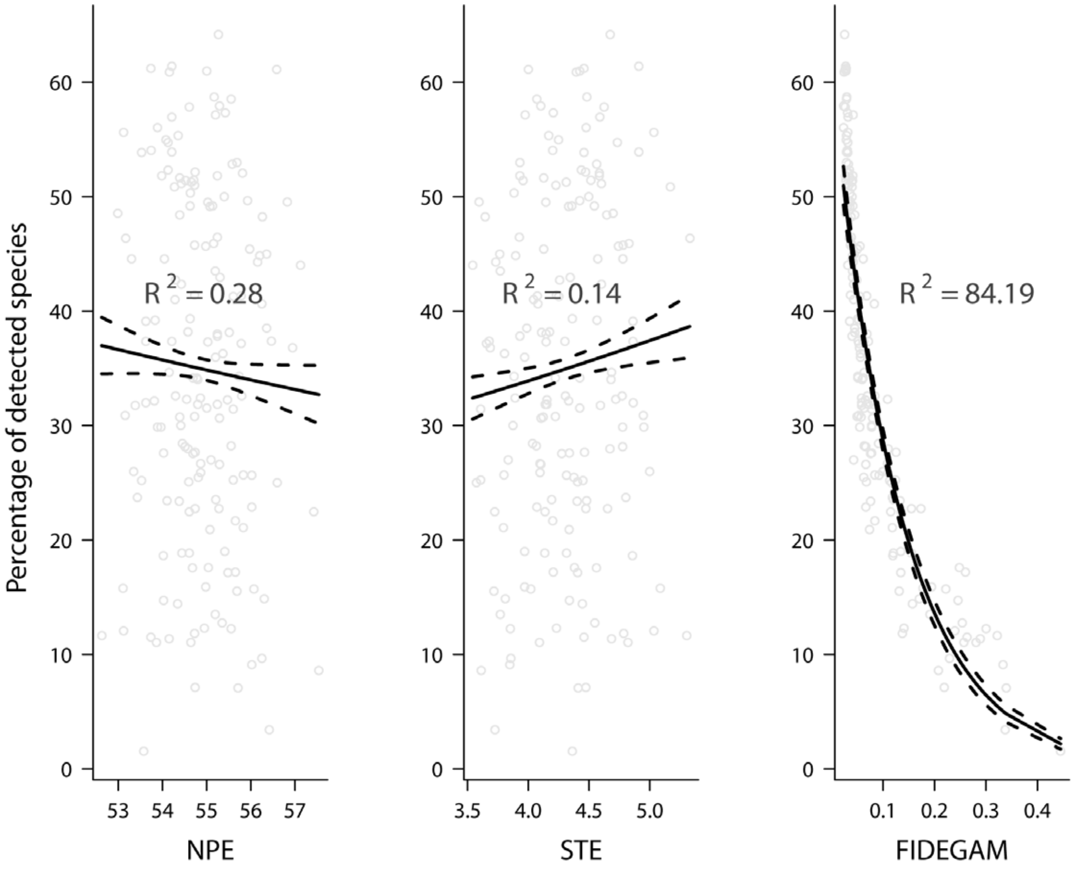

Figure 3. Relationship between the sampling completeness calculated using the FIDEGAM method and the percentage of detected species in a simulated scenario, where the true richness is known. Dashed lines state the 95% confidence intervals.
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severe limitations. Even when the statistical assumptions are not violated, not all methods are equally reliable because there are strong differences in their discrimination capability, as we have shown here. The novel method we have proposed in this study, FIDEGAM, outperforms others regardless of the sampling exhaustiveness, and both when true richness was known and unknown, evidencing its robustness. The most striking feature of the method is its excellent performance at low levels of exhaustiveness, because most regions and living groups worldwide are not exhaustively sampled [15]. In turn, other methods based on NPE and STE estimators, often misclassified well and poorly

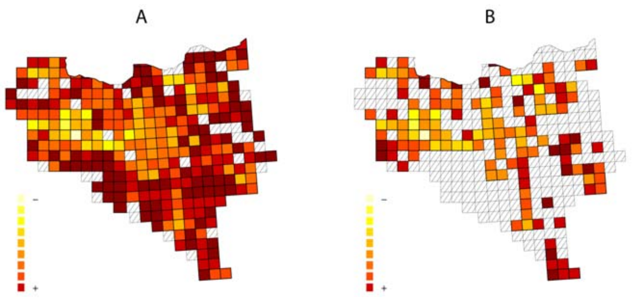

Figure 4. Taxonomic distinctiveness in the Ordesa-Monte Perdido National Park calculated using all (A) and selected (B) sampling units. Grid cells correspond to UTMs of 1 km^2. Striped cells indicates sampling units with less than three sampling records, where the quantification of sampling completeness is impossible using FIDEGAM method, in A, and poorly sampled units in B. Well and poorly sampled units were defined using their completeness value of FIDEGAM and according to a threshold value that maximize the discrimination capability between sampling units (see text for details).
doi:10.1371/journal.pone.0052786.g004

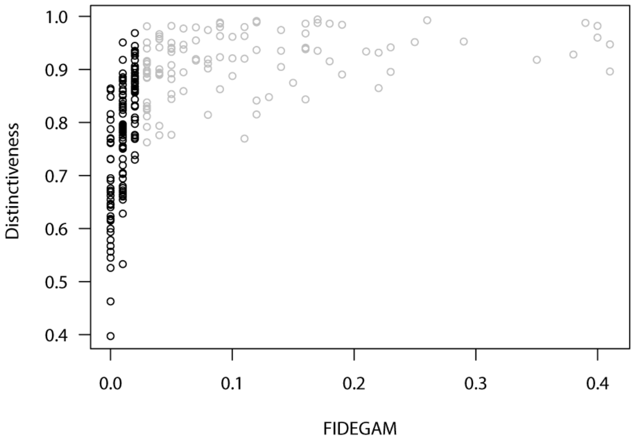

Figure 5. FIDEGAM values and taxonomic distinctiveness in the Ordesa-Monte Perdido National Park. Low values of FIDEGAM correspond to high sampling completeness. Black and grey dots indicate well and poorly sampled units (1 km^2) respectively, according to an optimum threshold value of FIDEGAM that maximizes discrimination capability.
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sampled units, which may constitute another source of bias to the original problem of sampling effort bias. As a result of this low discrimination capacity, NPE and STE also failed to represent the true proportion of detected species in a simulated scenario.

The sampling exhaustiveness of the database is an important constrain for the use of both non-parametric estimators and asymptotic methods [90]. Beyond discrimination capability, even the computation of sampling completeness values is limited by using asymptotic methods (e.g. Michaelis-Menten) in scarcely prospected areas. These areas ought to be ruled from the sampling effort assessment, and as a result, a large amount of information is susceptible to be lost. This situation is less dramatic using the FIDEGAM method, because it requires a lower number of sampling records (i.e. three) than the asymptotic ones.

The assessment of sampling completeness can be easily incorporated into biodiversity analyses to reduce the uncertainty of results. A promising procedure is to incorporate sampling completeness values as a covariate (or offset) in the analysis of biodiversity patterns (Pata et al., unpublished data), although the most frequent alternative is to only consider the areas that are well surveyed (i.e. those with a sampling effort above some threshold) [15,91,92,93]. If sampling effort is similarly distributed across space (regardless of the level of sampling exhaustiveness), the selection should be done according to comparable values of sampling effort rather than to high values [35,94]. The full interest of this procedure relies on how to define a threshold value in order to classify the suitability of different areas [30,95]. An arbitrary value may be justified when the knowledge of the studied system is robust, otherwise the subjectiveness should be avoided. In the example presented, the threshold value was defined according to the maximization of discrimination capability, thereby, minimizing in this way the potential bias intrinsic to method. The straightforward advantage of proceeding objectively is that the method can be equally used in other databases, regardless of the nature and spatial resolution of the information.

Correctly identifying well and poorly sampled areas is also of paramount importance for the interpretation of biodiversity distribution [27,92], and FIDEGAM has been proved to provide an accurate layer of uncertainty over results obtained from raw data. This would allow us to know at which locations results of biodiversity analysis is reliable, and where the prospective biological exploration is necessary if we want to extend results of standard analysis of biodiversity [53,92].

To summarize, our results have highlighted that an adequate selection of the assessment method is as important as the decision itself of taking into account the sampling effort for enhancing the reliability of database analyses. FIDEGAM provides the best discrimination capability and minor dependence on exhaustiveness. Therefore, we recommend this method to overcome sampling effort bias when analyzing the information gathered in biodiversity databases. By no means, a method for sampling completeness quantification will replace the advantages provided by further biological prospections. However, given the urgencies of biodiversity conservation and the limitations for intensive data gathering, we consider the quantification of sampling completeness the best alternative to enhance the reliability of biodiversity analyses based on non-exhaustive database.

Supporting Information

Figure S1 Diagram of the simulation procedure to create the ideal scenario, where the true richness is known (SIMULAU), and the scenario of low levels of sampling exhaustiveness (SIMULAU$_{\mathbf{sub}}$).
(PDF)

Appendix S1 Preliminary studies to establish an adequate threshold based on the number of sampling records for the discrimination analysis.
(PDF)

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Author Contributions

Conceived and designed the experiments: IP MPP. Analyzed the data: IP MPP. Contributed reagents/materials/analysis tools: DG. Wrote the paper: IP MPP DG MBG.

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Uncoupled changes in tree cover and field layer vegetation at two Pyrenean treeline ecotones over 11 years

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Background: The alpine treeline ecotone is regarded as a sensor of the effects of global change on alpine plant communities. However, little is known about how treeline dynamics influence the diversity and composition of alpine plant communities. Such information is necessary to forecast how ascending montane forests may affect the composition of alpine flora.
Aims: We analysed the temporal variations in tree cover, plant diversity and composition, and the effect of tree cover dynamics on field layer vegetation over a period of 11 years, at two alpine treeline ecotones in the central Pyrenees, Spain.
Methods: Tree and field layer vegetation was sampled in permanent transects in 1998 and 2009, using the point-intercept method. Temporal changes in tree cover, plant species richness and abundance were characterised along the ecotone by using a randomisation approach, rarefaction curves, and a non-parametric multivariate test, respectively.
Results: Tree cover increased significantly at one of the sites, whereas plant species richness only increased at the other site where tree cover had not changed. Vegetation composition changed significantly at both sites, but it was not spatially coupled with changes in tree cover along the ecotone.
Conclusions: A change of tree cover does not necessarily trigger changes in the ground flora at the treeline over relatively short periods (decade scale). The results challenge our ability to infer short-term biodiversity impacts from upslope advance of forests. Integrated tree and field layer monitoring approaches are necessary to produce a better understanding of the impact of ongoing global change on treeline ecotones.

Keywords: alpine plant diversity; climate change; forest limit; land-use change; monitoring; multivariate analysis; national parks; *Pinus uncinata*; species accumulation curves; upper montane forest; treeline ecotone

Introduction

Climate warming is causing the extension of plant species' ranges towards higher altitudes and shifts in community composition in mountain regions during the last decades (Grabherr et al. 1994; Peñuelas and Boada 2003; Walther 2003; Walther et al. 2005; Cannone et al. 2007; Lenoir et al. 2008, Kullman 2010, Pauli et al. 2012). The elevational treeline has been regarded as a reliable sensor of such climate effects worldwide (e.g. Hessl and Baker 1997; Kullman 2002; Daniels and Veblen 2003; Camarero and Gutiérrez 2004; Baker and Moseley 2007; Gehrig-Fasel et al. 2007; Mathisen et al. 2013) because its limit is expected to be determined by a thermal threshold (Jobbagy and Jackson 2000; Körner and Paulsen 2004; Holtmeier and Broll 2005; Körner 2012). Therefore, treelines should respond to ongoing climate warming by shifting upwards (Grace et al. 2002). However, some studies have reported tree cover increases within the ecotone while minor or no upward advances occurred, especially during the second half of the twentieth century (Camarero and Gutiérrez 2004). The reduction in grazing, especially since the 1960s, has promoted forest re-growth (Motta and Nola 2001; Holtmeier 2003; Ninot et al. 2008; Ameztegui et al. 2010), and it might override the effects of climate warming in treeline ecotones that have historically been below their natural threshold due to grazing (e.g. Gehrig-Fasel et al. 2007; Batllori and Gutiérrez 2008; Palombo et al. 2013).

Regardless of the main drivers of treeline responses, if the uppermost limits of montane forests ascend, adjacent alpine communities are expected to undergo changes. An overall assumption is that a treeline advance and an increase of tree density should lower species diversity and modify microenvironmental conditions in adjacent grasslands and in the field layer of the ecotone, respectively (Dirnböck et al. 2003; Walther 2003; Tinner and Kaltenrieder 2005, Illerbrun and Roland 2011). An increase of tree cover would modify light availability, moisture regime, soil properties and nutrient availability (Holtmeier and Broll 2005), creating forest-like microclimatic conditions in the treeline ecotone. Vegetation composition would therefore shift towards a greater dominance of shade-tolerant species, and the original shade-intolerant alpine flora would be gradually replaced by taxa typical of forest (Hofgaard and Wilmann 2002; Grytnes 2003; Sundqvist et al. 2008). However, the consequences of canopy closure are often based on studies of forest succession (e.g. Dahlgren et al. 2006; Laughlin et al. 2011), rather than on direct evidence from treeline ecotones (but see Illerbrun and Roland 2011).

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Despite the large amount of literature on treeline dynamics, relatively little attention has been paid to the consequences of treeline changes on plant diversity in the field layer (Camarero and Gutiérrez 2002; Hofgaard and Wilmann 2002; Camarero et al. 2006; Sundqvist et al. 2008; Batllori et al. 2009a). The scarce available information is from interpretations of chronosequences that use a space-for-time approach for assessing temporal dynamics, which has recently been criticised (Johnson and Miyanishi 2008). Therefore, empirical data are required for a reliable evaluation of the consequences of treeline dynamics on ground layer flora. Here, we report a detailed study on the dynamics of tree cover and associated field layer vegetation in the *Pinus uncinata* treeline ecotone in two national parks in the central Pyrenees (Spain) between 1998 and in 2009. The aim of this study was to assess the short-term impact of tree cover dynamics on field layer vegetation in the treeline ecotones. Specifically, we addressed the following questions: (i) did tree cover in two undisturbed treeline ecotones increase between 1998 and 2009? (ii) did field layer plant diversity and composition change significantly? and if so, (iii) were changes in tree and field vegetation layers coupled in space?

Materials and methods

Study area

We defined the treeline ecotone as the transition zone between the closed upper montane forest (hereafter forest limit) to the uppermost scattered and stunted trees (following Holtmeier 2003). The study sites were located in two protected areas: the Ordesa site (hereafter abbreviated as O) in the buffer zone of Ordesa-Monte Perdido National Park; the Tessó site (T) in the buffer zone of Aigüestortes-Estany de Saint Maurici National Park, central Pyrenees, Spain. On the basis of available historical data and comparison of aerial photographs taken in 1946 and 1988, these ecotones have been considered as undisturbed during the past century (Camarero and Gutiérrez 2002, 2004; Camarero et al. 2006). We examined orthophotographs taken in 1998 and 2009 to confirm that no wildfires, insect outbreaks, snow avalanches or logging occurred during this period. Repeated historical photographs also evidenced null elevational ascent of both treelines during the twentieth century (Camarero et al. 2000; Camarero and Gutiérrez 2004).

The sites were selected to represent the main contrasting structures of *Pinus uncinata* treeline ecotones (Camarero and Gutiérrez 2002). Site O was defined as a krummholz abrupt treeline, where the size and growth form of *P. uncinata* individuals changed abruptly upslope along the ecotone, from tall trees near the forest limit to a dense belt of krummholz individuals near the treeline (Camarero and Gutiérrez 1999) (Figure 1). In contrast, the density and height of *P. uncinata* individuals decreased gradually with increasing elevation at site T (Camarero and Gutiérrez 1999), a diffuse treeline ecotone (sensu Harsch et al. 2009). The two sites also differed in terms of climate, geology and vegetation composition. Site O (42º 37′ N, 0º 02′ W, 2110 m a.s.l.) was located on a south-facing gentle slope (5–10º), under continental and Mediterranean influence (1660 mm annual precipitation and 5 ºC mean temperature), with a yearly maximum snowpack of 1.0–2.0 m (Góriz Meteorological Station, 42º 39′ N, 00º 01′ E, 2215 m a.s.l.; 1981–1989 and 1992–1995 data [Camarero and Gutiérrez 2002]). The bedrock was mainly limestone, although it contained many sandstone patches (Camarero and Gutiérrez 2002). Rock outcrops were abundant in the uppermost part of the ecotone. Field layer was dominated by hemicryptophytes, such as *Calluna vulgaris*, *Carex sempervirens* and *Festuca rubra*. Site T (42º 28′ N, 01º 03′ E, 2330 m) was located on a north-east-facing steep slope (20–30º), under continental local climate (ca. 1600 mm of annual precipitation; 3 ºC of mean temperature), and with a yearly maximum snowpack of 1.5–3.0 m

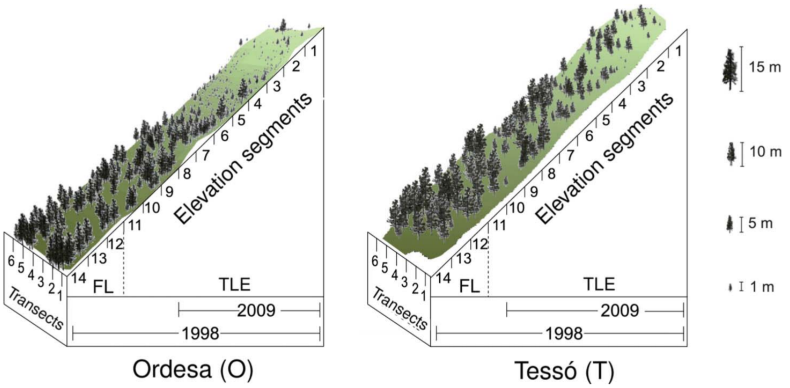

Figure 1. Schematic representation of the sampling design and tree layer structure at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain. *Pinus uncinata* individuals were located and their height was measured in 1995 (modified from Camarero and Gutiérrez, 1999). Vegetation transects were set from the treeline to the upper part of the forest in 1998, whereas in 2009 the study was restricted to the treeline ecotone (TLE), excluding the forest. The position of the forest limit (FL) was established according to Camarero et al. (2006).

(Esterri Meteorological Station, 42° 37′ N, 01° 07′ E, 1054 m a.s.l., 1970–1997 data [Camarero and Gutiérrez 2002]). The bedrock was shale, and the soil contained abundant organic matter (Camarero and Gutiérrez 2002). At this site, *Rhododendron ferrugineum* and *Vaccinium myrtillus* dwarf shrubs were dominant near the forest limit, whereas patchily distributed species, such as *Dryas octopetala* were more abundant in gaps near the treeline.

The mean temperature has increased at a rate of +0.3 °C per decade on average between 1950 and 2006 in the Pyrenees (López-Moreno et al. 2010), causing a decrease of snow depth (López-Moreno 2005). The same increasing rate of temperature has been recorded during the last decade at a Meteorological Station near to site O (R. García-González, unpublished data). This rate constitutes an accelerated warming in comparison with historical available temperature records before 1950 (Bücher and Dessens 1991). On the other hand, grazing activities have diminished along the central Pyrenees during the twentieth century, due to the phasing out of transhumance and the depopulation of rural areas (García-Ruiz et al. 1996; Lasanta-Martínez et al. 2005). For instance, the estimated number of sheep in the Ordesa-Monte Perdido National Park was reduced by 75% since 1960 to recent decades (Komac 2010). The mean stocking rate in 1990s was below 1.5 and 1 LAU (1 Large Animal Unit = eight sheep or one head of cattle) per month ha^{-1} in the Ordesa-Monte Perdido and Aigüestortes-Estany de Saint Maurici National Parks, respectively (Aldezábal et al. 1992; Bas et al. 1994). However, we assumed that grazing pressure was much lower at the study sites, based on their distant location away from the main summer pastures and livestock routes. Only a few goats were observed in the surroundings of site O (Aldezábal et al. 1992), their impact on *P. uncinata* individuals being negligible (Camarero and Gutiérrez 1999).

Sampling methods

In summer 1998, Camarero and Gutiérrez (2002) laid six line transects (each 140 m long) along the maximum slope from alpine grassland to the closed upper montane forest at both study sites. Transects were separated from each other by 6 m (Figure 1), and their position was permanently marked by using anchors, metal sticks and milestones. The abundance of *P. uncinata* and vascular plant taxa was estimated by using the point-intercept method (Barbour et al. 1987). A metal rod (diameter: 2 mm) was placed every 1 m along each transect, and all taxa contacted by the rod and its vertical projection were recorded (Camarero and Gutiérrez 2002). In 2009 transects were relocated and the tree and field layer vegetation was sampled in the same way as 11 years earlier (see Camarero and Gutiérrez 2002). As we were only interested on the dynamics of the treeline ecotone, we shortened transect length to the distance between the treeline and forest limit (i.e. the uppermost 80 and 100 m at sites O and T, respectively), to exclude the sampling points located within the forest (see Figure 1). More than 600 and 1000 plant records were gathered each year at sites O (486 sampling points) and T (606 sampling points), respectively. The botanical nomenclature of field layer species follows de Bolòs et al. (2005).

Statistical analyses

Changes over time in tree and field layer vegetation were first examined by considering each transect as a statistical unit after confirming the lack of spatial autocorrelation in preliminary analyses (Mantel test; $R = 0.41$ and 0.25 for Ordesa and Tessó sites, respectively; $P > 0.05$). In addition, we explored temporal changes along elevation by splitting transects into 10 m-long segments (see Figure 1).

Temporal changes in tree cover

We estimated the cover of *P. uncinata* individuals (hereafter tree cover) per transect ($n = 6$) by dividing the number of hits by the total number of sampling points in each survey (1998 and 2009). According to our previous knowledge of the treeline ecotones, we would expect an increase of tree cover rather than a decrease over 11 years. Therefore, we tested only for the increase in tree cover both at each treeline ecotone and along elevation segments, using one-tailed Wilcoxon tests.

To assess whether forest limit significantly shifted upwards over the 11-year study period, we tested whether the tree cover values along the elevational gradient in 2009 were the same as those observed below the forest limit in 1998. Different subjective criteria have been used to determine a threshold that separates the treeline ecotone from the forest below (e.g. Jobbagy and Jackson 2000; Holtmeier 2003). To overcome such limitations, we computed a site-specific threshold value on the basis of the tree cover values recorded below each forest limit in 1998 (Camarero et al. 2006) (Figure 1), and tested if the forest limit had shifted by using a randomisation procedure proposed by Harper and MacDonald (2001). First, we randomly selected one elevation segment below the forest limit (segments 12 to 14 in 1998, see Figure 1) in each transect and calculated the mean tree cover ($n = 6$) below the forest limit at each treeline ecotone. These steps were repeated 10,000 times for each ecotone, and the 2.5th percentile (two-tailed test, $\alpha = 0.5$) of the resulting distribution of mean cover values was selected as the threshold value. Finally, the tree cover values observed in 2009 were compared with such threshold values. We considered that the forest limit had shifted upwards when a set of consecutive segments of 2009 presented tree cover values that did not significantly differ ($P > 0.05$) from those observed in forest in 1998.

Temporal changes in plant diversity

Changes in plant diversity were analysed by considering the variation of species richness and evenness. We compared species richness between 1998 and 2009 by using sample-based rarefaction curves with 95% confidence intervals

(Gotelli and Colwell 2001; Colwell et al. 2004). Both curves and confidence intervals were calculated by using the EstimateS software (Colwell 2005), and following the analytical formulas detailed in Mao et al. (2005) (see also Colwell et al. 2004). The differences between sample-based rarefaction curves could be due to differences in species richness, but also to differences in the number of individuals recorded (Gotelli and Colwell 2001; Colwell et al. 2004). As we were only interested in the former and given that we recorded a different number of plant individuals in 1998 and 2009, we re-scaled the rarefaction curve to the number of plant individuals in the 2 years (Gotelli and Colwell 2001). Once rarefaction curves were plotted, significant differences ($P < 0.05$) between 1998 and 2009 were established when confidence intervals did not overlap (Colwell et al. 2004). In addition, we tested the changes of species richness between years at each elevation segments by using a two-tailed Wilcoxon test.

As an estimation of evenness we chose the Simpson index (1-D) (Legendre and Legendre 1998) which is preferable over other indices because it stabilises at small sample sizes (Magurran 2004). Differences of evenness between surveys were tested by using a two-tailed t-test with the Welch correction for groups with unequal variances (onwards Welch test), once normal distribution of the data was confirmed. We applied the same procedure for testing the changes in evenness along the elevation gradient.

Temporal changes in vegetation composition

We tested the change in the composition and abundance of field layer species (henceforth vegetation composition) in the treeline ecotone by using a non-parametric multivariate analysis of variance based on distance matrices (Anderson 2001; McArdle and Anderson 2001). This multivariate analysis was carried out by using the *adonis* function (Oksanen et al. 2010) in R (version 2.10.1; 2009), after excluding all unidentified species (see Table 1). This test is analogous to a MANOVA (Anderson 2001) and compares the variability in composition dissimilarity among transects from the same observation year to the variability in composition dissimilarity among transects from 1998 and 2009. We first calculated the dissimilarity among transects within and between observation years (the distance matrix), based on the Chao distance. This distance minimises the contribution of rare species (defined as those with only one or two hits in one of the sampling years) to the estimated dissimilarity (Chao et al. 2005, 2006); even so, the vegetation turnover might still be biased by our sampling limitation to track the changes of species occurring at low densities. To further explore this, we additionally calculated distance matrices by excluding less abundant species (defined as those species with abundance values lower than the observed third quartile; see further details in Appendix S1, online supplemental material). Next, the pseudo F-ratio statistic was computed for each distance matrix. A large value of this test statistic means that transects sampled in 1998 and 2009 differ in vegetation composition. The significance of this statistic was tested by using a permutation test ($n = 10{,}000$) carried out under the true null hypothesis of no difference between observation years. Each permutation transect was reassigned to one of the two possible observation years at random, and a new pseudo F-ratio was calculated. Finally, the significance of the test ($\alpha = 0.05$) was obtained by comparing the pseudo F-ratios obtained under permutation and those pseudo F-ratios corresponding to collected data (Anderson 2001; McArdle and Anderson 2001). As in similar analyses

Table 1. List of species recorded at the Ordesa (O) and Tessó (T) treeline ecotones, central Pyrenees, Spain, in 1998 and 2009. Nomenclature follows de Bolòs et al. (2005).

Site	Species
O	**Both in 1998 and 2009:** *Agrostis capillaris*, *Alchemilla* gr. *alpina, Allium senescens* subsp. *montanum*, *Antennaria dioica*, *Anthyllis montana*, *Calluna vulgaris*, *Campanula rotundifolia*, *Carex sempervirens*, *Deschampsia flexuosa*, *Festuca rubra, Galium pumilum* subsp. *marchandii*, *Geranium cinereum*, *Globularia cordifolia* subsp. *repens*, *Hieracium pilosella*, *Hieracium* sp., *Hippocrepis comosa*, *Jasione crispa*, *Juniperus communis* subsp. *communis*, *Nardus stricta*, *Pimpinella saxifraga*, *Plantago maritima* subsp. *alpina*, *Plantago monosperma*, *Potentilla alchemilloides, Potentilla crantzii*, *Sideritis hyssopifolia*, *Thymelaea tinctoria* subsp. nivalis, *Trifolium alpinum*, *Viola rupestris* subsp. *rupestris.* **Only in 1998:** *Arenaria purpurascens*, *Asperula pyrenaica*, *Astragalus monspessulanus*, *Phyteuma hemisphaericum.* **Only in 2009:** *Androsace villosa*, *Anthyllis vulneraria*, *Danthonia decumbens*, *Dianthus benearnensis*, *Erigeron uniflorus*, *Euphrasia salisburgensis* subsp. *salisburgensis, Hypochoeris radicata*, *Saponaria caespitosa*, *Sedum album*, *Thymus serpyllum* subsp. *nervosus*.
T	**Both in 1998 and 2009:** *Anemone narcissiflora*, *Botrychium lunaria*, *Carlina acaulis*, *Carex sempervirens*, *Dianthus hyssopifolius*, *Dryas octopetala*, *Festuca gautieri, Galium pumilum*, *Helianthemum nummularium*, *Hieracium lactucella*, *Juniperus communis* subsp. *communis*, *Lotus corniculatus* subsp. *alpinus*, *Pyrola chlorantha, Rhododendron ferrugineum*, *Saxifraga moschata*, *Vaccinium myrtillus*, *Veronica bellidioides*. **Only in 1998:** *Anemone nemorosa*, *Anemone vernalis*, *Daphne cneorum*, *Galium verum*, *Helictotrichon sedenense*. **Only in 2009:** *Alchemilla flabellata*, *Androsace villosa*, *Anemone hepatica*, *Antennaria dioica*, *Bupleurum ranunculoides* subsp. *gramineum*, *Campanula cochlearifolia*, *Cruciata glabra*, *Gentiana verna*, *Iberis sempervirens*, *Luzula nutans*, *Plantago monosperma*, *Polygonum viviparum*, *Primula elatior* subsp. *intrincata*, *Sempervivum montanum*, *Soldanella alpina*, *Vaccinium uliginosum* subsp. *microphyllum*.

of variance, the *adonis* test is sensitive to data heteroscedasticity (Anderson 2001, 2006). To confirm that significant differences between years were not caused by data heteroscedasticity across sampling years, we carried out a permutation test (10,000 randomisations), based on a multivariate dispersion analysis (Anderson 2006) by using the *permutest.betadisper* function (Oksanen et al. 2010).

The temporal turnover of vegetation was examined along the elevation gradient by using a dissimilarity index. We merged species abundances recorded at each elevation segment and computed the Chao index by using the *vegdist* function (Oksanen et al. 2010). High values indicate large changes in the composition of the assemblage between years. We also re-calculated Chao index by excluding the less abundant species (see Appendix S1) to determine whether the turnover pattern was affected by sampling limitations (i.e. insufficient capture of species occurring at low densities). Finally, temporal changes in the abundance of each taxon were tested by using a χ^2 test.

Results

Tree cover significantly increased from 20% to 35% at site O (one-tailed Wilcoxon test; $W = 4$, $P = 0.013$) between 1998 and 2009. Near the forest limit, tree cover in 2009 was not statistically different from the threshold values (randomisation test; $P > 0.05$), indicating that tree cover in these elevation segments of the treeline ecotone was similar to that observed within the forest (Figure 2). We also detected significant increases of tree cover in the third and fifth elevation segments (one-tailed Wilcoxon test, W-statistic ranges from 5.5–7, $P < 0.05$; Figure 2). Tree cover at site T was below 21% in 2009, and unlike at O, statistically the same as 11 years earlier (one-tailed Wilcoxon test; $W = 7.5$, $P = 0.054$). Tree cover did not significantly increase in any of the elevation segments either ($P > 0.05$ in all segments; Figure 2).

Plant richness of the field layer vegetation did not significantly differ between 1998 and 2009 at site O (Figure 3), but evenness significantly increased from 0.82 to 0.86 through time (two-tailed Welch test; $t = 3.60$, d.f. $= 8.18$, $P = 0.005$). At site T, plant richness increased significantly from 23 to 36 recorded taxa ($P < 0.05$; Figure 3), whereas evenness in 2009 was identical to that in 1998 (0.78; two-tailed Welch test; $t = 0.63$, d.f. $= 9.8$, $P = 0.54$). Neither richness nor evenness changed significantly (two-tailed Wilcoxon test and two-tailed Welch test, respectively, $P > 0.05$) along the elevation gradient at site T (Appendix S2, online supplemental material).

Vegetation composition changed significantly between 1998 and 2009 at both sites (O *adonis*; $F = 9.03$, $R^2 = 0.44$, $P = 0.003$; T *adonis*; $F = 17.01$, $R^2 = 0.63$, $P < 0.001$). None of these results were biased due to data heteroscedasticity (*permutest.betadisper;* $F = 1.31$, $P = 0.283$ in O and $F = 0.53$, $P = 0.487$ in T). At the two treeline ecotones, the species only detected in 2009 were typical of grasslands

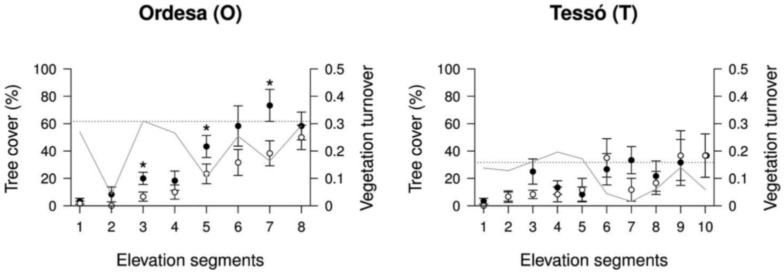

Figure 2. *Pinus uncinata* cover in 1998 (empty circles) and 2009 (filled circles) (means and standard errors), and vegetation turnover estimated as Chao index (grey line), at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain. Elevation segments are numbered from the treeline to the forest limit. Asterisks indicate significant (one-tailed Wilcoxon test, $P < 0.05$) increase of tree cover after 11 years. The dotted line corresponds to the threshold value of tree cover that separates the treeline ecotone from the forest limit.

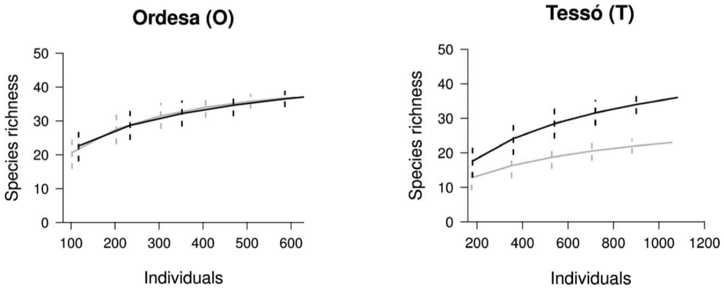

Figure 3. Individual-based rarefaction curves of the Mao-Tau expected species richness and 95% confidence intervals in 1998 (grey line) and 2009 (black line) at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain.

(Table 1). These shifts in composition were mostly driven by species with cover < 5% at site O (Figure 4). In particular, we only found significant increases (χ^2 test; $P < 0.05$) in the abundance of alpine grasses, such as *Nardus stricta*, *Danthonia decumbens* and *Agrostis capillaris* at this treeline ecotone (Figure 5), whereas the newly detected species at site T contributed the most to the observed change (Figure 4). However, when we repeated analyses excluding less abundant species ($n = 14$ and 12, O and T, respectively), we also found a significant shift in vegetation composition between years (O *adonis*; $F = 15.18$, $R^2 = 0.60$, $P = 0.002$; T *adonis*; $F = 36.05$, $R^2 = 0.78$, $P < 0.001$). Four species accounted for more than two-thirds of the hits both in 1998 and 2009 in the field layer at O: *Calluna vulgaris*, *Festuca rubra*, *Carex sempervirens* and *Hippocrepis comosa*, and only the abundance of the latter shifted significantly between 1998 and 2009 (Figure 5). At site T *Rhododendron ferrugineum*, *Vaccinium myrtillus*, *Dryas octopetala*, *Festuca gautieri* and *Carex sempervirens* accounted for 90% of the cover hits in 1998 and 89% in 2009, and only the abundance of two of the species changed significantly (Figure 5).

The highest values of vegetation turnover were patchily distributed along the elevation gradient at site O, whereas at site T, the highest turnover was restricted to the upper elevation segments (Figure 2). The same elevation pattern, though with lower turnover values, was found at both sites when the less abundant species were excluded,

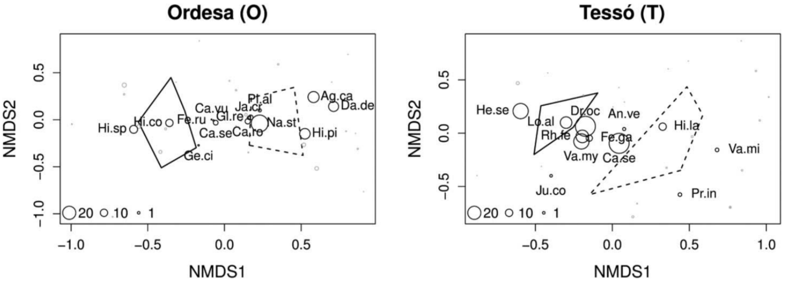

Figure 4. Non-metric multidimensional scaling ordination of field layer vegetation composition at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain. Convex hulls join vegetation samples from 1998 (solid lines) and 2009 (dashed line). Circles indicate the position of the less abundant (grey) and most abundant (black) plant species in the multivariate space. The size of the circles represents the absolute change in abundance of the species from 1998 to 2009. For clarity only the names of most abundant species are shown, names are abbreviated by writing the first two letter of the genus name and the first two letter of the (sub)species names (e.g. *Dryas octopelata* = Dr.oc). See Table 1 for a list of species names.

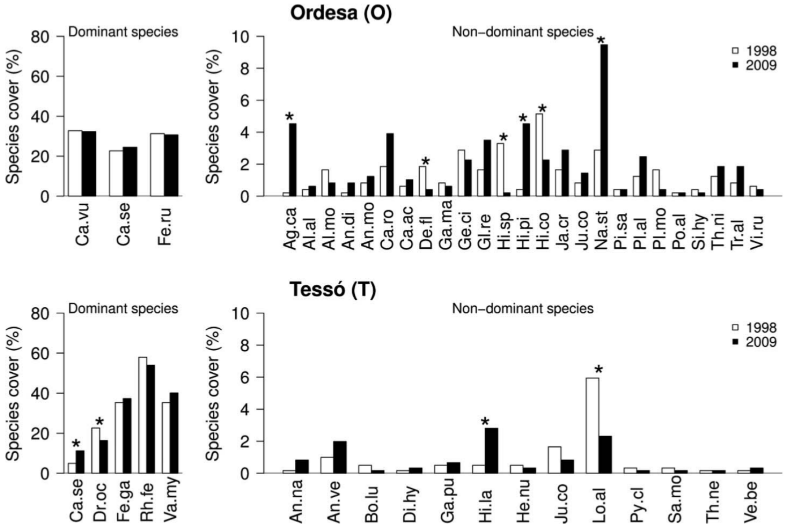

Figure 5. Changes in the cover of shared dominant (on the left) and non-dominant species (on the right) at the Ordesa and Tessó treeline ecotones, central Pyrenees, Spain, between 1998 and 2009. Asterisks indicate significant temporal changes in abundance (χ^2 test, $P < 0.05$, $n = 6$ transects). Codes for species correspond to the first two letter of the genus name and the first two letter of the (sub)species names (e.g. *Globularia cordifolia* subsp. *repens* = Gl.re). See Table 1 for a list of species names.

except for the uppermost segment in T (see Appendix S1). At both treeline ecotones, the patterns of highest vegetation turnover and significant increases in tree cover were spatially uncoupled along the elevation gradient (Figure 2).

Discussion

We found that changes in *Pinus uncinata* canopy cover and field layer plant communities were spatially uncoupled in Pyrenean treeline ecotones at a decadal scale. Even if the upwards shift of the forest limit at site O might have drastically modified field layer microsite conditions (e.g. by reducing the light reaching the field layer) (Smith et al. 2003), vegetation turnover was not higher there than in other parts of the treeline ecotone. Moreover, contrary to overall expectation, the species involved in the turnover were not those of shade-adapted, but grasses. Taken together, these results suggest that canopy closure did not trigger short-term changes in the composition of the field layer vegetation in the treeline ecotone.

The main driver of vegetation dynamics remains unclear after removing the effect of tree cover. Unlike some subarctic forest tundra ecotones (Pajunen et al. 2012), the shift in field layer vegetation cannot be attributed to denser shrub canopies, because none of the shrubs species (*Calluna vulgaris*, *Rhododendron ferrugineum* and *Vaccinium myrtillus*) increased in cover over the 11 years. At site O, *Nardus stricta*, *Agrostis capillaris* and *Danthonia decumbens* significantly increased at the expense of other grasses and non-dominant forbs. These species are abundant in grassland in the Pyrenees (Fillat et al. 2008), and their increase in abundance may be linked to the past decline in grazing intensity. Indeed, increasing abundance of *N. stricta* and *A. capillaris* has also been observed after two decades of grazing exclusion in nearby grasslands (D. Gómez and R. García-González, pers. comm.). However, climate-mediated responses cannot be discarded either, because these graminoids might have responded rapidly to the rising temperatures due to their modular organisation and high tissue turnover (Shaver et al. 1997). Whatever the driver was, the higher values of vegetation turnover was associated with the distribution of graminoids, whereas the lower turnover corresponded to localities where biotic conditions were little altered, such as rock outcrops or locations where shrubs and tussock-forming sedges (*Carex sempervirens*) were already established in 1998 (Camarero and Gutiérrez 2002). By contrast, a higher vegetation turnover was found in the upper half of the treeline ecotone at site T. In the uppermost elevation segment, we found a discordance between the turnover based on all species and that based on a subset of most abundant ones (Appendix S1), which indicates a higher contribution of newly detected species to vegetation turnover there. The fact that higher turnover values matched the distribution of the dwarf shrub that decreased the most, i.e. *Dryas octopetala* (Camarero and Gutiérrez 2002), led us to suggest that a competition release allowed the increase in abundance of less competitive species (*Plantago monosperma*, *Primula elatior* subsp. *intrincata*, *Soldanella alpina*) near the treeline (Choler et al. 2001; Virtanen et al. 2010). We cannot directly infer causality from our descriptive study; however, the low grazing since 1990s, and the decrease by *D. octopetala* similarly to that found in response to experimental warming (Klanderud and Totland 2005) suggests a potential role of rising temperature on the reduction of this dwarf shrub. In contrast, the stable dense layer of ericoid shrubs (*R. ferrugineum* and *V. myrtillus*) may have prevented the entrance of new species in the lower half of the elevation gradient at site T.

It is important to note that the reported changes in vegetation composition and species richness could also reflect stochastic processes that were not identified by our study, such as year-to-year fluctuations in the abundance of each species (Magurran et al. 2010). However, given that all recorded species were long-lived that buffer stochastic fluctuations to a great extent (García et al. 2008), the contribution of temporal variability to the observed vegetation changes may be regarded as minor. Another important caveat in our study was the number of sampling points per transect established in 1998 (Camarero and Gutiérrez 2002), which could be insufficient to capture species occurring at very low densities. To overcome such potential bias, we reduced the weight of species with low cover on vegetation analysis by using the dissimilarity distance that yielded the most conservative results (see Materials and methods). More importantly, we demonstrated that the observed results were robust to the removal of species with lower cover from analyses (except the aforementioned case in the uppermost elevation segment at site T; see Appendix S1). Finally, the detection of species not recorded in 1998 should be interpreted as an increase of their abundance in the treeline ecotone, rather than as a migration from adjacent belts. Taken together, the results indicate moderate changes in field layer plant diversity, supporting previous conclusions from high-latitude treelines (Virtanen et al. 2010).

Regarding the dynamics of *P. uncinata*, we only found a change in tree cover at site O. This result deviates from general predictions of lower sensitivity to rising temperature of abrupt treeline ecotones as compared with diffuse ones, which are less subjected to additional constrains (e.g. wind, snow) (Harsch et al. 2009; Harsch and Bader 2011). Wind has limited pine recruitment and growth through mechanical damage during the last decades (Camarero et al. 2000). Therefore, the negative impacts of the wind (winter desiccation, summer cooling, needle abrasion by snow particles or mechanical damage to exposed stems [Scott et al. 1993; Smith et al. 2003; Holtmeier and Broll 2010]) should be locally lessened to allow the increase in tree cover increase. In this context, a positive interaction between *P. uncinata* adults (Smith et al. 2003; Batllori et al. 2009b) may explain the upwards extension of the forest limit, whereas in the krummholz band (see Figure 1), the increased tree cover may be associated to more favourable microsite conditions under krummholz individuals (Germino et al. 2002; Smith et al. 2009; Holtmeier and Broll 2010), some field layer species (e.g. *Calluna vulgaris*) (Bartolomé et al. 2008), or/and the shelter of surface geomorphologic features (Resler 2006). By contrast, the dense patches of *R. ferrugineum* might have prevented the recruitment of *P. uncinata* at site T (Pornon and Doche 1996; Ninot et al. 2008; Batllori et al. 2009b).

Conclusions

Our study evidences an unexpected uncoupling between the dynamics of tree cover and field layer vegetation at the upper montane treeline ecotone over a decade. This result suggests that an upwards extension of the upper montane forest does not necessarily mean a loss of plant diversity in adjacent grasslands in the short term. So far, such local uncoupling between tree and field layer vegetation has not been evidenced from other sites. Thus, there is still little basis for robust generalisations based on comparative approaches. Further long-term studies that include field layer vegetation are required for assessing the full impact of ongoing global change on treeline dynamics and related plant diversity. Meanwhile, alpine plant diversity losses inferred from gross dynamics of treeline ecotones at large or regional scales should be cautiously interpreted.

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ORIGINAL PAPER

Long-term response of plant communities to herbivore exclusion at high elevation grasslands

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Abstract Understanding the effects of herbivores on plant communities is needed for anticipating how variation in grazing regimes will impact natural and semi-natural ecosystems. Prominent ecological hypotheses predict that drastic reductions of herbivory, as have occurred in many European mountains, will trigger fast diversity loss and structural changes in grasslands, because grazing tolerant species are expected to be rapidly replaced by taller and more competitive species. The aims of this study were to test generalizations of herbivory effects (mostly from lowlands) in high elevation grasslands over ecological relevant times scales, and to unravel the contribution of climatic conditions to observed changes. Species richness, canopy height, plant community structure, and transition probabilities among ecological groups were monitored throughout 19 years in herbivore exclusions and control plots in the Central Pyrenees. We used ordination analyses to track the long-term community response to herbivore exclusion, and generalized additive models

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to assess the non-linear effects of herbivore exclusion and climatic conditions on measured variables. Contrary to expectations, herbivore exclusion did not significantly affect species richness, and although canopy height increased, it was not drastic enough to suppress shade intolerant species. The strongest shifts in plant community structure and transition probabilities between ecological groups occurred during a sequence of warm and dry growing seasons, whereas in control plots, these changes were smaller, and largely reversed after cooler and wetter climatic conditions returned. Our results suggest that long-term effects of grazing cessation in high elevation grasslands can be weaker and slower than predicted. However, these effects can act synergistically with dry and warm events. Therefore, the maintenance of past grazing activities can be key in the face of ongoing climatic warming.

Keywords Alpine · Community monitoring · Generalized Grazing Model · Grassland management · Land abandonment · Land-use change

Introduction

Understanding the community effects of herbivory is a pivotal issue for the management of healthy plant communities. Many human activities shift the pattern of herbivore effects in both natural and semi-natural ecosystems worldwide. Therefore, these effects are of particular concern for understanding the optimal management of domestic herbivores as well as for defining conservation policies (Noy-Meir et al. 1989; Westoby et al. 1989; Hobbs and Huenneke 1992). In some areas, the main management question is how to lessen grazing pressure (e.g. Zhao and Zhou 1999), while in others, economic shifts have resulted in rapid reduction of herbivory that may have equally negative effects on plant diversity and foraging value of grasslands (e.g. Wells 1969; Stohlgren 1999; Tasser and Tappeiner 2002).

While herbivory has been one of the most intensively studied ecological interactions (McNaughton 1985; Milchunas et al. 1988; Huntly 1991), ecologists have crafted a wide range of sometimes opposing generalities about herbivore effects on plant communities and when and why these effects will be strong or weak (Milchunas et al. 1988; Westoby et al. 1989; Olff and Ritchie 1998; Proulx and Mazumder 1998). During the last decades, the Generalized Grazing Model (GGM) has provided the most prominent theoretical framework for understanding the variable impact of herbivory across different plant communities (Oesterheld and Semmartin 2011). The GGM is largely based on the hump shaped response of plant richness to disturbance (i.e. grazing) predicted by the 'Intermediate Disturbance Hypothesis' (Grime 1973). However, the GGM suggests that the strength of the response of plant communities to grazing will be different across gradients of precipitation and evolutionarily history of grasslands. In particular, the GGM predicts that competition between plant species shifts from soil resources to light along gradients of humidity (Milchunas et al. 1988), and primary productivity (Cingolani et al. 2005). Therefore, largest effects of herbivory on plant richness are expected in sub-humid and/or productive areas (Milchunas et al. 1988; Milchunas and Lauenroth 1993). The model also predicts that plant communities with long grazing histories will rapidly shift in response to large changes in grazing intensity, because they have evolved under divergent selection for grazing and canopy dominance, and developed pools of native species adapted to different levels of herbivory. Given that non-resilient species were eliminated from the initial species pool long ago, the predicted structural changes are likely to be more reversible than those occurring in communities with a short grazing history (Cingolani et al. 2005).

Empirical support for the GGM is mostly from low elevation grasslands and shrublands (Milchunas and Lauenroth 1993), whereas the herbivore impact on high elevation grasslands remains relatively less thoroughly studied (Erschbamer et al. 2003). This fact contrasts with the key role that wild and domestic herbivores play on the maintenance of highly valuable ecological and cultural landscapes at high elevations (Austrheim and Eriksson 2001; Chemini and Rizzoli 2014; Fillat et al. 2008), which in some cases are alarmingly endangered (Habel et al. 2013). In European mountains, the shift in socio-economic conditions of rural societies has weakened seasonal grazing to historical minimums, including the complete abandonment of many pastures (García-Ruiz et al. 1996; Luick 1998; MacDonald et al. 2000, Zervas 1998). As a result, forest and shrubs have encroached into semi-natural grasslands (Welch and Scott 1995; Gehrig-Fasel et al. 2007; Améztegui et al. 2010; Pajunen et al. 2012), and even more drastic changes to these ecosystems have been predicted (Sala et al. 2000).

Several studies conducted to address the specific impact of cessation of grazing on mountains have reported a decline in species richness (Rawes 1981; Tasser and Tappeiner 2002; Niedrist et al. 2008; Speed et al. 2013), whereas a few others have shown no changes in species richness, but rapid changes in the community composition and structure (Hill et al. 1992; Hope et al. 1996; Krahulec et al. 2001; Mayer et al. 2009). The lack of consistent results along with the particularly slow response of alpine plant species (Evju et al. 2009) limit our current understanding of the long-term effects of grazing reductions. To further complicate matters, the land-use change is coincident with climate warming in mountains (Dessens and Bücher 1997; Beniston 2006). As disentangling their separate effects is not easy under natural conditions, the interaction between these two drivers of change is still understudied at high elevation. However, this gap needs to be addressed to forecast the biological consequences of grazing reduction and to guide successful management of high elevation grasslands, particularly in the face of ongoing climate change.

In this study, we experimentally assess the long-term impacts of herbivore exclusion on plant species richness, canopy height, and relative abundance of species in some of the most common grassland communities of the Pyrenees (Fillat et al. 2008). The studied grasslands can be defined as sub-humid (annual precipitation ~1700) and long grazing history systems (Chocarro et al. 1990) following the classification criteria used in Milchunas and Lauenroth (1993). For this sort of grasslands, the GGM predicts a rapid shift in community structure towards taller and more competitive species in response to grazing cessation, and in some cases, a decline in species richness via competitive exclusion (Milchunas et al. 1988). If a few dominant grasses increase at the expense of forbs and shade intolerant grasses, we would also expect a decline in species richness (Milchunas et al. 1988; Hobbs and Huenneke 1992) and pasture value for livestock (Crawley 1983). We also expect that structural and diversity impacts of herbivore exclusion will be faster and more dramatic if climate-induced migration of high competitive lowland species (e.g. shrubs) alters the pool of available species (Olofsson et al. 2009; Speed et al. 2012). In contrast, whether climate-driven impacts would alter the equilibrium composition of grazed grasslands is more uncertain (Vittoz et al. 2009), and will depend on whether climate can override the effects of herbivory (Post and Pedersen 2008).

Methods

Study site

The study sites were located in the Ordesa-Monte Perdido National Park, Central Pyrenees, Spain (42°40′18″N, 0°3′20″E). This mountain park extends along five valleys radiating from the highest calcareous summit in Europe (3355 m a.s.l.), covering an elevational gradient of ~2400 m. Subalpine and alpine grasslands cover more than one third of the total area of the National Park (15608 Ha.), and have been used as summer pastures for livestock since at least the fifteenth century (Fillat et al. 2008), and probably into ancient pre-history. However, this traditional land-use has been dramatically modified owing to socio-economic shifts in the Pyrenean region during the last century, with the total number of domestic herbivores (mainly sheep) drastically diminished since the 1950s (García-Ruiz et al. 1996; Aldezabal 1997).

In 1992, two exclosures of 144 m^2 each were established to assess the effect of grazing abandonment on plant diversity and community composition and structure in high elevation grasslands. They were selected to represent the two most abundant types of grasslands along the Pyrenees (Fillat et al. 2008): *Bromion erecti* (henceforth Bromion) and *Nardion strictae* (henceforth Nardion). The former harbors high species richness (>40 sps) and plants of high fodder value (e.g. *Lotus corniculatus, Medicago suffruticosa and Trifolium* spp.) whereas the latter has lower species richness (<20), is usually dominated by the tussock-forming grass *Nardus stricta,* and poorer in terms of nutrient quality (Gómez 2008). In both communities *Festuca nigrescens* was the most abundant species at the beginning of the study. For Bromion communities, the mean aboveground net primary production was 483 g of dry matter per m^{-2} $year^{-1}$, and 378 for Nardion (García-González et al. 2002). Such values correspond to productive grasslands following the classification criteria of the GGM in Milchunas and Lauenroth (1993). Exclosures are located in a flat area (1930 m a.s.l.) 600 and 450 m distant from the nearest subalpine forest limit and shrubland, respectively, and separated each other by 125 m. The area was frequently visited by domestic herbivores (mostly cattle) for summer pasturing (from July to October) and a very few wild ungulates. Activity of other small herbivores (e.g., marmots and moles) was negligible in study area. The stocking rate was relative high throughout the study: 0.79 LU ha^{-1} (1LU = 1.25 cow = eight sheep) (Aldezabal et al. 1992).

The monitored grasslands share most topographic and environmental features, such as N aspect, length of vegetative period (Del Barrio et al. 1990), mean annual temperature (5 °C) and precipitation (~1700 mm). However, edaphic conditions were different: the soil of Bromion was basic, high permeable and with moderate levels of organic matter, whereas that of Nardion was acidic (pH 4), humic, with high levels of organic matter and water retaining capacity (Badía et al. 2002).

Climatic data

We gathered daily temperature, precipitation, and snow depth records between 1982 and 2012 from the nearby Góriz Meteorological Station (42°39′49’’N, 00°00′50′’E; 2215 m a.s.l.). In 2006, we also set up a Station at the Nardion site, which was used to validate the temperature records from Góriz, and to fill the gaps in the temperature series. We calculated the mean, maximum and minimum temperature, and the mean thermal sum (measured in degree days) for each year, summer (from June to September), snow free period, and growing season, defined as the span between the snow melt-out and the exact date of vegetation sampling (between July 28th and August 1st). For each of these periods, we also calculated the accumulated precipitation.

Vegetation sampling

Between 1993 and 2012 (with the exception of 1999), we annually monitored the vegetation community within the exclosures by using the point intercept method along two perpendicular transects (10 m long each) at each site. At each semi-permanent sample point ($n = 200$, spaced every 10 cm along the transects), we recorded the height and species of the tallest plant. When the point was not covered (e.g. bare soil, dung, or litter), it was registered as a 'non-plant'. Control transects were also established outside the exclosure, where the same sampling procedure was applied. This yielded a total of 400 sample points per site. The abundance of each species was calculated as the number of points covered by the species out of the total number of points per treatment (exclosure *vs* control). The sum of the heights of all recorded plant individuals was used as a surrogate of canopy height. Community structure was defined according to the composition and abundance of species.

Although the beginning and end of transects were permanently marked with sticks, we could not assume that each sample point was exactly relocated every year. Even if we did, it is unlike that the same plant individuals were always recorded, due to the small size of plants, their architecture, microtopography, and simple inaccuracy. While inaccuracy does not influence the relative abundance estimates, it might result in serious sampling error for analyses that track transitions between plants at each point (Conway-Cranos and Doak 2011). To account for such potential bias, we estimated the sampling error in point relocation, following Conway-Cranos and Doak (2011). One of the two transects at each control site was successively resampled three times in a single day in 2011. Each time, we removed and replaced the transects to reproduce the alignment process in annual resampling. Using the resulting data in combination with the annual sampling allowed us to correct transition estimates for sampling uncertainty (Conway-Cranos and Doak 2011).

Data analyses

Climate trends in annual mean temperature and precipitation were tested with the Mann–Kendall test (Mann 1945; Kendall 1975). For vegetation analyses, we first analyzed the effect of grazing exclusion on species richness, canopy height and abundance of dominant species over the 19 years by using the following Generalized Additive Model (GAM; Wood 2006; Hastie and Tibshirani 1990):

$$y_i = \alpha + \beta \text{ treatment } + f(x_i)\text{treatment} + \varepsilon_i \qquad ([1])$$

where y is the response variable in year i, α the intercept, β the factor indicating the treatment (control *vs* herbivore exclusion), $f(x_i)$ the smooth function of time, and ε_i the random error. Species richness and the abundance of dominant species were initially modeled using Poisson distribution, whereas data on canopy height was log transformed to fit into a Gaussian distribution. The effect of the grazing exclusion was evaluated as a smooth function of time by using cubic splines (Wood 2006) and the number of knots were selected from three to a maximum of five, iteratively. When we observed that an increase in the number of knots did not lead to a better explanation of the residuals, we selected the lowest number to minimize model overfitting (Wood 2006). Given that annual data within each plot is used as an independent sample, clearly, our sampling design is pseudo-replicated (with one single plot per treatment in each site), so P values may be inaccurate. For such reason, P values higher than 0.01 were not considered as significant. We used the same approach for testing the interactive effect of climate and herbivore exclusion by substituting the 'time' variable in Eq. (1) with temperature and/or precipitation variables. Inclusion of interaction effects between climatic variables was infeasible because of limited sample size. From the set of candidate climatic variables (see above), we selected thermal sum and accumulated precipitation during the growing season based upon their explanatory power (i.e. the proportion of deviance explained by the model).

To assess the effect of herbivory on the community structure over our 19 years of data, we performed a non-multidimensional scaling ordination based on the species abundances and the Bray-Curtis dissimilarity measure (Bray and Curtis 1957), comparing the trajectories of community structure in exclusion and control plots. Although the Bray-Curtis is a robust measure of plant community response (Faith et al. 1987), the use of any other

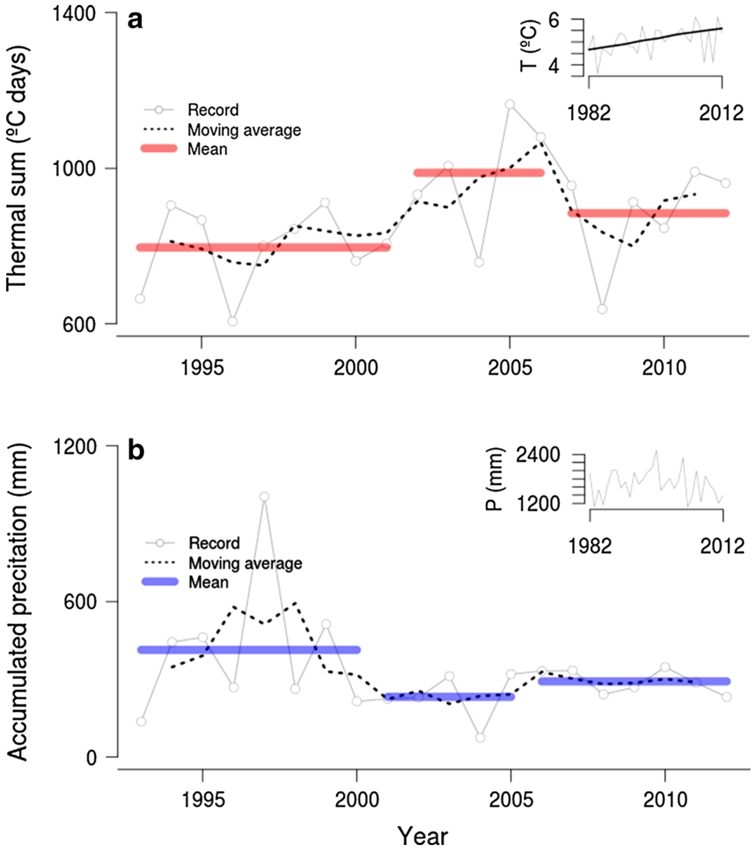

Fig. 1 Trends of thermal sum (**a**) and accumulated precipitation (**b**) in the growing season in the Ordesa-Monte Perdido National Park for the last decades. Moving averages (3-years fixed interval) of these variables indicate a warmer period between 2002 and 2006, and a dry period between 2001 and 2005. On average, corresponding bracketing time periods were cooler and more humid, respectively (*horizontal lines*). Embedded subplots show records of mean annual temperature (**a**) and precipitation (**b**) between 1983 and 2012. The *black line* indicates a significantly increasing trend in annual temperature ($P < 0.05$) according to the Mann–Kendall trend test

dissimilarity measures would lead to different ordination results (Anderson et al. 2006), and thus, to different interpretations. To assess the robustness of the observed trajectories of communities, we repeated ordination analyses with (i) two other dissimilarity measures (Euclidean and Horn-Morisita; Horn 1966), (ii) only common species, and (iii) incidence data (see Online Resource 1).

To further characterize the dynamics of grazed and ungrazed communities we used community transition models (Horn 1975; Hill et al. 2004). Because of the high number of species and specific functional groups (geophytes, rosette forbs) with low values at both sites, model construction with individual species or specific functional groups was problematic. Therefore, we decided to parameterize models using four ecological classes: (1) the dominant grass *F. nigrescens,* which was the only tall species (>35 cm based on field averaged field records); (2) other non-dominant grasses; (3) forbs; and (4) non-plants (i.e. bare soil, dung and litter). Non-dominant grasses were long-lived hemicriptophytes with similar leaf morphology, plant propagation and dispersal syndromes, whereas forbs were mostly dominated by highly palatable and small size hemicriptophytes. Nevertheless, and given the existent heterogeneity mostly within forbs (e.g. legumes, rosette forbs, thistles, all of which appeared in very low abundance), transition analyses were only used to identify broad effects of herbivory exclusion, such as gap creation or colonization (Non-plants ↔ other plants), competition relaxation (Dominant grass → other plants), stability (transition within groups) and ecological changes (Forbs ↔ Grasses). Transition probabilities between each set of groups were estimated using the approach of Conway-Cranos

Table 1 Summary of generalized additive models used to test the effect of herbivore exclusion and climate on species richness and log-transformed canopy height across 19-years

Response variable	Site	Predictor variable	Treatment parameter	Smooth term				Deviance explained (%)
				Control		Exclusion		
				Edf	Statistic	Edf	Statistic	
Species richness	Bromion	Year	−0.12	1	0.02	1	1.28*	40.7
		T_{sum}	−0.12	1	0.21	1	0.88	38.5
		P_{acc}	−0.12	1	0.06	1	0.63	35.0
	Nardion	Year	−0.14	1	1.07	1	0.72	48.6
		T_{sum}	−0.14	1	0.01	1	1.05	40.3
		P_{acc}	−0.14	1	0.98	1	0.10	41.2
Canopy height	Bromion	Year	0.69***	1.95	1.63	2.85	5.65**	78.6
		T_{sum}	0.69***	1.70	6.92**	1.77	1.23	75.7
		P_{acc}	0.69***	1	0.77	2.69	0.88	66.3
	Nardion	Year	0.32***	2.47	1.73	1	6.78*	50.4
		T_{sum}	0.32***	1.86	3.07	1.82	0.43	45.2
		P_{acc}	0.32***	1	2.06	1	1.50	35.7

Models were formulated using herbivore exclusion as a treatment, and time, growing thermal sum (T_{sum}) and accumulated precipitation (P_{acc}) as predictor variables. Cubic splines were used to account for potential non-linear relationships, with a maximum degrees of freedom of the model (edf) set to five to avoid overfitting. Note that test statistics of the smooth terms are *F* and Chi squared statistics for species richness' and canopy height's models, respectively

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

and Doak (2011). Estimation of error-corrected transition rates were conducted using Matlab programs modified from Conway-Cranos and Doak (2011). All other analyses were conducted using R 2.14.1 (R Development Core Team 2011), and 'kendall' (http://www.stats.uwo.ca/faculty/aim/), 'mgcv' (Wood 2006) and 'vegan' (Oksanen et al. 2013) R-packages.

Results

Meteorological records from Góriz Station indicated a significant increasing trend in annual mean temperature since 1981 ($\tau = 0.32$; $P < 0.05$) (Fig. 1). The period between 2001 and 2012 was on average warmer (+0.3 °C) than the previous decade. Most notably, the average (3-years fixed interval) of the thermal sum during the growing season evidenced a warm period between 2002 and 2006 (Fig. 1), whereas the prior and subsequent periods were markedly cooler. The pattern in annual mean precipitation over the last decades was less clear, with no linear trend in precipitation ($\tau = 0{,}06$; $P < 0{,}67$). However the driest growing seasons were concentrated in early 2000s (Fig. 1).

We did not find any significant ($P < 0.01$) effect of herbivore exclusion on species richness at any of the grasslands between 1993 and 2012 (Table 1). Temporal fluctuations in species richness were also unrelated to temperature and precipitation regimes in both cases. In contrast, canopy height increased significantly in response to herbivore exclusion (Table 1). In the Bromion exclusion, a significant decrease in canopy height was observed during the warm and dry period, which was reversed at the end of the study (Fig. 2). In the Nardion exclusion, on the contrary, canopy height gradually increased throughout the study (Table 1; Fig. 2), although in this case, the effect was not statistically significant ($P > 0.01$). The separate effects of thermal sum and accumulated precipitations on canopy height were significant ($P < 0.001$) in both grasslands, but only in the case of Bromion explained a reasonable amount of the observed variability (Table 1). In this case, the highest temperatures decreased canopy height only in the control plot.

Exclusion of herbivores affected the community structure at both sites, but the differences between control and exclusion plots after 19 years were stronger at Nardion (Fig. 3).

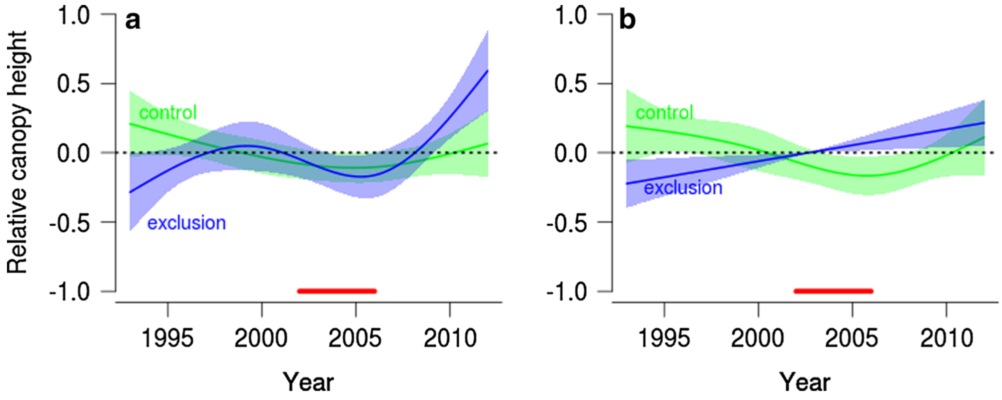

Fig. 2 Effect of herbivore exclusion on log-transformed canopy height in the Bromion (**a**) and Nardion (**b**) grasslands across 19-years. *Shaded area* represents 95 % confidence intervals for control and exclusion plots. Canopy height values are centered on 0 to facilitate comparison between sites and plots. *Red line* highlights an exceptionally warm and dry period detected between 2002 and 2006

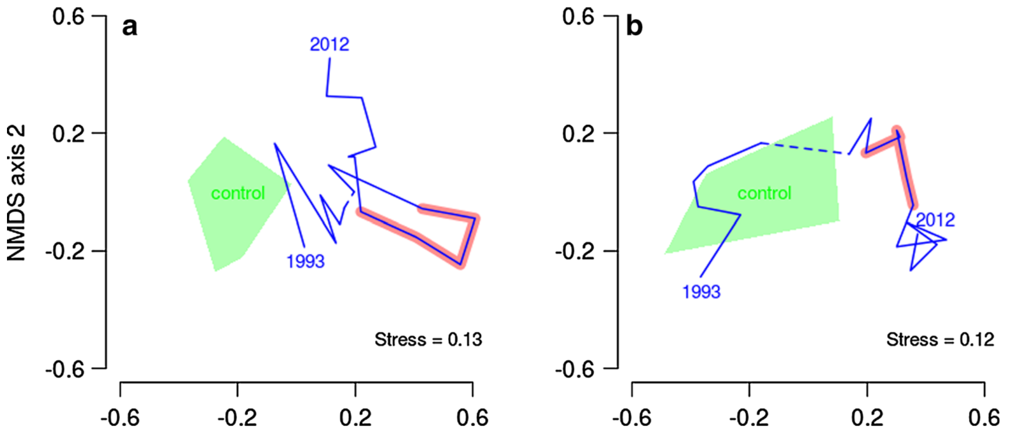

Fig. 3 Long-term trajectory of plant community structure in response to herbivore exclusion in the Bromion (**a**) and Nardion (**b**) grasslands. Ordination is based on non-metric multidimensional scaling (NMDS), and shows plant community dynamics in controls (convex hull) and exclusion plots (*solid line*) between 1993 and 2012 (no data was recorded in 1999; *dashed lines*). *Shade line* stresses the exceptionally warm and dry period between 2002 and 2006

The community structure of Bromion diverged considerably from the control in 2001, but it was partially reversed after 2006 (Fig. 3). In Nardion, changes in the community structure after herbivore exclusion were only noticeable after the 8th year (Fig. 3). At the end of the study, control plots approached initial community structures in both cases. Interestingly, interpretation of ordination analyses was consistent regardless of the dissimilarity measure used, and consideration or not of rare species, but not when incidence data was used (see Online Resource 1).

The dominant grass *Festuca nigrescens* underwent a markedly different shift in abundance in the different sites and treatments (Fig. 4). At the Bromion site, herbivore exclusion led to declining *F. nigrescens* during the warm and dry period, but that trend was reversed afterwards (Fig. 4). At the Nardion site, the decreasing trend of dominant *F. nigrescens* was gradual and significant since the first years of herbivore exclusion, and mirrored the increase of *Nardus stricta*. As a result, *N. stricta* became the most dominant species at the end of the study. In control plots, temporal fluctuations of the dominant

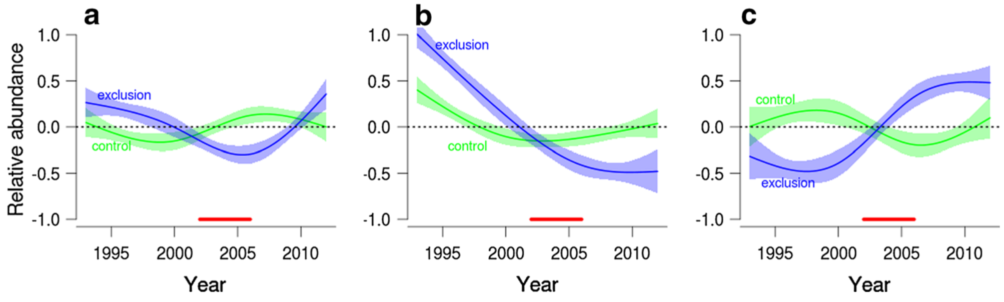

Fig. 4 Long-term dynamics of the most dominant species in response to herbivore exclusion. **a** Relative abundance of *Festuca nigrescens* at Bromion site. **b** Relative abundance of *F. nigrescens* at Nardion site. **c** Relative abundance of *Nardus stricta* at Nardion site. *Shaded areas* represent 95 % confidence intervals. Abundance values are re-scaled to 0 to ease visual comparison between sites and plots. *Red line* highlights an exceptionally warm and dry period detected between 2002 and 2006

species were less intense that in exclusion plots, and neither of these grasses showed substantial changes in abundance after 19-years.

Despite the permanent location of transects, our spatial sampling error rates were estimated as 0.64 and 0.33 at the Bromion and Nardion sites, respectively. For the sake of simplicity, and given the importance of the warm and dry period between 2002 and 2006 for community changes, transition probabilities were calculated separately for the warm period and the bracketing cooler time periods (1992–2001 and 2007–2012, respectively), and for each of the two treatments. The main difference in the transition probabilities of the two grasslands was related to grasses, whose probability to be replaced by forbs was higher than that of any other ecological groups in Bromion. In contrast, grasses were the most persistent group in Nardion. Regardless of the climatic period, we found more stable transitions (i.e. less transitions between groups) in presence of herbivores at both sites (Fig. 5). At the Bromion site, largest changes in transition probabilities in response to herbivore exclusion took place between 2002 and 2006, when the transition from the dominant grass and other grasses to forbs increased, as did the persistence of forbs relative to preceding time period. Conversely, there was a decrease in the transition from all groups towards *F. nigrescens* (Fig. 5). These changes were detectable at both control and exclusion plots, being higher in the latter case (Fig. 5). After 19-years of herbivore exclusion, however, only the persistence of forbs remained higher than initially seen. In Nardion, the clearest changes during the 2002–2006 period were the reduction of transition probabilities from all groups to the dominant grass, and the increasing persistence of grasses and forbs relative to that observed in previous time period. After 2006, the transition between ecological groups approached initial values in presence of herbivory (Fig. 5). Unlike in Bromion, the shifts in transition probabilities observed (except that between dominant and non-plants) during the warm and dry period continued afterwards in the exclusion plot (Fig. 5).

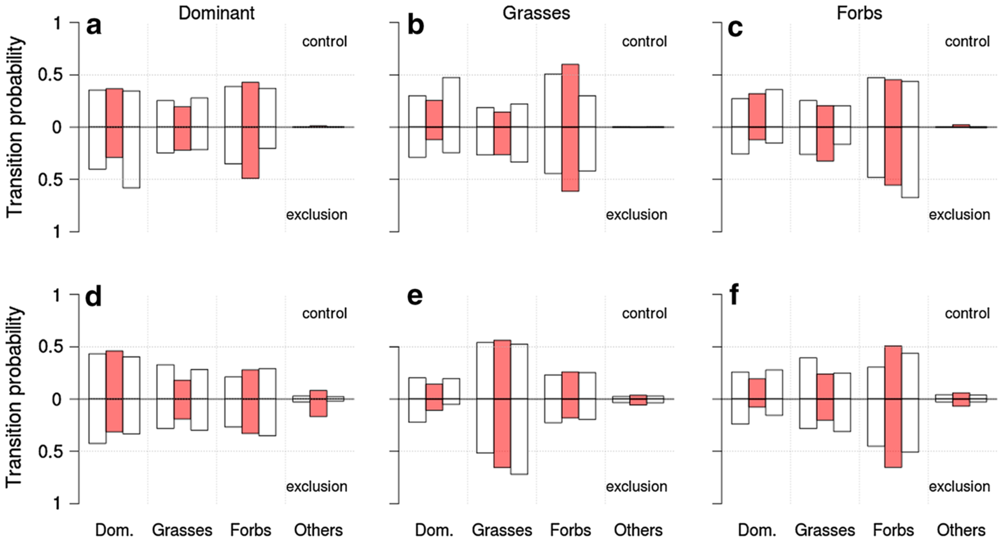

Fig. 5 Corrected mean transition probabilities between main ecological groups (dominant species, non-dominant grasses, forbs and other non-plants) in Bromion (**a**, **b**, **c**) and Nardion (**d**, **e**, **f**) grasslands. Each plot shows the averaged transition probabilities between a given group and the others for an exceptionally warm period (2002–2006; *bar* in *red*) and bracketing cooler time periods (1993–2000 and 2007–2012, respectively; *bars* in *white*)

Discussion

Contrary to expectations, we found that two decades of herbivore exclusion did not trigger large changes in community structure and diversity losses. Although the canopy height increased significantly within exclosures over 19 years as predicted by the GGM, this change was not drastic enough to modify competition for light and thereby, to suppress shade intolerant species. The lack of effect of herbivore exclusion on species richness may also be related to stronger effects of rates of local colonization and extinction on grassland structure (Glenn and Collins 1992; Olff and Ritchie 1998), or to the fact that herbivores were not selecting the most competitive species (Crawley 1983), and hence that their relative importance in altering interspecific competition was minor.

For productive subhumid grasslands, the GGM predicts rapid community shifts in response to changes in grazing regimes (Milchunas and Lauenroth 1993). However, we found that high-elevation grasslands responded slowly to the most extreme reduction of grazing possible. In fact, the response was mostly related to the shift in abundance of species, and marked divergences in community structure between control and exclusion plots did not happen before the ninth year of exclusion. This result is in disagreement with theoretical expectations, but also contrasts with previous findings from exclusion experiments in other mountain regions, where community changes (including those in composition) were stronger soon after excluding herbivores (Rawes 1981; Hill et al. 1992; Krahulec et al. 2001; Tasser and Tappeiner 2002; Mayer et al. 2009; Speed et al. 2013). Importantly, our results were irrespective to the dissimilarity measure used to characterize grasslands trajectories, and potential bias related to the detection of rare species.

Why was the community response to the exclusion of large herbivores so weak in this study? We speculate about four different possibilities, not mutually exclusive. First, the lack of response might be due to the absence of life forms such as dwarf-shrubs, which have been shown to fast modify community physiognomy and structure (e.g. De Bello et al. 2005; Olofsson et al. 2009). Second, the reduced growing period and predominance of long-lived species at high elevations might have slowed down the response of communities to herbivore exclusion (Tasser and Tappeiner 2002; Mayer et al. 2009). Third, given that the studied plots were surrounded by large grassland areas, immigration of species from lower elevations (including shrubs) might be reduced compared to grasslands close to the forest limit and/or shrublands (e.g. Speed et al. 2013). Likewise, the colonization of more competitive lowlands species (including shrubs) may also be limited in the absence of grazing (Austrheim and Eriksson 2003), because above ground biomass and species colonization and seedling emergence are negatively related (Eskelinen and Virtanen 2005). The fact that all the new species detected within the exclosure across the 19-years were also present in the surrounding grasslands support this explanation. Finally, continued intensive grazing throughout the last centuries might have depleted the pool of strictly non-tolerant species from the system, thus limiting the resilience mechanisms proposed by the GGM that allow for reversible floristic changes in response to grazing intensity (Cingolani et al. 2005).

A striking finding of this study was that hervibory played an important role in modulating the effect of climate in community structure and turnover between ecological groups. In both grasslands, plant assemblages become more vulnerable to a period of unusually warm and dry conditions in absence of herbivory. This was evidenced by higher changes in both the plant community structure and transition probabilities of ecological groups within exclosures between 2002 and 2006. The exceptionally warm and dry growing seasons affected negatively the dominant *Festuca nigrescens* in both sites, although it trigger different plant community responses. *Festuca nigrescens* was mostly replaced by forbs in Bromion, which explains the temporal shift in the community structure found in this grassland. Importantly, the initial community structure and group replacements were partially recovered once the exceptional climatic conditions reverted at this site, indicating a high resilience of this grasslands.

In Nardion site, *F. nigrescens* was mostly replaced by the *Nardus stricta,* which became the most dominant since the 2002. The replacement of one dominant species from another suggests a lower competitive release, and may explain why transition from grasses to forbs between 2002 and 2006 was lower than in Bromion. In this case the dominance of *N. stricta* continued after the end of the warm and dry event. This trend may constitute a potential risk from a management perspective, because *N. stricta* can spread in absence of herbivores up to form dense tussocks (Chadwick 1960; Hulme 1996; Tasser and Tappeiner 2002), which can be difficult to control even by restoring past grazing regimes (Rosef et al. 2004). As the low foraging value *N. stricta* spreads at the expense of more nutritive grasses and legumes, it can also result in an impoverishment of the quality of grassland for livestock production, and in some cases, in a diversity loss (Cernusca et al. 1996; Fillat et al. 2008). Our result do not corroborate such extremes, but they do warn about the potential influence of exceptional climatic conditions in favouring the spread of *N. stricta.*

As the community structure in all control plots was relatively stable across the 19-yr, we suggest that herbivory buffered changes of plant community in response to exceptional climatic events (Post and Pedersen 2008; Olofsson et al. 2009; Speed et al. 2012; Kaarlejärvi et al. 2013). A straightforward implication of this result is that the maintenance of past grazing stocking rates (or restoration in case of recent loss) may not only be valuable for the conservation of current biological diversity (Austrheim and Eriksson 2001), and productive communities (e.g. in the case of Nardion grasslands), but an efficient strategy to slow down some of the detrimental effects expected under ongoing climate warming (Theurillat and Guisan 2001).

Taken together, our results do not support the application of the GGM for guiding successful management of high elevation grasslands. As the GGM was mainly based on studies from low-elevation grasslands and shrubslands (Milchunas et al. 1988; Milchunas and Lauenroth 1993) it does not contemplate some particular aspects of high elevation grasslands (i.e. predominance of long-lived species, short growing periods) that may be critical in modulating the response of grasslands to grazing. At first glance, the weak long-term impact of the herbivory exclusion on grasslands may be perceived as good news for managers. However, caution is recommended when interpreting these results, because as we have shown, the relative slow response of plant communities to exclusion treatment would accelerate under climatic conditions, similar to those predicted for many European mountain (Engler et al. 2011).

Summarizing, we have shown that the long-term effect of herbivore exclusion on high elevation plant communities can be more moderate than expected, but also that they are modulated by the climatic conditions. Importantly, our results demonstrate that the strength of the response of grasslands to herbivory exclusion is likely to be enhanced under exceptional warm and dry periods, such as those expected under ongoing climate warming. Unfortunately, as in many other long-term experiments, the lack of replication limits our ability to generalize these results. However, we must be aware that the huge variability of grassland communities will make it difficult to establish accurate generalizations even if we had triplicated the number of grasslands. In absence of further detailed studies, our study provides novel insights into the long-term response of particular ecological communities under climate and land-use change. Grassland management should consider not only the direct effect of herbivore activity or climate warming, but also their combined effect across a variety of communities over ecological relevant times scales.

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