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Time lags in plant community assembly after forest encroachment into Mediterranean grasslands: drivers and mechanisms

Tesi doctoral

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per optar al grau de Doctor

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Certifiquen que:

Aquesta tesi duta a terme per Guillem Bagaria Morató al Departament de Biologia Animal, Biologia Vegetal i Ecologia i al Centre de Recerca Ecològica i Aplicacions Forestals, i titulada **Time lags in plant community assembly after forest encroachment into Mediterranean grasslands: drivers and mechanisms** ha estat realitzada sota la seva direcció.

Dr. Joan Pino Vilalta   **Dr. Ferran Rodà de Llanza**

Bellaterra (Cerdanyola del Vallès), març 2015

LO CEP

I

Al Cep, pare del vi,

li digué la pacífica Olivera:

—Acosta't a mon tronch,

de branca en branca enfila't, y barreja

als penjoys d'esmeragdes que jo duch

los teus rahims de perles—.

Y l'arbre de Noè

a l'arbre de la pau fa de contesta:

—Olivera que estàs prop de mi,

ni tu faràs oli, ni jo faré vi.

II

Ta brancada és gentil,

gentil y sempre verda,

mes, ay de mi! No em dexa veure el sol,

que ab sos raigs d'or més rossos m'enjoyella.

Nostres arrels se prenen la sahó

trobant-se furgadores dins la terra,

puix jo só vinater y tu oliera.

Olivera qui estàs prop de mi,

ni tu faràs oli, ni jo faré vi.

Jacint Verdaguer (Brins d'Espígol, 1902)

Agraïments

Una tesi és un projecte complex, gairebé tan complex com els mecanismes que intervenen en l'extinció i colonització d'espècies, i hi intervenen tants factors com espècies interaccionen en l'ensamblatge d'una comunitat. No és d'estranyar, doncs, que el camí sigui sovint complicat i inextricable, i que només amb l'ajuda de molta gent es pugui arribar a superar aquesta etapa, que és tota una experiència vital.

Primer de tot vull agrair a en Joan Pino i en Ferran Rodà l'oportunitat que em van donar d'involucrar-me en aquest projecte, fins i tot abans de llicenciar-me. He de dir que no ha estat complicat il·lusionar-se amb aquesta tesi amb ells com a directors. A més, el pragmatisme d'en Joan i la gran rigorositat d'en Ferran m'han ajudat molt en el desenvolupament de la tesi i en la meva formació com a ecòleg.

En aquesta tesi el treball de camp hi ha tingut un paper important, cosa que, com a naturalista, agraeixo. Part d'aquesta feina la vaig fer sol per zones de Prades on no hi passava ningú, però al Refugi de la Mussara sempre m'hi vaig trobar com a casa, gràcies a l'amabilitat de la gent que el porta. Tot i que la introspecció és agradable durant un temps, l'ajuda que em van donar en Moisès Guardiola, en Guillem Esparza i sobretot la Maria Clotet va fer el treball de camp més agradable.

Una de les tasques que m'ha portat més feina en la tesi, però de la qual també he après molt, és la determinació de plantes dels inventaris. Abans de començar la tesi coneixia ben poques de les més de 250 espècies amb què em vaig trobar en els tres mesos de mostreig la primavera del 2012 a Prades i, tot i anar assimilant-ne algunes, vaig generar una quantitat ingent de plecs d'herbari que no vaig poder acabar de determinar fins la primavera del 2014, tot i la inestimable ajuda sobretot d'en Moisès Guardiola, però també d'en Llorenç Sáez.

Un cop determinades les espècies, però, restava la tasca de fer les llistes de plantes especialistes, una feina complicada, com sempre que volem categoritzar la natura d'una manera artificial per tractar d'entendre-la millor, tot i que no sempre ens en sortim. En l'èxit d'aquesta llista vull destacar la contribució d'en Pep Ninot.

També vull remarcar la feina de la Paloma Vicente en la digitalització de l'ortofotomapa

de 1956 (té mèrit distingir-hi les diferents cobertes!) i la realització de punts d'entrenament per a la posterior reclassificació del mateix ortofotomapa.

La celeritat amb què he pogut tenir a punt l'últim capítol es deu en part a la Silvia Míguez, per la seva paciència en la minuciosa i acurada tasca d'avaluar la producció de llavors d'*Aphyllanthes monspeliensis* al laboratori. A ella també li vull agrair les idees que ha aportat a aquest capítol.

Per descomptat vull agrair a tota la gent del Jardí Botànic i del *Macroecology Workgroup* de la Universitat de Tartu (Estònia), i especialment a l'Aveliina Helm i en Meelis Pärtel, la seva gran acollida i l'ajuda en la tesi durant l'estada de poc més de tres mesos que hi vaig fer la primavera del 2013. M'emporto un bon record d'aquell petit país, amb una llengua, una cultura i un paisatge (molt pla) propis, que va lluitar per la seva independència i la va aconseguir. Gràcies especialment a en Rob, la Lena i la Liis, pels grans moments compartits (excursions, bicicletades, saunes, barbacoes, capbussades al riu, cerveses, i també discussions científiques). *Suur aitäh!* Crec que aquella estada va ser el punt d'inflexió per començar a veure la llum en la tesi.

D'altra banda, la resta de temps l'he passat al CREAF, on sempre m'hi he trobat com a casa. Tots els companys han contribuït a fer d'aquesta experiència professional també una gran experiència personal, amb el bon ambient que hi ha entre doctorands, postdocs i tècnics dins i fora del centre i que permet compartir coneixement, experiència, inquietuds, penúries i alegries. Per tots una forta abraçada!

Fora del CREAF, però, també he tingut recolzament i hi he trobat motivació per involucrar-me en altres projectes, tant naturalistes com castellers. Als amics de Ganàpies, de Nyerros i del Grup de Naturalistes d'Osona, gràcies pel suport i per deixar-me il·lusionar en el que he pogut contribuir.

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Moltes gràcies a tots!

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Table of contents

Resum

La pèrdua, fragmentació i transformació dels hàbitats representen una gran amenaça per a la biodiversitat en els ecosistemes terrestres d'arreu del món, desencadenant tant extincions com colonitzacions amb un resultat incert en la composició i la riquesa d'espècies. Els retards de diverses dècades, coneguts com a deute d'extinció i crèdit de colonització, són habituals després dels canvis en els usos del sòl. No obstant, hi ha una manca d'estudis que abordin l'ensamblatge de comunitats tenint en compte tant el deute d'extinció pels especialistes de l'hàbitat previ com el crèdit de colonització pels especialistes del nou hàbitat. L'objectiu d'aquesta tesi és, per tant, abordar les causes i els mecanismes dels efectes diferits del canvi en l'hàbitat i el paisatge sobre les comunitats vegetals en prats calcaris semi-naturals en ambients mediterranis des d'una aproximació inclusiva, tenint en compte tant els canvis en la riquesa com en la composició d'espècies i els seus retards potencials. El deute d'extinció i el crèdit de colonització han estat quantificats després de diverses dècades de canvis en l'hàbitat, i les seves causes han estat investigades (Capítol 1). Amb l'objectiu de desentrellar els processos que hi ha darrere els canvis de la comunitat, s'han investigat els patrons i causes de les extincions i colonitzacions d'espècies que ja s'han donat, a través dels dos components de la β-diversitat: recanvi d'espècies i diferències de riquesa (Capítol 2). D'altra banda, el paper dels atributs de les plantes com a mediadors de l'extinció d'espècies de l'hàbitat previ (Capítol 3), i els mecanismes poblacionals i individuals del retard en l'extinció d'un dels especialistes de prat més freqüents (*Aphyllanthes monspeliensis*; Capítol 4) han estat abordats després del canvi en l'hàbitat. Els estudis s'han dut a terme a les muntanyes del sud de Catalunya (nord-est de la Península Ibèrica), on la forestalització s'ha donat com a conseqüència de la reducció en la pastura durant la segona meitat del segle XX. Tot i que han passat més de 50 anys des de l'inici d'aquest procés de forestalització dels prats, s'ha detectat un important deute d'extinció per als especialistes de prat i crèdit de colonització per als especialistes de bosc. El deute d'extinció també ha estat confirmat per l'especialista de prat *A. monspeliensis*, ja que la seva abundància depèn de la connectivitat passada però no de l'actual. A més a més, un efecte rescat des dels prats circumdants i un lent decaïment vegetatiu i reproductiu després del deteriorament de l'hàbitat han estat identificats com a

mecanismes que afavoreixen el deute d'extinció. El crèdit de colonització de les plantes de bosc, al seu torn, probablement es manté per una limitació de la dispersió. Tot i que la riquesa ha canviat poc després de la forestalització, sí que s'ha donat un elevat recanvi d'espècies per al conjunt de la comunitat, fruit d'extincions i colonitzacions idiosincràtiques dels especialistes de prat i de bosc, respectivament. No obstant, mentre la fragmentació de l'hàbitat ha afectat negativament alguns especialistes de prat, no s'ha trobat un paper important dels atributs de les plantes en la mediació de les extincions per a aquest grup. S'espera que la pèrdua generalitzada d'espècies de prat i el guany generalitzat d'espècies de bosc, que resulten del pagament del deute d'extinció i el crèdit de colonització, continuïn a l'àrea d'estudi fins i tot en el cas que no avancés el procés de forestalització.

Abstract

Habitat loss, fragmentation and transformation are major threats for biodiversity in terrestrial ecosystems worldwide, driving both species extinctions and colonisations with an uncertain outcome on species composition and richness. Time lags of several decades, known as extinction debt and colonisation credit, often occur after land-use change events. Nevertheless, there is a lack of studies addressing community assembly taking into account both extinction debt for habitat specialists of the former habitat and colonisation credit for habitat specialists of the new habitat. The aim of this thesis is, therefore, to address the drivers and mechanisms of time-lagged effects of habitat and landscape change on plant communities in semi-natural Mediterranean calcareous grasslands from a comprehensive approach, taking both changes in species richness and composition and their potential time lags into account. Extinction debt and colonisation credit are quantified after several decades of habitat change, and their drivers are investigated (Chapter 1). In order to disentangle the processes behind community change, the patterns and drivers of species extinctions and colonisations that already occurred are investigated through the two components of β-diversity: species replacement and richness differences (Chapter 2). Moreover, the role of plant traits in mediating extinctions of species of the former habitat (Chapter 3), and the population and individual-level mechanisms of extinction delay for one of the most frequent grassland specialists (*Aphyllanthes monspeliensis*; Chapter 4) are assessed after habitat change. The studies were conducted in the southern mountains of Catalonia (northeastern Iberian Peninsula), where forest encroachment occurred following grazing reduction during the second half of the 20th century. Although more than 50 years elapsed since the beginning of forest encroachment into these grasslands, both an important extinction debt for grassland specialists and colonisation credit for forest specialists are detected. Extinction debt was also confirmed for the grassland specialist *A. monspeliensis*, since its abundance depends on historical but not current connectivity. In addition, a rescue effect from surrounding grasslands and a slow vegetative and reproductive decay after habitat deterioration were identified as mechanisms enhancing extinction debt. Colonisation credit of forest plants, in turn, was probably maintained by dispersal limitation. Although species richness changed little after

forest encroachment, high species replacement for the whole community occurred, resulting from idiosyncratic grassland specialists' extinctions and forest specialists' colonisations. However, while habitat fragmentation negatively affected some grassland specialists, no clear role of plant traits was found in mediating extinctions of this group. A generalised loss of grassland species and gain of forest species, resulting from the payment of extinction debt and colonisation credit, is expected to continue in the studied area even if no further forest encroachment occurs.

Introduction

General introduction

The anthropogenic global change, pushed by resource consumption, biotic exchange, nitrogen deposition, overexploitation and climate change, poses a major threat for biodiversity worldwide (Butchart *et al.*, 2010). Habitat loss, fragmentation and transformation are expected to be the most important impacts on terrestrial ecosystems (Sala *et al.*, 2000; Millennium Ecosystem Assessment, 2005), driving both species extinctions and colonisations with an uncertain outcome on species richness (McGill *et al.*, 2015). Small and isolated habitats usually result in the decline or local extinction of species, especially when the populations are small and the new habitat is not suitable for the establishment or growth of individuals (Maina & Howe, 2000). However, the formation of new habitat types creates opportunities for colonisation of new species that are able to reach the habitat, establish, grow and, eventually, persist as reproductive populations (Jackson & Sax, 2010).

Community assembly was initially conceived as the outcome of a complex set of abiotic and biotic filters that select species within certain trait combinations (Diamond, 1975; Weiher *et al.*, 1998). In contrast, Hubbell (2001) proposed the unified neutral theory of biodiversity and biogeography, which assumes that all species are equivalent. Later, it has been recognised that community assembly is driven by both niche-based and stochastic processes that act simultaneously (Leibold & McPeek, 2006), with a varying importance depending on each community. Thus, both environmental characteristics and stochasticity may contribute to shape communities over time.

Since processes involved in both species extinction and colonisation are numerous and sometimes they are slow or sequential, time lags of several decades or even centuries often occur after land-use change events (Vellend *et al.*, 2006; Pierik *et al.*, 2010; Cousins & Vanhoenacker, 2011), before the community reaches a new equilibrium or quasi-equilibrium in species composition (Jackson & Sax, 2010). Delayed extinctions and colonisations have been identified and even quantified for several groups of taxa in very different habitats (e.g. Helm *et al.*, 2006; Vellend *et al.*, 2006; Krauss *et al.*, 2010; Piqueray *et al.*, 2011; Hylander & Weibull, 2012; Lira *et al.*, 2012; but see Adriaens *et al.*, 2006; Cristofoli *et al.*, 2010), and they are known as extinction debt (Tilman *et al.*, 1994; Kuussaari *et al.*, 2009) and colonisation credit (Cristofoli *et al.*, 2010), respectively.

Although it is clear that extinctions and colonisations occur simultaneously after land-use change, there are no studies investigating the community assembly through both extinction debt for habitat specialists of the former habitat and colonisation credit for habitat specialists of the new habitat so far. In addition, the few studies accounting for both issues focused on

extinctions and colonisations of the same group of specialist species after habitat degradation and restoration, respectively (Hanski, 2000; Piqueray *et al.*, 2011; Hylander & Weibull, 2012; Lira *et al.*, 2012). Jackson & Sax (2010) stated that the rate of extinctions and colonisations after land-use change might differ, leading to transient richness situations of biodiversity surplus or deficit. Thus, beyond species richness, assessing both the magnitude and chronology of extinction debt and colonisation credit is key to understanding the balance of community richness over time following habitat change. At the same time, richness alone might not be a reliable indicator of habitat transformation impacts because it might remain constant after significant change in species composition, and this advocates for the need of quantifying turnover in community composition (McGill *et al.*, 2015). Moreover, little is known about the underlying processes leading to species extinctions and colonisations in this context. Environmental sorting or spatial and historical constraints would result in replacement of some species by others, while selective extinction/colonisation, habitat nestedness or interspecific variation in the breadth of environmental tolerance would lead to nested species loss or gain (Leprieur *et al.*, 2011). Hence, investigating the richness and β-diversity patterns resulting from these replacements and species gain or loss can contribute to disentangle the causes of biodiversity change in transient communities under the light of specific hypotheses (Baselga, 2010; Anderson *et al.*, 2011).

Colonisation lags can occur by the lack of propagule sources (Verheyen *et al.*, 2003b) or by habitat conditions that prevent establishment, survival or reproduction of individuals (Jacquemyn *et al.*, 2003; Vellend, 2005; Piqueray *et al.*, 2013). Regarding extinction debt, Hylander & Ehrlén (2013) stated that there are multiple mechanisms behind the delayed extinction of a species, acting at different levels: metapopulation, population and individual. Habitat loss and fragmentation reduce the size of habitat fragments, and increase the distance between them, leading to lower rescue effect of populations at extinction risk and lower recolonisation rates in unoccupied habitat patches (Hanski, 1999; Leibold *et al.*, 2004). However, if habitat loss and fragmentation are not much below the species extinction threshold, the metapopulation can persist for a long time before becoming extinct (Hanski & Ovaskainen, 2002). Moreover, the viability of small and isolated populations is also threatened by genetic drift, reduced pollination and environmental and demographic stochasticity that hamper survival and reproduction of individuals (Honnay *et al.*, 2005; Aguilar *et al.*, 2006). Finally, species persistence is also enhanced by biological characteristics that make individuals resistant to decreased habitat quality in particular life-cycle stages (Kuussaari *et al.*, 2009). Clonal plants, for instance, may persist slightly declining without recruitment for a long time, due to their extended life-span and long generation times (Eriksson, 1996; Young *et al.*, 1996; Lindborg, 2007; Saar *et al.*, 2012; but see Lindborg

et al., 2012). Other species persist in the seed bank and germinate after habitat improvement (Eriksson, 1996; Dupré & Ehrlén, 2002; Lindborg, 2007). Higher seed production increases the probability of finding suitable microsites and it might also promote species recolonisation (Dupré & Ehrlén, 2002; Saar *et al.*, 2012). However, the effect of dispersal mode appears to be variable and it might depend on landscape configuration (Dupré & Ehrlén, 2002; Saar *et al.*, 2012). Thus, both traits related with competitive ability and dispersal have been suggested to be important in determining species persistence (Jacquemyn *et al.*, 2003), and to mediate species responses to land-use change (Dupré & Ehrlén, 2002; Kahmen & Poschlod, 2004; Lindborg, 2007).

European semi-natural calcareous grasslands constitute a clear example of habitats that recently experienced an important decline and transformation. During the 20th century, an increase in arable fields and forests at the expense of semi-natural grasslands occurred throughout Europe, and this trend has been stronger in the last 70 years (Eriksson *et al.*, 2002; Adriaens *et al.*, 2006). In the Mediterranean basin, the depopulation of rural areas and the abandonment of traditional management practices that were no longer economically viable, resulted in forest encroachment into previously grazed open habitats. As a consequence, a decline of grassland specialists and a spread of forest specialists is expected (Debussche *et al.*, 1999). Although extinction debt has been widely studied in European semi-natural grasslands (e.g. Helm *et al.*, 2006; Adriaens *et al.*, 2006; Cousins, 2009; Krauss *et al.*, 2010; Guardiola *et al.*, 2013), its counterpart, colonisation credit, remains much more overlooked (but see Piqueray *et al.*, 2011). At the same time, the underlying causes and processes behind species extinctions and colonisations still need to be investigated.

Similarly, the mechanisms leading to extinction debts are still poorly known (Hylander & Ehrlén, 2013), while only metapopulation processes and the role of some plant traits have been addressed. Until now, most studies investigated extinction debt on the basis of island biogeography or metapopulation theories (Kuussaari *et al.*, 2009), and Hanski & Ovaskainen (2002) and Mouquet *et al.* (2011) advocate for the role of metacommunity dynamics and source-sink effects in extinction debt. Plant traits related to persistence (e.g. clonality) and dispersal (e.g. seed mass), have also been investigated in grasslands and forests after fragmentation with contrasting results (e.g. Lindborg, 2007; Saar *et al.*, 2012; Lindborg *et al.*, 2012). However, although some studies of plant fitness (e.g. Adriaens *et al.*, 2009; Takkis *et al.*, 2013) and stochastic population processes (e.g. Helm *et al.*, 2009) after habitat change have been conducted, individual and population-level mechanisms still remain poorly investigated.

In the present thesis I address the time-lagged effects of habitat and landscape change on plant communities from an integrated point of view. I investigate and quantify extinction

debt and colonisation credit, disentangle the processes behind community change, assess the role of plant traits and investigate population and individual-level mechanisms of delayed extinctions after forest encroachment, using several approaches. The studies are conducted in the southern mountains of Catalonia (northeastern Iberian Peninsula), mainly Prades Mountains, where an important grazing reduction and forest encroachment occurred during the second half of the 20th century (Guardiola *et al.*, 2013). The main aims of this thesis are:

1. To investigate the local and landscape drivers of plant community changes along forest encroachment in Mediterranean semi-natural calcareous grasslands.

2. To assess the mechanisms of time-lagged extinctions and colonisations of grassland and forest specialists, respectively, several decades after encroachment.

3. To explore the patterns and drivers of species extinctions and colonisations through the components of community change (species replacement and richness differences).

4. To assess the role of biological and ecological plant traits in mediating grassland specialists' responses to forest encroachment.

5. To evaluate vegetative and reproductive fitness of a grassland specialist after canopy closure and grassland isolation in order to assess population decay that might indicate an extinction debt.

Figure 1 summarises the multiple approach taken to study the time-lagged effects of habitat and landscape change on plant communities. At each of the study sites, 13 plant inventories were made and categorised as persistent grassland, wooded grassland or persistent forest, and the species richness (α-diversity) of grassland specialists and forest specialists in wooded grasslands was compared with persistent forests (outcome of the habitat change) to investigate the magnitude of extinction debt and colonisation credit, and their landscape and local-habitat drivers (Chapter 1). Total β-diversity and its species replacement and richness differences components were calculated between all sites along an habitat change gradient, for the total community, grassland specialists and forest specialists, in order to investigate the underlying processes leading to community change (Chapter 2). Moreover, for a subset of grassland specialists, the relationship between plant traits and climatic and landscape drivers potentially filtering them was investigated, in order to seek for plant characteristics that might mediate extinctions after land-use change (Chapter 3). Finally, plant fitness of 20 individuals of a grassland specialist (*Aphyllanthes monspeliensis* L.) were sampled at each site in order to assess the mechanisms causing an extinction debt following forest encroachment (Chapter 4).
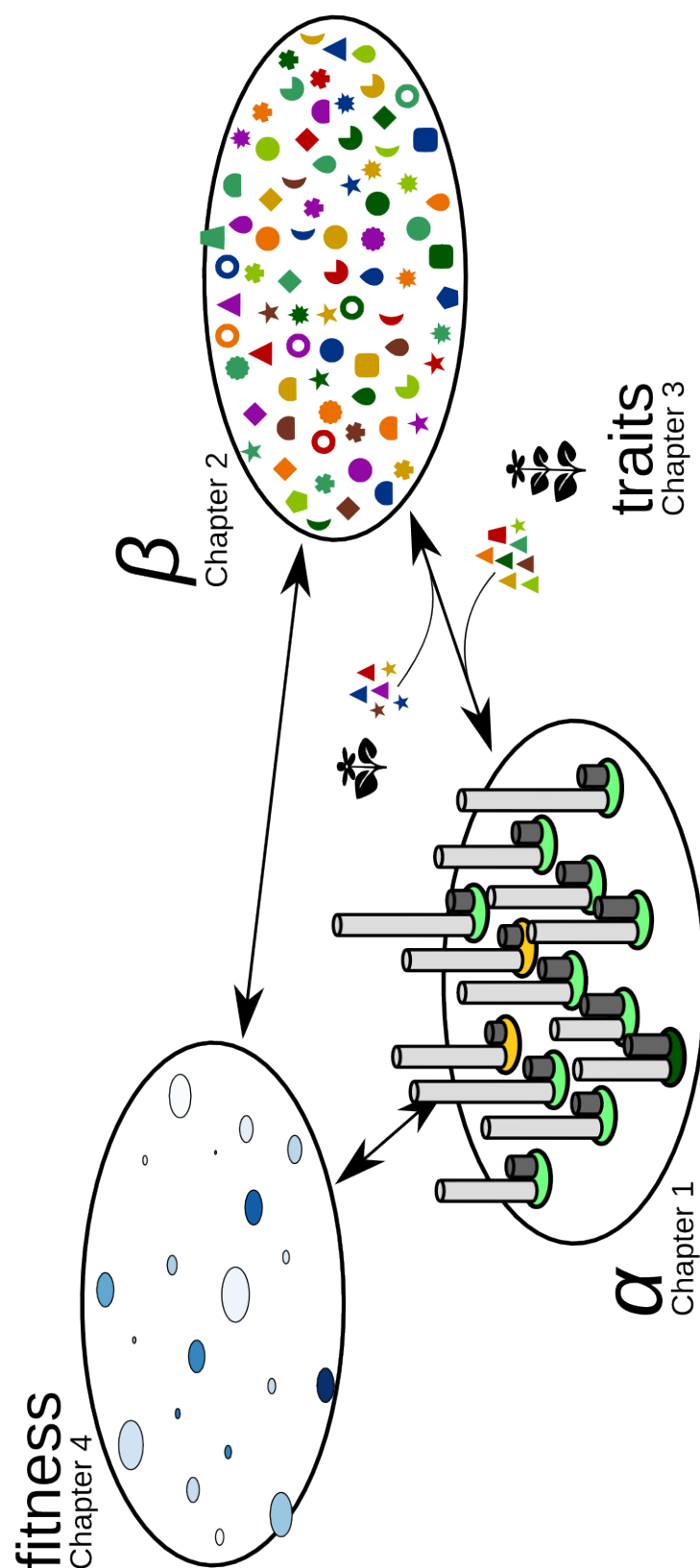
Fig. 1 Diagram of the four approaches taken to study the time lags in plant community assembly after habitat and landscape change (see text for more details). The diagram shows three study sites (large circles); the bottom one shows the 13 plots that were categorised as persistent grasslands (yellow circles), wooded grasslands (light-green circles) or persistent forests (dark-green circles), with their species richness of grassland specialists (light bars) and forest specialists (dark bars). The right large circle represents another of the study sites with its species composition at site scale (each small symbol corresponds to a species). The left large circle represents another of the study sites, showing 20 small circles that correspond to sampled individuals of a grassland specialist (*Aphyllanthes monspeliensis*). Circle size represents plant size, while colour intensity is related to flowering intensity, two of the fitness indicators that were investigated. The black pictures at each side of the right-bottom arrow represent plants differing in height, a trait that was investigated in environmental filtering of community composition.

Chapter 1

Assessing coexisting plant extinction debt and colonisation credit in a grassland-forest change gradient[1]

BAGARIA, G., HELM, A., RODÀ, F., PINO, J.

Abstract

Changes in species richness along ecological succession might be strongly determined by coexisting extinction debts of species from the original habitats and colonisation credits of those from the replacing habitats. The magnitude of these processes and their causes remain largely unknown. We explored the extinction debt and colonisation credit for grassland and forest specialist plants, respectively, and the local and landscape factors associated to richness of these species groups in a 50-year forest encroachment process into semi-natural Mediterranean grasslands. A set of sampling plots of persistent grasslands and forests and their transitional habitat (wooded grasslands) were selected within fixed-area sites distributed across the landscape. Results showed both extinction debt and colonisation credit in wooded grasslands, where a 53% decrease in grassland specialist richness and a 60% increase in forest specialist richness are expected comparing them with persistent forests, despite wooded grasslands and persistent forests having similar tree cover. Grassland connectivity and solar radiation oppositely affected richness of both grassland and forest specialists. Moreover, a rescue effect associated to grassland connectivity may enhance richness of grassland specialists in wooded grasslands. Thus, after more than 50 years of habitat change, extinction debt and colonisation credit still coexist, being contrastingly affected by local and landscape factors. These findings highlight the importance of documenting biodiversity time lags following habitat change when they are still in progress, in order to timely and adequately manage habitats of high conservation value as the studied grasslands.

1.1   Introduction

Land-use dynamics is driving the change of biodiversity patterns for many taxonomic groups all over the world, resulting in either species losses or gains (Baan *et al.*, 2012; Fahrig, 2003). Species extinctions and colonisations in dynamic landscapes and habitats often occur with time lags (Jackson & Sax, 2010). Both extinction debt (Kuussaari *et al.*, 2009; Tilman *et al.*, 1994) and colonisation credit (Cristofoli *et al.*, 2010; Jackson & Sax, 2010) have been identified following the changes in habitat quality, amount or connectivity. The relative rate at which extinctions and colonisations occur determines the existence of transient richness surpluses or deficits during the relaxation time (Jackson & Sax, 2010). The extinction of remnant populations that persist without completion of the whole life cycle (Eriksson, 1996) can be delayed by rescue effect from close populations or by biological characteristics, such as clonal growth or long life-span, which make individuals resistant to decreased habitat

quality in particular life-cycle stages (Kuussaari *et al.*, 2009; Hylander & Ehrlén, 2013; but see Lindborg *et al.*, 2012). Regarding colonisation, lags can occur by limited dispersal due to the lack of seed sources (Verheyen *et al.*, 2003b), or by suboptimal local conditions that prevent establishment, survival or reproduction (Jacquemyn *et al.*, 2003; Piqueray *et al.*, 2013; Sax & Brown, 2000; Vellend, 2005). Thus, both landscape and local environmental factors can determine the occurrence of time lags (Verheyen *et al.*, 2003a; Zulka *et al.*, 2014).

There is an extensive literature focusing on extinction debt following habitat loss, mainly for plants and butterflies in forests and semi-natural grasslands, and at different spatial and time scales (Cousins & Vanhoenacker, 2011; Guardiola *et al.*, 2013; Helm *et al.*, 2006; Krauss *et al.*, 2010; Vellend *et al.*, 2006), but the complementary topic of colonisation credit has received much less attention, and empirical evidence is relatively scarce (Pierik *et al.*, 2010; Piqueray *et al.*, 2011; Hylander & Weibull, 2012; Lira *et al.*, 2012). Furthermore, the few studies that have investigated the simultaneous occurrence of an extinction debt and a colonisation credit have focused on the dynamics within one habitat type and species group, i.e. the extinctions and colonisations of the same group of specialist species following either habitat degradation or restoration (Hanski, 2000; Hylander & Weibull, 2012; Lira *et al.*, 2012; Piqueray *et al.*, 2011).

European semi-natural grasslands have experienced a severe decline in habitat area due to the cessation of traditional management practices and expansion of arable fields or forests. This land-use change started more than a century ago, but it has become more accentuated over the past 70 years (Adriaens *et al.*, 2006; Eriksson *et al.*, 2002). The increase in forest cover following the depopulation of rural areas was widespread in the Mediterranean Basin during the 20th century, and increased occurrence of forest species and decline of grassland species are expected (Debussche *et al.*, 1999). Compared to the amount of studies from temperate European grasslands, the land-use changes in Mediterranean grasslands have received much less attention. However, due to their high biodiversity and conservational importance, time-lagged effects of habitat change on biodiversity need to be documented and quantified (Guardiola *et al.*, 2013). We focus on investigating the time lags in extinction of grassland specialists and in colonisation of forest specialists following forest encroachment into previously open grasslands. During forest encroachment, plant richness is expected to decrease because Mediterranean grasslands usually harbour more species than the resulting forests (Díaz-Villa *et al.*, 2003). However, it is not known to what extent recently encroached grasslands exhibit extinction debt for grassland species and colonisation credit for forest species, making the application of effective conservation measures more complicated.

In this study we aim to fill the gap in the current knowledge about the simultaneous processes of extinction debt and colonisation credit for habitat specialists of the original and

the replacing habitat type, respectively, after several decades of habitat change. We investigate the richness balance (i.e. the balance between extinctions and colonisations) for vascular plants and the drivers behind delayed species responses along the forest encroachment gradient of Mediterranean semi-natural grasslands, comparing transition (wooded) grasslands with persistent grasslands and forests (space-for-time substitution). As forest encroachment into Mediterranean landscapes leads to a continuous landscape gradient rather than to a patchy landscape with sharp habitat boundaries (Bagaria *et al.*, 2012), we used a sampling design that fits in with the habitat variegation concept (McIntyre & Barrett, 1992), considering gradients of target habitat transformation and suitability. Our main objectives were i) to investigate the magnitude of the extinction debt and colonisation credit for grassland specialists and forest specialists, respectively, following a forest encroachment process, and ii) to determine the local and landscape factors involved in extinction of grassland specialist species and colonisation of forest specialist species during forest encroachment.

1.2   Methods

Study system

The study was conducted on the calcareous plateau of Prades Mountains, a relatively small massif (20 × 16 km) in southern Catalonia, NE Iberian Peninsula (41°14′ – 41°23′N, 0°56′ – 1°10′E). Our focal habitat was Mediterranean semi-natural calcareous grasslands, which show a vegetation cover between 50% and 80% and consist mainly of hemicryptophytes and chamaephytes of <50 cm in height (Guardiola *et al.*, 2013).Historically, the study area was subjected to widespread sheep and goat grazing and the landscape was dominated by semi-natural grasslands with small forest patches at the beginning of the 20th century. The forest-grassland mosaic was relatively stable until the 1940s, when it is assumed that grasslands underwent a generalised decline due to great reduction or cessation of livestock grazing and forest encroachment (Guardiola *et al.*, 2013), resulting in open forests dominated by Scots pine (*Pinus sylvestris* L.) and holm oak (*Quercus ilex* L.) in variable proportions. The study area can be considered relatively homogeneous regarding topographic, geologic and land-use characteristics, and current grazing is sporadic and limited to the lowest areas. Semi-natural calcareous grasslands of Prades are located between 800 and 1200 m a.s.l. and show mean annual temperature around 11 °C, mean annual precipitation around 750 mm, and marked summer drought (Digital Climatic Atlas of Catalonia; www.opengis.uab.cat/acdc).

Site selection and sampling design

In order to study extinction debt and colonisation credit patterns and their associated drivers, plant richness in persistent (grasslands and forests) and in transitional wooded grasslands (i.e. previous grasslands that underwent an important woody encroachment process) was compared for different species groups, and its relationship with local environmental factors such as soil characteristics, solar radiation and grazing, and historical and current landscape was investigated. The continuous habitat transformation in our Mediterranean study system does not match the delimitation of distinctive habitat patches and, hence, a landscape sampling approach, consisting in a regular sampling within fixed-area sites, was used and provides a unique opportunity to study the effect of continuous habitat change on species extinctions and colonisations. We selected 20 sites corresponding to the maximum available landscape diversity in terms of forest and grassland cover, each with a diameter of 200 m. Site selection was performed on the basis of historical (1956) and recent (2009) orthophotomaps using a Geographical Information System (GIS) and a final selection based on preliminary fieldwork, seeking for a wide range in historical and current forest cover. The selected sites fulfilled the following requirements: (1) wide range in historical (19–96%) and current (16–70%) grassland proportion; (2) were naturally reforested; (3) had no crop fields in the past nor nowadays; (4) were not burned at least during the last 25 years, and (5) were at least 1-km apart from each other to avoid landscape overlap and increase data independence. Historical aerial photographs were obtained from Spanish Army photos with an original scale of ca. 1:30 000, and pixel size of the resulting 1-band orthophotomaps was 1 m. The present-day (2009) 3-band orthophotomaps were produced by the Cartographic Institute of Catalonia (www.icc.cat), with a scale of 1:5000 and a pixel size of 0.5 m.

Recording species occurrences

At each of the 20 selected sites, 13 circular plots of 3 m of diameter were sampled in a regular grid in spring and early summer 2011, each separated 33 m from neighbour plots (Fig. 1.1). The small size of the plots was chosen according to the fine-scale habitat changes that result from the forest encroachment process. At each plot, all vascular plant species were recorded. Each of the species found during the survey was assigned to one of the following categories, using regional floras (Bolòs *et al.*, 2005; Rivas-Martínez *et al.*, 2001) and expert advice: (1) grassland specialists, i.e. plants growing mainly in calcareous grasslands and sparse shrublands; (2) forest specialists, i.e. plants growing mainly in forests, and (3) other, i.e. plants growing both in grasslands and forests (generalists) or mainly in other habitats

(e.g. rocks, ruderal habitats). A total of 251 taxa were identified; 139 of them were grassland specialists and 56 were forest specialists (see Table A.1).

Site variables

Habitat availabilities in the historical and current landscapes were obtained as surrogates for habitat connectivity and the abundance of source populations that may contribute to rescue remnant populations (for grassland specialists) or act as propagule sources for colonising species (for forest specialists). From both past (1956) and present (2009) orthophotomaps with a pixel size of 1 m, 300 training points randomly distributed on the orthophotomaps were manually assigned by visual photo-interpretation to one of the following categories: forest, grassland, bare soil, crop fields or urban. Then, forest and grassland records from the training points were used to reclassify each orthophotomap based on a pruned classification tree. The classification tree was conducted with *rpart* package (Therneau *et al.*, 2013) in R programming language (R Development Core Team, 2014), and the reclassification was performed using MIRAMON GIS (www.miramon.uab.cat). Crop fields, bare soil and urban areas obtained from the land-cover map of Catalonia of 2009 (www.creaf.uab.cat/mcsc) and from a land-cover map of the study area of 1956 were combined respectively with the reclassifications of 2009 and 1956 orthophotomaps, to obtain maps with fine-scale transitions from grassland to forest and well-delimited areas for other habitats. The combination of land-cover with reclassified maps was performed using the MIRAMON GIS. Finally, a specific landscape (500 m radius from site centre) variable for each species group was calculated for both past (1956) and present (2009) combined maps: grassland/forest ratio (for all species), grassland ratio (grassland to non-grassland ratio; habitat availability for grassland specialists) and forest ratio (forest to non-forest ratio; habitat availability for forest specialists). Since sets of proportions can lead to intrinsic correlation of components (Aitchison, 1982), they are more properly expressed in terms of log-ratios (Aitchison, 1982; Kühn *et al.*, 2006).

Plot variables

At each plot, soil variables, solar radiation availability and grazing presence–absence were obtained to study the effect of local habitat conditions, which might delay or accelerate extinction or colonisation, on species richness of the different species groups along the forest encroachment gradient. Soil depth was obtained by the mean of three measures made at each plot, and a single soil sample was taken up to a depth of 10 cm to analyse soil pH and texture (clay to sand plus silt ratio, hereafter clay ratio). Solar radiation availability was measured
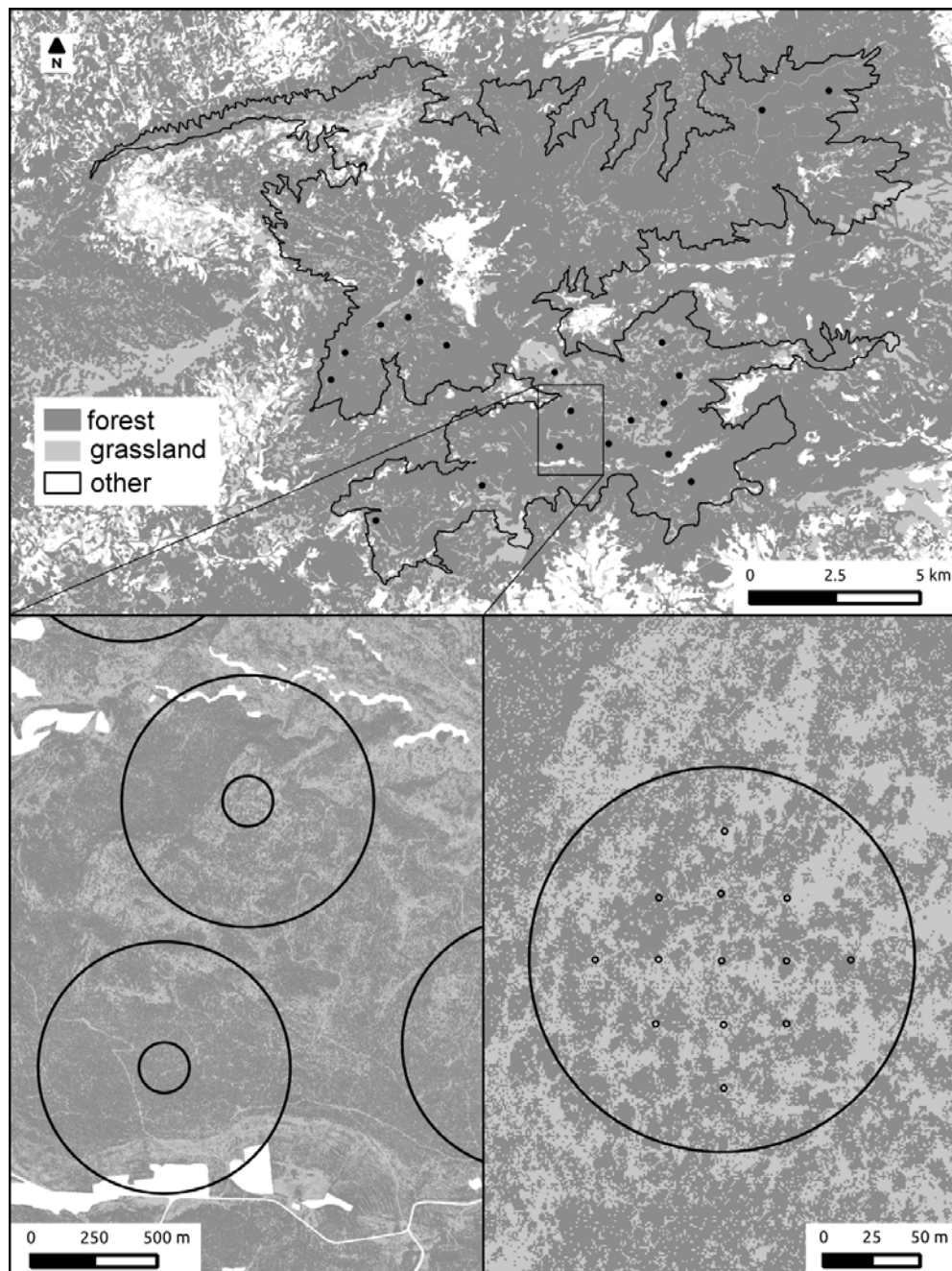
Fig. 1.1 Location of the study sites, fine-scale landscape maps and sampling method. The upper panel shows site distribution (black circles) in the Prades Mountains (800 m contour line) over the land-cover map of 2009; most of the area without sites (NW) is siliceous. The lower left panel is a close-up of two sites (small circles; 200 m of diameter) and surrounding landscapes (large circles; 500 m radius from the site centres) over the landscape combined map constructed by superposing reclassified and land-cover maps. The lower right panel shows the regular plot distribution (small circles) within a site (large circle), over the landscape combined map; plots measure 3 m of diameter and are separated 33 m from their neighbours.

using a ceptometer (AccuPAR LP-80; Decagon Devices, Inc.), and expressed as the ratio of the proportion of Photosynthetic Active Radiation (PAR) reaching understorey plants to the proportion absorbed or reflected by the tree cover. Grazing from livestock and/or roe deer was assessed at each plot as present or absent. In addition, historical and current tree cover proportion was obtained for each plot, in a radius of 5 m, from the combined maps (see Table A.2 for descriptive statistics of response variables and predictors).

Statistical analyses

In order to study extinction-colonisation dynamics and understand local habitat and landscape effects on species richness at plot scale, the 260 plots were categorised in three situations along the habitat change gradient: persistent grasslands (less than 50% tree cover in the past and nowadays; 83 plots), wooded grasslands (less than 50% tree cover in the past and more than 50% in the present; 148 plots)and persistent forests (more than 50% tree cover in the past and nowadays; 28 plots). Current tree cover in wooded grassland plots did not statistically differ from that in persistent forest plots ($P = 0.13$; tested using a general linear mixed model relating current tree cover with plot category, site as random factor, and a post-hoc Tukey test). One plot was deleted because it had more than 50% tree cover in the past and less than 50% in the present and it did not fit in any of the former categories. The continuous landscape gradient formed after forest encroachment results in the coexistence of persistent grasslands, wooded grasslands and persistent forests within sites(see Fig. 1.2).

For the purpose of investigating the plant extinction-colonisation balance after the forest encroachment process and testing for the existence of an extinction debt for grassland specialists and a colonisation credit for forest specialists, a general linear mixed model (LMM) for each species group (all species, grassland specialists, forest specialists and others) was conducted, with species richness as the response variable, site as random factor and plot category as the only fixed factor. Then, in order to test for the differences in richness between plot categories, a post-hoc Tukey test was performed for each model. General linear mixed models were conducted using *nlme* R package (Pinheiro *et al.*, 2014), and the post-hoc Tukey tests were performed using package *multcomp* in R (Hothorn *et al.*, 2008). A Moran's I test was conducted on each model to test for spatial autocorrelation in the residuals.

Finally, another set of general linear mixed models (LMMs) was constructed for all species (total richness) and for each of the specialist species groups to investigate the local and landscape drivers of species extinctions and colonisations along the forest encroachment process. Models included the relationship between species richness (response variable), site (random factor), and local and landscape variables in interaction with plot category (fixed
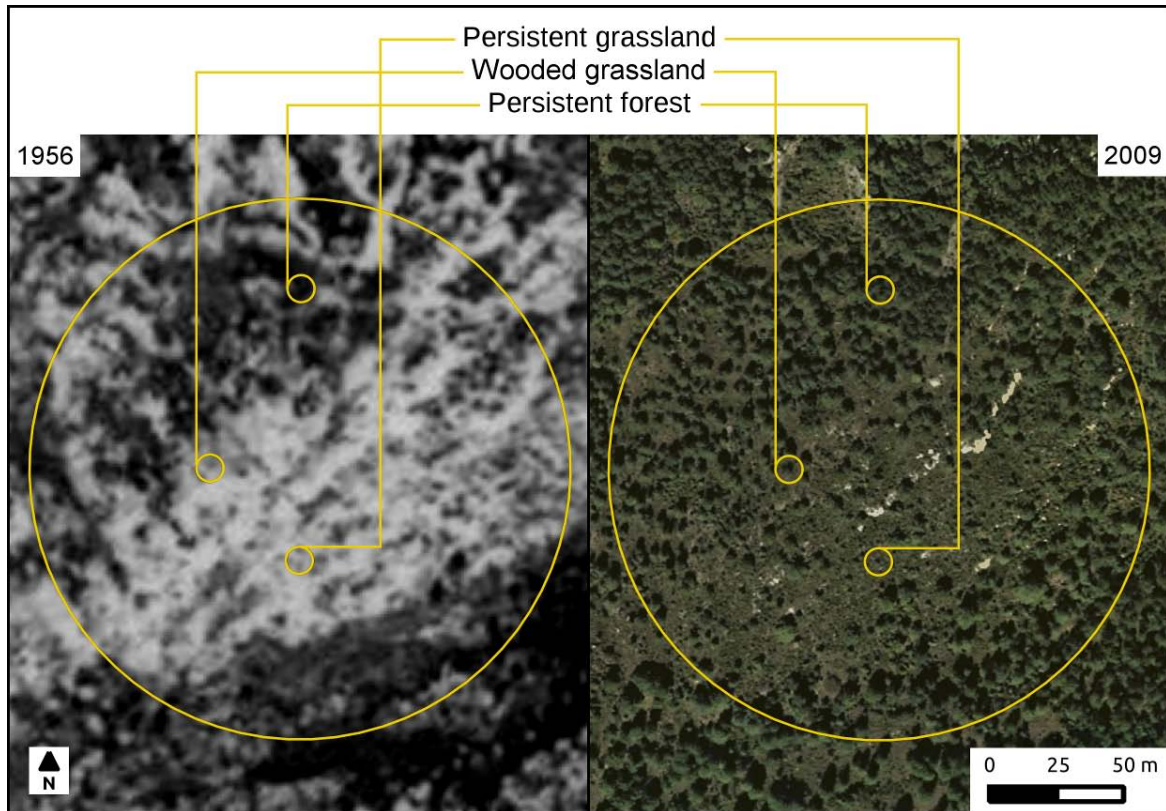
Fig. 1.2 Historical and current orthophotomaps from a study site (large circle), where the continuous landscape gradient can be observed. The left panel shows historical landscape and three of the 13 circles of 5-m radius at which plot tree cover was calculated (small circles), representing three different plot categories (persistent grasslands, wooded grasslands and persistent forests). The right panel shows the same circles over the current landscape, where different patterns of habitat change can be appreciated: few change from previous grasslands (persistent grasslands), woody encroachment into previous grasslands (wooded grasslands), and few change from previously wooded plots (persistent forests).

effects). Variables expressed as a ratio were log-transformed to improve their normality. A model selection using *dredge* function in the *MuMIn* R package (Bartoń, 2014) was conducted on each of the previous saturated models, fitted by maximum likelihood (ML), as comparisons between models fitted by restricted maximum likelihood (REML) that vary in their fixed effects are not valid (Weiss, 2005). The resulting models were ranked by AICc and the one with all parameters significant at $P = 0.05$ and lowest AICc was chosen and fitted by restricted maximum likelihood (REML). When interactions between plot category and a covariable were present, a post-hoc Tukey test was conducted in order to investigate significantly different covariable effects for different plot categories. In addition, spatial autocorrelation in the residuals of the three models was checked using Moran's I tests.

1.3   Results

We found no significant differences in total plant richness between any pair of plot categories (persistent grasslands, wooded grasslands and persistent forests), using a general linear mixed model with site as random factor, and a post-hoc Tukey test (Table 1.1; Fig. A.1). However, a marginally significant ($P = 0.056$; Table 1.1) decrease of 27% (8.6 species) in total species richness from persistent grassland to persistent forest plots was found (see Fig. 1.3).

As expected, the highest species richness of grassland specialists was found in persistent grassland plots (26.6 species), it was intermediate in wooded grasslands (18.3 species), and lowest in persistent forests (8.5 species; see Fig. 1.3). Using persistent forest plots as a reference, the extinction debt for grassland specialists in wooded grassland plots was 53.4% (9.8 species in average per plot).

Forest specialists showed the inverse pattern, having the highest richness in persistent forests (10.5 species) and the lowest in persistent grasslands (2.7 species), while also showing an intermediate richness in wooded grasslands (6.5 species). For wooded grasslands, the observed colonisation credit for forest specialists, taking persistent forest plots as a reference, was 60.3% (3.9 species in average per plot) of the forest specialist species currently present. Predicted values for each plot category are shown in Table A.3.

Additional linear mixed models were performed to identify the local and landscape variables that affect the species richness of all species, grassland specialists and forest specialists. According to the best model, total richness was determined by soil depth (negatively), solar radiation availability (positively), and interaction between plot category and current landscape (ratio of grassland/forest) and local predictors (soil clay content and soil pH) (Table 1.2, see also Fig. A.2 for effect plots). The model accounted for 53% of variation in total richness, according to the likelihood-ratio based pseudo-R^2 (Magee, 1990) Post-hoc Tukey tests revealed that grassland/forest ratio in the current landscape increased total richness in wooded grasslands significantly more than in persistent grassland or persistent forest plots (for the last two, the relationship was negative). Also, clay content had a higher positive effect in persistent grasslands than in persistent forests, and the positive effect of soil pH on total species richness was significantly higher in wooded grasslands than in persistent forests.

The best model for grassland specialist richness had a pseudo-R^2 of 0.73 and comprised solar radiation availability, soil pH (both having positive effects), interaction between plot category and grassland availability in the current landscape, and interaction between plot category and soil depth (Table 1.2). Grassland availability in the current landscape had

Table 1.1 Results of the post-hoc Tukey tests on the general linear mixed models (LMMs) comparing species richness between plot categories (PG, persistent grasslands; WG, wooded grasslands, and PF, persistent forests) for each species group. Estimates, standard errors (SE) and *P*-values are given for comparisons between all pairs of plot categories (columns). Each row corresponds to a model for a species group (all species, grassland specialists, forest specialists and other species). Significantly different comparisons are marked in bold, and likelihood-ratio based pseudo-R^2 for each model is given.

	Plot category (WG - PG)		Plot category (PF - PG)		Plot category (PF - WG)		R^2
	Estimate ± SE	*P*-value	Estimate ± SE	*P*-value	Estimate ± SE	*P*-value	
All species	–1.48 ± 1.13	0.376	–4.38 ± 1.92	0.056	–2.89 ± 1.74	0.211	0.31
Grassland specialists	**–3.26 ± 1.07**	**0.006**	**–8.68 ± 1.84**	**<0.001**	**–5.42 ± 1.66**	**0.003**	0.57
Forest specialists	**1.59 ± 0.35**	**<0.001**	**3.40 ± 0.60**	**<0.001**	**1.81 ± 0.54**	**0.002**	0.68
Other species	0.42 ± 0.22	0.133	**1.33 ± 0.37**	**0.001**	**0.91 ± 0.34**	**0.018**	0.31

significantly higher positive effect in wooded grasslands than in persistent forests. Soil depth had significantly stronger negative effect in persistent grasslands than in wooded grasslands.

For forest specialists, the selected model on species richness was much simpler, and had a pseudo-R^2 of 0.70. It only included the factor plot category and the covariables historical landscape forest availability (positively correlated) and solar radiation availability (negatively correlated).

1.4   Discussion

In this work we focus on the simultaneous extinction-colonisation dynamics of two specialist species groups in a successional habitat transition. Our landscape sampling approach, based on regularly distributed plots within selected sites across the landscape, has proven able to identify time lags and their drivers in a variegated landscape with fine-scale habitat transitions resulting from the forest encroachment process. Thus, our study detects both extinction debt and colonisation credit for grassland and forest specialists, respectively, at plot scale (7 m^2) after several decades of forest encroachment into semi-natural Mediterranean grasslands. The study also finds a marginally significant decline of 27% in total species richness as the outcome of this habitat change. Still, the study shows that species richness of all groups is influenced by both landscape and local habitat characteristics, but the effect of these characteristics differs between species groups.
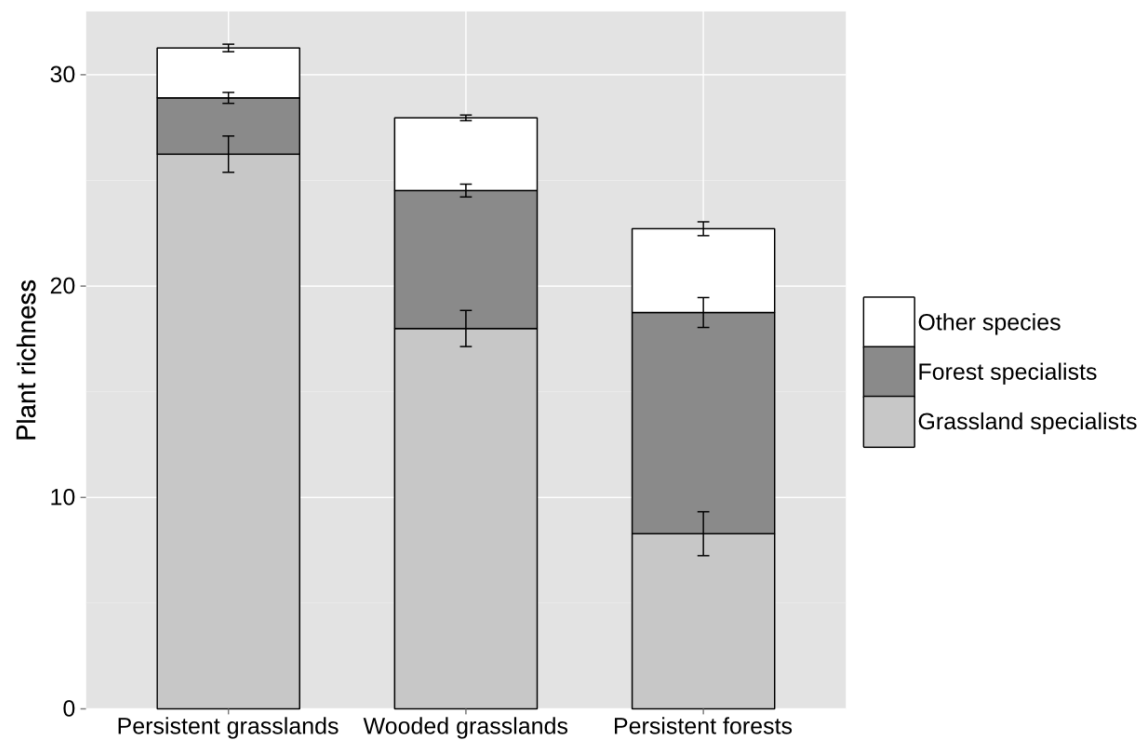
Fig. 1.3 Observed mean plant richness per sampling plot of different species groups (grassland specialists, forest specialists and other species) along the woody encroachment gradient (persistent grasslands, wooded grasslands and persistent forests categories). Error bars for each species group in each sampling plot category represent the standard error.

Table 1.2 Results of selected models for species richness in all species, grassland specialists and forest specialists. The model with all parameters significant and lowest AICc was selected and fitted by restricted maximum likelihood (REML). Significant predictors and interactions at $P < 0.05$ are marked in bold, and those that are significant only in an interaction term are also included as main effects. Estimates and P-values for all pair-wise comparisons between factor levels (for the factor plot category and interactions between it and a covariable) were obtained using post-hoc Tukey tests. PG, persistent grasslands; WG, wooded grasslands, and PF, persistent forests.

Predictor	All species	Grassland specialists	Forest specialists
	Estimate ± SE	Estimate ± SE	Estimate ± SE
(Intercept)	14.90 ± 10.71	6.41 ± 5.19	**7.53 ± 1.21**
Plot category (WG - PG)	−17.07 ± 11.73	−1.89 ± 2.14	**1.35 ± 0.35**
Plot category (PF - PG)	8.13 ± 16.89	**−14.07 ± 4.25**	**2.99 ± 0.60**
Plot category (PF - WG)	25.20 ± 14.45	**−12.18 ± 4.02**	**1.63 ± 0.53**
Historical landscape[a]			**2.26 ± 0.75**
Current landscape[b]	−7.27 ± 3.97	3.47 ± 4.99	
Solar radiation availability log-ratio	**1.60 ± 0.25**	**1.73 ± 0.22**	**−0.25 ± 0.08**
Clay log-ratio	**2.53 ± 0.89**		
Soil pH	2.47 ± 1.37	**3.06 ± 0.58**	
Soil depth	**−0.11 ± 0.04**	**−0.23 ± 0.07**	
Current landscape[b]*Plot category (WG - PG)	**10.49 ± 3.81**	6.14 ± 4.18	
Current landscape[b]*Plot category (PF - PG)	−3.19 ± 6.60	−5.65 ± 6.25	
Current landscape[b]*Plot category (PF - WG)	**−13.68 ± 5.70**	**−11.79 ± 4.99**	
Clay log-ratio*Plot category (WG - PG)	−2.38 ± 1.21		
Clay log-ratio*Plot category (PF - PG)	**−3.16 ± 1.31**		
Clay log-ratio*Plot category (PF - WG)	−0.78 ± 1.30		
Soil pH*Plot category (WG - PG)	2.49 ± 1.53		
Soil pH*Plot category (PF - PG)	−2.60 ± 2.20		
Soil pH*Plot category (PF - WG)	**−5.10 ± 1.91**		
Soil depth*Plot category (WG - PG)		**0.22 ± 0.08**	
Soil depth*Plot category (PF - PG)		0.20 ± 0.12	
Soil depth*Plot category (PF - WG)		−0.02 ± 0.10	
Likelihood-ratio based pseudo-R^2	0.53	0.73	0.70

[a]For all species model: grassland/forest log-ratio in historical landscape; for grassland specialists model: grassland/all other habitats log-ratio i.e. grassland availability in historical landscape; for forest specialists model: forest/all other habitats log-ratio i.e. forest availability in historical landscape.

[b]For all species model: grassland/forest log-ratio in current landscape; for grassland specialists model: grassland/all other habitats log-ratio i.e. grassland availability in current landscape; for forest specialists model: forest/all other habitats log-ratio i.e. forest availability in current landscape.

Patterns of richness change

As expected, species richness of grassland specialists is higher in persistent grasslands than in the other two studied habitats along the habitat change gradient. Beyond this trivial result, however, species richness of this group is significantly higher in wooded grasslands than in persistent forests, despite no significant differences were found in tree cover between these habitats. Moreover, 53% of grassland specialist species in wooded grasslands are expected to become extinct in the future, indicating the existence of a large extinction debt after forest encroachment, even though the process started more than 50 years ago. Extinction debt of grassland specialists has also been observed in grassland patches in the same region (Guardiola *et al.*, 2013) and in other calcareous grasslands (e.g. Helm *et al.*, 2006; Krauss *et al.*, 2010; Cousins & Vanhoenacker, 2011; but see Adriaens *et al.*, 2006). Vellend *et al.* (2006) and Cousins & Vanhoenacker (2011) found that extinction debt in plants might persist for more than a century following habitat fragmentation. Many grassland plants exhibit perennial life-cycles with long life-spans (Krauss *et al.*, 2010) and, consequently, long relaxation times after habitat change are expected (Kuussaari *et al.*, 2009) though other factors like rescue effect cannot be discarded. What is new in our study is the evidence that this extinction debt might persist for decades in the new wooded habitats resulting from grassland substitution.

Highest species richness of forest specialists is found, also as expected, in persistent forest plots. It should be noted, however, that wooded grasslands show significantly lower richness of forest specialists than persistent forests, and that a 60% increase in the species richness of this group is expected from future colonisation. This indicates the existence of a colonisation credit coexisting with the extinction debt of grassland specialists. Colonisation credits have not yet been widely studied, but they have been already identified in different habitats and for several taxonomic groups, such as plants (Pierik *et al.*, 2010; Piqueray *et al.*, 2011; Başnou *et al.*, in press), mosses (Hylander & Weibull, 2012), and birds and small mammals (Lira *et al.*, 2012). However, they have not been detected in some newly restored habitats like heathlands (Cristofoli *et al.*, 2010). Only few of these studies also investigated the coexistence of extinction debts and colonisation credits following habitat change, but they put the focus on a given habitat type instead of on the complete habitat series (Lira *et al.*, 2012; Piqueray *et al.*, 2011), or on the complete community instead of the specialist species groups (Hylander & Weibull, 2012). Our study extend these previous works through dealing with the whole habitat sequence along the successional transition from grasslands to forests, and doing so separately for the specialist species of each habitat.

Jackson & Sax (2010) indicated the importance of the magnitude and chronology of

extinction debt and colonisation credit on the community biodiversity balance over time. Although our study finds a marginally significant decrease in total species richness as the outcome of the process, it also suggests that extinctions and colonisations of plant specialists might have occurred simultaneously at a relatively similar rate during a 50-year period following forest encroachment, as total species richness did not significantly differ between wooded grasslands and neither persistent forest nor persistent grassland plots. However, it should be noted that the persistent forest plots used as a reference to calculate extinction debts and colonisation credits are not old-growth forests, and the fact that they were managed and cleared in the past may explain why a nonnegligible number of grassland specialist plant species are still present. Also, an unknown number of forest specialists might be absent from these historically managed forests (Grove & Rackham, 2001), thus limiting colonisation credit in wooded grasslands. If we considered that all grassland specialist species will ultimately go extinct and more forest specialists will still colonise persistent forests, the magnitude of both extinction debt and colonisation credit would be even greater.

Local and landscape drivers of richness change

Evaluating the separate response of each species group to the studied local habitat conditions and landscape factors can elucidate some of the richness patterns detailed above and the mechanisms involved in delayed extinctions and colonisations following forest encroachment.

For grassland specialists, high connectivity for the focal habitat in the landscape exerts a positive effect in wooded grasslands but has no relevant effect in persistent forests. Thus, grassland connectivity might help to maintain grassland specialist species in recently wooded plots thanks to a rescue effect, as supported by previous works (Hanski, 1999; Kuussaari *et al.*, 2009; Krauss *et al.*, 2010). In contrast, colonisation of grassland specialists from nearby populations in persistent forests might be constrained by habitat properties. Indeed, it is knownthat the establishment of grassland specialists is highly dependent on solar radiation (Öckinger *et al.*, 2006). Thus, habitat properties might offset the positive effect of grassland connectivity in persistent forests, leading to the progressive isolation and eventual extinction of the relict populations of grassland specialists.

Other local factors such as pH and soil depth also affect richness of grassland specialists. Positive association of pH with grassland species richness might be due to the fact that open habitats exhibit mildly alkaline soils in the study area, probably because of the negative association between pH and soil organic matter ($r = -0.55$), which mostly accumulates under tree canopies. In addition, soil depth shows a negative effect on the richness of grassland specialists in persistent grasslands, but it has no effect in wooded grasslands. This might be

due to a competition effect in suitable habitats, where the most competitive species could be able to exclude poor competitors when enough resources are available (deep soil), but in the suboptimal light conditions of wooded grasslands the less shade-tolerant species are probably already extinct, and competition effects may be strongly reduced (Grime, 2001).

For forest specialists, higher historical forest availability in the landscape and lower solar radiation availability determine higher species richness. The positive relationship with historical but not with current forest connectivity suggests that the colonisation credit previously mentioned in this group depends on the historical forest extent and it can persist for a very long time (Jacquemyn *et al.*, 2003; Vellend, 2003). Moreover, recently forested areas are likely to be less rich in forest specialist plants than historical forests (as observed in the studied wooded grasslands, see previous section) and, consequently, they might act as poorer seed sources, delaying the colonisation process. As expected, low solar radiation availability favours richness of forest specialists, as these plants thrive under tree canopies. There are no differences for the effect of predictors along the forest encroachment gradient, indicating that forest specialists respond to the same drivers in any transition point of the habitat change process.

Habitat condition and landscape factors have contrasting effects on total species richness, which depend on habitat type and are partially attributable to the effects on plant specialist groups reported above. Grassland connectivity increases richness in wooded grassland plots, probably due a rescue effect on declining grassland specialists previously suggested, but decreases it in persistent habitat plots. On the other hand, high forest connectivity in persistent grasslands can favour an increase in forest species. Also in persistent forests, higher forest connectivity may favour persistence of a higher number of forest species (Jacquemyn *et al.*, 2003; Verheyen *et al.*, 2003a). Regarding habitat characteristics, total species richness is positively related to solar radiation and a shallow soil along the entire gradient, and this can be explained by the higher contribution of grassland species compared to forest species to total richness in most plots. Moreover, open conditions and a shallow soil can increase the effect of recurrent droughts, leading to a reduction in the competitive effects of large and dominant plants and thus promoting species coexistence (Cousins, 2006; Grime, 2001). In addition, clay amount and soil pH show a positive effect on total richness in persistent grasslands or wooded grasslands, respectively, but their effect is negligible in persistent forests. The high cation exchange capacity of clay enhances soil fertility and water retention and might favour species richness in the driest places (Cornwell & Grubb, 2003). Still, high pH may favour persistence of grassland specialists, which are mainly calcicolous, especially in the transition habitat, where other environmental conditions are suboptimal.

Implications for management

Both extinction debts and colonisation credits constitute a great challenge for the conservation of biodiversity, but their integration has been rarely addressed, despite considerable literature on delayed extinctions and an increasing interest on delayed colonisations after the Jackson & Sax (2010) review. The simultaneous detection of these processes and the investigation of their potential correlates are needed to understand, assess, and mitigate biodiversity changes that might take place in the future and their effects. We have proven that both local and landscape drivers contribute to set species richness of the biodiversity groups involved in habitat change. Moreover, these drivers may strongly vary along the habitat change gradient, affecting to a different extent species persistence and, likely, dispersal at different stages of the process, and for different specialist species groups. Hence, considering variation in the drivers along habitat change gradients and across species groups is crucial to understand the overall process of extinction-colonisation following a forcing event.

Even though an important habitat change might have occurred, as long as the species predicted to become extinct still persist and those predicted to colonise have not arrived yet, there is time for conservation measures such as habitat restoration and landscape management. However, in order to conduct an effective restoration it is crucial to rely on information about the relation of extinction debt to habitat quality deterioration, connectivity loss, and small populations susceptibility to genetic deterioration or environmental and demographic stochasticity (Hylander & Ehrlén, 2013). This is especially important in habitats of high conservation value such as European semi-natural grasslands.

As the loss of semi-natural grasslands in favour of arable fields or forests has been a general trend during the last century in Europe (Adriaens *et al.*, 2006; Eriksson *et al.*, 2002), we still expect a general decline in grassland specialists' populations if no conservation measures are taken. Moreover, an increase in forest specialists is also expected in the following years or decades due to generalised forest encroachment (Debussche *et al.*, 1999). Therefore, extinctions and colonisations will continue to occur even if no more habitat loss takes place, causing a slow but steady biodiversity decline over several decades in these previously widespread semi-natural grasslands, while forest species become more and more common as extinction debt and colonisation credit are paid.

Chapter 2

Idiosyncratic extinctions and colonisations drive plant species replacement along forest encroachment into Mediterranean grasslands[1]

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[1] Submitted to *Oikos*

Abstract

Land-use change can cause both immediate and time-lagged increases and decreases in species richness. However, the associated patterns of species replacement remain largely unknown. We investigated β-diversity patterns for total plant species and for forest and grassland specialists along a forest encroachment gradient into semi-natural Mediterranean grasslands in Catalonia (NE Iberian Peninsula). We also investigated the landscape and geographic drivers of these patterns, and the existence of nestedness in species extinctions and colonisations. Total β-diversity and its components (species replacement and richness differences) were quantified for each species group along forest encroachment using general linear models, and considering the effect of landscape and geographic distance gradients. A nestedness analysis was used to test whether extinctions of grassland specialists and colonisations of forest specialists were either nested or idiosyncratic along forest encroachment. Following forest encroachment, a noticeable species replacement was found for the whole community, while richness differences were the main β-diversity component for habitat specialists. Landscape differences also contributed to explain compositional changes (both richness differences and replacement), while geographic distance contributed to a lesser extent to all species and forest specialists' replacement. For grassland and forest specialists, idiosyncratic rather than nested plant extinctions and colonisations were found. In conclusion, species replacement occurred along forest encroachment, triggered by grassland specialists' extinctions and forest specialists' colonisations. Nonetheless, the non-nested patterns of species loss and gain indicated that species extinctions and colonisations are not ordered following habitat change, although landscape characteristics and geographic distance may stand behind this pattern. From a methodological point of view, partitioning β-diversity into richness differences and replacement components and exploring the extinction and colonisation patterns of habitat specialists groups might provide relevant insights on the drivers and processes of community shift after land-use change.

2.1   Introduction

Land-use change is one of the main causes of biodiversity changes, driving extinctions and colonisations worldwide, which result in richness gains or losses, species replacement and biotic homogenisation or differentiation (Fahrig, 2003; Olden, 2006; Jackson & Sax, 2010; Baan *et al.*, 2012). Recently, there has been a wide interest in investigating both immediate and time-lagged effects of habitat loss and transformation with a focus on species richness,

while compositional changes remain much more overlooked (but see Larrea & Werner, 2010; Arroyo-Rodríguez *et al.*, 2013; Kopecký *et al.*, 2013; Trentanovi *et al.*, 2013; Lindborg *et al.*, 2014). Thus, biodiversity change and its time lags, namely extinction debts (Tilman *et al.*, 1994; Kuussaari *et al.*, 2009) and colonisation credits (Jackson & Sax, 2010; Cristofoli *et al.*, 2010) have been frequently identified following habitat change (but see Adriaens *et al.*, 2006; Cristofoli *et al.*, 2010). However, little is known about β-diversity patterns in these situations, largely ignoring whether extinctions and colonisations are idiosyncratic or dependent on the habitat change gradients as extinction debt and colonisation credit are paid. Since there are several changes of interest in transient communities, such as richness loss or gain and species replacement by other species, it is worth investigating them using different β-diversity measures driven by specific hypotheses (Anderson *et al.*, 2011). In the same vein, Baselga (2010) proposed additively partitioning total β-diversity into species replacement and species richness differences between nested sites, in order to account for the different processes leading to them: i) environmental sorting or spatial and historical constraints resulting in species replacement, and ii) selective extinction/colonisation, habitat nestedness or interspecific variation in the breadth of environmental tolerance, which result in nested species loss or gain (Leprieur *et al.*, 2011).

During the last century, there has been an important decline in European semi-natural grasslands, which have been either transformed into arable fields or experienced forest encroachment, and this trend has become more general over the past 70 years (Eriksson *et al.*, 2002; Adriaens *et al.*, 2006). The Mediterranean Basin experienced a generalised loss and fragmentation of its semi-natural grasslands, which are expected to result in a decline of grassland species and a spread of forest species (Debussche *et al.*, 1999). In fact, a partly paid extinction debt and colonisation credit and a tendency to total richness decrease have been recently found in northeastern Iberian Peninsula, after more than 50 years of forest encroachment into semi-natural grasslands (Chapter 1). Hence, changes in local-habitat conditions may promote richness gains and losses and species replacement, resulting in a decrease in compositional similarity between areas that underwent environmental differentiation, with this relationship being modified by landscape connectivity and by the distance decay of similarity (Nekola & White, 1999; Jacquemyn *et al.*, 2001a; Soininen *et al.*, 2007). However, little is known about the contribution of species replacement and richness differences to overall compositional change, their drivers and the chronology of extinctions and colonisations of particular species following habitat change. Species replacement due to environmental sorting has shown as a major process behind total community change along a gradient of environmental conditions (Leibold *et al.*, 2004). However, in Chapter 1 we pointed out strong richness gradients for habitat specialists. This suggests that richness

differences might be more important than replacement for explaining changes in specialist species composition, because selective extinctions or colonisations are likely to occur on the basis of the environmental tolerance of species (Kopecký *et al.*, 2013). Nevertheless, both historical and current landscape might modulate species extinctions and colonisations (Guardiola *et al.*, 2013).

Guardiola *et al.* (2013) suggested that grassland specialists' extinctions in these habitats were idiosyncratic following habitat loss, thus determining a poorly nested pattern for grassland specialists' composition along the habitat encroachment gradient. Forest specialists' colonisations, however, are expected to occur in a more ordered pattern, as they tend to be faster for very mobile and long-dispersed plants, while plants with large seeds, low fecundity and unassisted dispersal tend to be bad colonisers (Hermy *et al.*, 1999; Jacquemyn *et al.*, 2001b; Verheyen *et al.*, 2003b; Hermy & Verheyen, 2007). Nonetheless, forest connectivity may favour forest specialists' colonisation in certain areas, while distance decay may promote closer areas to share more species regardless of habitat differences. If habitat change strongly influences species composition, resulting in a highly nested pattern, a biotic homogenisation occurs (Kopecký *et al.*, 2013), but habitat change can also result in extinction and colonisation-driven biotic differentiation, through idiosyncratic species gains and losses, when fragmentation is high and seed dispersal is limited (Arroyo-Rodríguez *et al.*, 2013; Lindborg *et al.*, 2014).

In the present work, we investigated β-diversity patterns regarding species replacement and richness differences along habitat and landscape gradients in a forest encroachment process into Mediterranean semi-natural grasslands. Alongside, we studied grassland specialists' extinction and forest specialists' colonisation patterns following habitat change. We used a regular sampling approach at landscape scale, within constant-area sites selected across a grassland-forest gradient resulting from a forest encroachment process that started more than 50 years ago (Bagaria *et al.*, 2012). Our main aims were: i) to quantify species composition turnover and its components (species replacement and richness differences) along the habitat change gradient; ii) to assess its consistency between species groups (all species, grassland specialists and forest specialists); iii) to determine the landscape and geographic drivers modifying patterns of species replacement and richness differences along the habitat change gradient, and iv) to investigate whether species extinctions and colonisations following habitat change are idiosyncratic or nested.

2.2   Methods

Study area

The study system comprises Mediterranean semi-natural calcareous grasslands and the forests resulting from their encroachment in Prades Mountains in southern Catalonia (NE Iberian Peninsula; $41°14' - 41°23'$N, $0°56' - 1°10'$E). These grasslands are dominated by hemicryptophytes and chamaephytes up to 0.5 m high, and vegetation cover of 50% to 80% (see Guardiola *et al.*, 2013, for details). A forest-grassland mosaic persisted in the study area until the decade of 1940s, which was widely grazed by sheep and goats. During the second half of the 20th century, the semi-natural grasslands in the area experienced an important reduction due to livestock grazing reduction that led to forest encroachment (Guardiola *et al.*, 2013), resulting in relatively open, mixed forests of Scots pine (*Pinus sylvestris* L.) and holm oak (*Quercus ilex* L.). The study sites are located between 800 and 1200 m a.s.l. and have mean annual temperature around 11 °C, mean annual precipitation around 750 mm, and marked summer drought (Digital Climatic Atlas of Catalonia; www.opengis.uab.cat/acdc).

Site selection

With the aim of investigating β-diversity patterns shaped by species extinctions and colonisations and their local and landscape drivers along a forest encroachment gradient, we selected 18 sites, each with a diameter of 200 m, in a set of calcareous plateaus. A regular sampling approach was used within sites without distinguishing habitat patches, because habitat transitions in these Mediterranean systems show a high fuzziness, and the delimitation of habitat boundaries is not reliable (Bagaria *et al.*, 2012). Site selection was conducted using historical (1956) and recent (2009) orthophotomaps, according to the following criteria: (1) their historical (19–96%) and current (16–70%) grassland amount ranged considerably; (2) forest encroachment in these sites was natural (i.e. not due to forest plantation); (3) no crop fields existed in the past nor nowadays within them; (4) sites were not burned in the previous 25 years; (5) the distance between them was at least 1 km in order to avoid landscape overlap and increase data independence, and (6) they were located in a small area within the mountains (11 × 7 km) to prevent compositional variation due to a change in climatic conditions. After preliminary selection over orthophotomaps, fieldwork was used to discard unsuitable areas (see Chapter 1 for details). Two sites located in the northeastern part of the mountains and used in Chapter 1 were discarded for this study due to possible distance and climatic effects.

Sampling species composition

In spring and early summer 2011, all vascular plant species were recorded in 13 circular plots of 3 m of diameter at each of the 18 selected sites. Plots were arranged in a regular grid with a distance about 33 m between each other. Using regional floras (Rivas-Martínez *et al.*, 2001; Bolòs *et al.*, 2005) and expert advice, all species were classified as grassland specialists, i.e. plants growing mainly in calcareous grasslands and open shrublands; forest specialists, i.e. mainly growing in forests; and other. From 246 recorded taxa, there were 138 grassland specialists and 54 forest specialists (see Table B.1). In order to study β-diversity changes and colonisation and extinction patterns along the habitat change gradient, tables of species composition (presence–absence) at site level were obtained for each of the different plant groups (all species, grassland specialists and forest specialists) (see Table B.2 for the total composition table).

β-diversity measures

Compositional changes can be measured by tens of different β-diversity indices, and some of these indices produce very different results (Koleff *et al.*, 2003). The most widely used indices are measures of continuity ('broad-sense' measures, measures of total β-diversity), but indices specifically measuring either species replacement ('narrow-sense' measures) or richness differences are also available. A method to additively decompose total β-diversity into its components was proposed by Baselga (2010, 2012), who partitioned β-diversity into the replacement and richness differences components.

We calculated β-diversity measures for species presence–absence between all pairs of sites for the three different species groups (i.e. all species, grassland specialists, forest specialists) in order to investigate turnover in community composition (*sensu* Anderson *et al.*, 2011) along environmental and spatial gradients. Baselga (2010) used the term 'turnover' as a synonym of species replacement, but later the concept of turnover has been more widely used as compositional change along a spatial, temporal or environmental gradient (Anderson *et al.*, 2011; Legendre & De Cáceres, 2013). Total β-diversity and its two additive components (richness differences and species replacement) were calculated as proposed by Baselga (2010), using the function *designdist* in the *vegan* R package (Oksanen *et al.*, 2013). In this framework, for any pair of sites, total β-diversity (β_{sor}) is calculated by the Sørensen dissimilarity index:

$$\beta_{\text{sor}} = \frac{b+c}{2a+b+c} \tag{2.1}$$

The species replacement component of β-diversity (β_{sim}), in turn, is calculated by the

Simpson dissimilarity index:

$$\beta_{\text{sim}} = \frac{\min(b,c)}{a + \min(b,c)} \tag{2.2}$$

Finally, the nestedness-resultant dissimilarity (β_{sne}; a measure of richness differences between nested sites) is formulated as:

$$\beta_{\text{sne}} = \frac{\max(b,c) - \min(b,c)}{2a + b + c} \times \frac{a}{a + \min(b,c)} \tag{2.3}$$

where a, b and c are the shared species between both sites, species unique to the first site, and species unique to the second site, respectively (Krebs, 1999), and $\beta_{\text{sor}} = \beta_{\text{sim}} + \beta_{\text{sne}}$. Since β_{sne} is a measure of richness differences conditioned by the degree of nestedness between sites but not a nestedness measure itself (Baselga, 2012; Almeida-Neto *et al.*, 2012), an explicit nestedness analysis was conducted to investigate whether idiosyncratic or ordered extinctions and/or colonisations occur following forest encroachment.

Predictors of β-diversity

We obtained local-habitat variables that characterise the process of forest encroachment, which is expected to be the main driver of plant β-diversity changes through richness differences and species replacement. At each plot, soil variables, solar radiation and grazing intensity were measured *in situ*. Soil depth was calculated as the mean of three measures made at each plot and a soil sample up to a depth of 10 cm was taken to analyse soil pH, organic matter content and clay proportion. Solar radiation ($\text{MJm}^{-2}\text{day}^{-1}$) was calculated as the product between site spring solar radiation (obtained from the Digital Climatic Atlas of Catalonia; www.opengis.uab.cat/acdc) and the proportion of Photosynthetic Active Radiation (PAR) reaching understorey plants (measured using a ceptometer; AccuPAR LP-80; Decagon Devices, Inc.). Grazing intensity from livestock and/or wild ungulates (mostly roe deer) was measured at each plot in an ordinal way (0, no grazing signs; 1, one grazing sign; 2, more than one grazing sign). In order to reduce the number of local variables related to habitat to a single gradient, a principal components analysis (PCA) on the correlation matrix was conducted for soil depth, pH, organic matter proportion, clay proportion, solar radiation and grazing (taken as quantitative) at plot scale. The first axis of the PCA explained 37.4% of the total variance of the table (Fig 2.1), and it shows a gradient which was closely related (negatively) with forest encroachment, from forest areas in the negative side (high soil organic matter, clay texture and soil depth) to grassland areas in the positive side (high solar radiation, soil pH and grazing). The second axis explained 19.2% of the variance and

did not correspond to any clear gradient. Then, only the first axis was used in the statistical analysis as the habitat gradient resulting from forest encroachment.

In addition, geographic coordinates and habitat availability in both the historical and current landscapes were obtained for each site, as potential drivers of β-diversity gradients. Landscape measures were calculated at 500 m radius from each site centre, using maps produced by combining land-cover layers and reclassified orthophotomaps, for both historical (1956) and current (2009) contexts. These combined maps presented fine-scale transitions from grassland to forest (from reclassification of orthophotomaps) and well-defined crop fields, bare soil, and urban areas (from digitised land-cover maps) (see Chapter 1 for details). These landscape measures were species-group specific, i.e., grassland/forest ratio for all species, grassland ratio (grassland to non-grassland ratio) as habitat availability for grassland specialists, and forest ratio (forest to non-forest ratio) as habitat availability for forest specialists.

Statistical analyses

In order to quantify species composition turnover (*sensu* Anderson *et al.*, 2011) along forest encroachment and to determine the current landscape, historical landscape and geographic gradients modifying this relationship for its richness differences and replacement components, we related β-diversity from species presence–absence at site scale to Euclidean distances between sites in i) local characteristics (site centroids along axis 1 of the PCA); ii) landscape availability (log-transformed to improve normality), and iii) geographic coordinates.

For each of the plant groups (i.e. all species, grassland specialists, forest specialists), a general linear model was performed for total β-diversity (β_{sor}) and for each of its additive components (i.e. species richness differences [β_{sne}] and species replacement [β_{sim}]), relating compositional distances to distances in habitat, current landscape, historical landscape and geographical coordinates. The composition turnover rates along habitat (controlled by landscape and geographic confounding factors) were obtained for each model in order to compare the slope and strength of the relationship between species groups and β-diversity components.

Moreover, to investigate the relative contribution of habitat in front of other drivers affecting richness gradients or species replacement (i.e. historical landscape, current landscape and geographic distance), we obtained the variance explained by each variable and its standard error using *relaimpo* R package (Grömping, 2006) and *pmvd* metric in the models for the two different β-diversity components. Confidence intervals were calculated using 1000 bootstrap repetitions and the bias-corrected and accelerated method. The significance
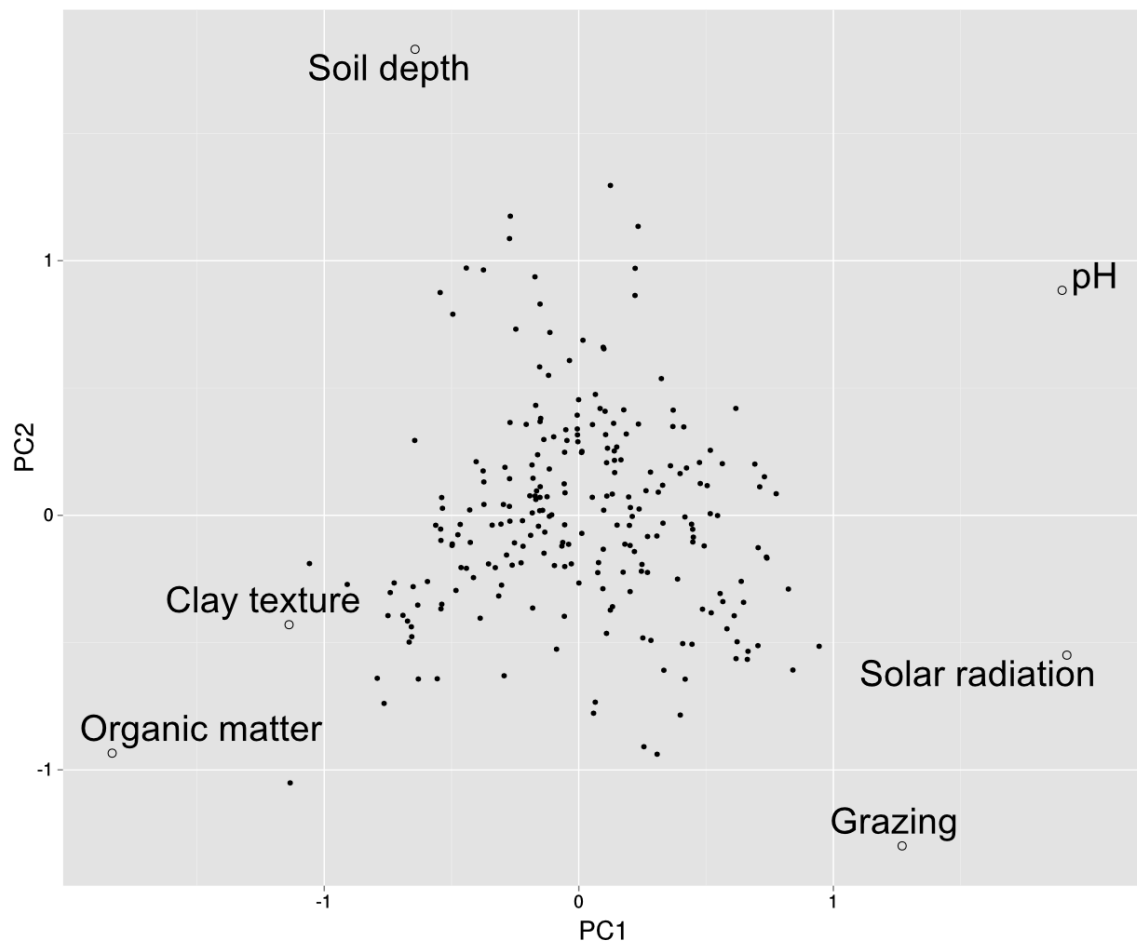
Fig. 2.1 Plot corresponding to the first and second axes from the PCA of habitat characteristics in the 234 sampled plots performed on the correlation matrix. Axis 1 explained 37.4% of the total variance and corresponds to a habitat gradient negatively related with forest encroachment, from forest in the negative side to grassland in the positive side. Filled circles show sampled plot positions, while open circles correspond to variables.

level for each predictor was corrected using error degrees of freedom equal to the number of sites (18), because of the non-independence of pair-wise site comparisons (Qian & Ricklefs, 2012).

Finally, a nestedness test was conducted to investigate extinction and colonisation patterns for, respectively, grassland specialists and forest specialists in two separate occurrence matrices. According to Ulrich *et al.* (2009), all the explanations for nestedness in species occurrence are variations of either ordered extinctions or colonisations along environmental and biological gradients (area, isolation, quality). Here sites were ordered following the habitat gradient, and species from higher to lower occurrence, in order to determine whether extinctions and colonisations were ordered following habitat change; i.e. whether forest sites were subsets of the progressively open ones for grassland specialists and grassland sites were subsets of progressively forested ones for forest specialists. NODF, a nestedness metric based on overlap and decreasing fill, and implemented in the *vegan* R package (Oksanen *et al.*, 2013), was used for this purpose. This metric allows validating hypotheses on the causes of nestedness by ordering columns or rows according to a coherent criterion (Almeida-Neto *et al.*, 2008), and ranges from 0 (no nestedness at all) to 100 (perfect nestedness). For each of the two matrices, 999 random null communities were constructed using the *quasiswap* method (fixed row and column marginal totals), which preserves type I error better than more liberal models (Ulrich *et al.*, 2009), and compared to the real ones in order to test for nestedness significance. All statistical analyses were conducted using R (R Development Core Team, 2014).

2.3 Results

Composition turnover along the habitat gradient

For all species, a noticeable turnover rate (slope of the regression of a β-diversity measure against the habitat gradient, controlled by landscape and geographic factors) was found for total β-diversity (estimate $\pm$ SE = 0.31 $\pm$ 0.04; r^2 = 0.28) and replacement (0.26 $\pm$ 0.04; r^2 = 0.20), but not for richness differences (0.05 $\pm$ 0.01; r^2 = 0.09). In the case of grassland specialists, the turnover rate for total β-diversity was similar to that of all species (0.35 $\pm$ 0.04; r^2 = 0.26), but it was mainly due to a species richness gradient (0.25 $\pm$ 0.04; r^2 = 0.23), rather than to species replacement (0.10 $\pm$ 0.04; r^2 = 0.03). For forest specialists, we found a very weak total β-diversity turnover rate along the habitat gradient (0.10 $\pm$ 0.05; r^2 = 0.03), but again the turnover rate of richness differences was stronger (0.15 $\pm$ 0.06; r^2 = 0.03) than that of species replacement (–0.05 $\pm$ 0.06; r^2 = 0), which was null (Fig. 2.2).

Total β-diversity (β_{sor}) turnover rate along habitat distances was similar for all species and grassland specialists ($P = 0.51$; t-test for the difference between slopes), but much lower for forest specialists than for all species ($P = 0.004$). The replacement component (β_{sim}) rate was much higher for all species than for both grassland ($P = 0.01$) and forest specialists ($P < 0.001$), and marginally significantly higher for grassland specialists than for forest specialists ($P = 0.051$), being null in the last (i.e. the slope was non-significant). Grassland specialists showed a much higher rate of the richness differences component (β_{sne}) than all species ($P < 0.001$), while forest specialists did not show significant differences with either grassland specialists ($P = 0.19$) or all species ($P = 0.11$) in this component of β-diversity.

Drivers of species richness differences and species replacement

For all species, the model for the β-diversity component summarising species richness differences between sites (β_{sne}) explained 17% of the variance, while habitat ($r^2 = 0.09$) and historical landscape ($r^2 = 0.06$) were positively correlated and explained a similar amount of variance. Both current landscape and geographic distance were not significantly associated with β_{sne} (Fig. 2.3a; Table 2.1). The model for all species replacement (β_{sim}) accounted for 45% of the variance. Habitat ($r^2 = 0.20$) and current landscape ($r^2 = 0.22$) explained the majority of this variance, while geographic distance ($r^2 = 0.03$) and historical landscape had almost no effect on species replacement, although geographic distance was significant (Fig. 2.3b). All the predictors were positively correlated with β_{sim}.

For grassland specialists, the model for the richness component of β-diversity (β_{sne}) between sites explained 41% of the variance, and showed that habitat ($r^2 = 0.23$), historical landscape ($r^2 = 0.11$) and current landscape ($r^2 = 0.06$) were the variables significantly and positively associated with richness differences for this species group. Geographic distance effect was not significant (Fig. 2.3c). Species replacement (β_{sim}) model for grassland specialists explained 29% of the variance, and included current landscape as the most important driver ($r^2 = 0.20$), followed by historical landscape ($r^2 = 0.05$) and habitat ($r^2 = 0.03$) (Fig. 2.3d). All these predictors but historical landscape dissimilarity were positively correlated with β_{sim}. In contrast, geographic distance was not significantly associated with β_{sim}.

For forest specialists, the model for the richness component of β-diversity (β_{sne}) explained 22% of the variance. Current landscape was the best predictor of this component ($r^2 = 0.18$), followed by habitat ($r^2 = 0.03$), and being both positively correlated with β_{sne}. In contrast, historical landscape and geographic distance were non-significantly associated with β_{sne} (Fig. 2.3e). The model for the species replacement component of β-diversity (β_{sim}) only
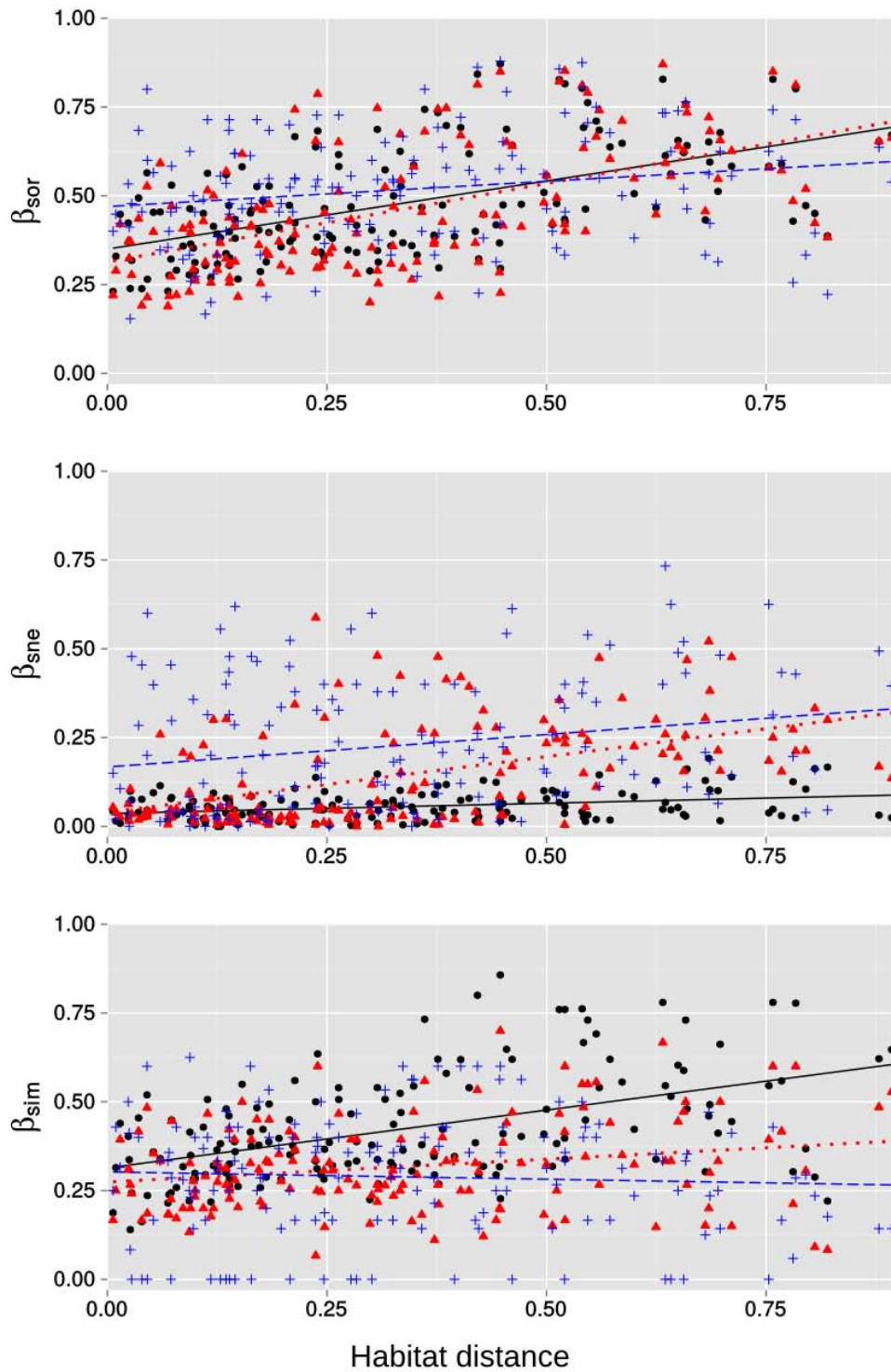
Fig. 2.2 β-diversity gradients along the habitat distance gradient for the different components (total β-diversity, β_{sor}; richness differences, β_{sne}; species replacement, β_{sim}) and different species groups: all species (dots, solid line), grassland specialists (triangles, dotted line) and forest specialists (crosses, dashed line). Each symbol is a pair of sites.

Table 2.1 Standardised coefficients and their standard error for each predictor in the linear model for each combination of species group (all species, grassland specialists, forest specialists) and β-diversity component (richness differences, β_{sne}; species replacement, β_{sim}) between pairs of sites. Significant predictors at $P < 0.05$ are marked in bold and total R^2 of each model is also given.

Species group	β-diversity index	Predictor				Total R^2
		Habitat	Current landscape	Historical landscape	Geographic distance	
All species	β_{sne}	**0.28 ± 0.08**	–0.01 ± 0.08	**0.21 ± 0.08**	0.13 ± 0.08	0.17
	β_{sim}	**0.39 ± 0.06**	**0.44 ± 0.06**	0.07 ± 0.06	**0.17 ± 0.06**	0.45
Grassland specialists	β_{sne}	**0.40 ± 0.07**	**0.22 ± 0.06**	**0.29 ± 0.06**	0.10 ± 0.06	0.41
	β_{sim}	**0.17 ± 0.07**	**0.44 ± 0.07**	**–0.25 ± 0.07**	0.09 ± 0.07	0.29
Forest specialists	β_{sne}	**0.19 ± 0.07**	**0.41 ± 0.07**	–0.07 ± 0.07	0.00 ± 0.07	0.22
	β_{sim}	–0.07 ± 0.08	–0.13 ± 0.08	0.08 ± 0.08	**0.21 ± 0.08**	0.08

explained 8% of the variance, and only geographic distance ($r^2 = 0.05$) was significant, being positively correlated with β_{sim} (Fig. 2.3f).

Nestedness analysis of extinction and colonisation patterns

The pattern of grassland specialists' extinctions and forest specialists' colonisations along the studied forest encroachment gradient was not significantly nested. The NODF metric for the composition of grassland specialists with sites ordered by the habitat gradient (Fig. 2.4) was 60.3, and the randomisation test failed to detect any significant nestedness ($P = 0.12$) along the forest encroachment gradient for these specialists. A similar pattern was found for the composition matrix of forest specialists along the habitat gradient (Fig. 2.4), whose NODF metric was 50.2. The randomisation test also failed to detect any significant nestedness along forest encroachment for forest specialists ($P = 0.91$).

2.4 Discussion

Our study has explored changes in the β-diversity turnover rate and its components (i.e. species richness differences and species replacement) across forest encroachment in a set of localities with a fixed number of plots in Mediterranean grasslands, and it shows that turnover rates are stronger for all species and for grassland specialists than for forest specialists in these localities. In addition, the study finds high replacement and few richness differences for all species, while species richness change was detected to be the main component behind composition turnover for habitat specialists along forest encroachment. Habitat and landscape properties are the primary drivers of β-diversity for both species replacement and richness
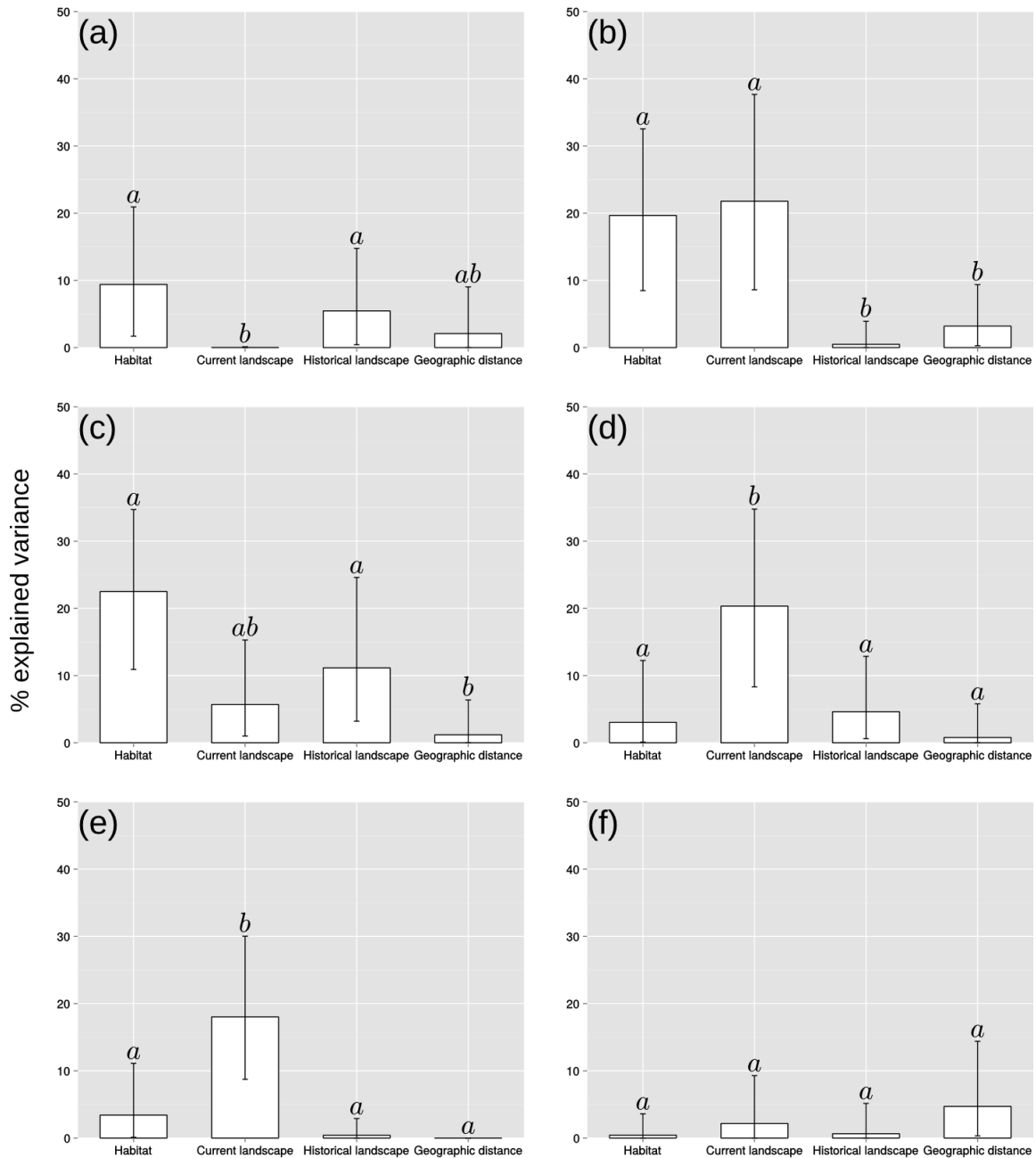
Fig. 2.3 Results of the variance partitioning on the models of species turnover for each species group and β-diversity component. Error bars depict 95% confidence intervals. Plot (a) shows results for all species and richness component (β_{sne}), plot (b) for all species and replacement component (β_{sim}), plot (c) for grassland specialists and richness component (β_{sne}), plot (d) for grassland specialists and replacement component (β_{sim}), plot (e) for forest specialists and richness component (β_{sne}), and plot (f) for forest specialists and replacement component (β_{sim}). Predictors that do not share a letter within a plot explain significantly different amount of variance, calculated from bootstrapping differences in the relative contribution between pairs of variables.
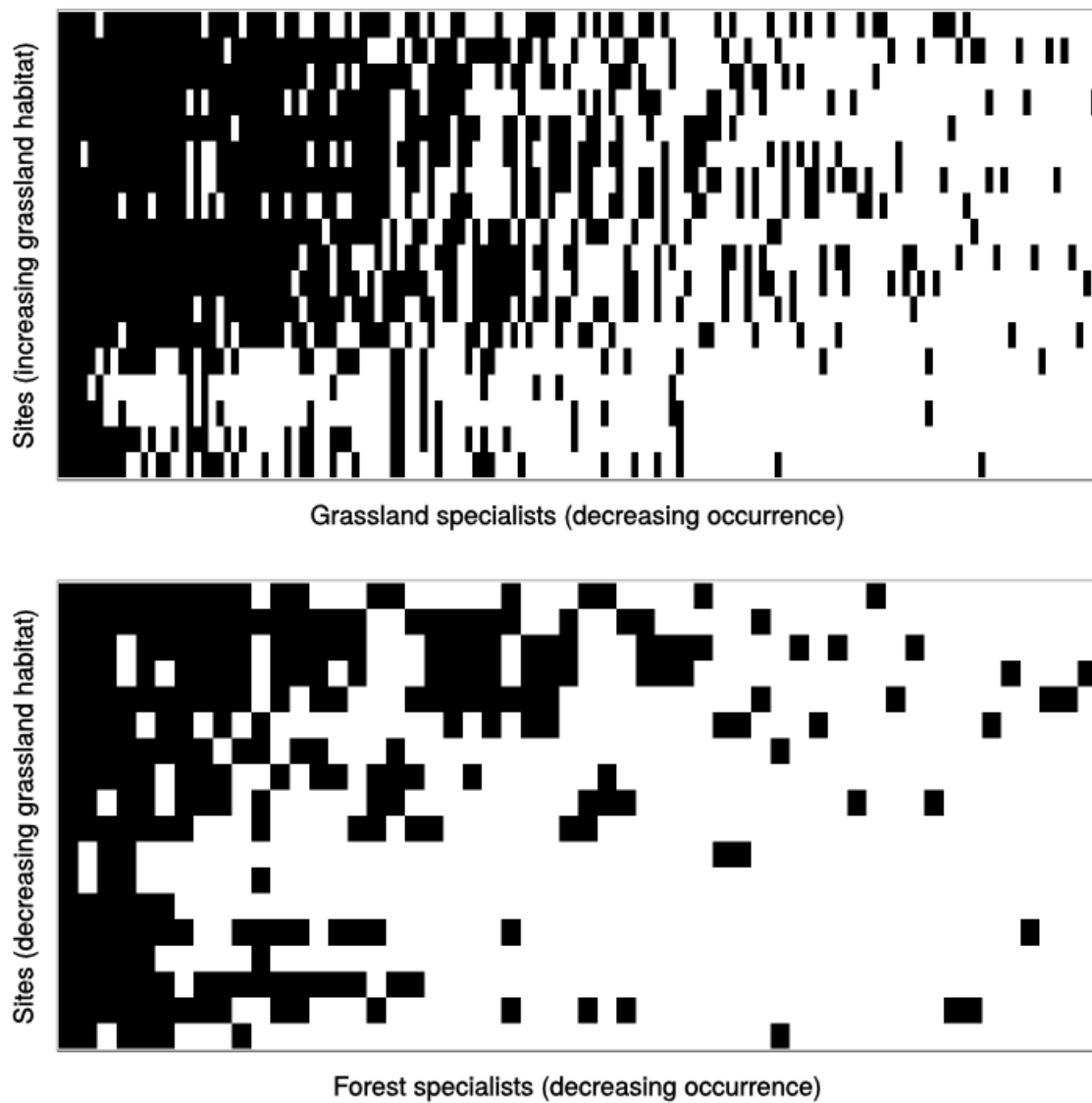
Fig. 2.4 Grassland specialists' and forest specialists' presence–absence matrices in the 18 sites. Sites are ordered by the habitat gradient (increasing grassland form the bottom to the top for grassland specialists, and decreasing grassland from the bottom to the top for forest specialists), and species by their occurrence (highest to lowest).

differences, while geographic distance only played a significant role in species replacement of both all species and forest specialists. Grassland specialists' extinctions and forest specialists' colonisations along the habitat gradient were, still, highly idiosyncratic, as no significantly nested patterns were found.

A noticeable plant turnover for all species is detected along the habitat gradient as a result of species replacement along forest encroachment. However, the contribution of richness differences of all species to total β-diversity is much weaker, indicating little loss of total richness independent from species replacement after habitat change. Analyses for specialist species groups reveal a noticeable richness gradient for grassland specialists and a weak, yet significant, richness gradient for forest specialists, but few or no replacement for any of these groups. This result, obtained for a set of sites in a forest encroachment gradient matches with the richness loss for grassland specialists and richness gain for forest specialists previously found at plot scale (Chapter 1).

Our study also shows that the studied components of β-diversity (i.e. richness differences and species replacement) are modulated by both current and historical landscape and, to a lesser extent, by geographic distance. A small effect of the historical landscape on richness differences of all species was found, indicating similar species richness in sites with similar grassland/forest ratio in the surroundings, but 50 years ago. Current landscape contributes as much as habitat to total species replacement, suggesting that the replacement of grassland specialists by forest specialists following forest encroachment can be modulated by the surrounding landscape. High grassland connectivity might delay grassland species extinctions thanks to a rescue effect (Hanski, 1999), while high forest connectivity would enhance forest specialists colonisation by increasing propagule sources (Verheyen *et al.*, 2003b).

Historical and current landscape distances also affect richness differences for grassland specialists, and they complement the richness gradient for this group along forest encroachment. Similar grassland availability makes sites more similar for the richness differences component of β-diversity, and the effect of historical landscape suggests that richness is not yet in equilibrium with the current environmental characteristics, indicating the existence of an extinction debt (Helm *et al.*, 2006; Kuussaari *et al.*, 2009; Jackson & Sax, 2010; Krauss *et al.*, 2010; Chapter 1). Grassland specialists' replacement was more dependent on current landscape than on habitat. Although habitat strongly drives richness differences between sites, current connectivity might favour the persistence of certain species irrespective of site habitat characteristics by mass effects (Mouquet & Loreau, 2003; Leibold *et al.*, 2004). Historical landscape distance also affects, to a lesser extent, grassland specialists' replacement but with a negative relationship, indicating that similar sites in historical grassland availability show higher replacement. This suggests that sites with very low connectivity in the past

are species-poor subsets of sites with high past connectivity, as supported by the richness differences component. In contrast, idiosyncratic extinctions might be occurring depending on other site characteristics, until the extinction debt will ultimately be paid (Marini *et al.*, 2013). Current landscape is the main predictor of richness differences for forest specialists, although habitat is also significant. Thus, forested landscapes might act as propagule sources able to increase forest species number at site scale. Forest specialists' replacement remains unexplained by habitat or landscape, and only geographic distance has a weak effect on it, indicating a distance decay of similarity (*sensu* Nekola & White, 1999).

We also detect idiosyncratic grassland specialists' extinctions and forest specialists' colonisations along forest encroachment. The nestedness analyses shows that species losses and gains largely depend on each site, but not on site habitat characteristics. The idiosyncratic nature of species extinctions was suggested by Guardiola *et al.* (2013) for grassland specialists in the same Mediterranean grasslands, but at patch scale in a patch area loss and fragmentation gradient. The difference in landscape connectivity is likely one of the underlying factors behind idiosyncratic extinctions, since it has been identified as the main driver of grassland specialists' replacement in our study. On the other hand, idiosyncratic forest specialists' colonisation may be explained by the lack of ancient forest species in Mediterranean forests due to a long history of forest management and exploitation (e.g. Grove & Rackham, 2001; Guirado *et al.*, 2007, 2008; Başnou *et al.*, in press). Ancient forest specialists tend to show low fecundity and unassisted dispersal and this might determine colonisation credits in these species as shown in temperate forests (Hermy *et al.*, 1999; Vellend, 2003). However, the geographic effect on forest specialists' replacement suggests that not all species are distributed throughout the area, and it is likely that colonisation is still in progress even in the most forested sites and landscapes (Jackson & Sax, 2010; Chapter 1). Habitat isolation has been proposed as a cause of biotic differentiation by seed dispersal limitation between populations (Jamoneau *et al.*, 2012; Arroyo-Rodríguez *et al.*, 2013). In our study system, the lack of biotic homogenisation due to the non-nested pattern of species extinctions and colonisations after forest encroachment might be enhanced by an extinction debt and a colonisation credit. However, when most extinctions and colonisations will have occurred, a biotic homogenisation will likely be observed if the colonising species are widely distributed (Olden & Poff, 2004) and only species with certain requirements persist, as found by Kopecký *et al.* (2013) after forest management cessation in European lowland forests. In any case, the arrival of ancient forest species at the end of the process might again promote a biotic differentiation (Vellend *et al.*, 2007; Jamoneau *et al.*, 2012).

The patterns of compositional turnover and the nestedness analyses for specialist species groups give insight into the processes shaping communities after habitat change. The partly

selective extinction of grassland specialists along the forest encroachment gradient and the poorly selective colonisation of forest specialists are the main drivers behind the noticeable replacement for all species after habitat change, while few richness changes occur because they partly compensate each other. Therefore, an environmental sorting of two groups of habitat specialists shapes the pattern found for the complete plant community. However, extinction of grassland specialists and colonisation of forest specialists are also modulated by landscape characteristics, suggesting that they might be delayed or accelerated depending on current habitat connectivity, and even a past connectivity signal remains for grassland specialists.

The lack of nestedness for grassland specialists and forest specialists might be related to species replacement due to current landscape differences (for grassland specialists) and geographic distance (for forest specialists), which might distort the order of species extinctions and colonisations by a rescue effect and distance decay of similarity, respectively.

To sum up, as a result of the forest encroachment process that affected the Mediterranean Basin during the last century (Debussche *et al.*, 1999), a species replacement is occurring, consisting in grassland specialists extinctions and forest specialists colonisations. However, the pattern in species loss and gain following habitat change is not nested, indicating that species extinctions and colonisations are idiosyncratic. Landscape characteristics and geographic distance are some of the drivers behind this pattern, as they promote grassland specialists replacement by forest specialists.

The additive decomposition of β-diversity has proven useful to disentangle community assembly processes (e.g. Leprieur *et al.*, 2011; Marini *et al.*, 2013), and here we depicted the patterns of species replacement and richness gradients, and their underlying processes, that drive total compositional change after forest encroachment. Likewise, investigating only the complete community without differentiating specialist species groups would not have provided insight into the processes leading to total species replacement. In fact, studying the β-diversity patterns for different plant groups has already proven useful to elucidate community assembly mechanisms (Marini *et al.*, 2013). Hence, partitioning total β-diversity into its replacement and richness differences components for different species groups, and completing it with a nestedness analysis for each specialist group, allowed clarifying how the extinction and colonisation processes occur after land-use change. However, more studies are needed to improve the knowledge on the complete process of compositional shift (richness differences and replacement) after land-use change in different systems, focusing on the environmental and species characteristics that might accelerate, delay or modify extinctions and colonisations.

Chapter 3

Species traits weakly involved in plant responses to landscape properties in Mediterranean grasslands[1]

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Abstract

We investigated the role of landscape structure and dynamics, compared with climatic and geographic factors, in determining species frequencies of grassland plant specialists under habitat loss. We also tested whether species traits mediate the relationship between plant community composition and environmental variables in the Mediterranean mountain grasslands of southern Catalonia (NE Iberian Peninsula), over an area of 100×20 km. Using redundancy analysis (RDA), we explored the association between frequency of broad plant specialists and both current and historical habitat patterns in the landscape (i.e. habitat amount and reduction over the period 1956–2003), after accounting for the effect of geographical location and climate in 29 grassland patches. Then, we constructed a database of biological and ecological plant traits potentially related to population persistence, in order to assess the role of these traits in explaining the found association between species composition and environmental variables. We used a single three-table ordination analysis (RLQ) of the species frequencies, environmental variables and species traits to relate species traits to environmental variables, after allowing for phylogenetic dependence of traits. The main environmental gradient explaining species frequencies was climatic and geographic. Habitat amount in the current landscape significantly affected species frequencies, while habitat amount in the historical landscape did not. A weak but significant association of species traits with environmental variables was detected. Taking into account the phylogenetic signal in plant traits did not change the results. Plant species in Mediterranean grasslands seem to respond quickly to landscape change, since no effect of historical landscape structure was observed on current species frequencies. Moreover, plant traits did not play a major role in mediating species response to environmental variation in these grasslands. Our findings differ from those obtained in northern and central European grasslands, probably due to differences in methodology but also to the smaller contrast in environmental conditions between grasslands and the adjacent forests and scrub in Mediterranean landscapes.

3.1 Introduction

Global land-use changes over the last century have had a large negative impact on Earth's biodiversity through habitat loss and fragmentation (Millennium Ecosystem Assessment, 2005), and these changes are expected to remain as the main extinction cause throughout the 21st century (Sala *et al.*, 2000). Plant species might decline or even disappear locally during or after land-cover changes, leading to smaller and more isolated habitat patches, especially

if populations are small and the new habitat is not suitable for establishment and growth (Maina & Howe, 2000; Lindborg & Eriksson, 2004; Lindborg *et al.*, 2005).

Habitat specialists are largely confined to remnant habitat fragments and hence are the species more affected by habitat loss and isolation (Dupré & Ehrlén, 2002; Adriaens *et al.*, 2006; Pino *et al.*, 2009). Furthermore, processes compromising the survival of small populations might result in non-random extinctions depending on species traits related with life-history and plant performance (Stöcklin & Fischer, 1999; Grime, 2002). Recent studies in grasslands and forests have identified several biological traits mediating species responses to landscape structure and dynamics. Plant height, specific leaf area, vegetative spread, flowering period and seed mass vary across successional gradients in abandoned grasslands, with contrasting results among studies (Kahmen & Poschlod, 2004; Lindborg & Eriksson, 2005). Plant and seed bank longevities, seed mass and dispersal type play an important role in explaining the effect of grassland area and connectivity on species composition (Bruun, 2000; Lindborg, 2007). Plant longevity, seed number and dispersal type also explain species' responses to habitat area and isolation in forests (Dupré & Ehrlén, 2002). In the only study of this kind conducted in a Mediterranean area, Chust *et al.* (2006) found that life form and dispersal type were related to landscape composition in scrubland but not in grassland. However, these studies did not take into account phylogenetic relatedness among species, and Tremlová & Münzbergová (2007) recommended conducting the analyses with and without phylogenetic correction, as the conclusions of trait responses to environment can sometimes be altered.

The extent of European grasslands, which harbour a high number of endangered plant species (WallisDeVries *et al.*, 2002), was substantially reduced following rural depopulation over the second half of the twentieth century; most of these grasslands were transformed to arable fields or forests (Eriksson *et al.*, 2002; Helm *et al.*, 2006; Adriaens *et al.*, 2006). The Mediterranean basin is not an exception to this trend (Debussche *et al.*, 1999) and, due to the high biodiversity found in these ecosystems, an important species pool is becoming endangered (Myers *et al.*, 2000). Despite this, little is known about the role of species traits on plant persistence in these grasslands (e.g. Chust *et al.*, 2006), unlike the thoroughly studied case of temperate and boreal European grasslands (e.g. Bruun, 2000; Kahmen & Poschlod, 2004; Adriaens *et al.*, 2006; Lindborg, 2007). Following those studies, we would expect plants with vegetative spread to be more frequent in reduced, small and isolated grasslands due to the maintenance of remnant populations that could persist for many years (Lindborg, 2007). In contrast, large-seeded species and plants without any specific seed dispersal mechanism should be less frequent in these reduced, small and isolated grasslands, as they generally have limited dispersal ability (Willson, 1993; van Dorp *et al.*, 1996).

However, results could differ in Mediterranean grasslands, where woody species are much more abundant than in other European grasslands and few strict habitat specialists exist.

The aim of this study is thus to investigate plant community composition and species traits in response to landscape changes, specifically habitat loss and fragmentation experienced by semi-natural Mediterranean communities in the last few decades. Our main objectives were: i) to determine the role of habitat spatio-temporal patterns at the landscape level (i.e. habitat amount and dynamics) in the distribution of grassland specialists in comparison with climatic and geographic factors; and ii) to investigate whether species traits mediate the relationship between species distribution and environmental variables.

We recorded plant frequencies in grassland patches with contrasting size and dynamics in the mountain ranges of southern Catalonia (NE Iberian Peninsula). Then, we selected broad habitat specialists and constructed a database of biological and ecological traits for these species. We used a three-table ordination analysis (RLQ) to relate plant traits to environmental variables through species frequencies (Dolédec *et al.*, 1996), taking into account the phylogenetic signal (Pavoine *et al.*, 2011). The approaches used by previous studies with similar purposes were less comprehensive, either defining a priori functional groups (Bruun, 2000; McIntyre & Lavorel, 2001; Verheyen *et al.*, 2003b; Kolb & Diekmann, 2005; Adriaens *et al.*, 2006), using the proportions or medians of traits at each plot (McIntyre & Lavorel, 2001; Lindborg, 2007), or analysing traits one by one (Dupré & Ehrlén, 2002; Jacquemyn *et al.*, 2003; Chust *et al.*, 2006). Our approach overcomes these limitations through simultaneous consideration of all plant traits over all sampled plots, while taking into account species composition and phylogeny.

3.2   Methods

Study area

We conducted the study in mountain ranges of southern Catalonia (NE Iberian Peninsula) aligned along a NE–SW axis between Prades and Ports massifs ($40°39'$ – $41°23'$N, $0°10'$ – $1°10'$E). We focused our study on semi-natural Mediterranean mountain grasslands, rich in dwarf shrubs and corresponding to the *Aphyllanthes* grasslands of the CORINE habitats classification (Moss *et al.*, 1990). These grasslands are, on average, equally dominated by hemicryptophytes and chamaephytes of less than 50 cm in height, and exhibit vegetation cover between 50% and 80%. In the study area, many of these grasslands are currently subject to woody encroachment due to grazing cessation. Also, they are

concentrated in calcareous plateaux between 800 and 1400 m a.s.l., with mean annual temperature between 10 and 13ºC, mean annual precipitation between 650 and 1050 mm, and marked summer drought (Digital Climatic Atlas of Catalonia; www.opengis.uab.cat/acdc).

Study patch selection

We digitised all grassland patches of the study area by on-screen photo-interpretation of historical (1956) and recent (2003) orthophotomaps using ARCGIS (version 9.2; Environmental Systems Research Institute, Redlands, CA, USA). We obtained historical aerial photographs from archives of the Spanish Army. Scale of the original photos was ca. 1:30 000, and pixel size of the resulting orthophotomaps was 1 m. The Cartographic Institute of Catalonia (www.icc.cat) produced present-day (2003) orthophotomaps, with a scale of 1:5000 and a pixel size of 0.5 m. Thus, there is a time period of 47 years between current and historical landscape information. We used fieldwork to select 29 of the above patches that: (1) represented a range in current patch area (from 0.1 to 26.8 ha) and in patch area reduction in recent decades (from 0 to 99.8%); (2) were grassland both in 1956 and 2003, thus excluding grasslands in 2003 resulting from abandonment of cultivated fields in 1956; (3) showed no signs of recent burning; and (4) whenever possible, were at least 1 km apart from each other to increase data independence and reduce overlap between the surrounding landscapes. Sample patches were distributed over an area of 100×20 km, and were surrounded by forest and dense scrubland.

Plant species composition

In each sample patch, we laid out 25 randomly distributed 0.5 m $\times$ 0.5 m plots during spring 2007 and recorded to subspecies level the presence of all vascular plants with any aerial part within the plot boundaries. For analysis we selected grassland specialist taxa in a broad sense, i.e. those that, according to the literature (Rivas-Martínez *et al.*, 2001; Bolòs *et al.*, 2005), mostly grow in grasslands and dwarf scrublands, as few plant species are specialists of this habitat in the strict sense. We excluded specialist taxa that were present in less than 3% of all sampled plots (pooled over all patches) from statistical analyses.

Phylogeny

To account for phylogenetic effects on plant traits, we constructed a phylogenetic tree using the last update (July 2010) in the topology of the new Angiosperm Phylogeny Group

classification (APG III Group, 2009) and other phylogenies from groups lacking good resolution. We used the *bladj* algorithm with a set of 20 dated nodes (mostly from Hedges & Kumar, 2009) to estimate tree branch lengths. Tree construction and dating were performed with the program PHYLOCOM (version 4; Webb *et al.*, 2008). See C for detailed information and sources.

Species traits

For the species encountered in the field sampling, we used literature, databases, unpublished studies, personal observations and communications from other plant researchers to compile a database of 13 biological and ecological traits related to plant and population persistence (Table C.2): seed size (maximum length without appendages, mm), dispersal type (anemochorous, wind-dispersed; zoochorous, animal-dispersed; barochorous, without any specific dispersal mechanism), corolla type (anemophilous; open entomophilous corolla; tubular; zygomorphic entomophilous), flower or pseudanthium (flower heads of *Asteraceae*, *Globulariaceae*, etc.), size (maximum length, mm), resprouting ability after fire (yes; no), life form (annual; herbaceous perennial; woody), mean plant height (small, <25 cm; intermediate, 25–50 cm; tall, >50 cm), vegetative spread (yes; no), leaf anatomy (aphyllous; succulent; mesomorphic, wrinkled once dry; scleromorphic, rigid once dry), leaf area (or leaflet in compound leaves, cm^2), spinescence (yes; no), phytogeography (strictly Mediterranean, species distributed almost only in the area of Mediterranean climate; broadly Mediterranean, species distributed in the Mediterranean climate and in colder and wetter areas; other, mostly pluri-regional species [based on Bolòs *et al.* (2005)]), and mammalian herbivore preference (preferred; indifferent; rejected). The above list was based on the traits suggested by Weiher *et al.* (1999) and Westoby *et al.* (2002) but extended with traits used in more recent studies (e.g. Kolb & Diekmann, 2005; Adriaens *et al.*, 2006) or considered by us to be important in the study area for resistance to herbivores (spinescence) or for pollination (flower size). Phytogeography was included because it is closely related to climate tolerances, with strictly Mediterranean species preferring dry and warm sites and broadly Mediterranean ones also being abundant in colder and wetter sites. These traits were obtained for 49 species. The remaining 13 species were not used in analyses because information on all of their traits was not available, and our analysis method does not allow missing values. Almost half of these 49 species were woody (24 species; 49%), 22 (45%) were herbaceous perennials and only three (6%) were annuals.

Environmental variables

Besides historical and current patch areas and their change over time, we obtained from the orthophotomaps of 1956 and 2003 three additional variables related to habitat amount and connectivity: percentage of historical and current grassland area in the landscape surrounding each patch (1-km buffer around the patch perimeter, including the patch itself), and percentage of grassland area reduction in this landscape. We consider this buffer distance to be sufficient for taking into account dispersal events in our abrupt landscape, as it was also used in similar studies in flat areas (Lindborg & Eriksson, 2004). In addition, we also considered four geographic and climatic variables for the analyses: longitude (UTMx), latitude (UTMy), mean annual temperature and mean annual precipitation (Table C.3). Both climatic variables were obtained from the Digital Climatic Atlas of Catalonia (www.opengis.uab.cat/acdc). We did not include other environmental variables due to high correlations to the previous ones (e.g. altitude, correlated with temperature and precipitation; mean summer precipitation, correlated to annual precipitation) or lack of information (e.g. grazing pressure, time since pasture abandonment). The correlation matrix between environmental variables is given in Table C.4.

Statistical analyses

The relationship between environmental variables and species traits or trait combinations can be assessed through the simultaneous analysis of three data tables (Dolédec *et al.*, 1996): a species abundance × site table is used as the link between an environment × site table and a trait × species table to investigate the so-called 'fourth-corner' table that is missing (i.e. the fourth table that would relate the environmental variables to species traits). Two methods have been described to tackle this problem. RLQ analysis (Dolédec *et al.*, 1996) provides a simultaneous multivariate ordination of the three tables, whereas fourth-corner analysis (Legendre *et al.*, 1997; Dray & Legendre, 2008) tests the correlation between the environmental variables and the species traits. Here we used the former approximation because ordination allows the visualisation of trait combinations and environmental gradients that combine more than one variable.

We constructed three data tables with the information on: (1) species frequencies (columns) in each patch (rows; table L, Table C.5); (2) the values of environmental variables (columns) at each patch (rows; table R, Table C.6); and (3) the traits (columns) for each species (rows; table Q, Table C.7). We transformed the species frequency table (L) using the Hellinger transformation (Rao, 1995) to reduce the effect of the most abundant species

(Lacourse, 2009) and solve the inherent problems of the Euclidean distance (Legendre & Gallagher, 2001). Then, we investigated the relationship between species composition and the main environmental gradients using an ordination analysis of species frequencies (L) constrained by environmental variables (R). Since a detrended correspondence analysis (DCA) on the frequency table resulted in gradient lengths <2 SD, we used a linear constrained ordination (redundancy analysis, RDA) to relate community composition to environmental variables. We applied a step-wise selection to obtain a model for the constrained ordination that avoided multicollinearity and to compare the variance explained by this model in to the variance explained by all ten environmental variables considered in the analysis. We performed ANOVA-like permutation tests (Legendre & Legendre, 1998) with 9999 permutations to assess the statistical significance of the association between species composition and the variables selected, for the entire model and for each axis. We conducted the RDA analysis using the *vegan* package (Oksanen *et al.*, 2013) in R (R Development Core Team, 2014).

We constructed a matrix of pair-wise phylogenetic distances among species. The distance metric used was the square root of the sum of branch lengths along the shortest path that connects species, which provides Euclidean distances (Pavoine *et al.*, 2011). To test whether species traits had a phylogenetic signal, we constructed a matrix of pairwise trait distances among species using the mixed- variables coefficient of distance (Pavoine *et al.*, 2009) and applied the root-skewness test developed in Pavoine *et al.* (2010) with 999 Monte Carlo simulations. Species traits show a phylogenetic signal if trait diversity is biased to the root of the phylogenetic tree, otherwise (trait diversity biased to the tips or at random) no phylogenetic signal in traits can be identified.

Then, in order to relate species traits to environmental variables through species composition, we conducted a RLQ analysis (Dolédec *et al.*, 1996), which consists of a simultaneous ordination of species traits (Q), environmental variables (R) and species frequencies (L). We also incorporated the phylogeny in the analysis, using the extended RLQ approach described in Pavoine *et al.* (2011). We first analysed the distance matrix of traits and the distance matrix of phylogeny by principal coordinates analysis (PCoA) with row weights equal to column weights of the correspondence analysis (CA) of table L, standardised the row coordinates by dividing them by the square root of the first eigenvalue of the respective analysis, and juxtaposed these two tables, obtaining the Q table of traits and phylogeny. Then we analysed the three tables (R, L and Q) separately to allow comparisons with the results of the joint analysis. We used a correspondence analysis (CA) of table L to obtain the simultaneous ordination of species and patches. We conducted a principal components analysis (PCA) of table R with row weights equal to row weights of the CA and

another PCA of table Q with row weights equal to column weights of the CA. Then we used a version of RLQ based on the CA of the species frequencies in patches to study the joint structure of the three tables. This method is a trade-off among the three separate ordinations and, since the frequency table is processed by CA, it maximises the covariance between patches and species scores (Dolédec *et al.*, 1996), resulting in the best joint combination of the ordination of the three data tables: patches–environment (R), species frequencies–patches (L) and species–traits and phylogeny (Q; Ribera *et al.*, 2001). We tested the global statistical significance of the analysis by comparing the total inertia value in the RLQ analysis obtained from the data to the distribution of the total inertias obtained from 999 Monte Carlo permutations of the rows of tables R and Q (Dolédec *et al.*, 1996). To investigate the strength of the association between tables of environmental variables and species traits, we compared the RLQ results with the results of the separate analyses of the three tables. The results of this RLQ analysis were also compared with the results of a standard RLQ analysis (without including phylogeny), as proposed by Tremlová & Münzbergová (2007) for analyses involving species traits and landscape attributes. In this analysis, table Q was the raw species traits table and it was analysed using a multivariate analysis with mixed quantitative variables and factors (Hill & Smith, 1976) with row weights equal to column weights of the CA. We obtained the distance matrices and performed RLQ analysis using the *ade4* package (Chessel *et al.*, 2004) in R.

3.3   Results

Constrained ordination of the species frequencies by the environmental variables

In the direct gradient analysis performed with RDA, the full model including ten environmental variables explained almost half (45.3%) of the total variance in the species frequencies. The reduced model obtained by the step-wise procedure still explained substantial variance (27.1%) and included only three environmental variables: UTMy, percentage of grassland area in the current landscape, and mean temperature (Table 3.1; Fig. 3.1). The permutation tests for this model revealed a highly significant overall association between species composition and constraining variables ($P < 0.0001$) as well as a statistical significance for the first two axes ($P < 0.0001$ and $P = 0.005$, respectively).

Axis 1 of this RDA explained 14.8% of the variance in species composition and had relatively high correlations with two geographic and climatic variables: latitude (positively

correlated) and mean temperature (negatively correlated; Fig. 3.1). Species such as *Genista hispanica* and *Satureja montana* plotted on the negative side, being significantly associated, respectively, with southern study sites ($r^2 = 0.20$) and warm sites ($r^2 = 0.14$). *Narcissus assoanus*, *Staehelina dubia*, *Helianthemum oelandicum*, *Arctostaphylos uva-ursi*, *Stipa offneri*, *Linum tenuifolium*, *Globularia vulgaris*, *Lithospermum fruticosum* and *Koeleria vallesiana* plotted on the positive side of the first axis, all significantly associated with northern and cooler sites (r^2 between 0.14 and 0.41 for latitude and between 0.17 and 0.43 for temperature), except *Staehelina dubia* that was only associated with northern sites ($r^2 = 0.36$). RDA axis 2 explained 7.8% of the variance and was most strongly correlated (positively) to percentage of grassland area in the current landscape. *Dorycnium pentaphyllum*, *Centaurea linifolia* and *Stipa offneri* plotted on the negative side, but none of them was significantly associated with current landscapes with small grassland area. On the other hand, *Erinacea anthyllis*, *Anthyllis vulneraria*, *Festuca* gr. *ovina*, *Carduus nigrescens* and *Serratula nudicaulis* plotted on the positive side of the second axis, and were significantly associated with current landscapes with abundant grassland (r^2 between 0.23 and 0.37; *Festuca* gr. *ovina* only marginally with $r^2 = 0.13$ and $P = 0.054$).

Table 3.1 Redundancy analyses (RDAs) of the species frequency table: variance explained by each environmental variable alone and results of the step-wise selection procedure.

Environmental variable	RDA models	Step-wise model	
	Variance (%)	F	P-value
Latitude (UTMy)	14.1	3.74	<0.001
Percentage of grassland area in the current landscape	9.0	2.76	<0.001
Mean annual temperature	7.6	1.70	0.033
Mean annual precipitation	11.7	1.15	0.27
Longitude (UTMx)	13.2	1.14	0.28
Percentage of grassland area in the historical landscape	6.5	0.99	0.45
Past patch area	4.8	0.83	0.66
Percentage of grassland area reduction in the landscape	4.8	0.84	0.69
Percentage of patch area reduction	4.7	0.82	0.73
Current patch area	7.5	0.73	0.81

Variance explained by the selected model[a] (%)

Axis 1	14.8
Axis 2	7.8
All three axes	27.1

[a]The selected model includes the first three environmental variables.
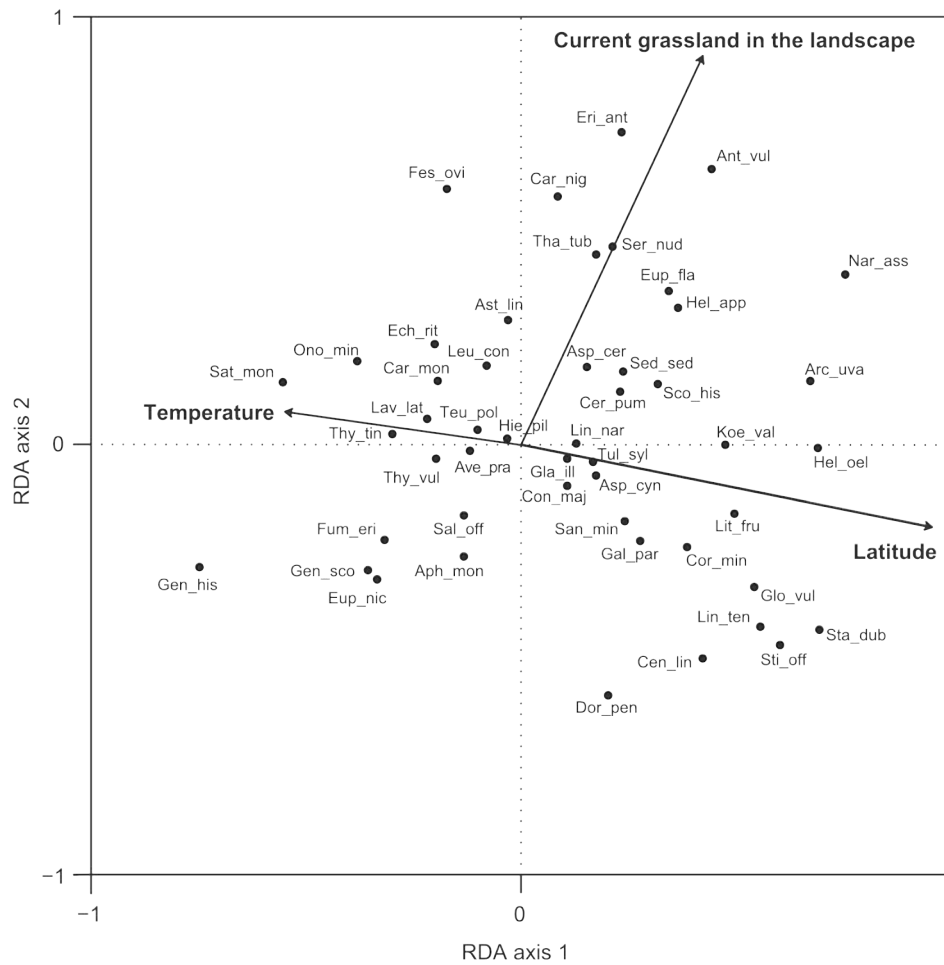
Fig. 3.1 Redundancy analysis (RDA) of species and environmental variables selected with the step-wise procedure. The first and second RDA axes, which explain 14.8% and 7.8% of the total variation, respectively, are plotted. The length of the projection of an arrow onto an axis indicates the correlation between the environmental variable and the RDA axis. Abbreviations are: Ant_vul, *Anthyllis vulneraria*; Aph_mon, *Aphyllanthes monspeliensis*; Arc_uva, *Arctostaphylos uva-ursi*; Asp_cyn, *Asperula cynanchica*; Asp_cer, *Asphodelus cerasiferus*; Ast_lin, *Asterolinon linum-stellatum*; Ave_pra, *Avenula pratensis*; Car_mon, *Carduncellus monspelliensium*; Car_nig, *Carduus nigrescens*; Cen_lin, *Centaurea linifolia*; Cer_pum, *Cerastium pumilum*; Con_maj, *Conopodium majus*; Cor_min, *Coronilla minima*; Dor_pen, *Dorycnium pentaphyllum*; Ech_rit, *Echinops ritro*; Eri_ant, *Erinacea anthyllis*; Eup_fla, *Euphorbia flavicoma*; Eup_nic, *Euphorbia nicaeensis*; Fes_ovi, *Festuca* gr. *ovina*; Fum_eri, *Fumana ericoides*; Gal_par, *Galium parisiense*; Gen_his, *Genista hispanica*; Gen_sco, *Genista scorpius*; Gla_ill, *Gladiolus illyricus*; Glo_vul, *Globularia vulgaris*, Hel_app, *Helianthemum apenninum*; Hel_oel, *Helianthemum oelandicum*; Hie_pil, *Hieracium pilosella*; Koe_val, *Koeleria vallesiana*; Lav_lat, *Lavandula latifolia*; Leu_con, *Leuzea conifera*; Lin_nar, *Linum narbonense*; Lin_ten, *Linum tenuifolium*; Lit_fru, *Lithospermum fruticosum*; Nar_ass, *Narcissus assoanus*; Ono_min, *Ononis minutissima*; Sal_off, *Salvia officinalis*; San_min, *Sanguisorba minor*; Sat_mon, *Satureja montana*; Sco_his, *Scorzonera hispanica*; Sed_sed, *Sedum sediforme*; Ser_nud, *Serratula nudicaulis*; Sta_dub, *Staehelina dubia*; Sti_off, *Stipa offneri*; Teu_pol, *Teucrium polium*; Tha_tub, *Thalictrum tuberosum*; Thy_tin, *Thymelaea tinctoria*; Thy_vul, *Thymus vulgaris*; Tul_syl, *Tulipa sylvestris*.

RLQ analysis relating species traits to environmental variables

Traits had a significant phylogenetic signal, as trait diversity was biased towards the root in the root-skewness test ($P < 0.001$). Thus, RLQ analysis was performed including phylogeny, but it yielded very similar results to the standard RLQ, although the phylogenetic approach explained less variance of the Q table (45-39% of the first and secondaxes, respectively, compared to 59-55% of the standard approach). Hence, only the results from the simpler, standard RLQ are reported in the text. Results from RLQ analysis including phylogeny are given in Table C.8.

The first and second axes of the RLQ analysis extracted 68.9% and 20.2%, respectively, of the total inertia of the cross-matrix of the environmental variables and species traits. Hence, we only considered these two axes, since together they accounted for 89.1% of the variance in the analysis. The results of the permutation test of the rows of the R and Q tables indicate a significant ($P < 0.001$) statistical association between environmental variables and species traits. Since the RLQ analysis represents the partial ordination between the three separate tables (R, L and Q), the variance corresponding to each table was compared to the variance from the separate analyses of the three tables (Table 3.2). RLQ axis 1 accounted for 96%, 42.6% and 59.3%, and axis 2 accounted for 87.4%, 32.7% and 54.8% of the variability explained in the separate analyses of patches–environment (R), species frequencies–patches (L) and species–traits (Q) tables, respectively. The variance of the Q table accounted for by the RLQ analysis is relatively low, but within the range of previous studies (Dolédec *et al.*, 1996; Ribera *et al.*, 2001; Choler, 2005; Cleary *et al.*, 2007; Lacourse, 2009). Since RLQ analysis maximises the covariance between R and Q tables, it accounted for more variability of these two tables than it did for the species frequency table (L). The species and patches scores in the RLQ analysis had a correlation of 0.159 and 0.116 in the first and second axes, respectively, compared to the maximum possible correlation of 0.373, given by the square root of the first eigenvalue of the CA of the frequency table. The first RLQ axis maximises the covariance between these two sets of scores, giving a value almost twice that in the second axis (0.446 and 0.241, respectively).

Climatic and geographic environmental variables, especially latitude and longitude (both negatively) and mean precipitation (positively), were correlated to the first RLQ axis (Fig. 3.2a). The species traits most correlated to this axis were plant spinescence, leaf succulence, zygomorphic corolla (all positively correlated), annual life form (negatively correlated), broadly Mediterranean phytogeography (positively correlated), tubular corolla and strictly Mediterranean phytogeography (both negatively correlated; Fig. 3.2b). The RLQ second axis, on the other hand, was most strongly correlated to variables related to habitat
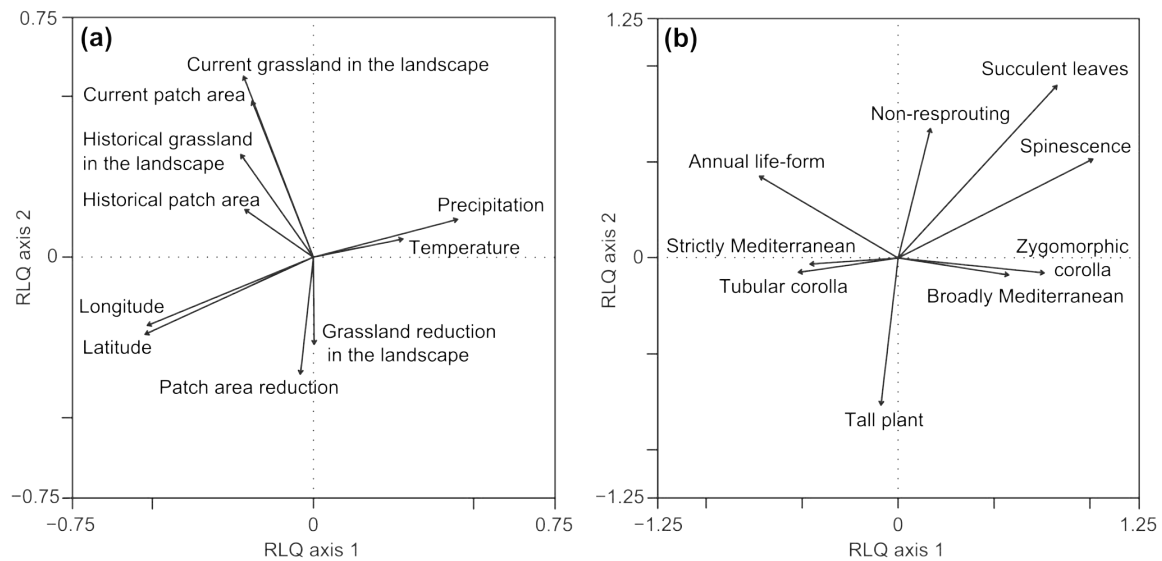
Fig. 3.2 RLQ analysis of environmental variables (a) and species traits (b). Only traits with a normed score > 0.45 on the first or second axis are shown.

amounts and dynamics: percentage of grassland area in the current landscape and current patch area (both positively correlated), resulting in a gradient from currently small patches with little grassland in the current surrounding landscape to patches of large size and having more grassland in the current landscape. This axis had the highest correlations with leaf succulence (positively correlated), large plant height (negatively correlated), non-resprouting ability after fire and spinescence (both positively correlated).

3.4   Discussion

The present study shows that current but not historical landscape composition explains species distributions in the study patches. Moreover, we found a weak but statistically significant relationship between biological and ecological species traits and environmental variables.

Current habitat amount in the landscape plays a significant role in explaining the frequencies of broad specialist species in the studied communities, as it was the variable first selected in the step-wise RDA model after accounting for the effects of geographical location, which was the factor explaining most variance alone (Table 3.1). This result suggests that the grasslands studied work as metacommunities, where dispersal between habitat fragments plays an important role in maintaining species composition (Holyoak *et al.*, 2005). Specifically, since some grassland species increased their within-patch frequency when the patch was surrounded by more grassland habitat in the landscape, propagules for

Table 3.2 Results of the RLQ analysis and the three separate analyses. Panel (a) shows the results of the separate analyses for the environmental variables table (R), the species composition table (L) and the species traits table (Q). Panel (b) gives the results of the RLQ analysis: variance explained by environmental variables and species traits, and covariance and correlation between sites and species for the first two axes. Panel (c) shows the comparison between the RLQ analysis and each separate analysis for the first and second axes.

a) Separate analyses

Analysis	Variance axis 1	Variance axis 2
PCA of R	3.635 (36.3%)	2.633 (26.3%)
CA of L	0.139 (16.0%)	0.126 (14.5%)
Mix model of Q	3.808 (17.3%)	3.424 (15.6%)

b) RLQ analysis

b.1) Variance

Analysis	Variance axis 1	Variance axis 2
R/RLQ	3.490	2.302
Q/RLQ	2.257	1.878

b.2) Covariance

Covariance axis 1	Covariance axis 2
0.446	0.241

b.3) Correlation

Correlation axis 1	Correlation axis 2
0.159	0.116

c) Comparison of RLQ and separate analyses

	Explained variance (%)	
	RLQ axis 1	RLQ axis 2
R/RLQ	96.0	87.4
L/RLQ	42.6	32.7
Q/RLQ	59.3	54.8
Inertia	68.9	20.2

these species probably originated partly in grassland habitat outside the patch. Increased within-patch frequencies likely result in enhanced population persistence. Moreover, none of the measures of historical landscape structure was important in our study, contrary to some previous studies, suggesting that plant species in our habitats have already responded to habitat change. Lindborg *et al.* (2005) and Vellend (2005) found effects of land-use history on patch occupancy and population size and dynamics in several grassland and forest plants, respectively. Past area and isolation are also important for explaining the richness of plant specialists in some grasslands (e.g. Helm *et al.*, 2006; Krauss *et al.*, 2010; but see Adriaens *et al.*, 2006). However, unlike the present study, none of these previous studies investigated the relationship between landscape structure and species composition of most plant specialists.

Even though we detected a phylogenetic signal in species traits, results did not change appreciably whether phylogeny was taken into account or not in the RLQ analysis, suggesting that the trait patterns observed are caused by ecological drivers. The lower variance of the separated analysis of table Q explained by the RLQ analysis including phylogeny may be due to the higher number of variables included in the traits and phylogeny Q table (68 variables) compared to the traits Q table (32 variables). Several plant traits were associated with habitat amount at the patch and landscape scales. However, we think the relationship between species traits and environmental variables, although statistically significant, was weak because: (1) the variance explained by the first two axes of the separate analysis of the species–traits table (Q) was relatively small; (2) the RLQ axes accounted for much less variance of the first two axes of species–trait table (Q; 59-55%) than of the first two axes of the patches–environment table (R; 96-87%); and (3) the contribution of the Q table to the joint ordination was small because environmental gradients in RDA and RLQ were almost identical. Besides, four out of the five traits most correlated to the RLQ second axis were represented by few species in our data set (i.e. succulent leaf anatomy, annual life form, spinescence and, to a lesser extent, tall plant). The fifth trait, the lack of resprouting ability, was most positively associated with current grassland area and negatively associated with grassland reduction. Non-resprouting species were regionally less frequent on average than resprouter species, and this could result in small populations vulnerable to local extinctions in small patches or in patches with little grassland in the landscape. In contrast, viable populations could persist in large patches and patches with more grassland in the landscape. Moreover, the most frequent non-resprouting species (*Fumana ericoides*, *Helianthemum apenninum*, *Helianthemum oelandicum* and *Ononis minutissima*) germinate after fire (Arnan *et al.*, 2007; Paula & Pausas, 2008) and could also take advantage of other disturbances prone to occur in open habitats, thus enhancing population regeneration in large patches. Tall

plant specialists were most negatively associated with current grassland area and positively associated with grassland reduction, suggesting that they could take some advantage of habitat reduction and woody encroachment. Tall plants might have better competitive advantage and dispersal capacity than small plants when patches become smaller, more isolated and encroached by woody plants. However, since RLQ is a correlational method, the relationships between the traits we used and species responses to landscape variables are not necessarily cause–effect.

Many previous studies have stressed the importance of several plant traits in mediating plant response to land-use change in grasslands of central and northern Europe (Adriaens *et al.*, 2006; Lindborg, 2007) and in other fragmented habitats (Dupré & Ehrlén, 2002; Jacquemyn *et al.*, 2003; Verheyen *et al.*, 2003b; Kolb & Diekmann, 2005). In our study, life form was not related with either current patch area or current grassland amount in the landscape. This result indicates that plant responses to landscape patterns and dynamics were, on average, similar between woody species and herbaceous perennials; although our data set contained only three annual species. Plants with no specific dispersal mechanism were not more associated with large and connected grasslands than anemochorous or zoochorous species, suggesting that dispersal limitation is not strong enough or that realised dispersal does not differ in the study area among these plant groups. As in our case, Chust *et al.* (2006) could relate neither life form nor dispersal to landscape structure in Mediterranean grasslands. Neither corolla type nor flower size was related to current landscape composition in our study, even though generalist corollas and large flowers or inflorescences could provide an advantage if specialised pollinators are more affected by habitat loss and fragmentation than generalist pollinators. However, other factors like autogamy could affect this relationship, since autogamous species could persist more easily in small and fragmented habitats (Aguilar *et al.*, 2006). Seed size has been related with dispersal strategy, establishment success and fecundity, although the advantage of larger seeds in seedling success is more important in shady habitats (Westoby *et al.*, 1996) than in the open grasslands studied here. Lindborg (2007), like us, did not find a significant relationship between a similar trait (seed mass) and either habitat area or connectivity in grasslands. The same study, in contrast, found that vegetative spread and seed bank longevity were negatively associated with historical connectivity, and vegetative spread was negatively associated with area. Our study did not find any correlation between vegetative spread and environmental gradients, possibly because of the low resolution of this attribute (yes/no). Moreover, the scarce information available for seed bank longevity in Mediterranean plant species prevented us from including this trait in our analysis.

These contrasting results with other studies could be attributed either to different

methodology or different study system. The RLQ analysis is not directly comparable with the methods used previously to assess the role of plant traits in land-use change, although it has proven useful to relate bird and insect traits to habitat and landscape fragmentation (Barbaro & van Halder, 2009) and plant traits to various treatments of grazing, mowing, mulching and abandonment in semi-natural grasslands (Römermann *et al.*, 2009; Pakeman & Marriott, 2010). Effects of species traits on species' responses to landscape changes have been poorly studied in Mediterranean grasslands, unlike other European grasslands. Perhaps the role of traits is not as marked in Mediterranean conditions, where grasslands are often rich in woody species that might show life strategies similar to those dominating the surrounding open-canopy forests or scrub. This might lessen the environmental and biological contrast between grasslands and adjacent habitats, and suggests that future analyses of these communities could benefit from the 'continuum' model (Fischer & Lindenmayer, 2006), which assumes individualistic species responses to either gradual or abrupt changes in environmental variables.

Additional variation in species composition may be explained by other climatic and geographic variables, edaphic factors or disturbance indicators for which information was not available in this study. More information on the landscape history of the study area, especially on grazing pressure and time since pasture abandonment, which are expected to affect landscape patterns and species composition, would provide a more complete view of vegetation dynamics. More information on poorly studied plant traits that possibly determine plant persistence in fragmented habitats is needed, especially on autogamy. Aguilar *et al.* (2006) showed that habitat fragmentation more negatively affected self-incompatible species than self-compatible ones. Seed bank longevity is also an important trait for plant persistence in fragmented boreal grasslands (Lindborg, 2007), where the proportion of plants with persistent seed banks was negatively associated with historical connectivity. Exploration of new plant traits or those known to be important in other communities is thus key to further our understanding of plant responses to habitat loss and fragmentation in Mediterranean grasslands.

Finally, if none of the considered traits succeeds in explaining species composition along environmental gradients, a different approach is needed. In relation to land-use change, we could measure demographic traits (e.g. vegetative regeneration, seed production, germination and survival rates) along a gradient of landscape composition and dynamics. This approximation would show the population performance of different specialist species in response to the generalised reduction and fragmentation of Mediterranean grasslands and would help to identify species vulnerable to landscape change.

We conclude that species composition is related to current landscape structure in

Mediterranean grasslands of southern Catalonia, although climatic and geographic variables play a primary role. Species responses to these changes in habitat amount seem to be relatively fast, as current habitat amount was a better predictor of current species frequencies than was habitat amount 50 years ago. A single, three-table ordination analysis used to relate biological and ecological plant traits to environmental variables yielded weak but significant relationships between environmental gradients and plant traits, indicating that species response to habitat reduction in these communities is not largely mediated by the traits we considered. These results suggest the need to investigate other factors like grazing pressure, other species traits like autogamy and seed bank persistence, and the role of demographic rates.

Chapter 4

Contrasting habitat and landscape effects on the fitness of a long-lived grassland plant under forest encroachment: do they provide evidence for extinction debt?[1]

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[1]In preparation

Abstract

Habitat loss, fragmentation and transformation threaten the persistence of many species worldwide. Population and individual fitness is often hampered in small, degraded and isolated habitats, but extinction can be a slow process and extinction debts can occur. Long-lived species are prone to persist as remnant populations in low quality habitats for a long time, but the population and individual-level mechanisms of extinction debt remain poorly explored so far. In the present work, we investigate the mechanisms involved in the long-term persistence of the common grassland specialist, long-lived, clonal plant *Aphyllanthes monspeliensis* L. after forest encroachment of semi-natural Mediterranean calcareous grasslands in Catalonia (NE Iberian Peninsula). For this purpose we assess vegetative (aboveground and belowground) and reproductive plant performance as affected by habitat characteristics and by current and historical connectivity. We confirm the existence of an extinction debt for the species, since current plant frequency is related with historical but not current landscape, and we also find a positive effect of historical landscape on seed set. In addition, current tree cover negatively affects individual size and aboveground/belowground biomass ratio, and soil acidification leads to a reduction in the flowering probability of individuals and stems. However, we find that current connectivity negatively affects flowering and that tree cover enhances seed set. The forestation process, thus, also exerts a positive effect on some fitness traits, probably by providing a moister environment. Habitat loss and deterioration result in a decreased vegetative performance of *Aphyllanthes monspeliensis*, but have contrasting effects on its reproductive performance. However, further forest encroachment would increase light competition and soil acidification, threatening its persistence and promoting the payment of the extinction debt if no conservation measures are taken.

4.1   Introduction

Habitat loss and transformation are major threats for biodiversity all over the world (Fahrig, 2003; Stockwell *et al.*, 2003; Ouborg *et al.*, 2006). Transformation or deterioration of the suitable habitat of a species threatens survival and/or reproduction of individuals, reducing fitness (Lienert, 2004; Mortelliti *et al.*, 2010). Altered habitat conditions due to edge effects become more important after fragmentation, which increases the transitional area between habitats (Lande, 1988; Lienert, 2004). Moreover, reduced habitat area and increased isolation, which result in decreased population size and restricted individual and gene

flows, respectively, also threaten population viability (Thrall *et al.*, 2000; Honnay *et al.*, 2005). However, species can persist for a long time in small, degraded and isolated habitats, especially when number, size and spatial configuration of habitat fragments are not much below their extinction thresholds for metapopulation functioning (Hanski & Ovaskainen, 2002). These populations that are not in equilibrium with current habitat conform the extinction debt (Tilman *et al.*, 1994; Kuussaari *et al.*, 2009), and they can be precariously maintained by metapopulation, population and individual-level mechanisms (Hylander & Ehrlén, 2013). For species showing good dispersal ability, populations from source habitats might act as propagule sources that rescue sink populations with otherwise limited recruitment (Hanski, 1999; Leibold *et al.*, 2004). On the other hand, plant biological characteristics that make individuals resistant to decreased habitat quality in particular life-cycle stages also enhance species persistence (Kuussaari *et al.*, 2009; Hylander & Ehrlén, 2013). Thus, both traits related with competitive ability and dispersal are important for the persistence of a species in a region (Jacquemyn *et al.*, 2003).

So far, the population and individual-level mechanisms behind extinction debt have been much less explored than metapopulation processes (Hylander & Ehrlén, 2013). However, it is known that long-lived species may persist for a long time in low quality habitats as remnant populations that, without recruitment, decline slowly over time (Eriksson, 1996). Due to extended life-span and long generation times, populations of clonal plants are usually more persistent than short-lived ones, even if they consist of few individuals (Young *et al.*, 1996; Honnay *et al.*, 2005; Saar *et al.*, 2012; but see Lindborg *et al.*, 2012; Bagaria *et al.*, 2012). As a consequence, these species with low extinction rates might constitute the main part of the extinction debt (Vellend *et al.*, 2006; Lindborg, 2007), probably because their survival and reproduction are influenced not only by the present conditions but by the environmental conditions in the preceding years through, for instance, the resources accumulated in perennial tissues (Stephenson, 1981; Mehrhoff, 1989; Lienert, 2004). Although population and individual performance might also show time-lagged responses to habitat change (Ewers & Didham, 2006; Takkis *et al.*, 2013), they proved useful to confirm population decay in an extinction debt context (Takkis *et al.*, 2013). Several drivers of population decay for long-lived species have been identified: soil moisture (Colling *et al.*, 2002), nutrients (Oostermeijer *et al.*, 1998; Colling *et al.*, 2002), vegetation cover (Oostermeijer *et al.*, 1994; Hutchings *et al.*, 1998), and also population size (Oostermeijer *et al.*, 1998; Jacquemyn *et al.*, 2002) have been found to be related with population structure and plant fitness. Clonal plants may change their architecture under limited resource availability (light or nutrients) as an expression of foraging behaviour to place ramets in more favourable microhabitats (Slade & Hutchings, 1987; Sutherland & Stillman, 1988). In response to shading many

plants show etiolation, and rhizomatous species tend to reduce branching intensity, but contrary to stoloniferous species, no clear internode elongation has been found (De Kroon & Hutchings, 1995). These architectural changes might be useful to assess plant performance under suboptimal conditions.

European semi-natural grasslands underwent an important reduction and fragmentation during the last century, and especially over the last 70 years, as a consequence of agricultural intensification or abandonment of traditional practices (Eriksson *et al.*, 2002; Adriaens *et al.*, 2006). Although richness patterns in these communities have been widely studied and delayed species extinctions have been reported at the community level (e.g. Helm *et al.*, 2006; Krauss *et al.*, 2010; Chapter 1; but see Adriaens *et al.*, 2006), few species-specific extinction debts have been studied (but see Herben *et al.*, 2006), and indicators of forthcomingextinctions are still needed (Helm *et al.*, 2009). The few works investigating the consequences of habitat and connectivity loss for the fitness of plants in these semi-natural systems found either that current connectivity was the main predictor of genetic diversity (Helm *et al.*, 2009), which in turn enhanced reproductive effort and plant height (Takkis *et al.*, 2013), or that tree cover negatively affected recruitment (Schleuning *et al.*, 2009) or flower production and fruit set (Adriaens *et al.*, 2009). However, some fitness traits, such as plant height and germination, were still related with historical factors (Takkis *et al.*, 2013). So, connectivity loss and habitat deterioration might threatenvlong-term population persistence long before changes in abundance are found, especially for long-lived plants.

In the Mediterranean Basin, an important forest encroachment resulting in the loss and fragmentation of semi-natural grasslands occurred during the 20th century, which is expected to cause extinctions of grassland specialists and colonisations of forest specialists (Debussche *et al.*, 1999). These biodiversity changes have partly occurred, but a deficit of forest specialists and a surplus of grassland specialists have been found after 50 years of forest encroachment and grassland area reduction (Guardiola *et al.*, 2013; Chapter 1). The predominance of long-lived species (hemicryptophytes and chamaephytes) in these grasslands might delay species extinctions, thus enhancing extinction debt (but see Bagaria *et al.*, 2012). Nevertheless, little is known about the individual and population-level mechanisms causing extinction debts for long-lived plants in these systems.

In this study we investigate the population and individual-level persistence mechanisms of the clonal plant *Aphyllanthes monspeliensis* L. (*Liliaceae*) after more than 50 years of forest encroachment into semi-natural Mediterranean grasslands. This species is a common grassland specialist able to persist along forest encroachment gradients due to its biological inertia (Summerfield, 1972), but potentially showing an extinction debt in these situations. In order to seek indicators of individual and population decay of this clonal plant, we assess

vegetative and reproductive performance in relation to environmental and landscape proxies of habitat loss and transformation.

We hypothesise that: i) *Aphyllanthes monspeliensis* will show an extinction debt in the encroached sites, since extinction debt was identified for the set of grassland specialists in the same area (Chapter 1); ii) vegetative performance and flower production should decrease under forest encroachment and canopy closure, as a result of resource limitation (Peri *et al.*, 2007; Adriaens *et al.*, 2009); iii) seed set should decrease with decreasing grassland connectivity, due to population isolation that can limit pollination and gene flow (Honnay *et al.*, 2005), and iv) rhizome branching intensity should decrease and aboveground/belowground biomass ratio and internode length might increase under canopy closure, as a consequence of foraging behaviour and because shade-dependent etiolation commonly occurs at the expense of underground growth (Kephart & Buxton, 1993).

4.2 Methods

Study area and system

The study was conducted in semi-natural Mediterranean calcareous grasslands and encroached grasslands of the Prades Mountains, in southern Catalonia (NE Iberian Peninsula; 41°14′ – 41°23′N, 0°56′ – 1°10′E), between 800 and 1200 m a.s.l. The mean temperature in this area is around 11 °C, and the mean precipitation around 750 mm, with a marked summer drought (Digital Climatic Atlas of Catalonia; www.opengis.uab.cat/acdc). The predominating life-forms in the studied grasslands are chamaephytes and hemicryptophytes, which usually do not grow higher than 0.5 m, and vegetation cover ranges from 50 to 80% (see Guardiola *et al.*, 2013, for details). A historically open landscape, widely grazed by sheep and goats, was maintained until the decade of 1940s, and was dominated by semi-natural grasslands with small forest areas. However, great grazing reduction during the second half of the 20th century led to generalized grassland reduction and forest encroachment (Guardiola *et al.*, 2013), which resulted in relatively open forests dominated by holm oak (*Quercus ilex* L.) and Scots pine (*Pinus sylvestris* L.).

Study species

Aphyllanthes monspeliensis is a rhizomatous, tussock-forming, evergreen graminoid plant. Its leaves are reduced to basal sheaths and its junciform, photosynthetic stems arise in groups from its horizontal and highly branched rhizome (Fig. 4.1). Flowers develop alone or in

groups of 2 or 3 at the stem apex; they are hermaphroditic, narrow at their base, insect pollinated, and show 6 tepals of 15–20 × 4–5 mm. The fruit is a trilocular capsule, which produces a maximum of one seed of 2–2.2 × 1.8–2 mm per locule (Rico, 2013). We selected *Aphyllanthes monspeliensis* as our study species because it is an abundant plant species in the area, it is a calcareous grassland specialist, but it is present even in rather closed-canopy forests even after more than 50 years since forest encroachment (authors' observation). Hence, it is a good model to study early indicators of decreased plant performance that would eventually trigger species extinction in the mid or long term.

Sampling design

In order to study the changes in *Aphyllanthes monspeliensis* performance along a habitat and landscape change gradient, we selected 20 sites of 200 m of diameter, which covered a wide range in historical (19–96%) and current (16–70%) grassland proportion. These sites experienced natural reforestation to varying degrees, did not contain any crop field neither in the past nor nowadays, were not burned at least in the 25 last years, and were separated at least 1 km to ensure data independence. The selection was made on basis of historical (1956) and current (2009) orthophotomaps and preliminary fieldwork.

At each of the 20 sites, the flowering state (flowering/non-flowering) of around 50 individuals (whenever possible, with a minimum of 37) was assessed in June–August 2012. Individuals form well-delimited tussocks that are easily recognisable. Alongside, up to 20 flowering individuals distributed throughout the site were sampled at each site in order to obtain stem number, individual size (area of an ellipse taking length and width of the tussock), flowers per stem and seeds per flower (seed set), as measures of plant performance in response to habitat and connectivity changes. Stem number, individual size, and number of flowers per stem were log-transformed in order to improve normality and homoscedasticity. Only flowering individuals were sampled to assess plant performance to ensure enough individuals per site were available for the analyses. One of the 20 sites was discarded because most plants showed a completely dead aerial part, probably as the combined result of low spring precipitation in 2012 (45 mm, while the climatic mean was 136.6 ± 79.7 [mean ± SD]; see Ogaya *et al.*, 2014, for details on meteorological data) and shallow soil (12 cm). A total of 329 flowering individuals of *Aphyllanthes monspeliensis* were sampled across the 19 sites, but since some capsules were partly eaten or broken, for nine individuals without complete capsules available seed set could not be estimated and they were excluded from the analysis of this variable.

In addition to the measures of plant performance, in 13 plots of 3 m of diameter distributed
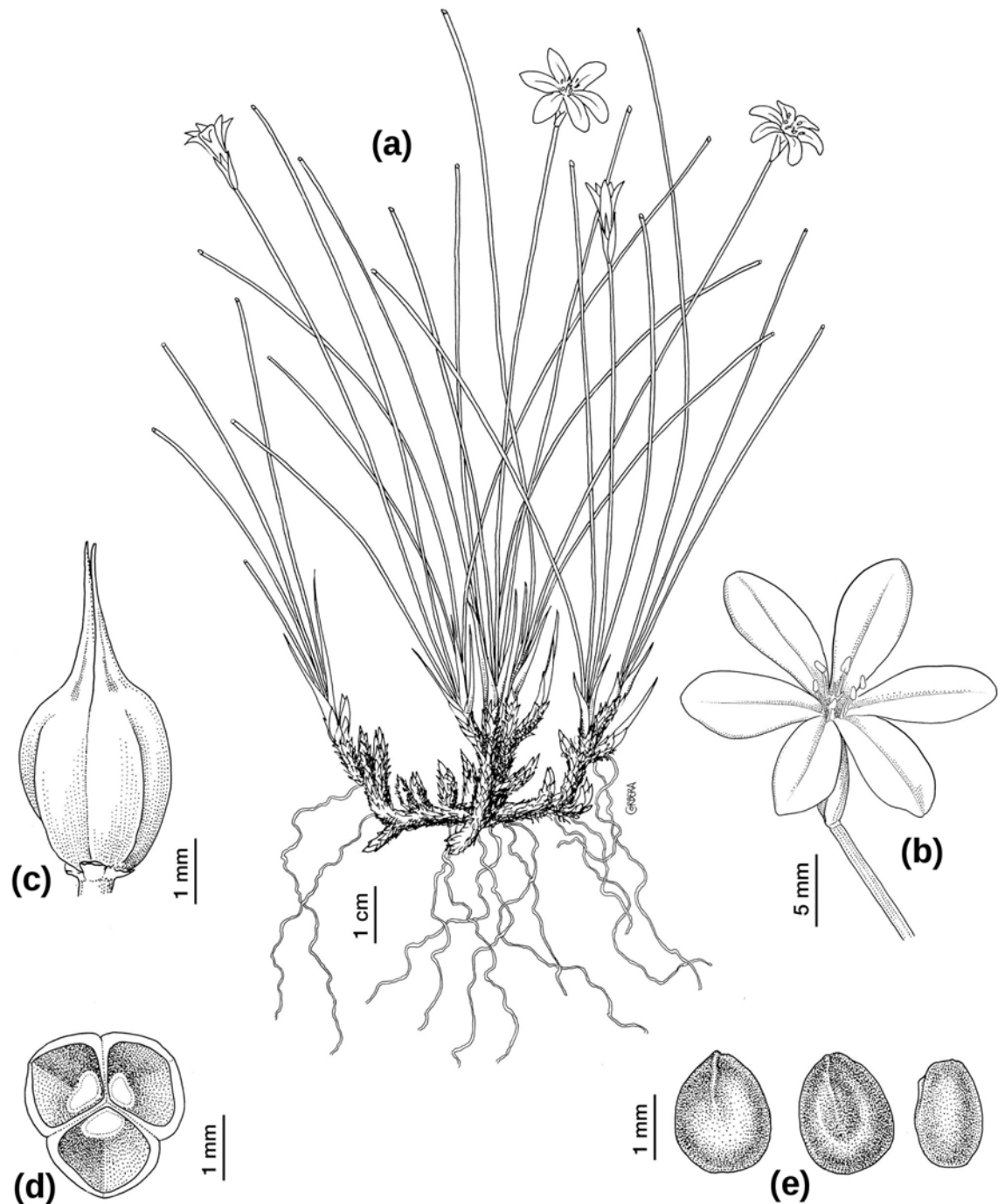
Fig. 4.1 *Aphyllanthes monspeliensis* morphology; (a) part of an individual, showing junciform stems arising in groups from its horizontal, branched rhizome; (b) flower; (c) capsule; (d) cross-section of a capsule, showing its three locules; (e) seeds.

in a regular grid at each site, the presence or absence of *Aphyllanthes monspeliensis* was recorded in the previous year (see Chapter 1), and the frequency for each site was calculated, from 0 to 13, in order to test for the existence of an extinction debt for this species. Moreover, with the aim of investigating belowground changes in plant performance, five individuals were taken from the field in six of the 19 sites, and cleaned in the laboratory. Rhizome branching intensity (number of ramifications per node) and internode length were taken as the mean of 20 measurements in each of these collected plants. Then, aerial (stems) and belowground (rhizome and roots) structures of these plants were separated and oven-dried at 80 °C during 24 h, and vegetative aboveground (stems after removing flowers)/belowground dry weight ratio was then obtained for each plant. All three plant measures were log-transformed in order to improve normality.

Environmental variables

In order to investigate the effects of forest encroachment and connectivity loss on the vegetative, flowering and fructification performance of *Aphyllanthes monspeliensis*, several landscape and habitat drivers were calculated. Tree cover was obtained for individual plants, while soil measures, grazing and habitat (grassland) amount in the landscape were obtained at site scale. No measure of tree cover at site scale was used when the focus were not individuals, because it showed a high negative correlation with grassland connectivity ($r < -0.7$).

For each of the up to 20 flowering individuals sampled at each site, three variables related to tree cover were obtained: (1) current tree cover, assessed in a circle of 5-m radius around the individual, from a combination of reclassified orthophotomaps and land-cover layers of 2009 (see Chapter 1, for details); (2) historical tree cover in the same circle, from a combination of reclassified orthophotomaps and land-cover layers of 1956; and (3) tree cover change (current minus historical tree cover proportion). In addition, historical and current grassland proportion in the landscape (500-m radius) was also obtained from the same maps for each site. Soil pH and clay proportion at each site were calculated as the mean of 13 soil samples up to a depth of 10 cm. Soil depth and grazing were the mean of 13 measures per site. Grazing was assessed as absent (0), only one sign (1) or more than one sign (2) at each of the measure points, and taken as numeric.

Statistical analyses

To identify the existence of an extinction debt for *Aphyllanthes monspeliensis* after more than 50 years of land-use change, we related the present frequency of the species at sites

with current and historical grassland connectivity in a linear model, including also potential confounding environmental factors (soil characteristics and grazing). The relative flowering frequency of the species at each of the 19 sites was investigated in relation to grassland connectivity, soil characteristics and grazing. The proportion of flowering individuals was high, 0.83 ± 0.13 (mean $\pm$ SD), but it showed variability. This analysis was conducted using a generalised linear model with binomial error with historical and current are and connectivity and site environmental characteristics as predictors, in R programming language (R Development Core Team, 2014). Since overdispersion was found, the model was constructed again using a quasi-binomial family to overcome the overdispersion problem.

Then, for each of the individual-level measures (i.e. stem number, individual size, flowers/stem and seeds/flower), a linear mixed model with current and historical grassland area and connectivity, site environmental variables and historical and current tree cover as fixed factors and site as random factor was conducted using *lme* function in the *nlme* R package (Pinheiro *et al.*, 2014). For stem number, individual size was also included as a predictor to control for it. A model selection using *dredge* function in the *MuMIn* R package (Bartoń, 2014) was performed for each of the saturated models with AICc as the rank criterion (QAICc for quasi-binomial models), and for each case the best model with all the variables being significant at $P = 0.05$ was presented. When ranking models that include random effects, the fitting method used was maximum likelihood (ML), as comparisons between models that vary in their fixed effects are not valid when they are fitted by restricted maximum likelihood (REML) (Weiss, 2005). The final models, however, were fitted by restricted maximum likelihood (REML). Finally, three sets of linear mixed models with site as random factor were constructed to relate belowground measures (rhizome branching intensity, rhizome internode length and aboveground/belowground dry weight ratio) to historical tree cover, current tree cover and tree cover change. For each set, the best significant model, ranked by AICc, was chosen.

Moreover, in order to estimate the variance accounted by each model, adjusted R^2, R^2_{D,γ_P} (which behaves satisfactorily when overdispersion is present; Heinzl & Mittlböck, 2003), and the adjusted likelihood-ratio based pseudo-R^2 (Magee, 1990) were calculated for linear models, generalised linear models and general linear mixed models, respectively.

4.3   Results

The model for the frequency of *Aphyllanthes monspeliensis* accounted for 40% of the variance. The only significant correlate of this model was the historical grassland cover in

the landscape, positively correlated with plant frequency (Table 4.1). In contrast, current grassland cover was not correlated with species frequency (Fig. 4.2). Hence, sites with higher grassland proportion in the landscape 50 years ago still harbour larger populations of *Aphyllanthes monspeliensis*. As for flowering frequency, the selected model accounted for 34% of the variance. It only included soil pH as significant variable, showing a positive association with flowering frequency.

The model for individual size (area) accounted for a small proportion of the variance (6%), and only included current tree cover as significant variable, with a negative effect, besides a significant site effect (Table 4.2). The model for stem number accounted for 64% of the variance, but it only included individual size (with positive association) and none of the environmental factors as predictors, although a significant site effect was found. The model for flowers per stem accounted, again, for a small proportion of the variation (9%), and included current connectivity, which was negatively correlated, and soil pH, which showed a positive effect. The model for seeds per flower accounted for 22% of the variance and was positively associated with current tree cover and historical connectivity, in addition to a significant site effect. However, no effects of grazing nor soil texture or depth were found for any of the vegetative or reproductive performance traits. Grazing differences between sites are probably small and not consistent through time, because few livestock grazing occurs nowadays. As for soil variables, only pH appeared as an important factor. Clay proportion presents little variation between sites (0.25 ± 0.07; mean ± SE), while soil depth shows high local variability and the site mean may not correlate to water availability for the sampled individual plants.

None of the tree cover measures predicted either branching intensity or internode length of *Aphyllanthes monspeliensis* rhizomes (Table 4.3). Nevertheless, the aboveground/belowground biomass ratio was predicted by tree cover change (with 21% of the variance explained): plants that experienced a greater increase in tree cover show lower stem biomass in relation to rhizome biomass.

4.4 Discussion

The study provides evidence of an extinction debt for the long-lived common plant *Aphyllanthes monspeliensis* in Mediterranean grasslands following more than 50 years of forest encroachment, using historical and current landscape characteristics, one of the approaches suggested by Kuussaari *et al.* (2009). The species frequency in the study sites was significantly and positively associated to historical grassland cover in the landscape, but

Table 4.1 Models for plant frequency and flowering frequency at sites. Standardised coefficients and their standard error are given for all the variables included in the model. Total adjusted R^2 for present frequency model and R^2_{D,γ_P} for flowering frequency model are also shown.

	Frequency (n=19)	Flowering frequency (n=19)
Proportion of historical grassland in the landscape	0.66 ± 0.18	
Proportion of current grassland in the landscape		
Soil depth		
Soil pH		0.03 ± 0.01
Clay proportion		
Grazing		
Total R^2	0.40	0.34
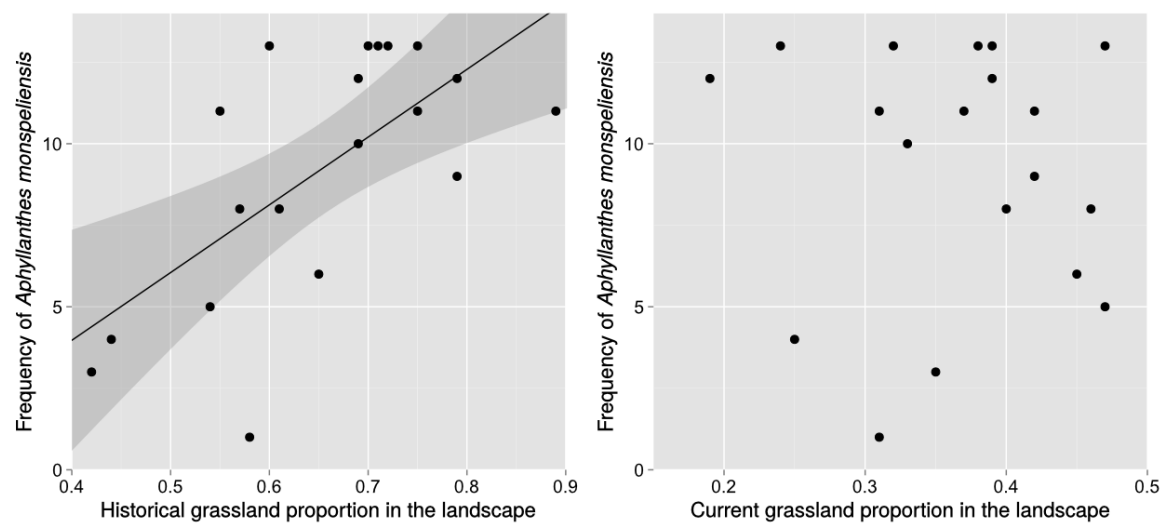
Fig. 4.2 Relationship between *Aphyllanthes monspeliensis* frequency at each of the 19 sites and historical and current grassland connectivity. Historical connectivity explains 40% of the variance in current plant frequency, while current connectivity shows no relationship with it. Regression line and confidence intervals at 95% are shown for the significant model.

Table 4.2 General linear mixed models (LMMs) for stem number, size, flowers/stem and seed set (seeds/flower) at individual level and site as random factor. Standardised coefficients and their standard error for all the selected variables and total adjusted likelihood-ratio based pseudo-R^2 of the model are presented.

	Stem number (n=329)	Size (area) (n=329)	Flowers/stem (n=329)	Seeds/flower (n=320)
Historical tree cover				
Current tree cover		–0.17 ± 0.06		0.16 ± 0.06
Proportion of historical grassland in the landscape				0.31 ± 0.11
Proportion of current grassland in the landscape			–0.25 ± 0.07	
Soil depth				
Soil pH			0.17 ± 0.07	
Clay proportion				
Grazing				
Individual size[a]	0.75 ± 0.03			
Total R^2	0.64	0.06	0.09	0.22

[a]Only for stem number, to control for area.

Table 4.3 General linear mixed models (LMMs) for aboveground/belowgroud biomass ratio, branching intensity and internode length at individual level and site as random factor. Standardised coefficients and their standard error for all the selected variables and total adjusted likelihood-ratio based pseudo-R^2 of the model are presented.

	Aboveground/belowground biomass ratio (n=30)	Branching intensity (n=30)	Internode length (n=30)
Current tree cover			
Tree cover change	–0.43 ± 0.17		
Total R^2	0.21	0	0

not with that of current grasslands (Fig. 4.2). Similarly, previous studies found an extinction debt for grassland specialists at the community level in these encroached Mediterranean grasslands (Guardiola *et al.*, 2013; Chapter 1). The main contribution of our study is the focus on how vegetative and reproductive indicators of species fitness respond to habitat and landscape factors associated to forest encroachment. Changes in these indicators, in turn, potentially affect the species demography and the likelihood of species persistence. Thus, changes in fitness associated to forest encroachment confirm the extinction risk of remnant populations showing an extinction debt (Adriaens *et al.*, 2009; Takkis *et al.*, 2013).

We show a negative impact of local habitat deterioration (i.e. increased tree cover and soil acidification) on plant vegetative performance and flowering, indicating population decay and confirming that extinction debt really occurs for this species after forest encroachment. Moreover, historical connectivity was positively related with current seed set, suggesting that genetic diversity and entomophilous pollination are still maintained thanks to large population sizes in habitats that showed high connectivity in the past. However, forestation of the current landscape also promoted flowering, and tree cover positively influenced seed set. Therefore, forest encroachment in these Mediterranean grasslands might exert contrasting effects on the demography of grassland specialists. On the one hand, connectivity loss, light competition and soil acidification threaten, respectively, seed production, vegetative performance and flowering of *Aphyllanthes monspeliensis*. On the other hand, a forested landscape and increased tree cover probably reduce summer water stress for understorey plants and avoid seed and flower abortion to some extent. These contrasting effects of habitat loss and deterioration suggest that, under the dry and hot summers prevailing at our sites, open forests might benefit plant fitness more than grasslands. However, further forest encroachment would continue decreasing both solar radiation under the tree canopy and soil pH, thus threatening even more survival and reproduction of grassland specialists as *Aphyllanthes monspeliensis*.

Local habitat quality (i.e. soil pH) was related with both the relative flowering frequency and flowers per stem of flowering individuals of *Aphyllanthes monspeliensis*. Soil pH, in turn, was negatively correlated with forest cover in the site ($r = -0.58$), suggesting that forest conditions that enhance the accumulation of organic matter result in soil acidification which, in turn, negatively affects fitness of this calcicolous species. Moreover, individuals were smaller under suboptimal light conditions, as found for various grassland plants (e.g. Sibbald *et al.*, 1991; Peri *et al.*, 2007), and they showed decreased stem biomass in relation to belowground biomass, contrasting with the etiolation found in other species (Kephart & Buxton, 1993). Since no change in stem density was found (stem number only depends on area), it suggests a clustered necrosis of rhizome areas, which likely lose stems but

persist belowground. Moreover, rhizome architecture remained fixed even in contrasting habitat conditions, contrary to what has been found in other rhizomatous species (De Kroon & Hutchings, 1995), although high plasticity between genets within a species can occur (Skálová *et al.*, 1997). Tree cover and a forested landscape, however, enhanced seed set (mean number of seeds per flower) and flowering, probably because shadow provides a moister environment than open grassland (Williams *et al.*, 1993; Payne & Norton, 2011), and likely promotes flower production (Akhalkatsi & Lösch, 2005) and prevents the abortion of flowers and seeds in drought conditions (Stephenson, 1981; Lee & Bazzaz, 1982).

As for landscape effects, seed set was promoted by historical grassland connectivity, which is likely to be linked to genetic diversity in this long-lived species, since population size is still dependent on historical landscape and because genetic drift acts more slowly in plants with long generation times (Young *et al.*, 1996). Thus, large population size and probably high genetic diversity would enhance reproductive fitness (Leimu *et al.*, 2006; Takkis *et al.*, 2013). Although positive effects of current connectivity on pollinator activity and seed set have been reported (Ghazoul, 2005; Schmucki & de Blois, 2009), currently isolated populations of *Aphyllanthes monspeliensis* would still benefit from maintained population size to attract pollinators (Lienert, 2004; but see Adriaens *et al.*, 2009).

The contrasting consequences of forest encroachment found for *Aphyllanthes monspeliensis* reproductive performance in the studied area suggest that, in a context of climate change, with increased temperature and summer drought in the Mediterranean Basin (IPCC, 2014), these populations might show lowered flowering and seed production in open grasslands. Further forestation, however, would increase light competition and soil acidification, which would threaten individual survival and also flowering. Moreover, habitat deterioration probably limits recruitment, since the species forms a transient seed bank germinating in the first favourable season after dispersal (Paula & Pausas, 2009) and seed germination rate was found to be low in similar grasslands (Ninot *et al.*, 2008). Other studies previously found comparable contrasting effects of habitat deterioration on different indicators of population fitness for clonal grassland plants. Adriaens *et al.* (2009) detected a positive effect of shrub cover on the number of flowers per plant, while shrub removal (and wood debris produced) favoured seed set and recruitment. Moreover, de Vere *et al.* (2009) found that bare soil promoted plant establishment, while flowering increased in unmanaged sites with tall vegetation.

In the case of *Aphyllanthes monspeliensis* in semi-natural Mediterranean grasslands, the death of individuals as a consequence of increased light competition and the limited recruitment would likely lead to a decrease in population size, and the chance of stochastic processes threatening population persistence (environmental stochasticity, demographic

stochasticity and genetic drift) would increase (Shaffer, 1987). Inbreeding depression, the reduction in fitness caused by the mating of related individuals, is another threat for small populations, which negatively affects flower and seed production, seed size and seed germination (Lienert, 2004, and references therein). However, ecological factors might be as important as inbreeding affecting fitness in the short term (Ellstrand & Elam, 1993). Thus, if isolation increases and plant density diminishes, pollinator efficiency might decrease (Rathcke & Jules, 1993; Ashworth *et al.*, 2004; Lienert, 2004), hampering reproductive success (Aguilar *et al.*, 2006; Aizen & Harder, 2007). Moreover, in a fragmented landscape, colonisation of new favourable habitats that could eventually appear would be limited by the relatively large ($\sim$2 mm) seed size of *Aphyllanthes monspeliensis* (Rico, 2013) and the lack of specific dispersal mechanisms (Bagaria *et al.*, 2012). Although regressive populations of long-lived plants may persist for a long period in unsuitable habitats (Eriksson, 1996), extinction debt is expected to be slowly paid if no habitat improvement occurs.

Conservation measures to allow the long-term persistence of this and likely other grassland specialists of semi-natural Mediterranean grasslands before extinction debt is paid would need to guarantee high habitat heterogeneity, by the preservation of open forests with grassland areas. This would promote light availability and high pH in gaps, but also the preservation of moisture by open forest conditions. However, since plant abundance and seed production are likely to decrease in the future even if no further forestation occurs because they depend on landscape properties that no longer exist, grassland connectivity should be enhanced by opening gaps in forested areas near persistent grassland habitat and managing them by means of livestock grazing, to promote restoration.

Discussion

General discussion

The mountains of southern Catalonia experienced more than 50 years of forest encroachment into semi-natural Mediterranean calcareous grasslands that led to tree cover increase and landscape forestation at the expense of traditionally managed grasslands. However, there is a lack of integrated views of the processes that are set in motion after these kinds of habitat changes, processes that shape communities over time (Jackson & Sax, 2010). The main aim of this thesis was to address the effects of forest encroachment into semi-natural grasslands on community assembly, time lags in extinctions and colonisations and their drivers and mechanisms.

Nowadays, plant communities of these Mediterranean habitats are still partly contingent on historical habitat and landscape characteristics. Both an extinction debt for grassland specialists and a colonisation credit for forest specialists of more than 50% were found in previous grasslands that are forested nowadays, indicating that the extinction of more than half of the grassland species and the colonisation of a number of forest species higher than the half of those already present in these habitats are expected. However, high species replacement already occurred following habitat change, yet little richness change for the whole community was found. At the same time, idiosyncratic grassland specialists' extinctions and forest specialists' colonisations resulted in opposite richness gradients of habitat specialists of the former and the new habitat that drove species replacement for the whole community. In addition, although some grassland specialists appear to be negatively affected by low current grassland connectivity, no clear role of plant traits was found in mediating compositional change of this group following forest encroachment. In contrast, other grassland specialists like *Aphyllanthes monspeliensis*, do not show a clear association with current connectivity. For *Aphyllanthes monspeliensis*, which is a common grassland specialist, an extinction debt was revealed by a clear relationship with historical grassland connectivity and by decreased vegetative and reproductive performance after habitat deterioration. However, the initial stages of forest encroachment might exert a transient positive effect on reproductive output for this species, which would be easily countered by further forest encroachment.

Both extinction debts and colonisation credits persist after more than 50 years of woody encroachment of previous semi-natural grasslands in the study area. Delayed extinctions have been identified in other calcareous grasslands that experienced habitat and connectivity loss (e.g. Helm *et al.*, 2006; Krauss *et al.*, 2010; Cousins & Vanhoenacker, 2011). Guardiola *et al.* (2013) found extinction debt in the same area at a larger scale (tens or hundreds of metres), but not at a smaller scale (less than a metre). However, they used a grassland patch approach, and the scale I used was in between (metres), which would account for the

contrasting results since the likelihood of detecting an extinction debt is scale-dependent. Delayed colonisations of vascular plants in the context of woody encroachment into semi-natural grasslands have not been previously assessed, but they have been identified in other studies on novel woody habitats or restored grasslands (Pierik *et al.*, 2010; Piqueray *et al.*, 2011; Başnou *et al.*, in press). In the studied Mediterranean habitats, as a result of delayed extinctions and colonisations after generalised forest encroachment into grasslands, a decline of grassland species and an increase of forest species are expected to occur (Debussche *et al.*, 1999).

Light availability and soil pH were the main drivers for calcareous grassland specialists' richness. The same drivers enhanced vegetative performance and flowering of a grassland specialist showing an extinction debt, *Aphyllanthes monspeliensis*. Thus, tree cover increase and soil acidification that result from the increase in soil organic matter after forest encroachment, could have already caused local extinctions of some grassland specialists, while others might be showing a slow decay that will ultimately result in more extinctions that will pay the extinction debt. Moreover, a rescue effect (Hanski, 1999) from nearby grasslands might also contribute to maintain populations in a deteriorated habitat (Mouquet & Loreau, 2003; Leibold *et al.*, 2004), such as grassland specialists in wooded grasslands. For *Aphyllanthes monspeliensis*, seed set was enhanced by historical connectivity, suggesting that genetic diversity and pollination are maintained thanks to a large population size (Lienert, 2004; Leimu *et al.*, 2006), which is still dependent on historical characteristics. However, in a context of climate change, some degree of forestation might enhance moisture and prevent flower and seed abortion for grassland species (Stephenson, 1981), although further forestation would probably have a strong negative impact on vegetative and reproductive performance. On the other hand, richness of forest specialists was enhanced by shadow and historical forest connectivity. Forest habitat conditions might facilitate the establishment of new forest species, but the historical effect suggests that recently established forests might act as poor seed sources and that colonisation credit can persist for a long time due to dispersal limitation (Jacquemyn *et al.*, 2003; Vellend, 2003).

Nevertheless, part of the extinction debt and colonisation credit are already paid, since transient habitats showed intermediate richness between persistent grasslands and persistent forests for both grassland and forest specialists. Thus, a species replacement of grassland species by forest species can be observed along the forestation process, with a marginally significant richness decline as an outcome of the process. This replacement was also identified when analysing β-diversity patterns along a current forestation gradient at a wider scale (hundreds of metres). The turnover β-diversity for all species was found to be mainly due to the replacement component and, at the same time, it was caused by richness gradients

of specialist species groups. An important richness gradient due to idiosyncratic grassland species extinctions and a weaker richness gradient of idiosyncratic forest species colonisations shaped species replacement for the whole community.

In addition to forest encroachment, connectivity also plays a role in community assembly. Current connectivity contributes to modulate richness gradients for both grassland and forest specialists, probably indicating the effect of nearby seed sources. Historical connectivity was also important for richness gradients of grassland specialists, suggesting again an extinction debt. Finally, idiosyncratic extinctions and colonisations regarding habitat change might rely on current landscape characteristics and geographic distance, respectively, since these were the main drivers of species replacement for habitat specialists. These analyses show that the study of β-diversity components contributed to disentangle the community assembly processes after forest encroachment, and it has also been useful in other contexts (e.g. Leprieur *et al.*, 2011; Marini *et al.*, 2013).

For a subset of common grassland specialists, community change along climatic, geographic and habitat area and connectivity gradients in a wider region was investigated, finding climatic and geographic gradients to be the main drivers of community composition, although some species were also favoured by current grassland connectivity. Again, not all grassland specialists seem to react fast to land-use change, although in this study no past effects were found and only a subset of the species was considered. Also, a wider region might introduce climatic and geographic confounding effects on community assembly.

Plant traits have been suggested to be important in mediating species extinctions after land-use change (Dupré & Ehrlén, 2002; Kahmen & Poschlod, 2004; Lindborg, 2007), but their effects are not always consistent (Tremlová & Münzbergová, 2007; Lindborg *et al.*, 2012). In the studied communities, the role of plant traits was weakly involved in mediating community change, and only tall plants and resprouting species were associated with forest encroachment. Resprouters were regionally more frequent and they might be less vulnerable to local extinctions. On the other hand, tall plants might be better competitors (Westoby, 1998) and show higher seed dispersal ability (Thomson *et al.*, 2011) than small ones, what would confer an advantage for persistence and dispersal in fragmented habitats. Other traits such as seed size or clonal growth were not related with forest encroachment in our study, although they have been related to dispersal strategy, establishment success and fecundity (Westoby, 1998), and to plant persistence (Lindborg, 2007; Saar *et al.*, 2012; but see Lindborg *et al.*, 2012), respectively.

With the studies conducted in this thesis I can conclude that, even if no further forest encroachment into the studied Mediterranean grasslands occurs, new grassland specialists' extinctions and forest specialists' colonisations will happen and species replacement leading

to community change will continue, because many plant species rely on habitat and landscape properties that no longer exist. Since this land-use change is generalised and Mediterranean grasslands are highly species rich, an important biodiversity pool is becoming endangered (Myers *et al.*, 2000). Habitat restoration would be needed to reverse this trend; extending livestock grazing in encroached grasslands would enhance habitat improvement for many grassland species and would promote grassland connectivity. Maintaining habitat heterogeneity has shown useful for persistence of some grassland species (Adriaens *et al.*, 2009; de Vere *et al.*, 2009), and in the studied area reproductive performance of *Aphyllanthes monspeliensis* also suggests that open forests with connected grassland areas might benefit some grassland plants in a context of climate change.

There is no doubt that this thesis still leaves questions about the drivers and mechanisms of community assembly and time lags in extinctions and colonisations after habitat change. More research is needed to check whether these results hold for other habitats, taxa and spatial scales. Extinction debt has been widely studied in very different systems, and the role of life-history traits in plant persistence and colonisation after habitat change has also been addressed in grasslands and forests, but there are many other issues that deserve future research. Studies investigating the existence of simultaneous extinction debt and colonisation credit after habitat change in other habitats and at other scales are needed to test for the generality of this pattern. Likewise, the population and individual-level mechanisms of extinction debt have been rarely addressed, and the assessment of vegetative and reproductive fitness for other habitat specialists with different degrees of extinction debt would be needed to elucidate strategies that increase persistence time. Moreover, the study of genetic diversity (e.g. Takkis *et al.*, 2013) and its association with functional processes like pollination (e.g. Schmucki & de Blois, 2009) have shown useful to complement plant fitness assessment in this context, although they have been rarely used so far. Beyond the fitness measures used in this thesis, investigating seed germination and plant recruitment, or conducting a population viability analysis, would improve the assessment of long-term population persistence after habitat change. In addition, the study of germination, survival, growth and reproduction of colonisers in the new habitat conditions would give more insight into the mechanisms of colonisation credit beyond dispersal limitation.

The analysis of species abundance patterns for the whole community would allow the detection of the most vulnerable and the most favoured species by forest encroachment, what would be useful for conservation purposes. Also, investigating functional and phylogenetic patterns of β-diversity and its components at the same time (Cardoso *et al.*, 2014) would allow a deeper understanding of community assembly after habitat change. The study of other plant traits such as self-compatibility and seed bank longevity might also give more

insight into mechanisms of plant persistence in the studied habitats. Finally, re-surveying the study plots approximately every 10 years would allow directly evaluating community change and the payment of extinction debt and colonisation credit after forest encroachment, without the need for using a space-for-time substitution. Additionally, the reintroduction of regular grazing in some of the study sites would allow the assessment of habitat restoration impact on the payment of extinction debt and colonisation credit compared to non-restored sites, and to evaluate the effectiveness of management practices for the conservation of semi-natural Mediterranean calcareous grasslands in the mid-term.

Conclusions

General conclusions

1. Both extinction debt and colonisation credit of more than 50% are detected after more
    than 50 years of forest encroachment into semi-natural Mediterranean calcareous
    grasslands. Thus, the extinction of more than half of the grassland species and the
    colonisation of a number of forest species higher than the half of those already present
    in wooded grasslands are expected (Chapter 1). Extinction debt was confirmed for a
    grassland specialist investigated in detail, since its abundance relies on historical but
    not on current connectivity (Chapter 4).

2. Metapopulation, population, and individual-level mechanisms of extinction debt were
    identified. Extinction debt in transient habitats is enhanced by a rescue effect from
    surrounding grasslands, which may act as propagule sources for grassland specialists
    (Chapter 1). For *Aphyllanthes monspeliensis*, a grassland specialist, extinction debt
    was maintained by a slow vegetative and reproductive (flowering) decay after grassland
    habitat deterioration, and by a positive effect of historical connectivity on seed set,
    which suggests that genetic diversity and pollination rely on large population size
    (Chapter 4).

3. Colonisation credit is probably maintained by dispersal limitation and the lack of
    seed sources of young forests, despite high current forest connectivity. Instead, high
    historical forest connectivity, resulting from the existence of older forests, contributed
    to the payment of forest species credit (Chapter 1).

4. Part of the extinction debt and colonisation credit is already paid, and community
    changes occurred after habitat change. An important species replacement along the
    forestation process is driven by grassland specialists' extinctions and forest specialists'
    colonisations. Total richness change was weaker than replacement, although it might
    be important at small spatial scale (Chapters 1, 2).

5. Grassland specialists' extinctions and forest specialists' colonisations after habitat change are idiosyncratic rather than nested. However, landscape characteristics and geographic distance might be behind this pattern of extinctions and colonisations, respectively (Chapter 2).

6. Plant traits do not largely mediate grassland specialists' extinctions after forest encroachment into Mediterranean grasslands (Chapter 3). Only tall plants and resprouting species were associated with higher persistence after forest encroachment.

7. A generalised loss of grassland specialists and gain of forest specialists will continue in the studied area even if no further forest encroachment occurs into semi-natural Mediterranean calcareous grasslands (Chapter 1). Although some degree of forestation might benefit some grassland specialists in a context of climate change, habitat restoration would be needed to preserve highly biodiverse semi-natural grasslands (Chapter 4).

Conclusions generals

1. Un deute d'extinció i un crèdit de colonització de més del 50% han estat detectats després de més de 50 anys de forestalització dels prats calcaris semi-naturals en ambients mediterranis. D'aquesta manera, s'espera l'extinció de més de la meitat de les espècies de prat i la colonització d'un nombre d'espècies de bosc superior a la meitat de les que trobem avui dia als prats emboscats (Capítol 1). El deute d'extinció ha estat confirmat per a un especialista de prat que es va investigar en detall, ja que la seva abundància depèn de la connectivitat passada però no de l'actual (Capítol 4).

2. S'han identificat mecanismes del deute d'extinció a nivell metapoblacional, poblacional i individual. El deute d'extinció en hàbitats en transició és afavorit per un efecte rescat que exerceixen els prats circumdants, els quals poden actuar com a fonts de propàguls per als especialistes de prat (Capítol 1). Per a *Aphyllanthes monspeliensis*, un especialista de prat, el deute d'extinció es manté per un lent decaïment vegetatiu i reproductiu (floració) després del deteriorament de l'hàbitat, i per un efecte positiu de la connectivitat passada en la producció de llavors, cosa que suggereix que la diversitat genètica i la pol·linització depenen d'una mida de població suficientment gran (Capítol 4).

3. El crèdit de colonització probablement es manté per una limitació en la dispersió i per la manca de fonts de llavors provinents dels boscos joves, tot i una alta connectivitat actual del bosc. A diferència d'això, una alta connectivitat passada del bosc, resultat de l'existència de boscos més vells, contribueix al pagament del crèdit per a les espècies de bosc (Capítol 1).

4. Part del deute d'extinció i del crèdit de colonització ja s'han pagat, i s'han donat canvis en les comunitats després del canvi d'hàbitat. Un important recanvi d'espècies al llarg del gradient de forestalització està impulsat per les extincions dels especialistes de prat i les colonitzacions dels especialistes de bosc. El canvi de riquesa total és més feble que el recanvi d'espècies, tot i que pot arribar a ser important a una escala espacial petita (Capítols 1, 2).

5. Les extincions dels especialistes de prat i les colonitzacions dels especialistes de bosc després del canvi d'hàbitat són idiosincràtiques més que no pas aniuades. No obstant, les característiques del paisatge i la distància geogràfica podrien estar darrere d'aquest patró d'extincions i colonitzacions, respectivament (Capítol 2).

6. Els atributs de les plantes no medien de forma important les extincions dels especialistes de prat després de la forestalització dels prats mediterranis (Capítol 3). Només les plantes altes i les espècies rebrotadores estan associades a una major persistència després de la forestalització.

7. Es continuarà donant una pèrdua d'especialistes de prat i un guany d'especialistes de bosc de forma generalitzada a l'àrea d'estudi, fins i tot si la forestalització dels prats calcaris semi-naturals mediterranis s'aturés (Capítol 1). Tot i que un cert grau de forestalització podria arribar a ser beneficiós per a alguns especialistes de prat en un context de canvi climàtic, la restauració de l'hàbitat seria necessària per a preservar aquests prats semi-naturals amb tanta biodiversitat (Capítol 4).

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Appendix A

Assessing coexisting plant extinction debt and colonisation credit in a grassland-forest change gradient

Table A.1 List of plant species found in the survey. The classification in grassland specialists and forest specialists was performed using regional floras (Bolòs *et al.*, 2005; Rivas-Martínez *et al.*, 2001) and expert advice. In addition to the plants in the list, two *Apiaceae*, two *Asteraceae*, one *Caryophyllaceae*, one *Cruciferae*, and four *Poaceae* species were found during the survey and included in the analyses, but we were not able to identify them to species or genus level.

Taxa	Grassland specialist	Forest specialist
Acer opalus Mill.		×
Aegilops triuncialis L.		
Aethionema saxatile (L.) R. Br.		
Allium senescens L.	×	
Allium sp. L.		
Althaea hirsuta L.	×	
Amelanchier ovalis Medik.		×
Anemone hepatica L.		×
Anthericum liliago L.	×	
Anthyllis montana L.	×	
Anthyllis vulneraria L.	×	
Aphyllanthes monspeliensis L.	×	
Aquilegia vulgaris L.		×
Arabis auriculata Lam.	×	
Arabis hirsuta (L.) Scop.	×	
Arabis scabra All.	×	
Arctostaphylos uva-ursi (L.) Spreng.	×	
Arenaria conimbricensis Brot.	×	
Arenaria tetraquetra L.	×	
Argyrolobium zanonii (Turra) P. W. Ball	×	
Arrhenatherum elatius (L.) P. Beauv. ex J. et C. Presl	×	
Asperula cynanchica L.	×	
Asphodelus cerasiferus Gay	×	
Asplenium adiantum-nigrum L.		×
Asplenium trichomanes L.		
Asterolinon linum-stellatum (L.) Duby in DC.	×	
Atractylis humilis L.	×	
Avenula pratensis (L.) Dumort.	×	
Biscutella laevigata L.	×	
Blackstonia perfoliata (L.) Huds.		
Brachypodium distachyon (L.) P. Beauv.	×	

Taxa	Grassland specialist	Forest specialist
Brachypodium phoenicoides (L.) Roem. et Schultes	×	
Brachypodium retusum (Pers.) P. Beauv.	×	
Brachypodium sylvaticum (Huds.) P. Beauv.		×
Briza media L.		
Bromus erectus Huds.	×	
Bupleurum baldense Turra	×	
Bupleurum fruticescens L.	×	
Bupleurum fruticosum L.		×
Bupleurum rigidum L.	×	
Buxus sempervirens L.		×
Campanula persicifolia L.		×
Campanula rotundifolia L.	×	
Campanula speciosa Pourr.		
Campanula trachelium L.		×
Carduncellus monspelliensium All.	×	
Carduus nigrescens Vill.	×	
Carex flacca Schreber		
Carex halleriana Asso	×	
Carex humilis Leysser	×	
Carlina vulgaris L.	×	
Centaurea linifolia L.	×	
Centaurium quadrifolium (L.) G. López et Ch. E. Jarvis	×	
Cephalanthera longifolia (L.) Fritsch		×
Cerastium pumilum Curtis	×	
Ceterach officinarum DC. in Lam. et DC.		
Chaenorhinum origanifolium (L.) Kostel.		
Cistus albidus L.		
Cistus clusii Dunal	×	
Clematis flammula L.		×
Clematis vitalba L.		×
Conopodium majus (Gouan) Loret in Loret et Barrandon	×	
Convolvulus arvensis L.		
Convolvulus lanuginosus Desr.	×	
Coris monspeliensis L.	×	
Coronilla emerus L.		×
Coronilla minima L.	×	
Crataegus monogyna Jacq.		×

Taxa	Grassland specialist	Forest specialist
Crepis albida Vill.	×	
Crucianella angustifolia L.	×	
Cruciata glabra (L.) Ehrend.		×
Crupina vulgaris Cass.	×	
Cytisophyllum sessilifolium (L.) O. Lang		×
Daphne gnidium L.		
Daphne laureola L.		×
Dianthus pungens L.	×	
Dipcadi serotinum (L.) Medik.	×	
Dorycnium pentaphyllum Scop.	×	
Echinops ritro L.	×	
Echium vulgare L.		
Epipactis sp. Zinn		×
Erica multiflora L.	×	
Erinacea anthyllis Link	×	
Eryngium campestre L.	×	
Erysimum grandiflorum Desf.	×	
Euphorbia amygdaloides L.		×
Euphorbia exigua L.	×	
Euphorbia flavicoma DC.	×	
Euphorbia minuta Loscos	×	
Euphorbia nevadensis Boiss. et Reut.	×	
Euphorbia nicaeensis All.	×	
Euphorbia serrata L.	×	
Festuca gr. *ovina* L.	×	
Festuca rubra L.		
Filipendula vulgaris Moench	×	
Fritillaria pyrenaica L.	×	
Fumana ericoides (Cav.) Gand.	×	
Galium parisiense L.	×	
Galium pumilum Murray		
Galium verum L.	×	
Genista hispanica L.	×	
Genista scorpius (L.) DC. in Lam. et DC.	×	
Geranium robertianum L.		×
Geum sylvaticum Pourr.		×
Gladiolus illyricus Koch	×	

Taxa	Grassland specialist	Forest specialist
Globularia vulgaris L.	×	
Gymnadenia conopsea (L.) R. Br.	×	
Hedera helix L.		×
Helianthemum apenninum (L.) Mill.	×	
Helianthemum hirtum (L.) Mill.	×	
Helianthemum ledifolium (L.) Mill.	×	
Helianthemum oelandicum (L.) DC. in Lam. et DC.	×	
Helichrysum stoechas (L.) Moench	×	
Helleborus foetidus L.		×
Hieracium gr. *murorum* L.		×
Hieracium pilosella L.	×	
Hippocrepis comosa L.	×	
Hornungia petraea (L.) Rchb.	×	
Ilex aquifolium L.		×
Inula montana L.	×	
Iris lutescens Lam.	×	
Jasminum fruticans L.		
Jasonia tuberosa (L.) DC.		
Juniperus communis L.		×
Juniperus oxycedrus L.		
Juniperus phoenicea L.		
Knautia arvensis (L.) Coult.	×	
Koeleria vallesiana (Honck.) Gaudin	×	
Lactuca perennis L.		
Laserpitium gallicum L.	×	
Lathyrus filiformis (Lam.) Gay	×	
Lavandula angustifolia Mill.	×	
Lavandula latifolia Medik.	×	
Leontodon taraxacoides (Vill.) Mérat	×	
Leuzea conifera (L.) DC. in Lam. et DC.	×	
Lilium martagon L.		×
Linaria supina (L.) Chaz.	×	
Linum narbonense L.	×	
Linum tenuifolium L.	×	
Lithospermum fruticosum L.	×	
Lonicera etrusca Santi		×
Lonicera implexa Aiton		×

Taxa	Grassland specialist	Forest specialist
Lonicera xylosteum L.		×
Lotus corniculatus L.	×	
Melica ciliata L.	×	
Micropus erectus L.	×	
Micropyrum tenellum (L.) Link		
Moehringia pentandra Gay		
Muscari neglectum Guss. ex Ten.	×	
Narcissus assoanus Dufour	×	
Odontides viscosus (L.) Clairv.		
Ononis minutissima L.	×	
Ononis spinosa L.		
Ophrys fusca Link	×	
Ophrys scolopax Cav.	×	
Orchis maculata L.		
Orobanche amethystea Thuill	×	
Orobanche gracilis Sm.	×	
Orobanche latisquama (F. W. Schultz) Batt.	×	
Orobanche sp. L.		
Petrorhagia prolifera (L.) P. W. Ball et Heywood	×	
Phillyrea latifolia L.		×
Phlomis lychnitis L.	×	
Phyteuma orbiculare L.	×	
Picris hieracioides L.	×	
Pinus halepensis Mill.		×
Pinus nigra Arnold		×
Pinus pinaster Aiton		×
Pinus pinea L.		×
Pinus sylvestris L.		×
Plantago lanceolata L.	×	
Platanthera bifolia (L.) Rich.		
Polygala calcarea F. W. Schultz	×	
Polygala rupestris Pourr.	×	
Polygala vulgaris L.	×	
Polygonatum odoratum (Mill.) Druce		×
Polypodium vulgare L.		
Potentilla hirta L.		
Potentilla neumanniana Rchb.	×	

Taxa	Grassland specialist	Forest specialist
Primula veris L.		×
Prunella grandiflora (L.) Scholler		
Prunus mahaleb L.		×
Prunus spinosa L.		
Pteridium aquilinum (L.) Kuhn		
Pyrola chlorantha Sw.		×
Quercus × *cerrioides* Willk. et Costa		×
Quercus coccifera L.		×
Quercus faginea Lam.		×
Quercus ilex L.		×
Ranunculus gramineus L.	×	
Rhamnus alaternus L.		×
Rhamnus saxatilis Jacq.		
Rosa pimpinellifolia L.		
Rosa sp. L.		
Rosmarinus officinalis L.	×	
Rubia peregrina L.		
Rubus ulmifolius Schott		
Ruscus aculeatus L.		×
Salvia officinalis L.	×	
Sanguisorba minor Scop.	×	
Santolina chamaecyparissus L.	×	
Saponaria ocymoides L.		
Satureja montana L.	×	
Scabiosa columbaria L.	×	
Scorzonera angustifolia L.	×	
Scorzonera hispanica L.	×	
Sedum acre L.	×	
Sedum album L.	×	
Sedum sediforme (Jacq.) Pau	×	
Senecio doronicum (L.) L.	×	
Serratula nudicaulis (L.) DC. in Lam. et DC.	×	
Sideritis hirsuta L.		
Silene nutans L.		
Smilax aspera L.		×
Solidago virgaurea L.		
Sorbus aria (L.) Crantz		×

Taxa	Grassland specialist	Forest specialist
Stachys officinalis (L.) Trevis.		
Staehelina dubia L.	×	
Stipa offneri Breistr.	×	
Stipa pennata L.	×	
Tanacetum corymbosum (L.) Schultz Bip.		
Taraxacum serotinum (Waldst. et Kit.) Poiret in Lam.	×	
Taxus baccata L.		×
Teucrium botrys L.	×	
Teucrium chamaedrys L.		×
Teucrium polium L.	×	
Thalictrum tuberosum L.	×	
Thesium humifusum DC.	×	
Thymelaea tinctoria (Pourr.) Endl.	×	
Thymus serpyllum L.	×	
Thymus vulgaris L.	×	
Trinia glauca (L.) Dumort.	×	
Tulipa sylvestris L.	×	
Ulex parviflorus Pourr.		
Viburnum lantana L.		×
Viburnum tinus L.		×
Vicia cracca L.		
Vicia sepium L.		×
Viola alba Besser		×
Viola rupestris F. W. Schmidt	×	
Viola willkommi Roem.		×

Table A.2 Basic descriptive statistics for response variables (plant richness for the different groups: all species, grassland specialists, forest specialists, other species), local and landscape predictors, and historical and current tree covers, by plot category. Mean and standard deviation for continuous variables, and counts for each level for factors are given.

	Persistent grasslands	Wooded grasslands	Persistent forests
All species' richness	31.3 ± 7.6	28.0 ± 9.3	22.7 ± 6.6
Grassland specialists' richness	26.6 ± 8.0	18.3 ± 10.6	8.5 ± 5.7
Forest specialists' richness	2.7 ± 2.4	6.5 ± 3.7	10.5 ± 3.7
Other species' richness	2.0 ± 1.5	3.2 ± 1.7	3.8 ± 1.7
Solar radiation availability ratio	5.85 ± 13.13	1.64 ± 5.47	0.55 ± 2.20
Soil depth (cm)	15.78 ± 10.46	21.13 ± 11.38	21.96 ± 11.37
Soil pH	7.72 ± 0.55	7.38 ± 0.73	7.18 ± 0.72
Clay ratio	0.32 ± 0.21	0.38 ± 0.19	0.43 ± 0.23
Historical grassland/forest ratio	5.15 ± 3.28	4.47 ± 3.72	1.99 ± 1.62
Current grassland/forest ratio	0.79 ± 0.21	0.57 ± 0.21	0.57 ± 0.13
Historical grassland availability ratio	2.46 ± 1.35	2.59 ± 1.72	1.27 ± 0.73
Current grassland availability ratio	0.73 ± 0.14	0.55 ± 0.19	0.53 ± 0.12
Historical forest availability ratio	0.22 ± 0.12	0.33 ± 0.21	0.57 ± 0.24
Current forest availability ratio	1.29 ± 0.37	1.95 ± 0.92	1.64 ± 0.39
Historical tree cover ratio	0.09 ± 0.17	0.24 ± 0.27	11.98 ± 26.90
Current tree cover ratio	0.48 ± 0.28	3.82 ± 2.87	4.31 ± 2.01
Grazing (Yes/No)	Yes: 25 / No: 58	Yes: 30 / No: 118	Yes: 4 / No: 24

Table A.3 Predicted values by general linear mixed models (LMMs) of species richness between plot categories for each species group. Estimates and standard errors (SE) are given. Each row corresponds to a model for a species group (all species, grassland specialists, forest specialists and other species).

	Persistent grasslands	Wooded grasslands	Persistent forests
All species	29.75 ± 1.50	28.27 ± 1.36	25.38 ± 2.00
Grassland specialists	22.66 ± 1.92	19.39 ± 1.82	13.98 ± 2.30
Forest specialists	4.45 ± 0.73	6.04 ± 0.70	7.85 ± 0.84
Other species	2.48 ± 0.28	2.89 ± 0.26	3.81 ± 0.38
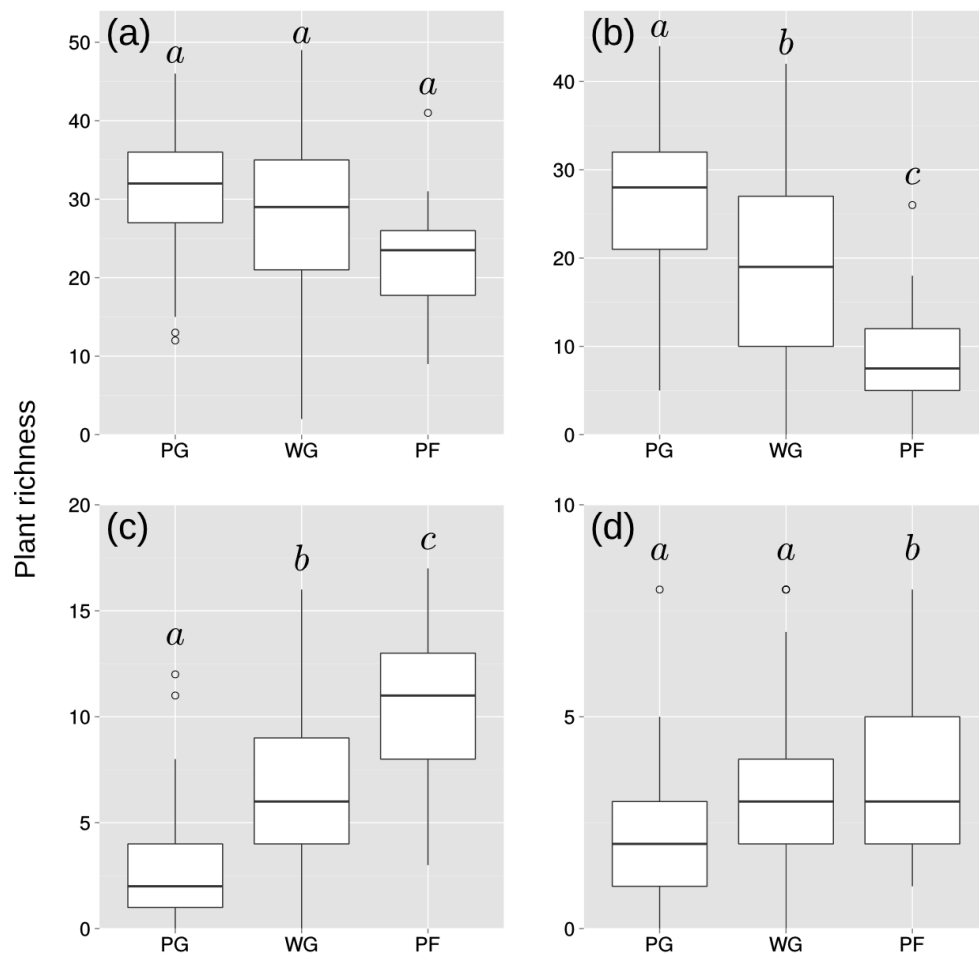
Fig. A.1 Box and whisker plots for observed species richness at sampling plots of 3 m of diameter for the different species groups between sampling plot categories: PG, persistent grasslands; WG, wooded grasslands, and PF, persistent forests. The main horizontal line shows the median, the bottom and the top of the box are the first and third quartiles, respectively, whiskers show extreme values or 1.5 times the inter-quartile range (whichever is smaller), and points are potential outliers. Letters a, b and c show significantly different species richness between sampling plot categories within each species group, according to post-hoc Tukey tests. Plot (a) shows species richness for all species, plot (b) for grassland specialists, plot (c) for forest specialists, and plot (d) for other species.
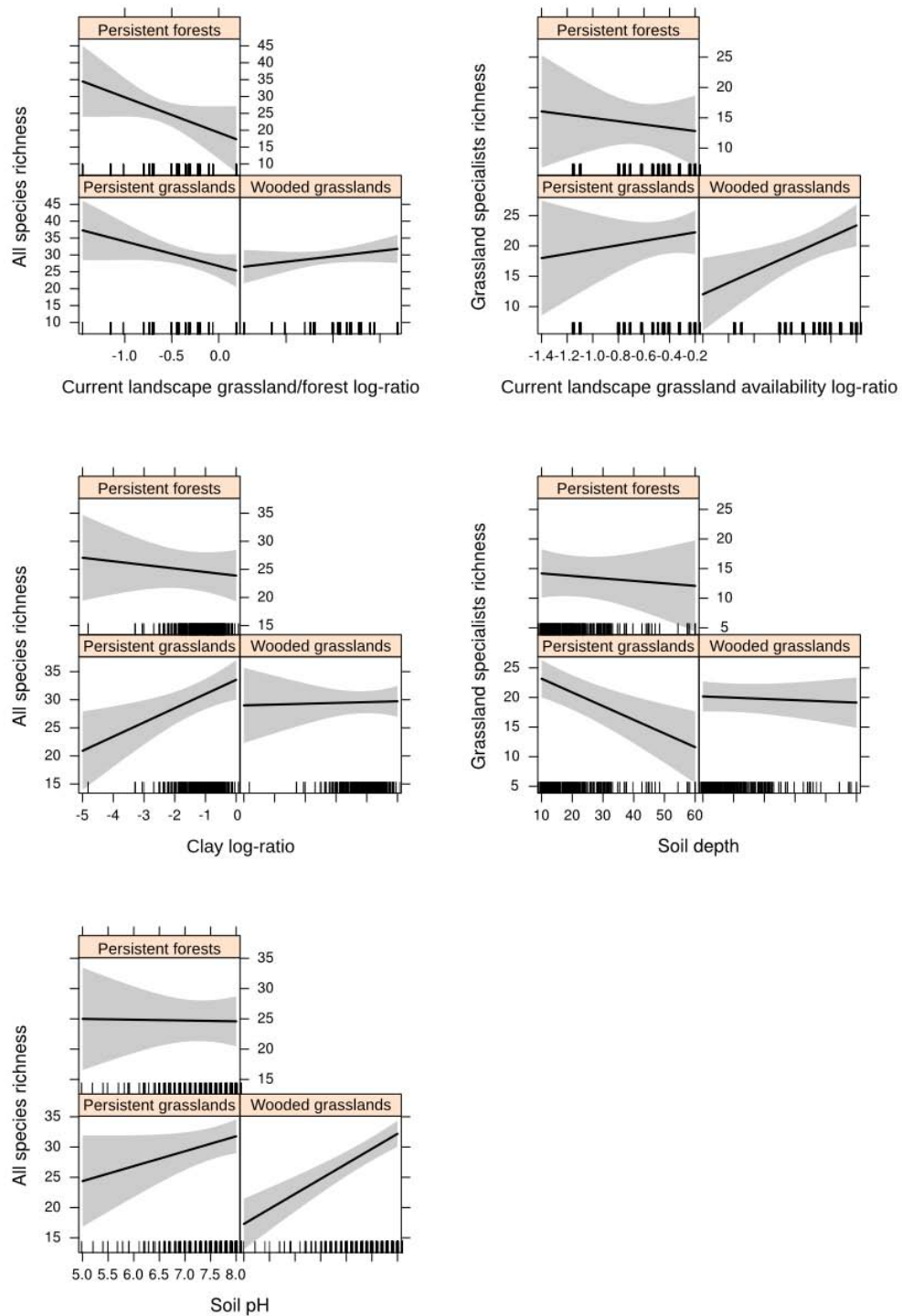
Fig. A.2 Effect plots of significant interactions between sampling plot category (persistent grasslands, wooded grasslands and persistent forests) and local and landscape variables for the general linear mixed models (LMMs). Shadowed area indicates 95% confidence intervals. Left column shows plots for all species model, and right column shows plots for grassland specialists model.

Appendix B

Idiosyncratic extinctions and colonisations drive plant species replacement along forest encroachment into Mediterranean grasslands

Table B.1 List of plant species found in the survey. The classification in grassland specialists and forest specialists was performed using regional floras (Bolòs *et al.*, 2005; Rivas-Martínez *et al.*, 2001) and expert advice. In addition to the plants in the list, two *Apiaceae*, two *Asteraceae*, one *Caryophyllaceae*, one *Cruciferae*, and three *Poaceae* species were found during the survey and included in the analyses, but we were not able to identify them to species or genus level.

Taxa	Grassland specialist	Forest specialist
Acer opalus Mill.		×
Aegilops triuncialis L.		
Aethionema saxatile (L.) R. Br.		
Allium senescens L.	×	
Allium sp. L.		
Althaea hirsuta L.	×	
Amelanchier ovalis Medik.		×
Anemone hepatica L.		×
Anthericum liliago L.	×	
Anthyllis montana L.	×	
Anthyllis vulneraria L.	×	
Aphyllanthes monspeliensis L.	×	
Aquilegia vulgaris L.		×
Arabis auriculata Lam.	×	
Arabis hirsuta (L.) Scop.	×	
Arabis scabra All.	×	
Arctostaphylos uva-ursi (L.) Spreng.	×	
Arenaria conimbricensis Brot.	×	
Arenaria tetraquetra L.	×	
Argyrolobium zanonii (Turra) P. W. Ball	×	
Arrhenatherum elatius (L.) P. Beauv. ex J. et C. Presl	×	
Asperula cynanchica L.	×	
Asphodelus cerasiferus Gay	×	
Asplenium adiantum-nigrum L.		×
Asplenium trichomanes L.		
Asterolinon linum-stellatum (L.) Duby in DC.	×	
Atractylis humilis L.	×	
Avenula pratensis (L.) Dumort.	×	
Biscutella laevigata L.	×	
Blackstonia perfoliata (L.) Huds.		
Brachypodium distachyon (L.) P. Beauv.	×	

Taxa	Grassland specialist	Forest specialist
Brachypodium phoenicoides (L.) Roem. et Schultes	×	
Brachypodium retusum (Pers.) P. Beauv.	×	
Brachypodium sylvaticum (Huds.) P. Beauv.		×
Briza media L.		
Bromus erectus Huds.	×	
Bupleurum baldense Turra	×	
Bupleurum fruticescens L.	×	
Bupleurum fruticosum L.		×
Bupleurum rigidum L.	×	
Buxus sempervirens L.		×
Campanula persicifolia L.		×
Campanula rotundifolia L.	×	
Campanula speciosa Pourr.		
Campanula trachelium L.		×
Carduncellus monspelliensium All.	×	
Carduus nigrescens Vill.	×	
Carex flacca Schreber		
Carex halleriana Asso	×	
Carex humilis Leysser	×	
Carlina vulgaris L.	×	
Centaurea linifolia L.	×	
Centaurium quadrifolium (L.) G. López et Ch. E. Jarvis	×	
Cephalanthera longifolia (L.) Fritsch		×
Cerastium pumilum Curtis	×	
Ceterach officinarum DC. in Lam. et DC.		
Chaenorhinum origanifolium (L.) Kostel.		
Cistus albidus L.		
Cistus clusii Dunal	×	
Clematis flammula L.		×
Clematis vitalba L.		×
Conopodium majus (Gouan) Loret in Loret et Barrandon	×	
Convolvulus arvensis L.		
Convolvulus lanuginosus Desr.	×	
Coris monspeliensis L.	×	
Coronilla emerus L.		×
Coronilla minima L.	×	
Crataegus monogyna Jacq.		×

Taxa	Grassland specialist	Forest specialist
Crepis albida Vill.	×	
Crucianella angustifolia L.	×	
Cruciata glabra (L.) Ehrend.		×
Crupina vulgaris Cass.	×	
Cytisophyllum sessilifolium (L.) O. Lang		×
Daphne gnidium L.		
Daphne laureola L.		×
Dianthus pungens L.	×	
Dipcadi serotinum (L.) Medik.	×	
Dorycnium pentaphyllum Scop.	×	
Echinops ritro L.	×	
Echium vulgare L.		
Epipactis sp. Zinn		×
Erica multiflora L.	×	
Erinacea anthyllis Link	×	
Eryngium campestre L.	×	
Erysimum grandiflorum Desf.	×	
Euphorbia amygdaloides L.		×
Euphorbia exigua L.	×	
Euphorbia flavicoma DC.	×	
Euphorbia minuta Loscos	×	
Euphorbia nevadensis Boiss. et Reut.	×	
Euphorbia nicaeensis All.	×	
Euphorbia serrata L.	×	
Festuca gr. *ovina* L.	×	
Festuca rubra L.		
Filipendula vulgaris Moench	×	
Fritillaria pyrenaica L.	×	
Fumana ericoides (Cav.) Gand.	×	
Galium pumilum Murray		
Galium verum L.	×	
Genista hispanica L.	×	
Genista scorpius (L.) DC. in Lam. et DC.	×	
Geranium robertianum L.		×
Geum sylvaticum Pourr.		×
Gladiolus illyricus Koch	×	
Globularia vulgaris L.	×	

Taxa	Grassland specialist	Forest specialist
Gymnadenia conopsea (L.) R. Br.	×	
Hedera helix L.		×
Helianthemum apenninum (L.) Mill.	×	
Helianthemum hirtum (L.) Mill.	×	
Helianthemum ledifolium (L.) Mill.	×	
Helianthemum oelandicum (L.) DC. in Lam. et DC.	×	
Helichrysum stoechas (L.) Moench	×	
Helleborus foetidus L.		×
Hieracium gr. *murorum* L.		×
Hieracium pilosella L.	×	
Hippocrepis comosa L.	×	
Hornungia petraea (L.) Rchb.	×	
Ilex aquifolium L.		×
Inula montana L.	×	
Iris lutescens Lam.	×	
Jasminum fruticans L.		
Jasonia tuberosa (L.) DC.		
Juniperus communis L.		×
Juniperus oxycedrus L.		
Juniperus phoenicea L.		
Knautia arvensis (L.) Coult.	×	
Koeleria vallesiana (Honck.) Gaudin	×	
Lactuca perennis L.		
Laserpitium gallicum L.	×	
Lathyrus filiformis (Lam.) Gay	×	
Lavandula angustifolia Mill.	×	
Lavandula latifolia Medik.	×	
Leontodon taraxacoides (Vill.) Mérat	×	
Leuzea conifera (L.) DC. in Lam. et DC.	×	
Lilium martagon L.		×
Linaria supina (L.) Chaz.	×	
Linum narbonense L.	×	
Linum tenuifolium L.	×	
Lithospermum fruticosum L.	×	
Lonicera etrusca Santi		×
Lonicera implexa Aiton		×
Lonicera xylosteum L.		×

Taxa	Grassland specialist	Forest specialist
Lotus corniculatus L.	×	
Melica ciliata L.	×	
Micropus erectus L.	×	
Micropyrum tenellum (L.) Link		
Moehringia pentandra Gay		
Muscari neglectum Guss. ex Ten.	×	
Narcissus assoanus Dufour	×	
Odontides viscosus (L.) Clairv.		
Ononis minutissima L.	×	
Ononis spinosa L.		
Ophrys fusca Link	×	
Ophrys scolopax Cav.	×	
Orchis maculata L.		
Orobanche amethystea Thuill	×	
Orobanche gracilis Sm.	×	
Orobanche latisquama (F. W. Schultz) Batt.	×	
Orobanche sp. L.		
Petrorhagia prolifera (L.) P. W. Ball et Heywood	×	
Phillyrea latifolia L.		×
Phlomis lychnitis L.	×	
Phyteuma orbiculare L.	×	
Picris hieracioides L.	×	
Pinus halepensis Mill.		×
Pinus nigra Arnold		×
Pinus pinea L.		×
Pinus sylvestris L.		×
Plantago lanceolata L.	×	
Platanthera bifolia (L.) Rich.		
Polygala calcarea F. W. Schultz	×	
Polygala rupestris Pourr.	×	
Polygala vulgaris L.	×	
Polygonatum odoratum (Mill.) Druce		×
Polypodium vulgare L.		
Potentilla hirta L.		
Potentilla neumanniana Rchb.	×	
Primula veris L.		×
Prunella grandiflora (L.) Scholler		

Taxa	Grassland specialist	Forest specialist
Prunus mahaleb L.		×
Prunus spinosa L.		
Pteridium aquilinum (L.) Kuhn		
Pyrola chlorantha Sw.		×
Quercus × cerrioides Willk. et Costa		×
Quercus coccifera L.		×
Quercus faginea Lam.		×
Quercus ilex L.		×
Ranunculus gramineus L.	×	
Rhamnus alaternus L.		×
Rhamnus saxatilis Jacq.		
Rosa pimpinellifolia L.		
Rosa sp. L.		
Rosmarinus officinalis L.	×	
Rubia peregrina L.		
Rubus ulmifolius Schott		
Ruscus aculeatus L.		×
Salvia officinalis L.	×	
Sanguisorba minor Scop.	×	
Santolina chamaecyparissus L.	×	
Saponaria ocymoides L.		
Satureja montana L.	×	
Scabiosa columbaria L.	×	
Scorzonera angustifolia L.	×	
Scorzonera hispanica L.	×	
Sedum acre L.	×	
Sedum album L.	×	
Sedum sediforme (Jacq.) Pau	×	
Senecio doronicum (L.) L.	×	
Serratula nudicaulis (L.) DC. in Lam. et DC.	×	
Sideritis hirsuta L.		
Silene nutans L.		
Solidago virgaurea L.		
Sorbus aria (L.) Crantz		×
Stachys officinalis (L.) Trevis.		
Staehelina dubia L.	×	
Stipa offneri Breistr.	×	

Taxa	Grassland specialist	Forest specialist
Stipa pennata L.	×	
Tanacetum corymbosum (L.) Schultz Bip.		
Taraxacum serotinum (Waldst. et Kit.) Poiret in Lam.	×	
Taxus baccata L.		×
Teucrium botrys L.	×	
Teucrium chamaedrys L.		×
Teucrium polium L.	×	
Thalictrum tuberosum L.	×	
Thesium humifusum DC.	×	
Thymelaea tinctoria (Pourr.) Endl.	×	
Thymus serpyllum L.	×	
Thymus vulgaris L.	×	
Trinia glauca (L.) Dumort.	×	
Tulipa sylvestris L.	×	
Viburnum lantana L.		×
Viburnum tinus L.		×
Vicia cracca L.		
Vicia sepium L.		×
Viola alba Besser		×
Viola rupestris F. W. Schmidt	×	
Viola willkommi Roem.		×

Table B.2 Species composition table for presence–absence of all species (rows) at the 18 sites (columns). A '0' indicates absence and a '1' indicates presence of the species.

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Acer opalus	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	0	0	0
Aegilops triuncialis	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Aethionema saxatile	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Allium senescens	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Allium sp.	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0
Althaea hirsuta	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Amelanchier ovalis	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
Anemone hepatica	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Anthericum liliago	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Anthyllis montana	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0
Anthyllis vulneraria	1	1	0	1	0	1	0	1	1	0	1	1	1	1	0	0	0	0
Aphyllanthes monspeliensis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Apiaceae 1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Apiaceae 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Aquilegia vulgaris	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Arabis auriculata	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Arabis hirsuta	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Arabis scabra	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Arctostaphylos uva-ursi	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	0	1	1
Arenaria conimbricensis	0	1	0	1	0	1	0	1	0	0	1	1	1	1	0	0	0	0
Arenaria tetraquetra	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
Argyrolobium zanonii	1	1	0	1	0	0	1	1	1	1	1	1	1	1	0	0	0	1
Arrhenatherum elatius	1	1	0	1	0	0	1	1	0	0	0	1	0	1	1	1	1	1
Asperula cynanchica	1	1	0	1	0	1	0	1	1	1	1	1	1	1	0	0	1	1
Asphodelus cerasiferus	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1
Asplenium adiantum-nigrum	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asplenium trichomanes	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Asteraceae 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Asteraceae 2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Asterolinon linum-stellatum	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Atractylis humilis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Avenula pratensis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Biscutella laevigata	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Blackstonia perfoliata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Brachypodium distachyon	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachypodium phoenicoides	1	0	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1
Brachypodium retusum	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1
Brachypodium sylvaticum	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Briza media	0	0	1	0	1	0	1	1	0	0	0	0	0	0	1	1	0	1
Bromus erectus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Bupleurum baldense	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Bupleurum fruticescens	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
Bupleurum fruticosum	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0
Bupleurum rigidum	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Buxus sempervirens	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1
Campanula persicifolia	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Campanula rotundifolia	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	1
Campanula speciosa	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Campanula trachelium	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Carduncellus monspelliensium	0	1	0	1	0	1	0	0	1	1	1	0	1	1	0	0	0	1
Carduus nigrescens	1	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1
Carex flacca	0	0	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	1
Carex halleriana	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
Carex humilis	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
Caryophyllaceae 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carlina vulgaris	0	0	1	1	1	0	1	0	0	1	0	0	0	1	0	1	1	1
Centaurea linifolia	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	0	0	1
Centaurium quadrifolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cephalanthera longifolia	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1	1	1	1
Cerastium pumilum	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Ceterach officinarum	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaenorhinum origanifolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cistus albidus	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0
Cistus clusii	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Clematis flammula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Clematis vitalba	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conopodium majus	1	0	1	1	0	0	0	1	0	0	1	1	0	0	0	0	1	1
Convolvulus arvensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Convolvulus lanuginosus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Coris monspeliensis	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1
Coronilla emerus	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Coronilla minima	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1
Crataegus monogyna	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Crepis albida	0	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	0	1
Crucianella angustifolia	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0
Cruciata glabra	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Cruciferae 1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Crupina vulgaris	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
Cytisophyllum sessilifolium	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0
Daphne gnidium	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Daphne laureola	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0
Dianthus pungens	1	1	0	1	0	0	1	0	0	0	1	0	0	0	0	1	0	0
Dipcadi serotinum	1	1	0	1	0	1	1	1	0	0	1	1	1	0	0	0	0	0

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Dorycnium pentaphyllum	1	0	0	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1
Echinops ritro	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1	0	1	1
Echium vulgare	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Epipactis sp.	0	0	1	0	1	0	1	1	1	1	0	0	0	0	1	1	1	1
Erica multiflora	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Erinacea anthyllis	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	1	0
Eryngium campestre	0	1	1	1	0	0	1	0	1	1	1	0	1	1	0	0	1	1
Erysimum grandiflorum	1	1	0	1	0	1	1	1	0	1	1	1	1	0	0	0	0	0
Euphorbia amygdaloides	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0
Euphorbia exigua	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Euphorbia flavicoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Euphorbia minuta	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Euphorbia nevadensis	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	1	1	1
Euphorbia nicaeensis	1	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	1
Euphorbia serrata	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Festuca gr. *ovina*	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1
Festuca rubra	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1
Filipendula vulgaris	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fritillaria pyrenaica	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0
Fumana ericoides	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1
Galium pumilum	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1
Galium verum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Genista hispanica	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	1
Genista scorpius	1	1	0	1	0	0	1	1	1	1	1	1	1	1	0	0	1	1
Geranium robertianum	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Geum sylvaticum	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	0	1	0
Gladiolus illyricus	0	0	0	1	1	0	0	1	1	0	1	0	1	0	1	0	1	1
Globularia vulgaris	1	0	0	0	0	1	1	1	1	1	0	0	0	1	0	0	0	1
Gymnadenia conopsea	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Hedera helix	1	0	1	0	1	0	0	1	1	1	0	0	0	0	1	1	1	1
Helianthemum apenninum	1	1	0	1	0	1	1	1	1	0	1	1	1	1	0	0	0	1
Helianthemum hirtum	0	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
Helianthemum ledifolium	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helianthemum oelandicum	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1
Helichrysum stoechas	0	0	0	1	0	0	1	1	1	1	1	0	1	1	0	0	0	1
Helleborus foetidus	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0
Hieracium gr. *murorum*	0	0	1	0	1	0	1	1	0	1	0	0	0	0	0	1	1	0

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Hieracium pilosella	0	0	0	1	0	0	1	0	1	1	1	0	0	1	0	0	1	1
Hippocrepis comosa	0	1	1	1	0	1	0	1	1	1	0	0	1	1	0	0	1	1
Hornungia petraea	0	1	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0
Ilex aquifolium	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0
Inula montana	0	1	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	0
Iris lutescens	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Jasminum fruticans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Jasonia tuberosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Juniperus communis	1	0	1	1	1	1	1	1	0	0	1	0	0	1	1	0	1	1
Juniperus oxycedrus	1	0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	0	1
Juniperus phoenicea	1	1	0	0	0	1	1	0	1	1	1	1	1	1	0	0	0	1
Knautia arvensis	0	0	1	0	1	0	1	1	1	1	0	0	0	1	1	1	1	0
Koeleria vallesiana	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1
Lactuca perennis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laserpitium gallicum	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Lathyrus filiformis	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Lavandula angustifolia	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Lavandula latifolia	1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1
Leontodon taraxacoides	0	1	0	1	0	1	1	0	1	0	0	1	0	0	0	1	0	0
Leuzea conifera	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1
Lilium martagon	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Linaria supina	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
Linum narbonense	0	1	1	1	0	0	1	0	0	1	1	1	1	1	1	0	0	1
Linum tenuifolium	0	0	0	0	0	0	1	0	1	1	0	0	1	0	1	0	0	0
Lithospermum fruticosum	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1
Lonicera etrusca	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	1
Lonicera implexa	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Lonicera xylosteum	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	1	0
Lotus corniculatus	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	1
Melica ciliata	1	1	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0
Micropus erectus	0	1	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0
Micropyrum tenellum	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Moehringia pentandra	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscari neglectum	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Narcissus assoanus	1	1	0	1	0	1	1	1	0	0	1	1	1	1	0	0	0	1
Odontides viscosus	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0
Ononis minutissima	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Ononis spinosa	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	1	1
Ophrys fusca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Ophrys scolopax	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Orchis maculata	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
Orobanche amethystea	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Orobanche gracilis	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Orobanche latisquama	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Orobanche sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Petrorhagia prolifera	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Phillyrea latifolia	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Phlomis lychnitis	1	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0
Phyteuma orbiculare	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1
Picris hieracioides	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0
Pinus halepensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pinus nigra	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1	0	0	1
Pinus pinea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pinus sylvestris	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
Plantago lanceolata	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Platanthera bifolia	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1
Poaceae 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Poaceae 2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Poaceae 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Polygala calcarea	0	0	1	0	1	0	1	1	0	1	1	0	0	0	1	1	1	1
Polygala rupestris	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0
Polygala vulgaris	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Polygonatum odoratum	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polypodium vulgare	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Potentilla hirta	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Potentilla neumanniana	1	1	1	1	0	0	0	1	0	1	1	1	0	1	1	0	1	1
Primula veris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Prunella grandiflora	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1
Prunus mahaleb	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prunus spinosa	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0
Pteridium aquilinum	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyrola chlorantha	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Quercus × cerrioides	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Quercus coccifera	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Quercus faginea	0	0	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	0
Quercus ilex	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ranunculus gramineus	1	1	0	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0
Rhamnus alaternus	1	1	0	1	0	0	1	1	1	0	0	0	1	1	0	0	1	0
Rhamnus saxatilis	1	1	0	1	0	1	0	1	0	0	1	1	1	1	0	0	1	0
Rosa pimpinellifolia	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosa sp.	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Rosmarinus officinalis	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1	0	0	1
Rubia peregrina	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
Rubus ulmifolius	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0
Ruscus aculeatus	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0
Salvia officinalis	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Sanguisorba minor	0	1	1	1	1	0	1	0	1	1	0	0	1	1	1	0	1	1
Santolina chamaecyparissus	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0
Saponaria ocymoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Satureja montana	0	0	0	1	0	1	1	0	0	0	1	0	1	1	0	0	0	0
Scabiosa columbaria	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1
Scorzonera angustifolia	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1
Scorzonera hispanica	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
Sedum acre	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sedum album	1	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	0
Sedum sediforme	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	0	0	0
Senecio doronicum	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Serratula nudicaulis	1	1	0	1	0	1	0	1	1	1	1	1	1	0	0	0	0	0
Sideritis hirsuta	0	1	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0
Silene nutans	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Solidago virgaurea	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0
Sorbus aria	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1
Stachys officinalis	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staehelina dubia	1	1	0	1	0	1	1	1	1	1	1	0	1	1	0	0	0	1
Stipa offneri	0	0	0	0	0	1	1	1	1	1	0	1	1	1	0	0	0	1
Stipa pennata	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	0
Tanacetum corymbosum	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Taraxacum serotinum	0	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Taxus baccata	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Teucrium botrys	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Teucrium chamaedrys	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1

Taxa	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Teucrium polium	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1
Thalictrum tuberosum	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1
Thesium humifusum	0	0	0	0	0	1	1	1	1	1	0	0	0	1	0	0	0	1
Thymelaea tinctoria	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1
Thymus serpyllum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Thymus vulgaris	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
Trinia glauca	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	0	0	1
Tulipa sylvestris	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Viburnum lantana	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Viburnum tinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Vicia cracca	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Vicia sepium	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0
Viola alba	0	0	1	1	1	0	0	1	0	1	0	0	0	0	1	1	1	1
Viola rupestris	1	0	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
Viola willkommi	1	0	1	1	1	0	1	0	1	1	0	0	0	1	1	1	1	1

Appendix C

Species traits weakly involved in plant responses to landscape properties in Mediterranean grasslands

Supplementary information on the phylogeny construction

Tree topology was constructed using the last update (July 2010) in the topology of the new Angiosperm Phylogeny Group classification (APG III Group, 2009) as the basic structure, and then joining other subtrees from some groups lacking a good resolution: *Asparagales* (McPherson *et al.*, 2004), *Poaceae* (Bouchenak-Khelladi *et al.*, 2010) and *Cardueae* (Susanna *et al.*, 2006). In addition, an unresolved node (*Lamiales*, *Boraginaceae* and *Rubiaceae*) was modified according to Bremer (2009).

We used the timetree database (Hedges *et al.*, 2006; www.timetree.org) to obtain the age of 20 nodes dated in recently published studies. These nodes and their references are shown in Table C.1 and in Fig. C.1. Then the *bladj* algorithm was applied to the phylogeny to estimate branch lengths using the program PHYLOCOM (version 4; Webb *et al.*, 2008).

Table C.1 Estimated age and source for the 20 dated nodes.

| Node	Estimated age (Mya)	Reference
1	147.8	Magallón, 2009
2	135.0	Forest & Chase, 2009a
3	125.0	Forest & Chase, 2009a
4	124.0	Anderson & Janssen, 2009
5	122.0	Forest & Chase, 2009a
6	122.0	Anderson & Janssen, 2009
7	121.0	Forest & Chase, 2009a
8	114.0	Bremer, 2009
9	109.0	Forest & Chase, 2009b
10	108.0	Bremer, 2009
11	107.0	Bremer, 2009
12	98.0	Forest & Chase, 2009b
13	94.0	Forest & Chase, 2009b
14	76.0	Bremer, 2009
15	69.0	Forest & Chase, 2009b
16	67.0	Bremer, 2000
17	61.0	Leebens-Mack *et al.*, 2005
18	51.0	Forest & Chase, 2009c
19	50.5	Bremer, 2000
20	36.5	Kim *et al.*, 2005
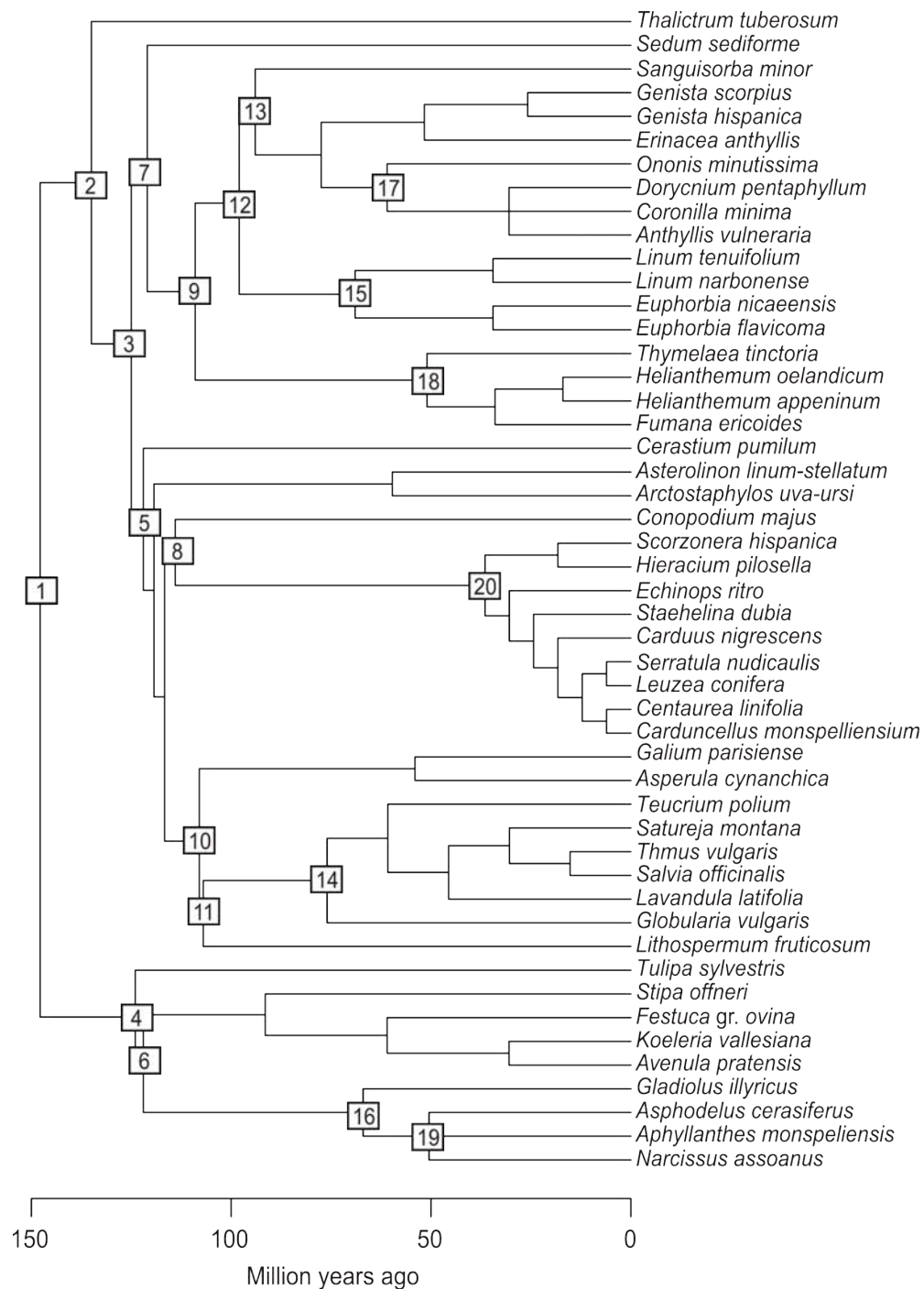
Fig. C.1 Phylogenetic tree of the plant species. Dated nodes are numbered in the tree and their estimated ages are given in Table C.1.

Table C.2 Species traits and sources. The studied traits are related to [a]dispersal, [b]establishment and/or [c]persistence.

Trait	Description	Sources
Reproductive traits		
Seed size[abc]	Maximum length of a seed (mm)	Bolòs & Vigo (1984); Grime *et al.* (1988); Bishop & Davy (1994); García-Fayos & Cerdà (1997); Klotz *et al.* (2002); Bolòs *et al.* (2005); J.M. Ninot (pers. comm.); Flora Iberica (www.floraiberica.es); personal observation on fresh and herbarium material
Dispersal type[a]	Anemochorous; Barochorous (or without any specific dispersal mechanism); Zoochorous	Julve (1998); Paula & Pausas (2009); Paula *et al.* (2009); personal observation on fresh and herbarium material
Corolla type[c]	Based on flower morphology: Anemophilous corolla; Open entomophilous; Tubular; Zygomorphic	Personal observation on fresh and herbarium material
Flower or pseudanthium size[c]	Maximum length of a flower or inflorescence when it acts as a unit of attraction (mm)	Bolòs & Vigo (1984); Dixon (1991); Bolòs *et al.* (2005); Flora Iberica (www.floraiberica.es); personal observation on fresh and herbarium material
Vegetative traits		
Resprouting ability after fire[c]	Yes; No	Papió (1994); Guerrero-Campo (1998); Arnan *et al.* (2007); Paula & Pausas (2008, 2009); Saura (2008); Paula *et al.* (2009); A. Rodrigo (pers. comm.); X. Arnan (pers. comm.); personal observation on fresh and herbarium material
Life-form[c]	Annual; Herbaceous perennial; Woody	Based on Bolòs & Vigo (1984)
Mean plant height[c]	Small (<25cm); Intermediate (25-50cm); Tall (>50cm)	Bolòs *et al.* (2005); J.M. Ninot (pers. comm.)
Vegetative spread[c]	Yes; No	Fitter & Peat (1994); Klotz *et al.* (2002); Hill *et al.* (2004); Klimešová & Klimeš (2006); Klimešová & de Bello (2009); personal observation on fresh and herbarium material
Leaf anatomy[c]	Aphyllous; Succulent; Mesomorphic; Scleromorphic	Klotz *et al.* (2002); J.M. Ninot (pers. comm.); personal observation on fresh and herbarium material
Leaf area[c]	Area of a leaf or leaflet in the case of compound leaves, without considering lobulation (cm2)	Bolòs & Vigo (1984); Dixon (1991); Bishop & Davy (1994); Bolòs *et al.* (2005); J.M. Ninot (pers. comm.); Flora Iberica (www.floraiberica.es); personal observation on fresh and herbarium material
Spinescence[c]	Yes; No	Personal observation on fresh and herbarium material
Ecological traits		
Phytogeography[bc]	Strictly Mediterranean; Broadly Mediterranean; Other	Based on Bolòs *et al.* (2005)
Mammal herbivore preference[c]	Preferred; Indifferent; Rejected	J. Bartolomé (pers. comm.); P. Casals (pers. comm.)

Table C.3 Environmental variables and sources.

Environmental variable	Units	Source
Habitat amount and dynamics		
Past patch area	ha	Photo-interpretation on ortho-corrected 1956 aerial photographs
Current patch area	ha	Photo-interpretation on 2004 ICC ortho-images
Percentage of patch area reduction	% ((historical area-current area)/historical area*100)	Calculation
Percentage of historical grassland area in the landscape	% (grassland area in patch+buffer/patch+buffer area)	Photo-interpretation on ortho-corrected 1956 aerial photographs
Percentage of current grassland area in the landscape	% (grassland area in patch+buffer/patch+buffer area)	Photo-interpretation on 2004 ICC ortho-images
Percentage of grassland area reduction in the landscape	% ((historical percentage-current percentage)/historical percentage*100)	Calculation
Geographical location and climate		
Longitude (UTMx)	m	GIS
Latitude (UTMy)	m	GIS
Mean annual temperature	°C	Digital Climatic Atlas of Catalonia (Pons, 1996; Ninyerola *et al.*, 2000)
Mean annual precipitation	mm	Digital Climatic Atlas of Catalonia (Pons, 1996; Ninyerola *et al.*, 2000)

Table C.4 Environmental variables correlation matrix; Pearson correlation coefficients.

	Longitude (UTMx)	Latitude (UTMy)	Mean annual temperature	Mean annual precipitation	Past patch area	Current patch area	% patch area reduction	% historical grassland in the landscape	% current grassland in the landscape
Latitude (UTMy)	0.958								
Mean annual temperature	-0.210	-0.361							
Mean annual precipitation	-0.820	-0.904	0.202						
Past patch area	0.362	0.316	0.035	-0.292					
Current patch area	0.280	0.226	-0.126	-0.259	0.341				
% patch area reduction	0.209	0.149	0.167	0.024	0.185	-0.452			
% historical grassland in the landscape	0.375	0.318	0.047	-0.250	0.691	0.236	0.028		
% current grassland in the landscape	0.253	0.234	-0.158	-0.272	0.347	0.818	-0.539	0.553	
% grassland reduction in the landscape	0.218	0.135	0.379	0.031	0.141	-0.378	0.810	0.318	-0.306

Table C.5 Table L: Species frequencies by patches.

	Anthyllis vulneraria	Aphyllanthes monspeliensis	Arctostaphylos uva-ursi	Asperula cynanchica	Asphodelus cerasiferus	Asterolinon linum-stellatum	Avenula pratensis	Carduncellus monspelliensium	Carduus nigrescens	Centaurea linifolia	Cerastium pumilum	Conopodium majus	Coronilla minima	Dorycnium pentaphyllum	Echinops ritro	Erinacea anthyllis	Euphorbia flavicoma	Euphorbia nicaeensis	Festuca gr. ovina	Fumana ericoides	Galium parisiense	Genista hispanica	Genista scorpius	Gladiolus illyricus	Globularia vulgaris	Helianthemum apeninum	Helianthemum oelandicum	Hieracium pilosella	Koeleria vallesiana	Lavandula latifolia	Leuzea conifera	Linum narbonense	Linum tenuifolium	Lithospermum fruticosum	Narcissus assoanus	Ononis minutissima	Salvia officinalis	Sanguisorba minor	Satureja montana	Scorzonera hispanica	Sedum sediforme	Serratula nudicaulis	Staehelina dubia	Stipa offneri	Teucrium polium	Thalictrum tuberosum	Thymelaea tinctoria	Thymus vulgaris	Tulipa sylvestris
Patch 01	0	15	5	5	0	2	4	3	0	0	0	0	5	15	5	0	7	0	0	5	7	1	3	0	1	2	5	1	1	2	2	1	7	0	0	4	0	6	0	3	0	0	11	4	8	0	0	20	0
Patch 02	15	20	0	2	1	4	7	3	0	0	1	0	3	2	2	2	7	1	3	3	12	0	5	2	3	3	9	1	13	3	2	0	2	0	5	3	0	0	0	0	1	2	5	520	8	1	0	22	0
Patch 03	0	23	4	0	0	7	0	0	0	13	0	12	0	4	2	0	21	0	0	12	0	11	0	0	0	0	0	0	6	4	0	3	4	0	0	7	0	1	0	9	0	0	4	2	5	5	0	22	0
Patch 04	14	0	0	1	3	6	0	0	0	0	3	0	2	0	2	4	1	0	0	4	0	1	0	0	0	1	3	0	7	1	3	0	4	0	7	0	1	0	0	0	0	0	4	2	2	5	0	17	0
Patch 05	13	14	1	7	10	0	6	9	9	4	3	0	2	2	2	15	20	0	7	14	1	0	2	0	0	13	6	0	9	2	6	0	0	1	1	3	1	1	0	6	3	0	10	16	4	4	2	20	1
Patch 06	2	8	10	2	13	5	10	5	1	0	1	0	1	0	0	2	1	0	2	4	0	2	2	2	0	7	10	1	9	2	6	0	3	0	13	1	0	3	6	0	2	3	2	0	7	4	0	21	1
Patch 07	4	20	12	3	7	0	19	1	1	6	0	1	1	0	2	1	12	0	8	12	2	5	5	2	1	10	10	0	10	4	0	5	0	0	7	1	0	3	6	7	0	3	11	13	14	19	1	20	3
Patch 08	0	25	1	0	0	17	5	0	0	2	0	0	5	7	0	4	12	0	13	3	6	6	8	12	10	4	15	5	13	0	0	1	5	0	7	1	0	2	5	7	0	0	6	4	5	2	3	7	0
Patch 09	0	9	0	4	0	0	5	0	0	3	0	0	7	0	4	0	11	2	3	1	0	0	6	6	0	2	2	7	15	0	0	0	0	5	2	0	0	6	0	0	3	0	1	1	0	0	0	16	0
Patch 10	18	14	8	4	14	0	7	5	2	0	2	0	5	8	5	8	8	0	11	5	0	1	1	0	0	5	3	11	13	3	0	2	0	0	0	3	0	6	0	0	3	8	10	0	20	3	1	2	19	0
Patch 11	4	23	19	1	3	0	17	1	1	2	0	0	0	6	10	5	7	0	3	8	2	4	3	1	2	1	1	1	5	3	1	2	7	5	2	4	1	0	1	1	0	1	2	18	5	4	4	17	0
Patch 12	0	25	18	2	1	0	15	4	0	0	0	0	1	6	10	6	0	11	0	12	1	16	13	12	3	9	9	0	19	1	6	6	8	4	7	13	0	0	0	1	0	12	9	16	0	0	0	22	0
Patch 13	2	19	14	2	4	0	16	5	0	5	2	0	0	9	6	0	11	0	4	7	0	0	8	0	5	10	12	0	19	0	0	6	4	11	23	0	4	0	0	1	8	10	0	20	3	0	2	19	0
Patch 14	1	23	16	3	4	3	19	4	1	4	4	0	8	0	1	0	16	0	4	10	0	2	16	0	13	6	11	14	14	1	6	0	8	5	3	13	5	4	1	2	2	1	7	18	20	0	1	18	1
Patch 15	15	15	14	1	0	13	1	9	3	0	0	12	0	0	0	0	0	0	4	11	0	1	5	0	7	16	2	1	8	0	4	0	1	4	15	5	13	0	0	0	5	5	1	18	5	0	0	21	11
Patch 16	5	22	9	4	1	2	22	5	0	5	2	0	6	8	4	0	17	0	16	6	1	9	10	9	9	9	12	0	17	0	13	0	6	4	15	16	1	0	0	5	2	0	11	11	13	2	0	23	1
Patch 17	0	22	3	2	3	0	20	6	0	3	3	0	0	1	2	0	7	0	2	10	0	17	1	0	8	8	11	0	17	0	12	11	11	2	11	0	4	0	2	0	0	0	12	7	6	4	7	21	0
Patch 18	15	4	0	1	2	15	16	12	0	11	1	0	0	19	7	19	13	1	0	0	0	0	0	0	0	3	11	2	1	4	1	2	13	0	13	12	1	1	0	4	2	12	0	1	5	2	0	13	0

Species	Patch 19	Patch 21	Patch 22	Patch 23	Patch 24	Patch 25	Patch 26	Patch 27	Patch 28	Patch 29	Patch 30
Anthyllis vulneraria	5	3	1	1	0	0	0	0	0	6	0
Aphyllanthes monspeliensis	23	14	12	1	10	23	25	24	13	13	16
Arctostaphylos uva-ursi	0	0	0	0	0	0	0	0	3	0	3
Asperula cynanchica	6	0	4	1	13	20	0	3	4	0	0
Asphodelus cerasiferus	0	1	0	0	0	0	0	5	0	0	0
Asterolinon linum-stellatum	1	0	9	9	7	12	0	0	0	0	0
Avenula pratensis	18	12	23	8	5	12	8	15	11	11	12
Carduncellus monspeliensium	3	11	10	1	1	1	0	1	9	4	6
Carduus nigrescens	20	1	0	0	2	3	0	0	0	0	0
Centaurea linifolia	1	0	0	2	0	0	0	0	0	0	0
Cerastium pumilum	0	16	2	0	0	0	0	0	0	0	0
Conopodium majus	1	0	0	0	1	0	0	0	0	0	0
Coronilla minima	0	0	0	0	0	0	0	0	0	0	0
Dorycnium pentaphyllum	1	0	0	0	10	4	0	2	2	0	2
Echinops ritro	0	17	5	10	5	0	4	2	2	5	2
Erinacea anthyllis	3	21	3	1	0	0	0	0	0	0	0
Euphorbia flavicoma	13	11	3	0	1	8	8	0	9	18	1
Euphorbia nicaeensis	0	4	13	13	0	13	0	1	0	0	0
Festuca gr. ovina	2	13	13	0	8	21	13	0	15	6	3
Fumana ericoides	11	3	5	0	2	13	17	12	0	10	19
Galium parisiense	0	22	12	18	0	0	13	20	0	0	0
Genista hispanica	1	2	2	8	1	6	13	20	8	17	17
Genista scorpius	7	0	0	7	8	2	3	9	8	7	12
Gladiolus illyricus	0	0	0	0	0	0	0	0	0	2	0
Globularia vulgaris	0	0	0	0	0	0	2	0	0	0	0
Helianthemum apeninum	6	6	6	4	0	0	0	0	2	3	3
Helianthemum oelandicum	5	0	0	2	1	0	0	0	0	3	0
Hieracium pilosella	0	4	12	0	0	0	1	0	11	1	0
Koeleria vallesiana	2	8	12	0	0	2	4	0	11	4	6
Lavandula latifolia	1	5	8	0	6	3	0	1	1	4	3
Leuzea conifera	4	0	6	14	1	2	2	2	5	1	0
Linum narbonense	0	1	0	0	0	2	2	1	1	0	3
Linum tenuifolium	0	0	0	0	0	0	0	0	0	0	0
Lithospermum fruticosum	0	0	0	0	0	0	0	0	0	0	0
Narcissus assoanus	4	2	10	2	14	0	0	0	0	0	0
Ononis minutissima	6	0	10	14	7	13	5	4	8	3	8
Salvia officinalis	0	0	0	1	0	0	0	6	7	5	0
Sanguisorba minor	2	0	0	0	0	14	15	2	6	0	0
Satureja montana	20	0	0	0	4	15	1	6	0	0	5
Scorzonera hispanica	5	1	0	0	0	0	0	1	0	0	1
Sedum sediforme	0	4	1	0	2	0	0	0	0	0	0
Serratula nudicaulis	3	0	0	0	2	0	0	1	0	1	6
Staehelina dubia	4	0	0	0	0	0	15	3	0	1	0
Stipa offneri	3	0	0	0	0	15	3	0	1	7	12
Teucrium polium	14	7	0	5	7	11	1	1	5	13	12
Thalictrum tuberosum	2	16	0	0	0	0	0	3	1	5	5
Thymelaea tinctoria	0	0	0	0	0	5	0	10	13	0	0
Thymus vulgaris	15	8	18	22	14	13	13	12	15	21	24
Tulipa sylvestris	0	0	0	0	0	0	0	0	5	1	0

Table C.6 Table R: Environmental variables by patches.

	UTMx (m)	UTMy (m)	MAT (°C)	MAP (mm)	Past patch area (ha)	Current patch area (ha)	% of patch area reduction	% of historical grassland area in the landscape	% of current grassland area in the landscape	% of grassland area reduction in the landscape
Patch 01	341938	4580866	11.5	744.9	4.177	0.101	97.58	1.50	0.03	97.92
Patch 02	341223	4578632	11.4	760.6	15.038	0.500	96.68	4.41	0.42	90.53
Patch 03	343168	4578442	11.7	751.8	1.830	0.339	81.48	1.31	0.10	92.68
Patch 04	339022	4572346	11.8	764.9	14.037	3.105	77.88	4.40	0.76	82.74
Patch 05	336397	4570859	11.1	786.1	19.276	19.276	0.00	16.69	6.29	62.31
Patch 06	334567	4570998	11.4	753.4	8.785	7.194	18.11	21.77	3.18	85.40
Patch 07	333653	4573827	11.6	714.1	94.662	0.232	99.75	23.97	1.57	93.44
Patch 08	331949	4568815	11.4	742.6	3.011	0.677	77.52	1.59	0.42	73.60
Patch 09	331391	4573218	11.7	695.5	5.713	1.191	79.15	1.52	0.32	79.15
Patch 10	329737	4574387	10.7	736.3	35.623	26.845	24.64	4.81	3.62	24.64
Patch 11	327792	4572380	10.9	724.6	33.159	20.744	37.44	5.31	3.32	37.44
Patch 12	323888	4573418	10.6	711.9	4.178	3.412	18.33	3.52	1.46	58.41
Patch 13	322806	4573833	10.7	698.0	2.513	0.400	84.08	3.02	0.81	73.31
Patch 14	320193	4571790	10.2	730.4	0.397	0.397	0.00	0.18	0.18	0.00
Patch 15	319376	4572619	10.1	726.6	4.003	4.003	0.00	1.01	1.01	0.00
Patch 16	314791	4571338	10.4	685.7	10.877	10.877	0.00	2.78	2.40	13.78
Patch 17	313813	4571191	10.6	669.6	0.629	0.629	0.00	2.29	2.29	0.00
Patch 18	320799	4553177	11.5	747.8	16.875	15.441	8.50	2.88	2.63	8.50
Patch 19	321156	4550023	12.1	741.1	18.165	18.165	0.00	2.91	2.91	0.00
Patch 21	277846	4535078	11.7	798.8	2.074	1.336	35.58	4.16	2.06	50.60
Patch 22	279171	4535598	12.0	774.9	5.927	4.626	21.95	3.13	1.53	51.07
Patch 23	276009	4523613	11.4	933.7	0.968	0.252	73.97	0.29	0.08	73.97
Patch 24	278272	4523038	12.3	883.5	2.463	0.743	69.83	1.61	0.34	78.99
Patch 25	274308	4518955	11.0	1003.9	2.627	1.382	47.39	0.90	0.46	49.31

	UTMx (m)	UTMy (m)	MAT (°C)	MAP (mm)	Past patch area (ha)	Current patch area (ha)	% of patch area reduction	% of historical grassland area in the landscape	% of current grassland area in the landscape	% of grassland area reduction in the landscape
Patch 26	272676	4517860	10.3	1049.6	1.697	0.278	83.62	1.27	0.48	61.90
Patch 27	273347	4516205	12.9	913.5	0.339	0.339	0.00	0.44	0.22	48.86
Patch 28	270596	4513099	11.3	1015.1	0.214	0.214	0.00	1.30	1.30	0.00
Patch 29	269626	4512764	11.6	986.6	7.905	7.905	0.00	3.65	1.93	47.06
Patch 30	269082	4510211	11.6	990.7	1.538	0.592	61.51	0.73	0.31	56.88

Table C.7 Table Q: Biological and ecological traits by species[a].

	Seed size (mm)	Dispersal type	Corolla type	Flower/pseudanthium size (mm)	Resprouting ability after fire	Life-form	Mean plant height	Vegetative spread	Leaf anatomy	Leaf area (cm²)	Spinescence	Phytogeography	Herbivore preference
Anthyllis vulneraria	2.3	Ba	Zg	14.0	No	HP	S	No	M	6.0	No	SM	P
Aphyllanthes monspeliensis	2.1	Ba	Tb	23.5	Yes	HP	I	Yes	Ap	0.0	No	BM	P
Arctostaphylos uva-ursi	4.5	Zo	Tb	6.0	Yes	W	S	Yes	S	1.6	No	O	R
Asperula cynanchica	2.3	Ba	Tb	3.3	Yes	HP	I	Yes	M	0.6	No	O	I
Asphodelus cerasiferus	6.0	Ba	Oc	21.0	No	HP	T	Yes	M	80.0	No	SM	R
Asterolinon linum-stellatum	1.2	Ba	Oc	0.7	No	A	S	No	M	0.1	No	SM	I
Avenula pratensis	5.2	Zo	An	13.2	Yes	HP	I	Yes	M	5.5	No	O	P
Carduncellus monspelliensium	4.5	An	Tb	40.0	No	HP	S	No	S	42.0	Yes	BM	R
Carduus nigrescens	4.5	An	Tb	35.0	No	HP	T	No	S	9.3	Yes	SM	R
Centaurea linifolia	4.0	Zo	Tb	11.0	Yes	W	S	No	M	1.5	No	SM	R
Cerastium pumilum	0.6	Ba	Oc	4.8	No	A	S	No	M	0.7	No	O	I
Conopodium majus	4.6	Ba	Oc	10.6	No	HP	I	No	M	0.3	No	SM	P
Coronilla minima	3.4	Ba	Zg	8.0	Yes	W	I	No	M	0.3	No	SM	P
Dorycnium pentaphyllum	2.2	Ba	Zg	5.0	Yes	W	T	No	M	0.1	No	SM	P

	Seed size (mm)	Dispersal type	Corolla type	Flower/pseudanthium size (mm)	Resprouting ability after fire	Life-form	Mean plant height	Vegetative spread	Leaf anatomy	Leaf area (cm²)	Spinescence	Phytogeography	Herbivore preference
Echinops ritro	6.7	An	Tb	30.0	No	HP	I	No	S	32.0	Yes	SM	R
Erinacea anthyllis	3.0	Ba	Zg	16.8	No	W	I	Yes	Ap	0.3	Yes	BM	R
Euphorbia flavicoma	2.5	Zo	Oc	2.7	Yes	W	I	Yes	M	0.8	No	SM	R
Euphorbia nicaeensis	2.5	Zo	Oc	2.8	No	W	I	Yes	M	2.4	No	BM	R
Festuca gr. *ovina*	2.7	Zo	An	8.6	Yes	HP	I	Yes	S	0.7	No	O	P
Fumana ericoides	2.2	Ba	Oc	12.5	No	W	S	No	S	0.1	No	SM	R
Galium parisiense	1.0	Zo	Tb	0.9	No	A	S	No	M	0.1	No	SM	I
Genista hispanica	2.4	Ba	Zg	7.0	Yes	W	S	No	M	0.2	Yes	BM	I
Genista scorpius	2.7	Ba	Zg	8.5	Yes	W	T	No	S	0.1	Yes	BM	R
Gladiolus illyricus	5.1	An	Oc	39.7	Yes	HP	I	No	M	20.0	No	SM	R
Globularia vulgaris	1.6	Ba	Tb	17.5	Yes	HP	I	Yes	M	4.1	No	BM	I
Helianthemum apenninum	1.5	Ba	Oc	26.0	No	W	I	No	M	0.4	No	BM	R
Helianthemum oelandicum	1.8	Ba	Oc	9.2	No	W	S	No	M	0.4	No	O	R
Hieracium pilosella	1.9	An	Tb	8.5	Yes	HP	S	Yes	M	4.5	No	O	R
Koeleria vallesiana	3.0	Zo	An	4.3	Yes	HP	S	Yes	S	1.5	No	O	I
Lavandula latifolia	2.1	Ba	Zg	9.0	No	W	T	No	M	0.3	No	BM	I
Leuzea conifera	4.0	An	Tb	40.0	No	HP	S	No	S	12.2	No	SM	R
Linum narbonense	2.6	Ba	Tb	22.5	Yes	W	I	No	M	0.4	No	BM	R
Linum tenuifolium	2.0	Ba	Tb	18.4	Yes	W	T	No	S	0.1	No	SM	R
Lithospermum fruticosum	3.6	Zo	Tb	13.0	Yes	W	I	No	S	0.2	No	SM	P
Narcissus assoanus	1.9	Ba	Tb	15.0	Yes	HP	S	No	M	3.0	No	SM	R
Ononis minutissima	1.9	Ba	Zg	9.0	No	W	S	No	M	0.1	No	BM	I
Salvia officinalis	2.8	Ba	Zg	22.5	Yes	W	I	No	M	8.3	No	BM	R
Sanguisorba minor	2.8	Ba	An	15.7	Yes	HP	I	Yes	M	1.6	No	BM	P
Satureja montana	1.3	Ba	Zg	8.0	No	W	I	No	S	0.8	No	BM	R
Scorzonera hispanica	14	An	Tb	36.8	No	HP	S	No	M	41.2	No	SM	P

	Seed size (mm)	Dispersal type	Corolla type	Flower/pseudanthium size (mm)	Resprouting ability after fire	Life-form	Mean plant height	Vegetative spread	Leaf anatomy	Leaf area (cm²)	Spinescence	Phytogeography	Herbivore preference
Sedum sediforme	1.0	Ba	Oc	36.1	No	W	I	Yes	Sc	0.4	No	BM	R
Serratula nudicaulis	3.8	An	Tb	22.5	Yes	HP	I	Yes	S	25.0	No	BM	R
Staehelina dubia	4.5	An	Tb	17.5	Yes	W	I	No	M	0.6	No	SM	R
Stipa offneri	11.5	An	An	20.0	Yes	HP	T	Yes	S	1.3	No	BM	P
Teucrium polium	2.3	Ba	Zg	8.0	Yes	W	I	Yes	M	0.5	No	SM	R
Thalictrum tuberosum	6.0	Ba	Oc	23.4	Yes	HP	I	Yes	M	0.6	No	SM	R
Thymelaea tinctoria	2.9	Zo	Tb	4.5	Yes	W	I	No	Sc	0.3	No	SM	R
Thymus vulgaris	0.8	Ba	Zg	5.5	Yes	W	S	No	S	0.1	No	BM	R
Tulipa sylvestris	4.8	Ba	Oc	22.5	Yes	HP	S	No	M	8.7	No	O	R

[a] Abbreviations are, for Dispersal type: An, anemochorous; Ba, barochorous; Zo, zoochorous; for Corolla type: An, anemophilous; Oc, open entomophilous corolla; Tb, tubular; Zg, zygomorphic; for Life-form: A, annual; HP, herbaceous perennial; W, woody; for Plant height: S, small (<25cm); I, intermediate (25-50cm); T, tall (>50cm); for Leaf anatomy: Ap, aphyllous; Sc, succulent; M, mesomorphic; S, scleromorphic; for Phytogeography: SM, strictly Mediterranean; BM, broadly Mediterranean; O, other, and for Herbivore preference: P, preferred; I, indifferent; R, rejected.

Table C.8 Results from the RLQ analysis including phylogeny. Panel (a) shows the results of the separate analyses for the environmental variables table (R), the species composition table (L) and the species traits and phylogeny table (Q). Panel (b) gives the results of the RLQ analysis: variance explained by environmental variables and species traits and phylogeny, and covariance and correlation between sites and species for the first two axes. Panel (c) shows the comparison between the RLQ analysis and each separate analysis for the first and second axes.

a) Separate analyses

Analysis	Variance axis 1	Variance axis 2
PCA of R	3.635 (36.3%)	2.633 (26.3%)
CA of L	0.139 (16.0%)	0.126 (14.5%)
PCA of Q	1.826 (17.8%)	1.225 (12.0%)

b) RLQ analysis

b.1) Variance

Analysis	Variance axis 1	Variance axis 2
R/RLQ	3.529	2.188
Q/RLQ	0.820	0.476

b.2) Covariance

Covariance axis 1	Covariance axis 2
0.299	0.148

b.3) Correlation

Correlation axis 1	Correlation axis 2
0.176	0.145

c) Comparison of RLQ and separate analyses

	Explained variance (%)	
	RLQ axis 1	RLQ axis 2
R/RLQ	97.1	83.1
L/RLQ	47.2	40.8
Q/RLQ	44.9	38.9
Inertia	70.5	17.3