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

Guillem Bagaria[1,2]*, Aveliina Helm[3], Ferran Rodà[1,2], Joan Pino[1,2]

[1]CREAF, Cerdanyola del Vallès 08193, Spain

[2]Univ Autònoma Barcelona, Cerdanyola del Vallès 08193, Spain

[3]Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, 51005 Tartu, Estonia

*Correspondence: Guillem Bagaria, CREAF, Cerdanyola del Vallès 08193, Spain.

E-mail: g.bagaria@creaf.uab.cat

Phone: +34 935814851

FAX: +34 93 5814151

Author Contributions: GB, AH, FR and JP conceived the ideas. GB conducted the field sampling. GB, AH, FR and JP analysed the data. GB wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Abstract

Changes in species richness along ecological succession might be strongly determined by coexisting extinction debts of species from the original habitats and colonization credits of those from the replacing habitats. The magnitude of these processes and their causes remain largely unknown. We explored the extinction debt and colonization 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 confirmed extinction debt and suggested colonization credit (according to observed trends and model predictions) in wooded grasslands 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, the availability of seed sources from old forests may accelerate the payment of colonization credit in wooded grasslands. Thus, extinction debt and colonization credit have driven species turnover across 50 years of forest encroachment, but at different rates and 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.

Keywords Biodiversity change, Habitat change, Mediterranean grasslands, Variegated landscape, Vascular plants.

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 (Fahrig 2003; Baan et al. 2012). Species extinctions and colonizations in dynamic landscapes and habitats often occur with time lags (Jackson and Sax 2010). Both extinction debt (Tilman et al. 1994; Kuussaari et al. 2009) and colonization credit (Jackson and Sax 2010; Cristofoli et al. 2010) have been identified following the changes in habitat quality, amount or connectivity. The relative rate at which extinctions and colonizations occur determines the existence of transient richness surpluses or deficits during the relaxation time (Jackson and 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 and Ehrlén 2013; but see Lindborg et al. 2012). Regarding colonization, 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 (Sax and Brown 2000; Jacquemyn et al. 2003; Vellend 2005; Piqueray et al. 2013). 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 (Helm et al. 2006; Vellend et al. 2006; Krauss et al. 2010; Cousins and Vanhoenacker 2011; Guardiola et al. 2013), but the complementary topic of colonization credit has received much less attention, and empirical evidence is relatively scarce (Pierik et al. 2010; Piqueray et al. 2011; Hylander and Weibull 2012; Lira et al. 2012). Furthermore, the few studies that have investigated the simultaneous occurrence of an extinction debt and a colonization credit have focused on the dynamics within one habitat type and species group, i.e. the extinctions and colonizations of the same group of specialist species following either habitat degradation or restoration (Hanski 2000; Piqueray et al. 2011; Hylander and Weibull 2012; Lira et al. 2012).

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 (Eriksson et al. 2002; Adriaens et al. 2006). 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 colonization 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 colonization 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 colonization 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 colonizations) 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 and Barrett 1992), considering gradients of target habitat transformation and suitability. Our main objectives were i) to investigate the magnitude of the extinction debt and colonization 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 colonization of forest specialist species during forest encroachment.

Materials and methods

Study system

The study was conducted on the calcareous plateau of Prades Mountains, a relatively small massif (20 x 16 km) in southern Catalonia, NE Iberian Peninsula (41º14'-41º23'N, 0º56'-1º10'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 generalized 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 colonization 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 colonizations. We selected 20 sites corresponding to the maximum available landscape diversity in terms of forest and grassland cover proportions, each with a diameter of 200 m. Preliminary site selection was performed on the basis of historical (1956) and current (2009) orthophotomaps and the final selection was made during preliminary fieldwork, seeking for a wide range in historical and current forest cover to allow the study of delays in species extinctions and colonizations. The 20 finally selected sites fulfilled the following requirements: (1) considerably ranged 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. 1:30000, 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, and we converted them to a pixel size of 1 m to make them comparable with the historical ones. From both past (1956) and present (2009) orthophotomaps, 300 training points randomly distributed on the orthophotomaps were manually assigned by visual photo-interpretation to one of the following categories: forest, grassland, bare ground, 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 misclassification error rate for the historical map was 24%, while it was 19% for the current map, which has a better quality, but the same inherent problems of complex Mediterranean landscapes and mountainous areas. 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 ground 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.

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). The small size of the plots was chosen according to the fine-scale habitat changes that result from the forest encroachment process. Each plot was marked with a precision GPS, and all vascular plant species with any aerial part within its boundaries were recorded. Each of the species found during the survey was assigned to one of the following categories, using regional floras (Rivas-Martínez et al. 2001; Bolòs et al. 2005) 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 Online Resource Table S1).

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 increase species richness for a specific group, rescue remnant populations of grassland specialists and delay extinction (grassland connectivity) (Hanski 1999) or act as propagule sources for colonizing species of forest specialists and accelerate colonization (forest connectivity) (Jacquemyn et al. 2003; Verheyen et al. 2003a). Thus, 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 that might be relevant to species richness of some species groups and might delay or accelerate extinction of grassland specialists or colonization of forest specialists in different situations 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 proportion). Soil depth is related to water availability and pH may favour grassland specialists (mainly calcicolous), while the high cation exchange capacity of clay enhances soil fertility and water retention and might favour species richness where water is limiting (Cornwell and Grubb 2003). Solar radiation availability, wich is important for the establishment of grassland specialists (Öckinger et al. 2006), was measured 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, which might favour grassland species, was assessed at each plot as present or absent. All these drivers of species richness can potentially delay or accelerate extinctions and colonizations of habitat specialists during forest encroachment. In addition, historical and current tree cover proportion was obtained for each plot, in a radius of 10 m, from the combined maps (see Online Resource Table S2 for descriptive statistics of response variables and predictors). In order to study extinction-colonization 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; 84 plots), wooded grasslands (less than 50% tree cover in the past and more than 50% in the present; 156 plots) and persistent forests (more than 50% tree cover in the past and nowadays; 20 plots). Current tree cover in wooded grassland plots (0.72 ± 0.12; mean ± SD) did not statistically differ from that in persistent forest plots (0.75 ± 0.07; *P*=0.57; tested using a general linear mixed model relating current tree cover with plot category, site as random factor, and a post-hoc Tukey test). The continuous landscape gradient formed after forest encroachment results in the coexistence of persistent grasslands, wooded grasslands and/or persistent forests within almost all sites (see Fig. 2).

Statistical analyses

For the purpose of investigating the plant extinction-colonization balance after the forest encroachment process and testing for the existence of an extinction debt for grassland specialists and a colonization credit for forest specialists, a general linear mixed model (LMM) for each species group (all species, grassland specialists and forest specialists) 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 situations along the habitat change gradient and detect extinction debt and colonization credit, a post-hoc Tukey test was performed for each model. Significantly higher richness of grassland specialists at wooded grasslands than at persistent forests would reveal an extinction debt. Similarly, significantly lower richness of forest specialists at wooded grasslands than at persistent forests would indicate a colonization credit. 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 the residuals of each model to test for spatial autocorrelation in the residuals. In order to estimate the magnitude of extinction debt and colonization credit, we calculated the difference between mean richness of each specialist group at wooded grasslands and stable forests, for grassland and forest specialists, respectively.

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 richness and to elucidate the mechanisms of species extinctions and colonizations 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 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.

Results

We found a significant decrease of 32% (9.8 species on average) in total plant richness from persistent grassland to persistent forest plots (see Fig. 3), but no richness differences between wooded grasslands and any persistent habitat, tested using a general linear mixed model with site as random factor, and a post-hoc Tukey test (Table 1; Online Resource Fig. S1).

As expected, the highest species richness of grassland specialists was found in persistent grassland plots (26.6 species), it was intermediate in wooded grasslands (17.3 species), and lowest in persistent forests (7.9 species; see Fig. 3). Using persistent forest plots as a reference, the extinction debt for grassland specialists in wooded grassland plots was 54.6% (9.4 species on average per plot).

Forest specialists showed almost the inverse pattern, having the lowest observed richness in persistent grasslands (2.3 species on average per plot). However, richness of forest specialists did not statistically differ between wooded grasslands (7.0 species) and persistent forests (10.2 species) according to the general linear mixed model (Table 1), even though there is a tendency to richness increase along the forest encroachment process (Online Resource Fig. S1). Although no significant colonization credit for forest specialists was found, the tendency to richness increase from wooded grasslands to persistent forests (3.2 species on average per plot) was supported by a relatively low *P*-value (*P*=0.13), and the average species richness increase represented 45.7% of the forest specialist species currently present in wooded grasslands. Predicted values for each plot category are shown in Online Resource Table S3.

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 and that may delay or accelerate extinctions of grassland specialists and colonizations of forest specialists. According to the best model, total richness was determined by soil pH, solar radiation availability (both positively correlated), and interaction between plot category and current landscape (ratio of grassland/forest) and local predictors (soil clay proportion and soil depth) (see also Online Resource Table S4, Fig. S2 for results and effect plots). The model accounted for 52% 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 decreased total richness in persistent grasslands significantly more than in wooded grasslands (for the latter, the relationship was slightly positive). Clay proportion had a higher positive effect in persistent grasslands than in wooded grasslands, and the negative effect of soil depth on total species richness was significantly higher in persistent grasslands than in wooded grasslands, although no much difference can be observed.

The best model for grassland specialist richness had a high pseudo-R^2 of (0.71) and comprised solar radiation availability, soil pH, grassland availability in the current landscape (all three having positive effects), soil depth (negatively correlated), and the factor plot category (Table 2). No interaction between plot category and any of the variables was included in the model, indicating that the drivers of the richness of grassland specialists are held constant along the forest encroachment process.

For forest specialists, the selected model on species richness had a very similar pseudo-R^2 to the model for grassland specialists. It included solar radiation availability (negatively correlated), and the interaction between plot category and historical landscape forest availability and clay proportion. Historical forest connectivity had a positive effect on richness in persistent grasslands and wooded grasslands, while it was slightly negative in persistent forests. Clay proportion had a slightly positive effect on richness of forest specialists in persistent grasslands and a slightly negative effect in wooded grasslands, although the differences are barely noticeable (Online Resource Fig. S2). No significant spatial autocorrelation was found for any of the models.

Discussion

In this work we focus on the simultaneous extinction-colonization 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 extinction debt but not significant colonization 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 significant decline of 32% 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.

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, 55% 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 and Vanhoenacker 2011; but see Adriaens et al. 2006). Vellend et al. (2006) and Cousins and 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, although it is not significantly different from that in wooded grasslands. High variability in forest species' richness, the low number of persistent forest plots and the fact that many of these plots belong to one site might explain the non-significance of this trend. Thus, we do not find evidence of a significant colonization credit coexisting with the extinction debt of grassland specialists. Colonization 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. 2015), mosses (Hylander and 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 colonization credits following habitat change, but they put the focus on a given habitat type instead of on the complete habitat series (Piqueray et al. 2011; Lira et al. 2012), or on the complete community instead of the specialist species groups (Hylander and 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 and Sax (2010) indicated the importance of the magnitude and chronology of extinction debt and colonization credit on the community biodiversity balance over time. Although our study finds a significant decrease in total species richness as the outcome of the process, it also suggests that extinctions and colonizations of plant specialists might have occurred simultaneously following forest encroachment, as no richness surplus or deficit was observed in total species across the encroachment process (plant richness in wooded grasslands does not statistically differ from any of the stable habitats), although at not identical rate. Our results suggest that colonization credit of forest specialists is being paid faster than extinction debt of grassland specialists (actually, forest specialists' richness does not statistically differ between wooded grasslands and persistent forests, while that of grassland specialists does), probably due to its smaller magnitude. Helm et al. (2015) showed that with decreasing grassland size, total number of species on sites can even increase due to influx of generalist species and species not characteristic to a given habitat, while the number of characteristic species remains the same due to extinction debt. This indicates that expected colonizations can occur (at least partly) faster than extinctions, resulting in temporal enrichment of flora due to simultaneous coexistence of different species pools. It should be noted that the persistent forest plots used in our study as a reference to calculate extinction debts and colonization 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 and Rackham 2001; Başnou et al. 2015), thus limiting forest species richness in persistent forests and colonization credit in wooded grasslands. If we considered that all grassland specialist species will ultimately go extinct and more forest specialists will still colonize persistent forests, the magnitude of both extinction debt and colonization credit would be even greater. However, a thorough study would be needed to predict the species richness of old-growth forests in the area that would allow the accurate calculation of extinction debt and colonization credit, because species richness can be influenced by very long-term historical effects (e.g. Dambrine et al. 2007). The study of extinction debt and colonization credit dynamics following a land-use change event is needed to understand how community change occurs over time (Jackson and Sax 2010). A good knowledge of initial and final habitat situations is also crucial to assess the magnitude of both debts and credits and to identify potential transient situations of richness surplus or deficit that would allow further understanding on the interaction of both processes.

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 colonizations following forest encroachment.

For grassland specialists, high connectivity for the focal habitat in the landscape exerts a positive effect on species richness for this group in all situations along the habitat encroachment gradient. Thus, grassland connectivity might enhance persistence of grassland specialist species both in optimal and degraded habitats. In previous works, a rescue effect from nearby populations was suggested as a mechanism for delayed extinctions (Kuussaari et al. 2009; Krauss et al. 2010), but we did not find a specific effect on transient habitats that might point to that mechanism. Solar radiation and soil pH enhance species richness for this group; the former is known to promote the establishment of grassland specialists, while the 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, probably due to the already known competition effects of high resource availability (Grime 2001).

For forest specialists, higher historical forest availability in the landscape enhances plant richness in wooded and persistent grasslands, while it has no effect in persistent forests. It suggests that colonization is enhanced by connectivity where there is still a lack of forest species, reinforcing the idea that a colonization credit might exist despite richness of forest specialists was not significantly lower in wooded grasslands than in persistent forests. The positive relationship with historical but not with current forest connectivity suggests that the potential colonization credit depends on the historical forest extent and it can persist for a 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 and, consequently, they might act as poorer seed sources, delaying the colonization process. Clay proportion has a slightly negative effect on forest species richness in wooded grasslands compared to persistent grasslands. Despite these differences are very small, it might indicate that increased soil fertility and water retention due to clay could improve habitat quality for forest species in persistent grasslands, where water is limiting. Finally, as expected, low solar radiation availability favours richness of forest specialists, as these plants thrive under tree canopies, and this effect is independent of the habitat gradient situation.

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 (see Online Resource Table S4, Fig. S2).

Implications for management

Both extinction debts and colonization 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 colonizations after the Jackson and 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-colonization following a forcing event.

Although an important habitat change might have occurred, as long as the species predicted to become extinct still persist and those predicted to colonize 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 and 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 (Eriksson et al. 2002; Adriaens et al. 2006), we still expect a general decline in grassland specialists' populations if no conservation measures are taken. Moreover, an increase in forest specialists might be also expected in the following years or decades due to generalised forest encroachment (Debussche et al. 1999). Therefore, extinctions and probably colonizations 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 common as extinction debt and colonization credit are paid. However, since extinction debt exceeds colonization credit in these habitats, the community is expected to undergo net species loss after all extinctions and colonizations occur, as pointed out by the strong total richness decline from persistent grasslands to persistent forests.

Acknowledgements We thank M. Guardiola for fieldwork and plant identification support, L. Sáez for aid in plant identification, G. Esparza for fieldwork assistance, J.M. Ninot for advice on plant specialists lists, P. Vicente for digitising the orthophotomap of 1956 and performing the training points for orthophotomaps' reclassification, M. Pärtel for advice in conducting the analyses, and him and two anonymous referees for valuable comments on the manuscript. This study was funded by MICINN (Spain) in the project LANDPOLNET (CGL2009-12646) and by the Spanish Consolider-Ingenio 2010 programme in the project MONTES (CSD2008-00040). G. Bagaria was supported by a Pre-doctoral FPU fellowship (AP2009-4599) from the Ministerio de Educación y Ciencia (Spain). A. Helm was supported by Estonian Research Council (grant no 9223) and by the EU through the European Regional Development Fund (Centre of Excellence FIBIR). The

experiments comply with the current laws of the country (Spain) in which the experiments were performed.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Adriaens D, Honnay O, Hermy M (2006) No evidence of a plant extinction debt in highly fragmented calcareous grasslands in Belgium. Biol Conserv 133:212–224. doi: 10.1016/j.biocon.2006.06.006

Aitchison J (1982) The statistical analysis of compositional data. J R Stat Soc Ser B 44:139–177. doi: 10.2307/2345821

Baan L, Alkemade R, Koellner T (2012) Land use impacts on biodiversity in LCA: a global approach. Int J Life Cycle Assess 18:1216–1230. doi: 10.1007/s11367-012-0412-0

Bagaria G, Pino J, Rodà F, Guardiola M (2012) Species traits weakly involved in plant responses to landscape properties in Mediterranean grasslands. J Veg Sci 23:432–442. doi: 10.1111/j.1654-1103.2011.01363.x

Bartoń K (2014) MuMIn: Multi-model inference. R package version 1.10.0. http://cran.r-project.org/package=MuMIn

Başnou C, Vicente P, Espelta JM, Pino J (2015) Of niche differentiation, dispersal ability and historical legacies: what drives woody community assembly in recent Mediterranean forests? Oikos. doi: 10.1111/oik.02534

Bolòs O, Vigo J, Masalles RM, Ninot JM (2005) Flora manual dels Països Catalans. Pòrtic, Barcelona

Cornwell WK, Grubb PJ (2003) Regional and local patterns in plant species richness with respect to resource availability. Oikos 100:417–428. doi: 10.1034/j.1600-0706.2003.11697.x

Cousins SAO, Vanhoenacker D (2011) Detection of extinction debt depends on scale and specialisation. Biol Conserv 144:782–787. doi: 10.1016/j.biocon.2010.11.009

Cristofoli S, Piqueray J, Dufrene M, Bizoux J, Mahy G (2010) Colonization credit in restored wet heathlands. Restor Ecol 18:645–655. doi: 10.1111/j.1526-100X.2008.00495.x

Dambrine E, Dupouey J-L, Laüt L, Humbert L, Thinon M, Beaufils T, Richard H (2007) Present forest biodiversity patterns in France related to former Roman agriculture. Ecology 88:1430–1439. doi: 10.1890/05-1314

Debussche M, Lepart J, Dervieux A (1999) Mediterranean landscape changes: evidence from old postcards. Glob Ecol Biogeogr 8:3–15.

Díaz-Villa MD, Marañón T, Arroyo J, Garrido B (2003) Soil seed bank and floristic diversity in a forest-grassland mosaic in southern Spain. J Veg Sci 14:701–709. doi: 10.1111/j.1654-1103.2003.tb02202.x

Eriksson O (1996) Regional dynamics of plants: A review of evidence for remnant, source-sink and metapopulations. Oikos 77:248–258. doi: 10.2307/3546063

Eriksson O, Cousins SAO, Bruun HH (2002) Land-use history and fragmentation of traditionally managed grasslands in Scandinavia. J Veg Sci 13:743–748. doi: 10.1111/j.1654-1103.2002.tb02102.x

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annu Rev Ecol Evol Syst 34:487–515. doi: 10.1146/annurev.ecolsys.34.011802.132419

Grime JP (2001) Plant strategies, vegetation processes, and ecosystem properties. John Wiley and Sons, Chichester

Grove AT, Rackham O (2001) The nature of Mediterranean Europe: An ecological history. Yale

University Press, New Haven

Guardiola M, Pino J, Rodà F (2013) Patch history and spatial scale modulate local plant extinction and extinction debt in habitat patches. Divers Distrib 19:825–833. doi: 10.1111/ddi.12045

Hanski I (2000) Extinction debt and species credit in boreal forests: modelling the consequences of different approaches to biodiversity conservation. Ann Zool Fennici 37:271–280

Hanski I (1999) Metapopulation ecology. Oxford University Press, Oxford

Helm A, Hanski I, Pärtel M (2006) Slow response of plant species richness to habitat loss and fragmentation. Ecol Lett 9:72–77. doi: 10.1111/j.1461-0248.2005.00841.x

Helm A, Zobel M, Moles AT, Szava-Kovats R, Pärtel M (2015) Characteristic and derived diversity: implementing the species pool concept to quantify conservation condition of habitats. Divers Distrib 21:711–721. doi: 10.1111/ddi.12285

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346–363. doi: 10.1002/bimj.200810425

Hylander K, Ehrlén J (2013) The mechanisms causing extinction debts. Trends Ecol Evol 28:341–346. doi: 10.1016/j.tree.2013.01.010

Hylander K, Weibull H (2012) Do time-lagged extinctions and colonizations change the interpretation of buffer strip effectiveness? - a study of riparian bryophytes in the first decade after logging. J Appl Ecol 49:1316–1324. doi: 10.1111/j.1365-2664.2012.02218.x

Jackson ST, Sax DF (2010) Balancing biodiversity in a changing environment: extinction debt, immigration credit and species turnover. Trends Ecol Evol 25:153–160. doi: 10.1016/j.tree.2009.10.001

Jacquemyn H, Butaye J, Hermy M (2003) Influence of environmental and spatial variables on regional distribution of forest plant species in a fragmented and changing landscape. Ecography 26:768–776. doi: 10.1111/j.0906-7590.2003.03620.x

Krauss J, Bommarco R, Guardiola M, Heikkinen RK, Helm A, Kuussaari M, Lindborg R, Öckinger E, Pärtel M, Pino J, Pöyry J, Raatikainen KM, Sang A, Stefanescu C, Teder T, Zobel M, Steffan-Dewenter I (2010) Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. Ecol Lett 13:597–605. doi: 10.1111/j.1461-0248.2010.01457.x

Kühn I, Bierman SM, Durka W, Klotz S (2006) Relating geographical variation in pollination types to environmental and spatial factors using novel statistical methods. New Phytol 172:127–139. doi: 10.1111/j.1469-8137.2006.01811.x

Kuussaari M, Bommarco R, Heikkinen RK, Helm A, Krauss J, Lindborg R, Öckinger E, Pärtel M, Pino J, Rodà F, Stefanescu C, Teder T, Zobel M, Steffan-Dewenter I (2009) Extinction debt: a challenge for biodiversity conservation. Trends Ecol Evol 24:564–571. doi:10.1016/j.tree.2009.04.011

Lindborg R, Helm A, Bommarco R, Heikkinen RK, Kühn I, Pykälä J, Pärtel M (2012) Effect of habitat area and isolation on plant trait distribution in European forests and grasslands. Ecography 35:356–363. doi: 10.1111/j.1600-0587.2011.07286.x

Lira PK, Ewers RM, Banks-Leite C, Pardini R, Metzger JP (2012) Evaluating the legacy of landscape history: extinction debt and species credit in bird and small mammal assemblages in the Brazilian Atlantic Forest. J Appl Ecol 49:1325–1333. doi: 10.1111/j.1365-2664.2012.02214.x

Magee L (1990) R^2 measures based on Wald and likelihood ratio joint significance tests. Am Stat 44:250–253. doi: 10.2307/2685352

McIntyre S, Barrett GW (1992) Habitat variegation, an alternative to fragmentation. Conserv Biol 6:146–147. doi: 10.1046/j.1523-1739.1992.610146.x

Öckinger E, Eriksson AK, Smith HG (2006) Effects of grassland abandonment, restoration and management on butterflies and vascular plants. Biol Conserv 133:291–300. doi:

10.1016/j.biocon.2006.06.009

Pierik M, van Ruijven J, Bezemer T, Berendse F (2010) Travelling to a former sea floor: colonization of forests by understorey plant species on land recently reclaimed from the sea. J Veg Sci 21:167–176. doi: 10.1111/j.1654-1103.2009.01134.x

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2014) nlme: Linear and nonlinear mixed effects models. R package version 3.1-117. http://cran.r-project.org/package=nlme

Piqueray J, Cristofoli S, Palm R, Bisteau E, Mahy G (2011) Testing coexistence of extinction debt and colonization credit in fragmented calcareous grasslands with complex historical dynamics. Landsc Ecol 26:823–836. doi: 10.1007/s10980-011-9611-5

Piqueray J, Saad L, Bizoux J-P, Mahy G (2013) Why some species cannot colonise restored habitats? The effects of seed and microsite availability. J Nat Conserv 21:189–197. doi: 10.1016/j.jnc.2012.12.005

R Development Core Team (2014) R: A language and environment for statistical computing. R package version 3.1.0. http://www.r-project.org

Rivas-Martínez S, Fernández-González F, Loidi J, Lousa M, Penas A (2001) Syntaxonomical checklist of vascular plant communities of Spain and Portugal to association level. Itinera Geobot 14:5–341

Sax DF, Brown JH (2000) The paradox of invasion. Glob Ecol Biogeogr 9:363–371. doi: 10.1007/s00442-011-2203-x

Therneau T, Atkinson B, Ripley B (2013) rpart: Recursive Partitioning. R package version 4.1-1. http://cran.r-project.org/package=rpart

Tilman D, May R, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. Nature 371:65–66. doi: 10.1038/371065a0

Vellend M (2005) Land-use history and plant performance in populations of *Trillium grandiflorum*.

Biol Conserv 124:217–224. doi: 10.1016/j.biocon.2005.01.027

Vellend M (2003) Habitat loss inhibits recovery of plant diversity as forests regrow. Ecology 84:1158–1164. doi: 10.1890/0012-9658(2003)084[1158:HLIROP]2.0.CO;2

Vellend M, Verheyen K, Jacquemyn H, Kolb A, Van Calster H, Peterken G, Hermy M (2006) Extinction debt of forest plants persists for more than a century following habitat fragmentation. Ecology 87:542–548. doi: 10.1890/05-1182

Verheyen K, Guntenspergen GR, Biesbrouck B, Hermy M (2003a) An integrated analysis of the effects of past land use on forest herb colonization at the landscape scale. J Ecol 91:731–742. doi: 10.1046/j.1365-2745.2003.00807.x

Verheyen K, Honnay O, Motzkin G, Hermy M, Foster DR (2003b) Response of forest plant species to land-use change: a life-history trait-based approach. J Ecol 91:563–577. doi: 10.1046/j.1365-2745.2003.00789.x

Weiss RE (2005) Modeling longitudinal data. Springer, New York. doi: 10.3109/02813439309045499

Zulka KP, Abensperg-Traun M, Milasowszky N, Bieringer G, Gereben-Krenn B-A, Holzinger W, Hölzler G, Rabitsch W, Reischütz A, Querner P, Sauberer N, Schmitzberger I, Willner W, Wrbka T, Zechmeister H (2014) Species richness in dry grassland patches of eastern Austria: A multi-taxon study on the role of local, landscape and habitat quality variables. Agric Ecosyst Environ 182:25–36. doi:10.1016/j.agee.2013.11.016

Table 1. Results of 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 and forest specialists). 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	-1.24±1.20	0.540	**-6.43±2.66**	**0.038**	-5.18±2.59	0.103	0.31
Grassland specialists	**-4.05±1.14**	**0.001**	**-10.64±2.60**	**<0.001**	**-6.59±1.66**	**0.022**	0.57
Forest specialists	**1.99±0.38**	**<0.001**	**3.60±0.88**	**<0.001**	1.61±0.86	0.134	0.66

Table 2. Results of selected models for determinants of species richness for 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, those that are significant only in an interaction term are included as main effects, and those not selected are not included. 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

	Grassland specialists	Forest specialists
Predictor	Estimate ± SE	Estimate ± SE
(Intercept)	5.62±4.86	**8.01±1.67**
Plot category (WG - PG)	-1.56±0.99	2.62±1.28
Plot category (PF - PG)	**-6.87±2.20**	-3.84±3.28
Plot category (PF - WG)	**-5.32±2.10**	-6.47±3.07
Historical landscape[a]	---	**3.04±0.98**
Current landscape[b]	**7.20±3.29**	---
Solar radiation availability log-ratio	**1.71±0.22**	**-0.22±0.09**
Clay proportion	---	3.46±2.50
Soil pH	**3.08±0.58**	---
Soil depth	**-0.10±0.04**	---

Historical landscape[1]*Plot category (WG - PG)	---	-0.45±0.65
Historical landscape[1]*Plot category (PF - PG)	---	**-4.64±1.60**
Historical landscape[1]*Plot category (PF - WG)	---	**-4.19±1.51**
Clay proportion*Plot category (WG - PG)	---	**-7.49±3.19**
Clay proportion*Plot category (PF - PG)	---	5.30±6.82
Clay proportion*Plot category (PF - WG)	---	12.79±6.61
Likelihood-ratio based pseudo-R^2	0.71	0.70

[a] 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 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.

FIGURE LEGENDS

Fig. 1 Maps showing a) location of the study area in the Iberian Peninsula and b) distribution of the study sites in the Prades Mountains (800 m contour line) over the land-cover map of 2009. Specific site locations showing c) the spatial scale of study sites and d) the distribution of sampling plots within a site

Fig. 2 a) Historical and b) current orthophotomaps from a study site showing how plot tree cover was calculated to determine plots with few changes from previous grasslands (persistent grasslands), woody encroachment into previous grasslands (wooded grasslands), and few changes from previously wooded plots (persistent forests)

Fig. 3 Observed richness per sampling plot of different plant species groups along the woody encroachment gradient. Bars are means ±1SE for each species group

FIGURES

Fig. 1

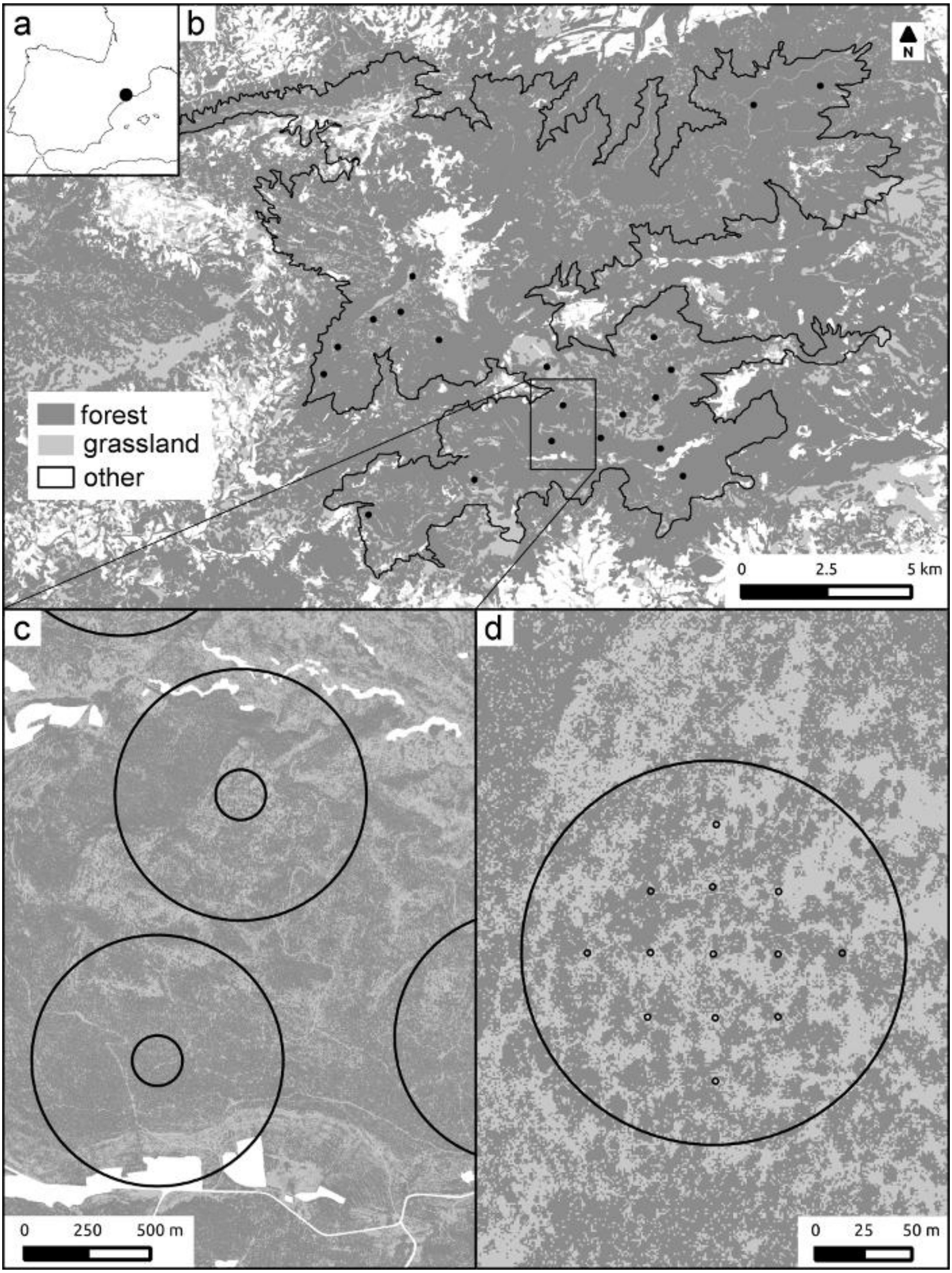


Fig. 2

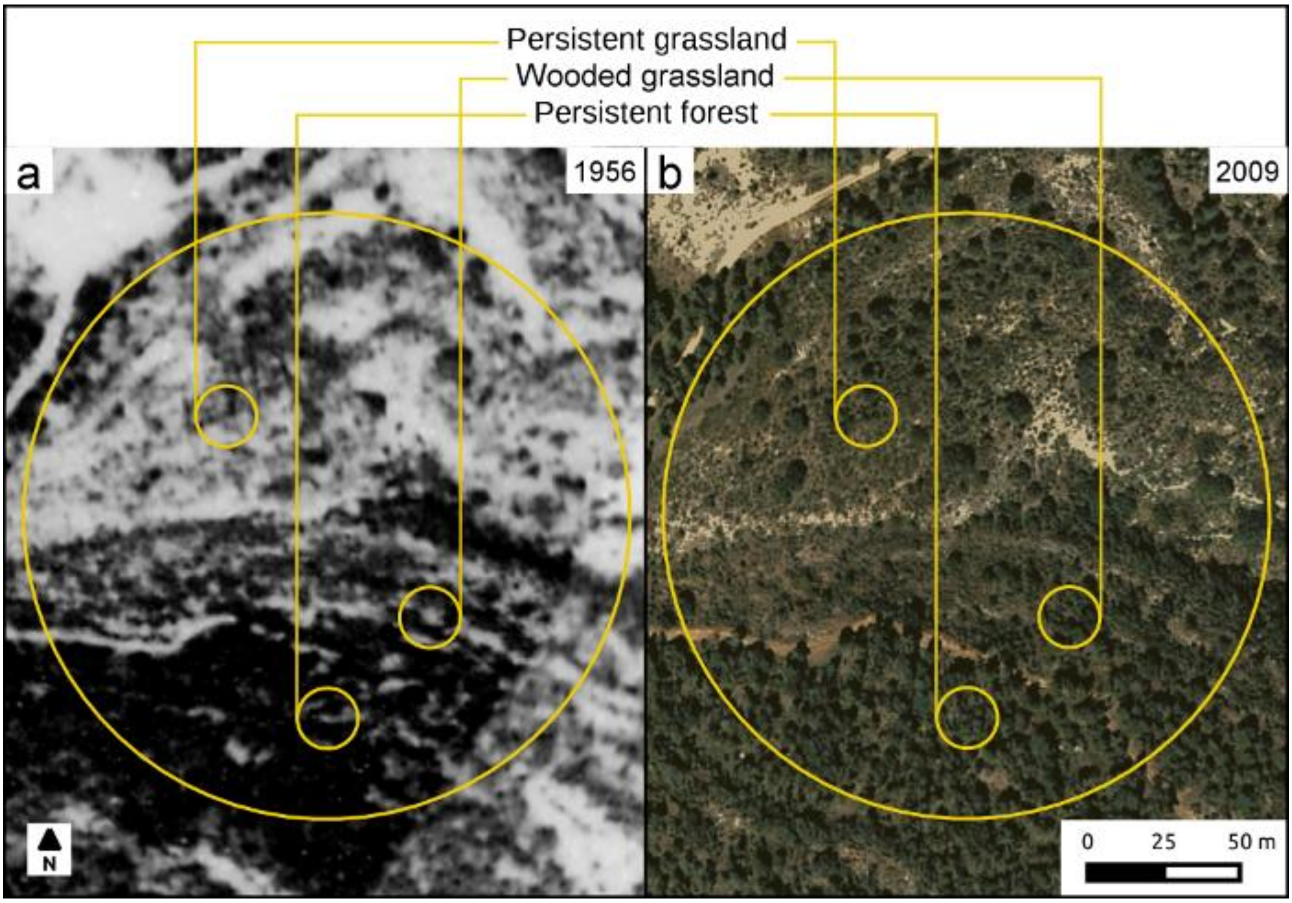
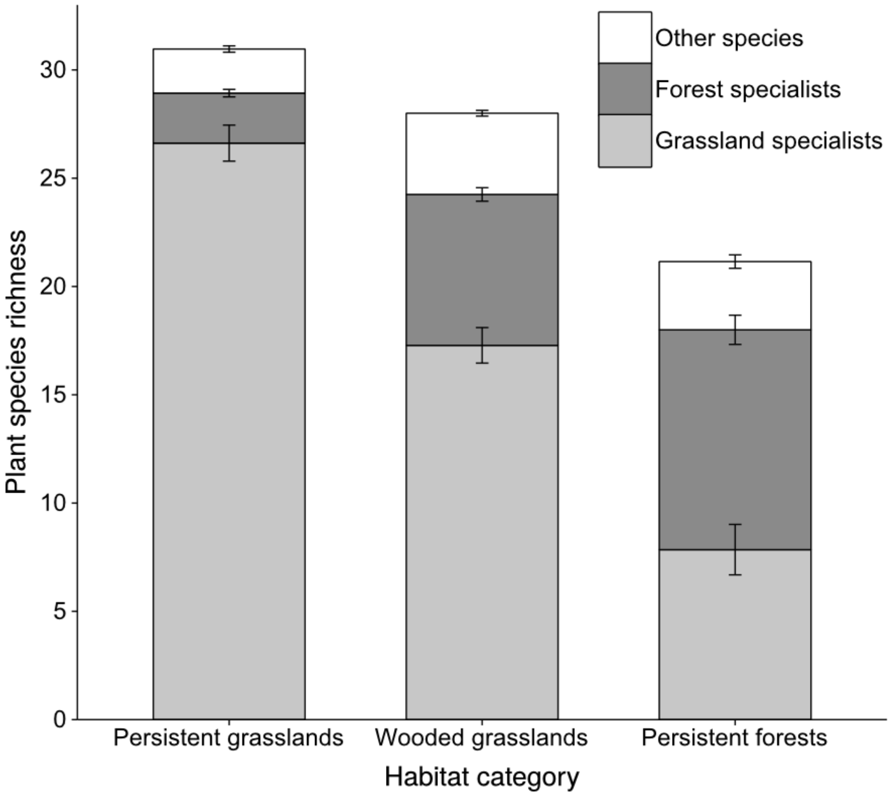

Persistent grassland
Wooded grassland
Persistent forest
a
1956
b
2009
N
0
25
50 m

Fig. 3


Other species
Forest specialists
Grassland specialists
Plant species richness
0
5
10
15
20
25
30
Persistent grasslands
Wooded grasslands
Persistent forests
Habitat category