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1 **Extinction and colonization of habitat specialists drive plant species replacement along a**
2 **Mediterranean grassland-forest succession.**

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4 Running title: **β -diversity along succession**

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20

21 **Abstract**

22 **Questions:** Land-use change causes shifts in species richness, which can be delayed. However,
23 beta-diversity patterns and especially the relative role of species replacement and nestedness in
24 these situations with time-lagged extinctions and colonizations remain unknown. We aim to (1)
25 quantify beta-diversity change, species replacement and nestedness for vascular plants along a
26 grassland-forest succession with time-lagged biodiversity change for more than 50 years; (2) check
27 its consistency between all species, grassland specialists and forest specialists, and (3) identify the
28 role of forest encroachment relative to other drivers.

29 **Location:** Prades Mountains, Catalonia (NE Iberian Peninsula).

30 **Methods:** We sampled 18 sites representing a gradient in past and current grassland area and
31 connectivity, and in forest encroachment intensity, to obtain plant composition of all species,
32 grassland specialists and forest specialists. We quantified overall beta-diversity and its components
33 at each species classification group along the forest encroachment gradient and other drivers. Then,
34 we used general linear models to study (1) the change rate of beta diversity along the forest
35 encroachment gradient and (2) the relative importance of the drivers in explaining beta diversity.

36 **Results:** Following forest encroachment gradient, we found an overall noticeable species
37 replacement, while nestedness was the main component for habitat specialists. Landscape
38 differences contributed to explain most compositional differences (both nestedness and
39 replacement), while soil characteristics and geographic distance had a more restricted contribution.

40 **Conclusions:** Species replacement due to environmental sorting occurred along succession,
41 triggered by selective grassland specialists' extinctions and selective forest specialists' colonizations.
42 Nonetheless, historical landscape characteristics, current landscape characteristics and geographic
43 distance modulate plant extinctions and colonizations, suggesting biological inertia, mass effects
44 and habitat isolation, respectively. Partitioning beta-diversity into nestedness and replacement
45 components and exploring the extinction and colonization patterns of habitat specialists groups

46 might provide relevant insights on the drivers and processes of community shift after land-use
47 change.

48

49 **Keywords**

50 Beta-diversity; Habitat change; Habitat specialists; Nestedness; Species replacement; Vascular
51 plants

52

53 **Nomenclature** Bolòs et al. (2005)

54

55 **β -diversity after forestation**

56

57 **Introduction**

58 Land-use change is one of the main causes of biodiversity changes, driving extinctions and
59 colonizations worldwide, which result in richness gains or losses (Fahrig 2003; Jackson & Sax
60 2010; Baan et al. 2012), species replacement (Fahrig 2003; Jackson & Sax 2010) and biotic
61 homogenization or differentiation (Olden 2006). Recently, there has been a wide interest in
62 investigating both immediate and time-lagged effects of habitat loss and transformation with a focus
63 on species richness, while compositional changes remain much more overlooked (but see Larrea &
64 Werner 2010; Arroyo-Rodríguez et al. 2013; Kopecký et al. 2013; Trentanovi et al. 2013; Lindborg
65 et al. 2014). Biodiversity change and its time-lags, namely extinction debt (Tilman et al. 1994;
66 Kuussaari et al. 2009) and colonization credit (Jackson & Sax 2010; Cristofoli et al. 2010) have
67 been frequently identified following habitat change (for counterexamples see Adriaens et al. 2006;
68 Cristofoli et al. 2010). However, little is known about β -diversity patterns, largely ignoring whether
69 the payment of these debts and credits follows any pattern along the habitat change gradients.
70 Investigating β -diversity patterns is particularly interesting across large environmental changes

71 (Mori et al. 2018), e.g. in ecological communities along succession. In this situation, a species
72 replacement is expected, promoted by extinctions of species of the former habitat type and
73 colonizations of species specific to the new habitat type, and resulting in a directional change in
74 composition. Composition differences can only be due to species replacement and nestedness
75 between sites, or a combination of both. Species replacement means a substitution of species by
76 others, while nestedness implies net species gain or loss. Both changes usually occur together but
77 are due to different ecological processes (Legendre 2014) and, then, they must be investigated
78 separately in order to unravel the processes behind composition changes. Baselga (2010) proposed
79 additively partitioning total β -diversity into species replacement and species nestedness between
80 sites, in order to account for the different processes leading to them: (i) environmental sorting or
81 spatial and historical constraints are the processes resulting in a pattern of species replacement, and
82 (ii) selective extinction/colonization, habitat nestedness or interspecific variation in the breadth of
83 environmental tolerance are the processes which result in a pattern of species loss or gain (Leprieur
84 et al. 2011). Species replacement due to environmental sorting has shown as a major process behind
85 total community change along a gradient of environmental conditions (Leibold et al. 2004).

86 However, little is known about the relative contribution and drivers of both species replacement and
87 nestedness to species compositional change following habitat change, especially along succession.

88 During the last century and especially over the past 70 years, there has been an important decline
89 in European semi-natural grasslands, which experienced encroachment by forest (Eriksson et al.
90 2002; Adriaens et al. 2006). In the Mediterranean Basin, semi-natural grasslands also experienced a
91 widespread loss and fragmentation which was predicted to result in a decline of grassland species
92 and a spread of forest species (Debussche et al. 1999), and evidence for a partly paid extinction debt
93 and colonization credit, and a tendency to total richness decrease was already found (Bagaria et al.
94 2015).

95 Regarding habitat specialists, Bagaria et al. (2015) pointed out strong richness gradients which

96 suggest that nested species gain or loss might be more important than replacement for explaining
97 changes in specialist species composition. Selective extinctions or colonizations are likely to occur
98 on the basis of the breadth of environmental tolerance of species (Kopecký et al. 2013), with few or
99 no replacement (substitution of some species by others) within each species group along a habitat
100 change. However, Guardiola et al. (2013) suggested that grassland specialists' extinctions in the
101 same habitats were idiosyncratic following habitat loss, thus determining a poorly nested pattern for
102 grassland specialists' composition along the forest encroachment gradient. Forest specialists'
103 colonizations, on the other hand, are expected to occur in a more ordered pattern, as they tend to be
104 faster for very mobile and long-dispersed plants, while plants with large seeds, low fecundity and
105 unassisted dispersal tend to be poor colonizers (Verheyen et al. 2003; Ozinga et al. 2005; Hermy &
106 Verheyen 2007; Svenning et al. 2009). Moreover, both historical and current landscape might
107 modulate species extinctions and colonizations (Bagaria et al. 2015). Forest connectivity, among
108 other factors, enhances forest specialists' colonization (Honnay et al. 2005), but for some species
109 showing limited dispersal, the signal of historical landscape composition might be found after
110 several decades or even more than a century (Vellend et al. 2006; Bañnou et al. 2016). If habitat
111 change strongly influences species composition, resulting in a highly nested pattern, a biotic
112 homogenization occurs (Kopecký et al. 2013), but habitat change can also result in extinction and
113 colonization-driven biotic differentiation, through idiosyncratic species gains and losses, when
114 fragmentation is high and seed dispersal is limited (Arroyo-Rodríguez et al. 2013; Lindborg et al.
115 2014).

116 In the present work, we investigated β -diversity patterns regarding species replacement and
117 nested species loss or gain in a forest encroachment process that drives strong habitat change from
118 Mediterranean semi-natural grasslands to forests, which is a representative successional gradient of
119 a common process (Debussche et al. 1999). We selected 18 sites across a grassland-forest gradient
120 resulting from a forest encroachment process that started more than 50 years ago (Bagaria et al.

121 2012). Our main aims were: i) to quantify β -diversity and its components (species replacement and
122 nestedness) along the forest encroachment gradient; ii) to assess its consistency between species
123 groups (all species, grassland specialists and forest specialists), and iii) to quantify the relative
124 importance of forest encroachment, soil characteristics, landscape and geographic distance on
125 species replacement and nestedness.

126

127 **Methods**

128 **Study area**

129 The study system comprises Mediterranean semi-natural calcareous grasslands and forests resulting
130 from grassland encroachment in Prades Mountains in southern Catalonia (NE Iberian Peninsula;
131 41°14'-41°23'N, 0°56'-1°10'E). These grasslands are dominated by hemicryptophytes and
132 chamaephytes up to 0.5 m high, and vegetation cover ranges from 50% to 80% (see Guardiola et al.
133 2013 for details). A forest-grassland mosaic persisted in the study area until the decade of 1940s,
134 which was grazed by sheep and goats. During the second half of the 20th century, the semi-natural
135 grasslands in the area experienced an important decrease due to livestock grazing reduction that led
136 to forest encroachment (Guardiola et al. 2013), resulting in relatively open, mixed forests of Scots
137 pine (*Pinus sylvestris* L.) and holm oak (*Quercus ilex* L.). The study sites are located between 800
138 and 1200 m a.s.l. and have mean annual temperature around 11 °C, mean annual precipitation
139 around 750 mm, and marked summer drought (Digital Climatic Atlas of Catalonia;
140 www.opengis.uab.cat/acdc).

141 **Site selection**

142 We selected 18 sites, each with a diameter of 200 m, in a set of calcareous plateaus. All sites were
143 located in a small area within the mountains (11 x 7 km) to prevent compositional variation due to a
144 change in climatic conditions. Sampling within sites was conducted without distinguishing habitat
145 patches, because habitat transitions in these Mediterranean systems show a high fuzziness (very

146 gradual and irregular transitions), and the delimitation of habitat boundaries is not reliable (Bagaria
147 et al. 2012). Site selection was conducted using historical (1956) and recent (2009) orthophotomaps
148 and other topographic and thematic maps (e.g. wildfires, soil types), according to the following
149 criteria: (1) it was required that site current grassland cover and grassland cover change ranged
150 considerably (Fig. 1); (2) forest encroachment had to be spontaneous (i.e. not due to forest
151 plantation); (3) no crop fields could exist in the past nor nowadays within them; (4) sites could not
152 have been burned in the previous 25 years; (5) slope was required to be lower than 20°, and (6) the
153 distance between them had to be at least 1 km in order to avoid landscape overlap and increase data
154 independence. After preliminary selection over orthophotomaps, a preliminary field survey was
155 used to discard unsuitable areas (see Bagaria et al. 2015 for details). In the 18 selected sites, current
156 grassland cover ranged from 16 to 70%, while grassland cover change varied from 61% loss to 8%
157 increase.

158 **Sampling species composition**

159 In spring and early summer 2011, all vascular plant species were recorded in 13 circular plots of 3
160 m in diameter at each of the 18 selected sites (see Appendix S1 for details). Plots were arranged in a
161 regular grid with a distance about 33 m between each other. Using regional floras (Rivas-Martínez
162 et al. 2001; Bolòs et al. 2005) and expert advice, all species were classified as grassland specialists,
163 i.e. plants growing mainly in calcareous grasslands and open shrublands; forest specialists, i.e.
164 mainly growing in forests; and other (either generalist species or specialists from other habitats,
165 e.g., ruderal or rocky habitats). From 246 recorded taxa, there were 138 grassland specialists and 54
166 forest specialists (see Appendix S2). In order to study β -diversity changes and colonization and
167 extinction patterns along the forest encroachment gradient, tables of species composition (presence-
168 absence) at site level were obtained for each of the different plant groups (all species, grassland
169 specialists and forest specialists).

170 **β -diversity measures**

171 We calculated β -diversity metrics for species presence-absence between all pairs of sites for the
172 three different species groups (i.e. all species, grassland specialists, forest specialists) in order to
173 investigate changes in community composition along environmental and spatial gradients. β -
174 diversity and its two additive components (nestedness and species replacement) were calculated as
175 proposed by Baselga (2010), using the function 'designdist' in the *vegan* R package (Oksanen et al.
176 2013). In this framework, for any pair of sites, total β -diversity (β_{sor}) is calculated by the Sørensen
177 dissimilarity index:

$$178 \quad \beta_{\text{sor}} = \frac{b+c}{2a+b+c} \quad (1)$$

179 The species replacement component of β -diversity (β_{sim}) is calculated by the Simpson dissimilarity
180 index:

$$181 \quad \beta_{\text{sim}} = \frac{\min(b, c)}{a + \min(b, c)} \quad (2)$$

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

$$184 \quad \beta_{\text{sne}} = \frac{\max(b, c) - \min(b, c)}{2a+b+c} \times \frac{a}{a + \min(b, c)} \quad (3)$$

185 where a , b and c are the shared species between both sites, species unique to the first site, and
186 species unique to the second site, respectively (Krebs 1999). Total β -diversity is the sum of the two
187 components, replacement and nestedness-resultant dissimilarity; $\beta_{\text{sor}} = \beta_{\text{sim}} + \beta_{\text{sne}}$.

188 **Habitat and landscape characteristics**

189 We obtained local-habitat variables that describe site characteristics and the process of forest
190 encroachment, which is expected to be the main driver of plant β -diversity changes through species
191 replacement and nestedness. Soil depth was calculated as the mean of three measures made at each
192 plot and a soil sample up to a depth of 10 cm was taken to analyze soil pH, organic matter content
193 and clay proportion. Solar radiation ($\text{MJm}^{-2}\text{day}^{-1}$) was calculated as the product between site spring

194 solar radiation (obtained from the Digital Climatic Atlas of Catalonia; www.opengis.uab.cat/acdc)
195 and the proportion of Photosynthetic Active Radiation (PAR) reaching understory plants (measured
196 using a ceptometer at plot centre; AccuPAR LP-80; Decagon Devices, Inc.). Grazing intensity from
197 livestock and/or wild ungulates (mostly roe deer) was measured at each plot, based on excrement
198 groups and eaten plants, in an ordinal way (0, no grazing signs; 1, one grazing sign; 2, more than
199 one grazing sign). Tree cover in a 10-m radius was obtained for each plot as the proportion of forest,
200 from reclassified orthophotomaps of 2009 (pixel size of 1 m). In order to reduce redundancy among
201 local variables related to habitat, a principal components analysis (PCA) on the Pearson correlation
202 matrix was conducted for tree cover, soil depth, pH, organic matter proportion, clay proportion,
203 solar radiation and grazing (taken as quantitative) at plot scale. The first axis of the PCA explained
204 38.6% of the total variance of the table (Fig. 2), and it shows a gradient which was closely related
205 (negatively) with forest encroachment, from forest habitat in the negative side (high tree cover, soil
206 organic matter, clay texture and soil depth) to grassland habitat in the positive side (high solar
207 radiation, soil pH and grazing). The second axis explained 16.6% of the variance and corresponded
208 to a gradient in soil characteristics, from high soil depth and pH on the positive side to high organic
209 matter in the negative side. Then, site centroids (from all plots within a site) for these two axes were
210 used in the statistical analysis as forest encroachment and soil characteristics proxies. Scores from
211 environmental variables on the first and second PCA axes are shown in Appendix S3.

212 In addition, geographic coordinates and connectivity in both the historical and current landscapes
213 were obtained for each site. Landscape connectivity measures were calculated at 500 m radius from
214 each site centre, using maps produced by combining land-cover layers and reclassified
215 orthophotomaps, for both historical (1956) and current (2009) contexts. The combined maps
216 presented fine-scale transitions from grassland to forest (from reclassification of orthophotomaps)
217 and well-defined crop fields, bare soil, and urban areas (from digitized land-cover maps; see
218 Bagaria et al. 2015 for details). These landscape connectivity measures were species-group specific,

219 i.e., grassland/forest ratio for all species, grassland ratio (grassland to non-grassland ratio) as
220 connectivity for grassland specialists, and forest ratio (forest to non-forest ratio) as connectivity for
221 forest specialists.

222 **Statistical analyses**

223 We performed a set of general linear models relating β -diversity to Euclidean distances between
224 sites in (a) forest encroachment (site centroids along axis 1 of the PCA); (b) soil characteristics (site
225 centroids along axis 2 of the PCA); (c) current landscape connectivity (log-transformed to improve
226 normality); (d) historical landscape connectivity (log-transformed), and (e) geographic coordinates.

227 For each of the plant groups (i.e. all species, grassland specialists, forest specialists), and β -
228 diversity measures (total β -diversity [β_{sor}], nestedness-resultant dissimilarity [β_{sne}] and species
229 replacement [β_{sim}]), the composition change rate along forest encroachment (slope of the regression
230 of a β -diversity measure against the forest encroachment gradient) was obtained in order to compare
231 the slope (standardized coefficients) and strength (contribution to total R^2) of the relationship
232 between species groups and β -diversity components.

233 To investigate the relative contribution of forest encroachment and the other drivers affecting
234 nestedness or species replacement (i.e. soil characteristics, historical landscape, current landscape
235 and geographic distance), we decomposed total R^2 of each model into the contribution of each
236 predictor using *relaimpo* R package (Grömping 2006) and 'pmvd' metric (Feldman 2005) in the
237 models for the two different β -diversity components. Confidence intervals were calculated using
238 1000 bootstrap repetitions and the bias-corrected and accelerated method. The significance level for
239 each predictor was corrected using error degrees of freedom equal to the number of sites (18),
240 because of the non-independence of pair-wise site comparisons (Qian & Ricklefs 2012). No model
241 selection was performed for these models because the aim was to study the relative contribution of
242 all the included factors to beta-diversity, and to compare it between models. All statistical analyses
243 were conducted using R (R Core Team 2016).

244

245 **Results**

246 **β -diversity along the forest encroachment gradient**

247 For the whole species composition, a noticeable rate of change along the forest encroachment
248 gradient was found for total β -diversity (standardized coefficient \pm SE = 0.48 ± 0.06 ; R^2
249 contribution=0.36) and replacement (0.44 ± 0.06 ; R^2 contribution=0.29), but not for nestedness
250 (0.18 ± 0.08 ; R^2 contribution=0.04). In the case of grassland specialists, the total β -diversity change
251 rate along the forest encroachment gradient was similar to that of all species (0.47 ± 0.06 ; R^2
252 contribution=0.33), but it was mainly due to nestedness (0.35 ± 0.07 ; R^2 contribution=0.19), rather
253 than to species replacement (0.27 ± 0.07 ; R^2 contribution=0.08). For forest specialists, we found
254 rather weak total β -diversity (0.20 ± 0.08 ; R^2 contribution=0.06), but noticeable nestedness ($0.25 \pm$
255 0.08 ; R^2 contribution=0.06) change rates and no effect of species replacement (-0.08 ± 0.08 ; R^2
256 contribution=0.01) along the forest encroachment gradient (Fig. 3). The effect of forest
257 encroachment on β -diversity is significant in all nine models, except for forest specialists'
258 replacement.

259 **Drivers of nestedness and species replacement**

260 For all species composition, the model for the β -diversity component accounting for species
261 nestedness between sites (β_{sne}) explained 16% of the variance, while historical landscape (R^2
262 contribution=0.07), forest encroachment (R^2 contribution=0.04) and soil characteristics (R^2
263 contribution=0.03) were positively correlated and had a similar contribution to total R^2 . Both
264 current landscape and geographic distance were not significantly associated with β_{sne} (Table 1;
265 Appendix S4a). As expected, the model for all species replacement (β_{sim}) accounted for higher
266 amount of total variance, 49%. Forest encroachment (R^2 contribution=0.29) and current landscape
267 (R^2 contribution=0.16) explained the majority of this variance, while geographic distance (R^2
268 contribution=0.03), historical landscape and soil characteristics had almost no effect on species

269 replacement, although geographic distance was significant (Appendix S4b). All the predictors were
270 positively correlated with β_{sim} .

271 For grassland specialists, the model for the nestedness component of β -diversity (β_{sne}) between
272 sites explained 38% of the variance, and showed that forest encroachment (R^2 contribution=0.19),
273 historical landscape (R^2 contribution=0.12) and current landscape (R^2 contribution=0.04) were the
274 variables significantly and positively associated with species nestedness for this group. Soil
275 characteristics and geographic distance effects were not significant (Appendix S4c). Regarding
276 species replacement (β_{sim}), the model for grassland specialists explained 32% of the variance, and
277 included current landscape as the most important driver (R^2 contribution=0.18), followed by forest
278 encroachment (R^2 contribution=0.08) and historical landscape (R^2 contribution=0.05) (Appendix
279 S4d). All these predictors but historical landscape dissimilarity were positively correlated with β_{sim} .
280 Again, geographic distance and soil characteristics effects were not significant.

281 For forest specialists, the model for the nestedness component of β -diversity (β_{sne}) explained
282 25% of the variance. Current landscape was the best predictor of this component (R^2
283 contribution=0.17), followed by forest encroachment (R^2 contribution=0.06), and being both
284 positively correlated with β_{sne} . In contrast, soil characteristics, historical landscape and geographic
285 distance were non-significantly associated with β_{sne} (Appendix S4e). Finally, the model for the
286 species replacement component of β -diversity (β_{sim}) only explained 9% of the variance, and only
287 geographic distance (R^2 contribution=0.05) was significant, being positively correlated with β_{sim}
288 (Appendix S4f).

289

290 **Discussion**

291 Our study has explored changes in β -diversity and its components (i.e. species nestedness and
292 replacement) across forest encroachment in Mediterranean grasslands. Species replacement for the
293 whole community is driven by ordered extinctions and colonizations of grassland and forest

294 specialists, respectively, along the forest encroachment gradient. This is consistent with the richness
295 loss for grassland specialists and richness gain for forest specialists without total richness change
296 found by Bagaria et al. (2015) at plot scale in the same sites, after 50 years of forest encroachment.
297 Also Kopecký et al. (2013) found a similar pattern of nested extinctions, after forest management
298 cessation, corresponding to light-demanding species. On the other hand, Guardiola et al. (2013)
299 found an idiosyncratic (non-nested) extinction of grassland specialists at local scale in the same
300 region of our study in grassland patches that decreased in size. However, strong habitat
301 transformation similar to the present study had not occurred in their study sites.

302 Forest encroachment, but also landscape connectivity, are the overall primary drivers of β -
303 diversity for both species replacement and nestedness, while soil characteristics only played a
304 significant role in all species nestedness, and geographic distance was significantly related with
305 species replacement of both all species and forest specialists. A small effect of the historical
306 landscape on nestedness of all species was found, indicating that part of the nowadays weak nested
307 composition pattern still relies on grassland/forest ratio 50 years ago. Current landscape markedly
308 contributes, altogether with forest encroachment, to the replacement of total species, suggesting that
309 the replacement of grassland specialists by forest specialists following forest encroachment can be
310 modulated by the surrounding landscape. However, to elucidate the mechanisms under these
311 patterns, the study of nestedness and replacement of grassland and forest specialists is needed.

312 Historical and, to a lesser extent, current landscape distances affect nestedness for grassland
313 specialists, and they complement nestedness for this group along forest encroachment. Hence, there
314 also exists an ordered loss of grassland specialists (nestedness) as grassland prevalence in the
315 landscape decreases. In the same vein, many studies stressed the role of landscape connectivity in
316 promoting richness of grassland specialists in a grassland fragmentation context (e.g. Adriaens et al.
317 2006; Lindborg et al. 2014). On the other hand, the effect of historical landscape suggests that
318 richness is not yet in equilibrium with the current environmental characteristics, indicating the

319 existence of an extinction debt (Helm et al. 2006; Kuussaari et al. 2009; Krauss et al. 2010; Jackson
320 & Sax 2010; Bagaria et al. 2015) caused by biological inertia of grassland specialist plants, as found
321 for a grassland specialist species in the study area (Bagaria et al. 2018). Although habitat conditions
322 strongly drive nestedness of grassland specialists between sites, grassland specialists' replacement
323 was more dependent on current landscape than on the forest encroachment gradient, indicating that
324 different grassland specialists appear for different current landscape compositions regardless of
325 habitat characteristics. Plant populations in the surrounding landscapes likely reinforce site
326 populations by mass effects (Mouquet & Loreau 2003; Leibold et al. 2004), providing new
327 individuals to otherwise non-viable populations. Moreover, since species substitutions occur along
328 this gradient, species with different biological characteristics and/or competitive and dispersal
329 abilities might be favoured by different landscape compositions, as shown in previous works
330 (Lindborg 2007; Saar et al. 2012). Historical landscape distance also affects, to a lesser extent,
331 grassland specialists' replacement but with a negative relationship, indicating that sites similar in
332 historical grassland availability show higher replacement. This suggests that sites with very low
333 connectivity in the past are species-poor analogues of sites with high past connectivity, as supported
334 by the nestedness component. In contrast, idiosyncratic extinctions might be occurring depending
335 on other site characteristics (Marini et al. 2013), until the extinction debt will ultimately be paid.

336 Current landscape is the main predictor of nestedness for forest specialists, although forest
337 encroachment is also significant. Thus, forested landscapes might act as propagule sources able to
338 increase forest species number at site scale in a nested way, likely starting with high dispersability
339 plants and ending with those having large seeds, low fecundity or unassisted dispersal (Verheyen et
340 al. 2003). However, this process is expected to last at least some more decades, since ancient forest
341 species are known to rely on the amount of ancient forest in the landscape and not in current forest
342 connectivity (Vellend 2003). Contrary to grassland specialists, no historical effects were found,
343 probably because relevant historical effects act at longer time scales. Forest specialists' replacement

344 remains unexplained by forest encroachment or landscape, and only geographic distance has a weak
345 effect on it, indicating a distance decay of similarity (*sensu* Nekola & White 1999). That might
346 occur because colonization is still in progress even in the most forested sites and landscapes
347 (Jackson & Sax 2010; Bagaria et al. 2015) and dispersal limitation plays an important role
348 (Jamoneau et al. 2012) due to a long history of forest management and exploitation of
349 Mediterranean forests (e.g. Grove & Rackham 2001; Guirado et al. 2007, 2008; Başnou et al. 2016).

350 In our study system, some biotic homogenization is likely to occur for grassland specialists as
351 forest encroachment increases, increasing similarity in species composition for this group between
352 sites, which is supported by the nestedness of grassland specialists' composition along the forest
353 encroachment gradient. However, replacement, although weaker, is also significant, which
354 advocates for being cautious about this possibility. For forest specialists, the nestedness rate along
355 forest encroachment is weaker, and replacement occurs as distance increases, likely due to habitat
356 isolation, which has been proposed as a cause of biotic differentiation by seed dispersal limitation
357 between populations (Jamoneau et al. 2012; Arroyo-Rodríguez et al. 2013). A specific study would
358 be needed in order to fully investigate homogenization/differentiation patterns and their causes in
359 this system.

360 In conclusion, the patterns of β -diversity analysis for specialist species groups give insight into
361 the processes shaping communities after habitat change. As a result of the forest encroachment
362 process that affected the Mediterranean Basin during the last century (Debussche et al. 1999), a
363 noticeable species replacement occurred with few richness change (few overall nestedness along the
364 forest encroachment gradient), and it was driven by the mainly selective extinction of grassland
365 specialists and the weaker selective colonization of forest specialists, which partly compensated
366 each other. Therefore, an environmental sorting exists for the complete plant community, driven by
367 two species groups with opposite habitat requirements. However, extinction of grassland specialists
368 and colonization of forest specialists are also modulated by landscape characteristics, suggesting

369 that they might be delayed or accelerated depending on current habitat connectivity, and even a past
370 connectivity signal remains for grassland specialists.

371 The additive decomposition of β -diversity has proven useful to disentangle community assembly
372 processes (e.g. Leprieur et al. 2011; Marini et al. 2013), and here we depicted the patterns of species
373 replacement and nestedness, and their underlying processes, that drive total compositional change
374 after forest encroachment. Likewise, investigating only the complete community without
375 differentiating specialist species groups would not have provided insight into the processes leading
376 to total species replacement. Although previous studies showed the usefulness of investigating
377 richness gradients for habitat specialists compared to all species after grassland loss and
378 fragmentation (e.g. Adriaens et al. 2006; Öster, Cousins & Eriksson 2007; Öckinger et al. 2012),
379 they were limited to former habitat specialists, and no replacement patterns were investigated. Also,
380 studying the β -diversity patterns for different plant groups has already proven useful to elucidate
381 community assembly mechanisms in a plant invasion context (Marini et al. 2013). Hence,
382 partitioning total β -diversity into its replacement and nestedness components for different species
383 groups allowed clarifying how the extinction and colonization processes occur after land-use
384 change, being selective extinction and colonization the causes under plant replacement for the
385 whole community which, in turn, is driven by environmental sorting.

386 The processes under community change in this system could well hold for other communities
387 experiencing strong habitat transformation due to forestation. The study of a well delimited system
388 and habitat transition allowed us to deal with reduced species pools and environmental variability,
389 since larger geographic gradients might mask diversity patterns (e.g. Guardiola et al., 2013).
390 However, β -diversity patterns might be different if different processes occur, like random
391 extinctions or colonizations resulting in high replacement within habitat specialists' groups, or
392 strong total richness gain or loss resulting in nestedness for the whole community. Then, more
393 studies are needed to improve the knowledge on the complete process of compositional shift

394 (nestedness and replacement) after land-use change in different systems, focusing on the
395 environmental and species characteristics that might accelerate, delay or modify extinctions and
396 colonizations.

397

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404

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536 577.

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538

539 **Supporting Information**

540 Additional Supporting Information may be found in the online version of this article:

541

542 **Appendix S1.** Details on sampling design procedure.

543 **Appendix S2.** List of plant species found in the survey.

544 **Appendix S3.** Environmental variables scores on PCA axes.

545 **Appendix S4.** Figure showing variance decomposition on models for each species group and β -
546 diversity component.

547 Table 1. Standardized coefficients and their standard error for each predictor in the linear model for
 548 each combination of species group (all species, grassland specialists, forest specialists) and β -
 549 diversity component (total, β_{sor} ; richness differences, β_{sne} ; species replacement, β_{sim}) between pairs
 550 of sites. Total R^2 of each model is given and significant predictors at $P < 0.05$ are marked in bold
 551 (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

552

Species group	β -diversity index	Predictor					Total R^2
		Δ Forest encroachment	Δ Soil characteristics	Δ Current landscape	Δ Historical landscape	Geographic distance	
All species	β_{sor}	0.48±0.06***	0.09±0.05	0.35±0.06***	0.12±0.05*	0.19±0.05**	0.58
	β_{sne}	0.18±0.08*	0.17±0.08*	-0.04±0.08	0.23±0.08*	0.12±0.08	0.16
	β_{sim}	0.44±0.06***	0.05±0.06	0.37±0.06***	0.06±0.06	0.16±0.06*	0.49
Grassland specialists	β_{sor}	0.47±0.06***	0.10±0.05	0.42±0.05***	0.06±0.05	0.14±0.05*	0.61
	β_{sne}	0.35±0.07***	0.12±0.07	0.18±0.07*	0.31±0.07***	0.10±0.07	0.38
	β_{sim}	0.27±0.07**	0.02±0.07	0.39±0.07***	-0.26±0.07**	0.08±0.07	0.32
Forest specialists	β_{sor}	0.20±0.08*	-0.01±0.08	0.30±0.08**	0.00±0.08	0.22±0.08*	0.20
	β_{sne}	0.25±0.08**	-0.12±0.07	0.37±0.07***	-0.08±0.07	0.01±0.07	0.25
	β_{sim}	-0.08±0.08	0.12±0.08	-0.12±0.08	0.09±0.08	0.19±0.08*	0.09

553

554 Figure 1. Relation between current site grassland cover and site grassland cover change (current
555 minus historical grassland cover). Both measures are only weakly and non-significantly correlated
556 ($R^2 = 0.18$; $P = 0.08$).

557

558 Figure 2. Plot corresponding to the first and second axes from the PCA of habitat characteristics in
559 the 234 sampled plots performed on the correlation matrix. Axis 1 explained 38.6% of the total
560 variance and corresponds to a habitat gradient negatively related with forest encroachment, from
561 forest in the negative side to grassland in the positive side. Axis 2 accounted for 16.6% of the
562 variance and is consistent with a gradient in soil characteristics, from high soil depth and pH on the
563 positive side to high organic matter in the negative side. Circles show sampled plot positions, while
564 arrows correspond to variables.

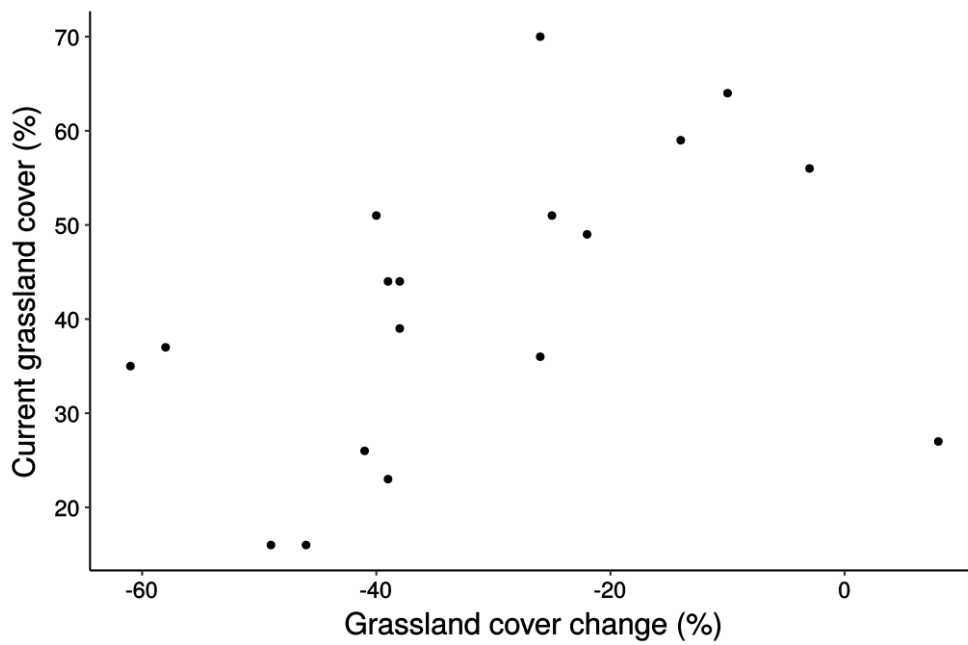
565

566 Figure 3. β -diversity gradients along the forest encroachment distance gradient for the different
567 components: (a) total β -diversity, β_{sor} ; (b) nestedness, β_{sne} , and (c) species replacement, β_{sim} . The
568 three species groups are depicted with different symbols: all species (dots, solid line), grassland
569 specialists (triangles, dotted line) and forest specialists (crosses, dashed line). β -diversity values are
570 partial residuals, and each symbol is a pair of sites.

571

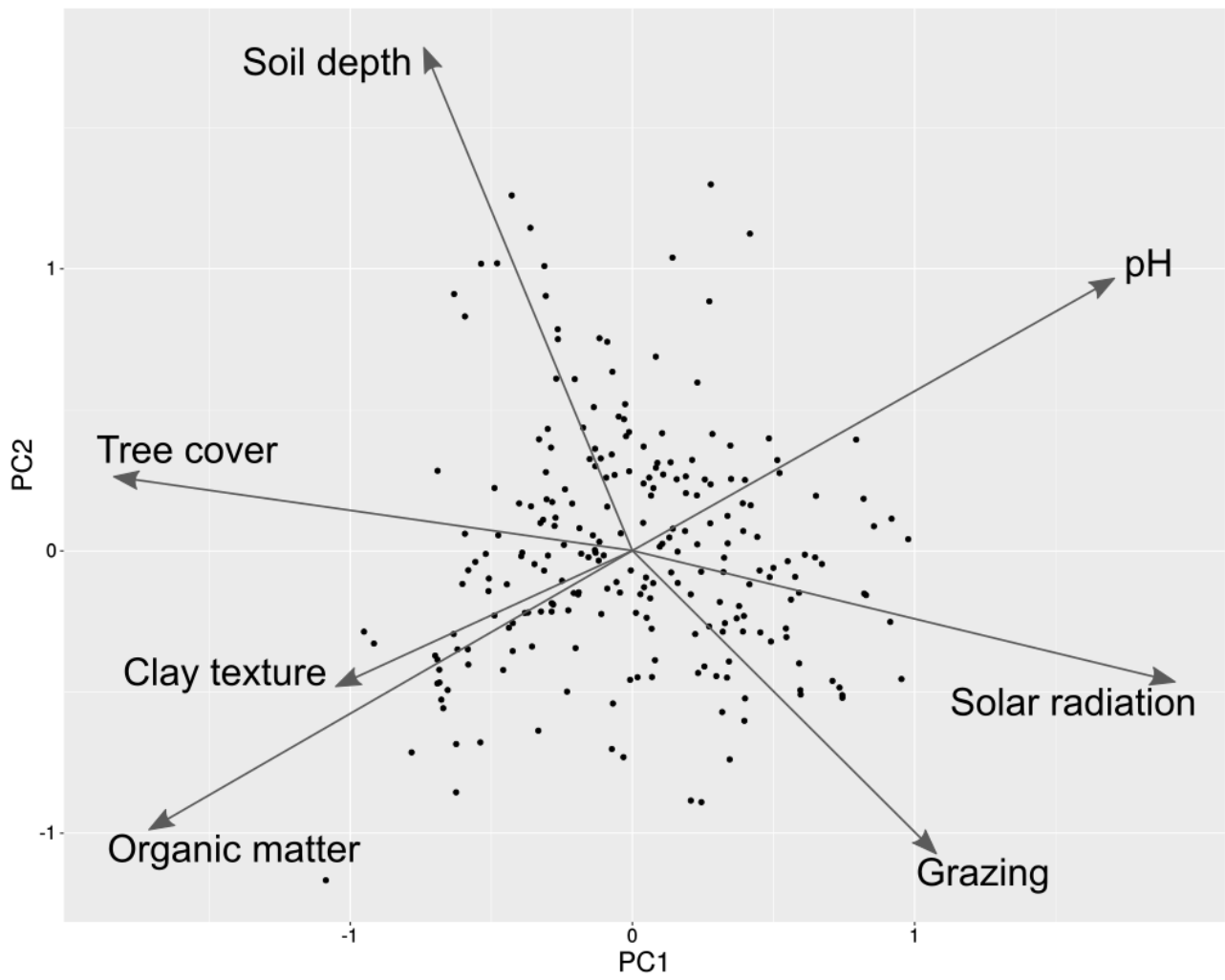
572 Figure 1

573



574 Figure 2

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576 Figure 3

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