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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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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

3

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

4

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; Başnou 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 41º14'-41º23'N, 0º56'-1º10'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

6

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 20º, 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
$$\beta_{sor} = \frac{b+c}{2a+b+c}$$
(1)

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

180 index:

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

182 Finally, the nestedness-resultant dissimilarity (β_{sne}; a measure of richness differences between

183 nested sites) is formulated as:

184
$$\beta_{sne} = \frac{\max(b,c) - \min(b,c)}{2a+b+c} \times \frac{a}{a+\min(b,c)}$$
(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; $\beta_{sor} = \beta_{sim} + \beta_{sne}$.

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 ($MJm^{-2}day^{-1}$) 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.

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 (c) 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

Results

β-diversity along the forest encroachment gradient

For the whole species composition, a noticeable rate of change along the forest encroachment

gradient was found for total β-diversity (standardized coefficient ± SE = 0.48 ± 0.06; R^2

contribution=0.36) and replacement (0.44 ± 0.06; R^2 contribution=0.29), but not for nestedness

(0.18 ± 0.08; R^2 contribution=0.04). In the case of grassland specialists, the total β-diversity change

rate along the forest encroachment gradient was similar to that of all species (0.47 ± 0.06; R^2

contribution=0.33), but it was mainly due to nestedness (0.35 ± 0.07; R^2 contribution=0.19), rather

than to species replacement (0.27 ± 0.07; R^2 contribution=0.08). For forest specialists, we found

rather weak total β-diversity (0.20 ± 0.08; R^2 contribution=0.06), but noticeable nestedness (0.25 ±

0.08; R^2 contribution=0.06) change rates and no offect of species replacement (-0.08 ± 0.08; R^2

contribution=0.01) along the forest encroachment gradient (Fig. 3). The effect of forest

encroachment on β-diversity is significant in all nine models, except for forest specialists'

replacement.

Drivers of nestedness and species replacement

For all species composition, the model for the β-diversity component accounting for species

nestedness between sites (β_{sne}) explained 16% of the variance, while historical landscape (R^2

contribution=0.07), forest encroachment (R^2 contribution=0.04) and soil characteristics (R^2

contribution=0.03) were positively correlated and had a similar contribution to total R^2. Both

current landscape and geographic distance were not significantly associated with β_{sne} (Table 1;

Appendix S4a). As expected, the model for all species replacement (β_{sim}) accounted for higher

amount of total variance, 49%. Forest encroachment (R^2 contribution=0.29) and current landscape

(R^2 contribution=0.16) explained the majority of this variance, while geographic distance (R^2

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

15

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

16

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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405 **References**

406 Adriaens, D., Honnay, O., & Hermy, M. 2006. No evidence of a plant extinction debt in highly
407   fragmented calcareous grasslands in Belgium. *Biological Conservation* 133: 212–224.

408 Arroyo-Rodríguez, V., Rös, M., Escobar, F., Melo, F.P.L., Santos, B.A., Tabarelli, M., & Chazdon,
409   R. 2013. Plant β-diversity in fragmented rain forests: testing floristic homogenization and
410   differentiation hypotheses. *Journal of Ecology* 101: 1449–1458.

411 Baan, L., Alkemade, R., & Koellner, T. 2012. Land use impacts on biodiversity in LCA: a global
412   approach. *The International Journal of Life Cycle Assessment* 18: 1216–1230.

413 Bagaria, G., Helm, A., Rodà, F., & Pino, J. 2015. Assessing coexisting plant extinction debt and
414   colonization credit in a grassland-forest change gradient. *Oecologia* 179: 823–834.

415 Bagaria, G., Pino, J., Rodà, F., & Guardiola, M. 2012. Species traits weakly involved in plant
416   responses to landscape properties in Mediterranean grasslands. *Journal of Vegetation Science*
417   23: 432–442.

418 Bagaria, G., Rodà, F., Clotet, M., Míguez, S., & Pino, J. 2018. Contrasting habitat and landscape
419   effects on the fitness of a long-lived grassland plant under forest encroachment: do they
420   provide evidence for extinction debt? *Journal of Ecology* 106: 278–288.

421 Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. *Global*
422   *Ecology and Biogeography* 19: 134–143.

423 Başnou, C., Vicente, P., Espelta, J.M., & Pino, J. 2016. Of niche differentiation, dispersal ability and
424   historical legacies: what drives woody community assembly in recent Mediterranean forests?

17

425   *Oikos* 125: 107–116.

426 Bolòs, O., Vigo, J., Masalles, R.M., & Ninot, J.M. 2005. *Flora manual dels Països Catalans*. Pòrtic,
427   Barcelona, ES.

428 Cristofoli, S., Piqueray, J., Dufrene, M., Bizoux, J., & Mahy, G. 2010. Colonization credit in
429   restored wet heathlands. *Restoration Ecology* 18: 645–655.

430 Debussche, M., Lepart, J., & Dervieux, A. 1999. Mediterranean landscape changes: evidence from
431   old postcards. *Global Ecology and Biogeography* 8: 3–15.

432 Eriksson, O., Cousins, S.A.O., & Bruun, H.H. 2002. Land-use history and fragmentation of
433   traditionally managed grasslands in Scandinavia. *Journal of Vegetation Science* 13: 743–748.

434 Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology,*
435   *Evolution, and Systematics* 34: 487–515.

436 Feldman, B. 2005. *Relative Importance and Value*.

437 Grömping, U. 2006. Relative Importance for Linear Regression in R : The Package relaimpo.
438   *Journal of Statistical Software* 17: 1--27.

439 Grove, A.T., & Rackham, O. 2001. *The nature of Mediterranean Europe: An ecological history*.
440   Yale University Press, New Haven, CT, US.

441 Guardiola, M., Pino, J., & Rodà, F. 2013. Patch history and spatial scale modulate local plant
442   extinction and extinction debt in habitat patches. *Diversity and Distributions* 19: 825–833.

443 Guirado, M., Pino, J., & Rodà, F. 2007. Comparing the role of site disturbance and landscape
444   properties on understory species richness in fragmented periurban Mediterranean forests.
445   *Landscape Ecology* 22: 117–129.

446 Guirado, M., Pino, J., Rodà, F., & Başnou, C. 2008. Quercus and Pinus cover are determined by
447   landscape structure and dynamics in peri-urban Mediterranean forest patches. *Plant Ecology*
448   194: 109–119.

449 Helm, A., Hanski, I., & Pärtel, M. 2006. Slow response of plant species richness to habitat loss and
450   fragmentation. *Ecology Letters* 9: 72–77.

451 Hermy, M., & Verheyen, K. 2007. Legacies of the past in the present-day forest biodiversity: a
452   review of past land-use effects on forest plant species composition and diversity. *Ecological*
453   *Research* 22: 361–371.

454 Honnay, O., Jacquemyn, H., Bossuyt, B., & Hermy, M. 2005. Forest fragmentation effects on patch
455   occupancy and population viability of herbaceous plant species. *New Phytologist* 166: 723–
456   736.

457 Jackson, S.T., & Sax, D.F. 2010. Balancing biodiversity in a changing environment: extinction debt,
458   immigration credit and species turnover. *Trends in Ecology & Evolution* 25: 153–160.

459 Jamoneau, A., Chabrerie, O., Closset-Kopp, D., & Decocq, G. 2012. Fragmentation alters beta-

diversity patterns of habitat specialists within forest metacommunities. *Ecography* 35: 124–133.

Kopecký, M., Hédl, R., & Szabó, P. 2013. Non-random extinctions dominate plant community changes in abandoned coppices. *Journal of Applied Ecology* 50: 79–87.

Krauss, J., Bommarco, R., Guardiola, M., Heikkinen, R.K., Helm, A., Kuussaari, M., Lindborg, R., Öckinger, E., Pärtel, M., Pino, J., Pöyry, J., Raatikainen, K.M., 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. *Ecology Letters* 13: 597–605.

Krebs, C. 1999. *Ecological methodology*. Addison-Wesley, California, US.

Kuussaari, M., Bommarco, R., Heikkinen, R.K., 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 in Ecology & Evolution* 24: 564–571.

Larrea, M.L., & Werner, F.A. 2010. Response of vascular epiphyte diversity to different land-use intensities in a neotropical montane wet forest. *Forest Ecology and Management* 260: 1950–1955.

Legendre, P. 2014. Interpreting the replacement and richness difference components of beta diversity. *Global Ecology and Biogeography* 23: 1324–1334.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M., & Gonzalez, A. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7: 601–613.

Leprieur, F., Tedesco, P.A., Hugueny, B., Beauchard, O., Dürr, H.H., Brosse, S., & Oberdorff, T. 2011. Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. *Ecology Letters* 14: 325–334.

Lindborg, R. 2007. Evaluating the distribution of plant life-history traits in relation to current and historical landscape configurations. *Journal of Ecology* 95: 555–564.

Lindborg, R., Plue, J., Andersson, K., & Cousins, S.A.O. 2014. Function of small habitat elements for enhancing plant diversity in different agricultural landscapes. *Biological Conservation* 169: 206–213.

Marini, L., Bertolli, A., Bona, E., Federici, G., Martini, F., Prosser, F., & Bommarco, R. 2013. Beta-diversity patterns elucidate mechanisms of alien plant invasion in mountains. *Global Ecology and Biogeography* 22: 450–460.

Mori, A.S., Isbell, F., & Seidl, R. 2018. β-Diversity, Community Assembly, and Ecosystem Functioning. *Trends in Ecology & Evolution*. doi: 10.1016/J.TREE.2018.04.012

Mouquet, N., & Loreau, M. 2003. Community patterns in source-sink metacommunities. *American Naturalist* 162: 544–557.

496 Nekola, J.C., & White, P.S. 1999. The distance decay of similarity in biogeography and ecology.
497   *Journal of Biogeography* 26: 867–878.

498 Öckinger, E., Lindborg, R., Sjödin, N.E., & Bommarco, R. 2012. Landscape matrix modifies
499   richness of plants and insects in grassland fragments. *Ecography* 35: 259–267.

500 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L.,
501   Solymos, P., Stevens, M.H.H., & Wagner, H. 2013. vegan: Community ecology package. R
502   package version 2.0-10.

503 Olden, J.D. 2006. Biotic homogenization: A new research agenda for conservation biogeography.
504   *Journal of Biogeography* 33: 2027–2039.

505 Öster, M., Cousins, S.A.O., & Eriksson, O. 2007. Size and heterogeneity rather than landscape
506   context determine plant species richness in semi-natural grasslands. *Journal of Vegetation
507   Science*. doi: 10.1658/1100-9233(2007)18[859:SAHRTL]2.0.CO;2

508 Ozinga, W.A., Schaminée, J.H.J., Bekker, R.M., Bonn, S., Poschlod, P., Tackenberg, O., Bakker, J.,
509   & Van Groenendael, J.M. 2005. Predictability of plant species composition from
510   environmental conditions is constrained by dispersal limitation. *Oikos*. doi: 10.1111/j.0030-
511   1299.2005.13632.x

512 Qian, H., & Ricklefs, R.E. 2012. Disentangling the effects of geographic distance and
513   environmental dissimilarity on global patterns of species turnover. *Global Ecology and
514   Biogeography* 21: 341–351.

515 R Core Team. 2016. R: A language and environment for statistical computing.

516 Rivas-Martínez, S., Fernández-González, F., Loidi, J., Lousa, M., & Penas, A. 2001.
517   Syntaxonomical checklist of vascular plant communities of Spain and Portugal to association
518   level. *Itinera Geobotanica* 14: 5–341.

519 Saar, L., Takkis, K., Pärtel, M., & Helm, A. 2012. Which plant traits predict species loss in
520   calcareous grasslands with extinction debt? *Diversity and Distributions* 18: 808–817.

521 Svenning, J.-C., Baktoft, K.H., & Balslev, H. 2009. Land-use history affects understorey plant
522   species distributions in a large temperate-forest complex, Denmark. *Plant Ecology* 201: 221–
523   234.

524 Tilman, D., May, R., Lehman, C.L., & Nowak, M.A. 1994. Habitat destruction and the extinction
525   debt. *Nature* 371: 65–66.

526 Trentanovi, G., Lippe, M., Sitzia, T., Ziechmann, U., Kowarik, I., & Cierjacks, A. 2013. Biotic
527   homogenization at the community scale: disentangling the roles of urbanization and plant
528   invasion. *Diversity and Distributions* 19: 738–748.

529 Vellend, M. 2003. Habitat loss inhibits recovery of plant diversity as forests regrow. *Ecology* 84:
530   1158–1164.

531 Vellend, M., Verheyen, K., Jacquemyn, H., Kolb, A., Van Calster, H., Peterken, G., & Hermy, M.

532   2006. Extinction debt of forest plants persists for more than a century following habitat
533   fragmentation. *Ecology* 87: 542–548.

534   Verheyen, K., Honnay, O., Motzkin, G., Hermy, M., & Foster, D.R. 2003. Response of forest plant
535   species to land-use change: a life-history trait-based approach. *Journal of Ecology* 91: 563–
536   577.
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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
		ΔForest encroachment	ΔSoil characteristics	ΔCurrent landscape	ΔHistorical 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
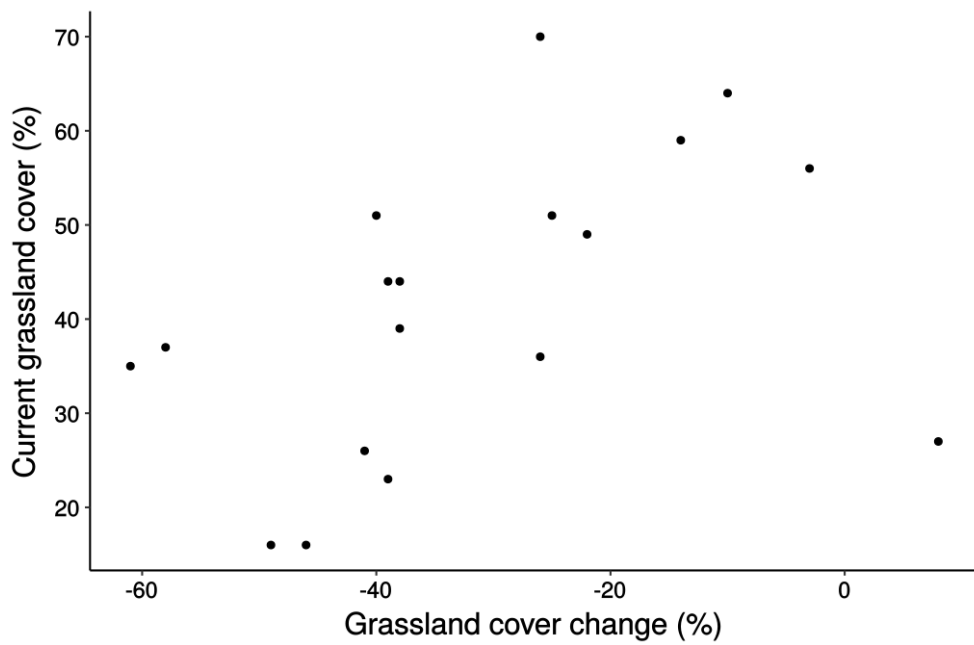
556   ($R^2 = 0.18$; $P = 0.08$).

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

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

572   Figure 1
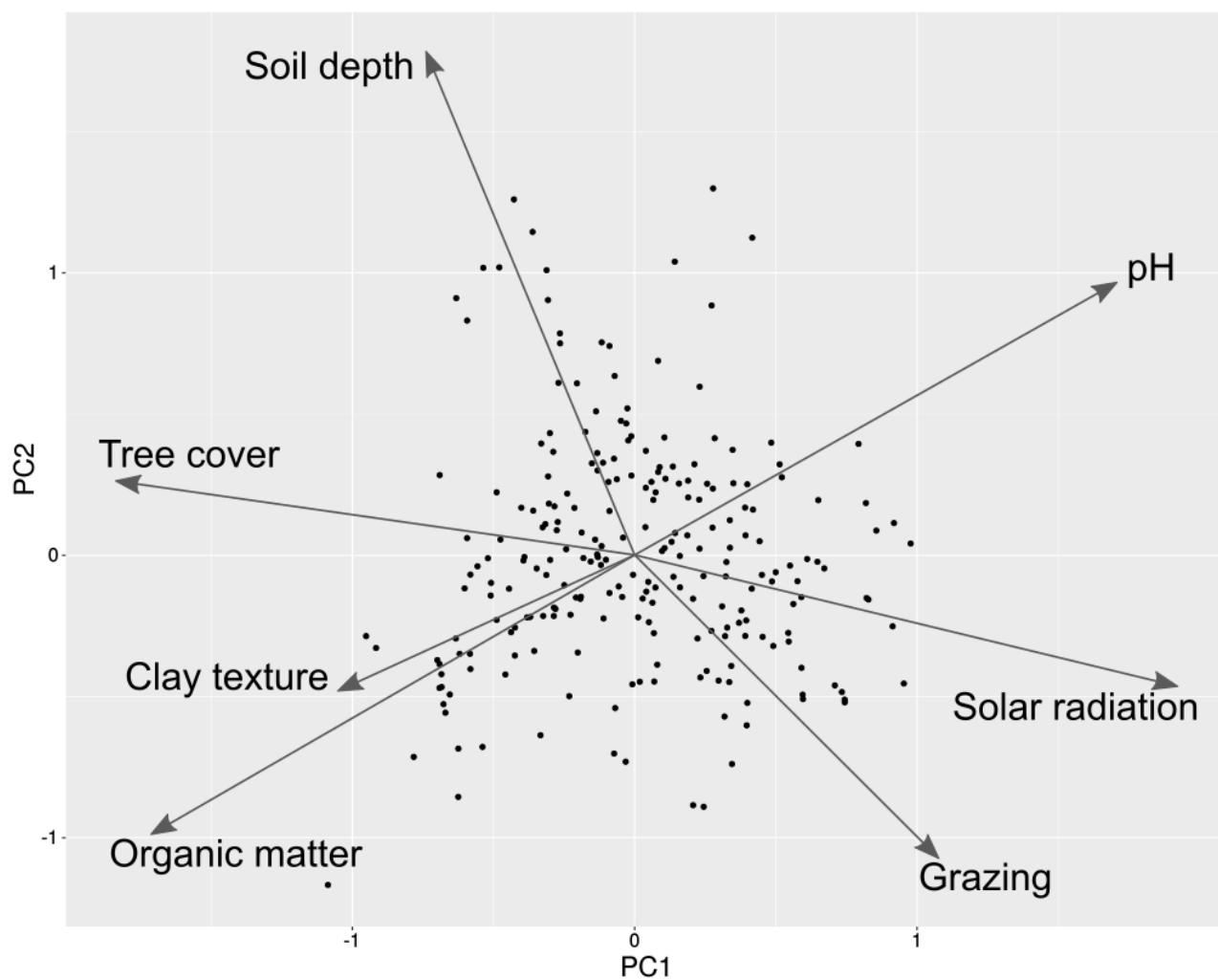
573

574   Figure 2

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