1 **Contrasting habitat and landscape effects on the fitness of a long-lived grassland plant under**

2 **forest encroachment: do they provide evidence for extinction debt?**

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14 **Running title:** Plant fitness and extinction debt

15

16   **Summary**

17   **1.** Habitat loss, fragmentation and transformation threaten the persistence of many species

18   worldwide. Population and individual fitness are often compromised in small, degraded and isolated

19   habitats, but extinction can be a slow process and extinction debts are common.

20   **2.** Long-lived species are prone to persist as remnant populations in low quality habitats for a long

21   time, but the population and individual-level mechanisms of extinction debt remain poorly explored

22   so far.

23   **3.** We here investigate the mechanisms involved in the long-term persistence of the common

24   grassland specialist, long-lived, clonal plant *Aphyllanthes monspeliensis* L. (*Asparagaceae*) after

25   forest encroachment into semi-natural Mediterranean calcareous grasslands in Catalonia (NE

26   Iberian Peninsula). For this purpose we assess vegetative (aboveground and belowground) and

27   reproductive plant performance indicators and their habitat and landscape (current and historical)

28   drivers.

29   **4.** We confirm the existence of an extinction debt for this species, since current plant frequency is

30   related to historical but not current connectivity, and we also find a positive effect of historical

31   connectivity on seed set. In addition, current tree cover negatively affects individual size and

32   aboveground/belowground biomass ratio, and biotic soil acidification leads to a reduction in the

33   flowering probability of individuals and stems.

34   **5.** However, we also find that current connectivity negatively affects flowering and that tree cover

35   enhances seed set. The forestation process, thus, also exerts a positive effect on some fitness traits,

36   probably by providing a moister environment.

37   **6.** *Synthesis*. Habitat loss and deterioration result in a decreased vegetative performance of

38   *Aphyllanthes monspeliensis*, a grassland specialist, but show contrasting effects on its reproductive

39   performance. This suggests that the species would perform better in open forests than grasslands in

40   a context of climate change. However, further forest encroachment would increase light competition

41 and soil acidification, threatening its persistence and promoting the payment of the extinction debt if

42 no conservation measures are taken, like opening gaps in forests and enhancing grassland

43 connectivity.

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45 **Key-words:** Biomass, clonal plant, flowering, grassland specialist, habitat fragmentation, land-use

46 change, Mediterranean, seed set

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48

49 **Introduction**

50 Habitat loss and transformation are major threats for biodiversity all over the world (Fahrig 2003;

51 Stockwell, Hendry & Kinnison 2003; Ouborg, Vergeer & Mix 2006). Transformation or

52 deterioration of the suitable habitat of a species threatens survival and/or reproduction of

53 individuals, reducing fitness (Lienert 2004; Mortelliti, Amori & Boitani 2010). In addition, altered

54 habitat conditions due to edge effects become more important after fragmentation, which increases

55 the transitional area between habitats (Lande 1988; Lienert 2004). Another threat to population

56 viability is the reduced habitat area and increased isolation, which result in decreased population

57 size and restricted individual and gene flows, respectively (Thrall, Burdon & Murray 2000; Honnay

58 *et al.* 2005). But species can persist for a long time in small, degraded and isolated habitats. This

59 especially happens when number, size and spatial configuration of habitat fragments are not much

60 below their extinction thresholds for metapopulation functioning (Hanski & Ovaskainen 2002).

61 However, for metapopulations that are not in equilibrium with current habitat, a number of local

62 populations are expected to become extinct by deterministic or stochastic processes. During the

63 time until the equilibrium is reached (relaxation time), the metapopulation shows an extinction debt,

64 computed as the number or proportion of populations predicted to become extinct (Tilman *et al.*

65 1994; Hanski, Moilanen & Gyllenberg 1996; Bulman *et al.* 2007; Kuussaari *et al.* 2009). These

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66　populations can be precariously maintained by metapopulation, population and individual-level

67　mechanisms (Hylander & Ehrlén 2013). For species with good dispersal ability, populations from

68　source habitats might act as propagule sources that rescue sink populations with otherwise limited

69　recruitment (Hanski 1999; Leibold *et al.* 2004). On the other hand, plant biological characteristics

70　that make individuals resistant to decreased habitat quality in particular life-cycle stages also

71　enhance species persistence (Kuussaari *et al.* 2009; Hylander & Ehrlén 2013). For example, long-

72　lived species may show biological inertia (Summerfield 1972), i.e., they may persist for a long time

73　as remnant populations that, without recruitment, decline slowly over time (Eriksson 1996). Thus,

74　both traits related with competitive ability and dispersal are important for the persistence of a

75　species in a landscape (Jacquemyn, Butaye & Hermy 2003).

76　　So far, the population and individual-level mechanisms behind extinction debt have been much

77　less explored than metapopulation processes (Hylander & Ehrlén 2013). Due to extended life-span

78　and long generation times, populations of clonal plants are usually more persistent than short-lived

79　ones, even if they consist of few individuals (Young, Boyle & Brown 1996; Honnay et al. 2005;

80　Saar et al. 2012; but see Lindborg et al. 2012; Bagaria et al. 2012). As a consequence, these species

81　with low extinction rates might constitute the main part of the extinction debt (Vellend *et al.* 2006;

82　Lindborg 2007). This probably occurs because their survival and reproduction are influenced not

83　only by the present environmental conditions but also by past ones through, for instance, the

84　resources accumulated in perennial tissues (Stephenson 1981; Mehrhoff 1989; Lienert 2004).

85　Although population and individual performance might also show time-lagged responses to habitat

86　change (Ewers & Didham 2006; Takkis *et al.* 2013), they proved useful to confirm population decay

87　in an extinction debt context (Takkis *et al.* 2013). Soil moisture (Colling, Matthies & Reckinger

88　2002), nutrients (Oostermeijer *et al.* 1998; Colling *et al.* 2002), vegetation cover (Oostermeijer,

89　Van't Veer & Den Nijs 1994; Hutchings, Mendoza & Havers 1998), and also population size

90　(Oostermeijer *et al.* 1998; Jacquemyn, Brys & Hermy 2002) have been identified as drivers of

91 population decay for long-lived species, through effects on population structure and plant fitness.

92 Clonal plants may change their architecture under limited resource availability (light or nutrients) as

93 an expression of foraging behaviour to place ramets in more favourable microhabitats (Slade &

94 Hutchings 1987; Sutherland & Stillman 1988). In response to shading many plants show etiolation,

95 and rhizomatous species tend to reduce branching intensity, but contrary to stoloniferous species, no

96 clear internode elongation has been found (De Kroon & Hutchings 1995). These architectural

97 changes might be useful to assess plant performance under suboptimal conditions.

98   European semi-natural grasslands underwent an important reduction and fragmentation during

99 the last century, and especially over the last 70 years, as a consequence of agricultural

100 intensification or abandonment of traditional practices (Eriksson, Cousins & Bruun 2002; Adriaens,

101 Honnay & Hermy 2006). Although richness patterns in these communities have been widely studied

102 and delayed species extinctions have been reported at the community level (e.g. Helm, Hanski &

103 Pärtel 2006; Krauss et al. 2010; Bagaria et al. 2015; but see Adriaens et al. 2006), few species-

104 specific extinction debts have been studied (but see Herben et al. 2006), and indicators of

105 forthcoming extinctions are still needed (Helm *et al.* 2009). The few works investigating the

106 consequences of habitat and connectivity loss for the fitness of plants in these semi-natural systems

107 found either that current connectivity was the main predictor of genetic diversity (Helm *et al.* 2009),

108 which in turn enhanced reproductive effort and plant height (Takkis *et al.* 2013), or that tree cover

109 negatively affected recruitment (Schleuning *et al.* 2009) or flower production and fruit set (Adriaens

110 *et al.* 2009). However, some fitness traits, such as plant height and germination, were still related

111 with historical factors (Takkis *et al.* 2013). So, connectivity loss and habitat deterioration might

112 threaten long-term population persistence long before changes in abundance are found, especially

113 for long-lived plants.

114   In the Mediterranean Basin, an important forest encroachment resulting in the loss and

115 fragmentation of semi-natural grasslands occurred during the 20th century, which is expected to

116 cause extinctions of grassland specialists and colonisations of forest specialists (Debussche, Lepart

117 & Dervieux 1999). These biodiversity changes have partly occurred, but a deficit of forest

118 specialists and a surplus of grassland specialists do still occur even after 50 years of forest

119 encroachment and grassland area reduction (Guardiola, Pino & Rodà 2013; Bagaria *et al.* 2015).

120 The predominance of long-lived species (hemicryptophytes and chamaephytes) in these grasslands

121 might delay species extinctions, thus enhancing extinction debt (but see Bagaria et al. 2012).

122 Nevertheless, little is known about the individual and population-level mechanisms causing

123 extinction debts for long-lived plants in these systems.

124   In this study we investigate the population and individual-level persistence mechanisms of the

125 clonal plant *Aphyllanthes monspeliensis* L. (*Asparagaceae*) after more than 50 years of forest

126 encroachment into semi-natural Mediterranean grasslands. This species is preferably found in

127 calcareous dry grasslands, stony soils and clearings in shrublands or forests of the western

128 Mediterranean, from 0 to 1600 m a.s.l. (Rico 2014). It is a grassland specialist able to persist along

129 forest encroachment gradients (authors' observation), and it potentially shows an extinction debt in

130 encroached grasslands. In order to seek indicators of individual and population decay for this clonal

131 plant, we assess vegetative and reproductive performance in relation to environmental and

132 landscape proxies of habitat loss and transformation.

133   We hypothesise that: i) *Aphyllanthes monspeliensis* will show an extinction debt in the

134 encroached sites, with its abundance depending more on past than current connectivity, since

135 extinction debt was identified for the set of grassland specialists in the same area (Bagaria *et al.*

136 2015); ii) vegetative performance and flower production (and consequently seed production) will

137 decrease under forest encroachment and canopy closure, as a result of resource limitation (Peri,

138 Lucas & Moot 2007; Adriaens *et al.* 2009); iii) seed set will decrease with decreasing grassland

139 connectivity, due to population isolation that can limit pollination and gene flow (Honnay *et al.*

140 2005), and iv) rhizome branching intensity will decrease and aboveground/belowground biomass

141 ratio and internode length might increase under canopy closure, as a consequence of foraging

142 behaviour and because shade-dependent etiolation commonly occurs at the expense of underground

143 growth (Kephart & Buxton 1993).

144

Materials and methods

Study area and system

147 The study was conducted in semi-natural Mediterranean calcareous grasslands in southern Catalonia

148 (NE Iberian Peninsula; 41º14'-41º23'N, 0º56'-1º10'E), between 800 and 1200 m a.s.l. The mean

149 temperature in this area is around 11 ºC, and the mean precipitation around 750 mm, with a marked

150 summer drought (Digital Climatic Atlas of Catalonia; www.opengis.uab.cat/acdc). The

151 predominating life-forms in the studied grasslands are chamaephytes and hemicryptophytes, which

152 usually do not grow higher than 0.5 m, and vegetation cover ranges from 50 to 80% (see Guardiola

153 et al. 2013 for details). A historically open landscape, widely grazed by sheep and goats, was

154 maintained until the decade of 1940s, and was dominated by semi-natural grasslands with small

155 forest areas. However, great grazing reduction during the second half of the 20th century led to

156 generalized grassland reduction and natural forest encroachment (Guardiola *et al.* 2013), which

157 resulted in relatively open forests dominated by holm oak (*Quercus ilex* L.) and Scots pine (*Pinus*

158 *sylvestris* L.). After more than 50 years of grassland loss and habitat transformation, a plant richness

159 decline at small scale was detected, leading to a rarefaction of grassland specialists (Guardiola *et al.*

160 2013). However, a community-level extinction debt is still present at patch and sub-patch scales

161 (Guardiola *et al.* 2013; Bagaria *et al.* 2015).

Study species

163 *Aphyllanthes monspeliensis* is a rhizomatous, tussock-forming, evergreen graminoid plant. Its

164 leaves are reduced to basal sheaths and its junciform, photosynthetic stems arise in groups from its

165 horizontal and highly branched rhizome (Fig. 1). Flowers develop alone or in groups of 2 or 3 at the

166 stem apex; they are hermaphroditic, narrow at their base, insect pollinated, and show 6 tepals of 15-

167 20 x 4-5 mm. The fruit is a trilocular capsule, which produces a maximum of one seed of 2-2.2 x

168 1.8-2 mm per locule (Rico 2014). We selected *Aphyllanthes monspeliensis* as our study species

169 because it is an abundant calcareous grassland specialist in the area, but it is still present in rather

170 closed-canopy forests even after more than 50 years since forest encroachment. Hence, it is a good

171 model to study early indicators of decreased plant performance that would eventually trigger local

172 species extinction in the mid or long term.

173 **Sampling design**

174 In order to study the changes in *Aphyllanthes monspeliensis* performance along a habitat and

175 landscape change gradient, we selected 20 sites of 200 m of diameter, which covered a wide range

176 in historical (19-96%) and current (16-70%) grassland proportion. These sites experienced natural

177 reforestation to varying degrees, did not contain any crop field neither in the past nor nowadays,

178 were not burned at least in the 25 last years, and were separated at least 1 km to ensure data

179 independence (see Fig. 2b for a map of site locations). A preferential selection was made on basis of

180 historical (1956) and current (2009) orthophotomaps and preliminary fieldwork, in order to cover a

181 wide range of habitat change situations.

182   At each of the 20 sites, the flowering state (flowering/non-flowering) of around 50 randomly

183 selected individuals (whenever possible, with a minimum of 37) was assessed in June-August 2012.

184 Plant individualisation is feasible because each individual forms a well-delimited tussock that is

185 easily distinguishable. Alongside, up to 20 flowering individuals distributed throughout the site

186 were sampled at each site in order to obtain stem number, individual size (area of an ellipse taking

187 length and width of the tussock), flowers per stem and seeds per flower (seed set), as measures of

188 plant performance in response to habitat and connectivity changes (see Fig. 2c for a map showing

189 the location of individual plants in a site). Stem number, individual size, and number of flowers per

190 stem were log-transformed in order to improve normality and homoscedasticity. Only flowering

191   individuals were sampled to assess plant performance to ensure enough individuals per site were

192   available for the analyses. The proportion of flowering individuals was generally high, but it

193   showed variability (0.83 ± 0.13; mean ± SD). One of the 20 sites was discarded because most plants

194   showed a completely dead aerial part, probably as the combined result of low late-spring and

195   summer precipitation (May - July) in 2012 (45 mm, while the climatic mean was 136.6 ± 79.7;

196   mean ± SD; see Ogaya et al. 2014 for details on meteorological data) and shallow soil (12 cm). A

197   total of 329 flowering individuals of *Aphyllanthes monspeliensis* were sampled across the 19 sites,

198   but since some capsules were partly eaten or broken, for 9 individuals without complete capsules

199   available seed set could not be estimated and they were excluded from the analysis of this variable.

200   In addition to the measures of plant performance, in 13 plots of 3 m of diameter distributed in a

201   regular grid at each site (see Fig. 2d), the presence or absence of *Aphyllanthes monspeliensis* was

202   recorded in the previous year (see Bagaria et al. 2015), and the frequency for each site was

203   calculated, from 0 to 13, in order to test for the existence of an extinction debt for this species.

204   Moreover, with the aim of investigating belowground changes in plant performance, five

205   individuals per site were taken from the field in six of the 19 sites, and cleaned in the laboratory.

206   The selection of these plants was preferential, in order to encompass a wide range of tree cover at

207   each site. Rhizome branching intensity (number of ramifications per node) and internode length

208   were taken as the mean of 20 measurements in each of these collected plants. Then, aerial (stems)

209   and belowground (rhizome and roots) structures of these plants were separated and oven-dried at

210   80ºC during 24 h, and vegetative aboveground (stems after removing flowers)/belowground dry

211   weight ratio was then obtained for each plant. All three plant measures were log-transformed in

212   order to improve normality.

213   **Environmental variables**

214   In order to investigate the effect of forest encroachment and connectivity loss on the vegetative,

215   flowering and fructification performance of *Aphyllanthes monspeliensis*, several landscape and

216 habitat drivers were calculated. Tree cover was obtained for individual plants (in a 10 m radius),

217 while soil measures, grazing and habitat (grassland) amount in the landscape were obtained at site

218 scale. No measure of tree cover at site scale was used when the focus were not individuals, because

219 it showed a high negative correlation with grassland connectivity ($r < -0.7$).

220   For each of the up to 20 flowering individuals sampled at each site, three variables related to tree

221 cover were obtained: (1) current tree cover, assessed in a circle of 10 m in radius around the

222 individual, from a combination of reclassified orthophotomaps (scale 1:5000; pixel size of 0.5 m)

223 and land-cover layers of 2009 (see Bagaria et al. 2015 for details); (2) historical tree cover in the

224 same circle, from a combination of reclassified orthophotomaps (scale 1:30000; pixel size of 1 m)

225 and land-cover layers of 1956; and (3) tree cover change (current minus historical tree cover

226 proportion). In addition, historical and current grassland proportion in the landscape (500 m in

227 radius) was also obtained from the same maps for each site, as a proxy for habitat connectivity (see

228 Fig. 2c,d for cover maps). Soil pH and clay proportion at each site were calculated as the mean of

229 13 soil samples (taken in the same plots where plant presence or absence was recorded) up to a

230 depth of 10 cm, in accordance with plant rooting depth (see Fig. 1). Soil depth and grazing

231 (presence of excrements) were also the mean of 13 measures per site. Grazing was assessed as

232 absent (0), only one group of excrements (1) or more than one group (2) at each of the measure

233 points, and taken as numeric.

Statistical analyses

235 To identify the existence of an extinction debt for *Aphyllanthes monspeliensis* after more than 50

236 years of land-use change, we related the present frequency of the species at each site with current

237 and historical grassland connectivity in a linear model, including also potential confounding

238 environmental factors (soil characteristics and grazing). The relative flowering frequency of the

239 species at each of the 19 sites was investigated in relation to grassland connectivity, soil

240 characteristics and grazing. This analysis was conducted using a generalised linear model with

241 binomial error with historical and current area and connectivity and site environmental

242 characteristics as predictors, in R programming language (R Core Team 2016). Since overdispersion

243 was found, the model was constructed again using a quasi-binomial family to overcome the

244 overdispersion problem. Then, for each of the individual-level measures (i.e. stem number,

245 individual size, flowers/stem and seeds/flower), a linear mixed model with current and historical

246 grassland area and connectivity, site environmental variables and historical and current tree cover as

247 fixed factors and site as random factor was conducted using 'lme' function in the *nlme* R package

248 (Pinheiro *et al.* 2014). For stem number, individual size was also included as a predictor to control

249 for it. A model selection using 'dredge' function in the *MuMIn* R package (Bartoń 2014) was

250 performed for each of the saturated models with AICc as the rank criterion (QAICc for quasi-

251 binomial models), and for each case the best model with all the variables being significant at

252 *P*=0.05 was presented. When ranking models that include random effects, the fitting method used

253 was maximum likelihood (ML), as comparisons between models that vary in their fixed effects are

254 not valid when they are fitted by restricted maximum likelihood (REML) (Weiss 2005). The final

255 models, however, were fitted by restricted maximum likelihood (REML). Finally, three sets of

256 linear mixed models with site as random factor were constructed to relate belowground measures

257 (rhizome branching intensity, rhizome internode length and aboveground/belowground dry weight

258 ratio) to historical tree cover, current tree cover and tree cover change. For each set, the best

259 significant model, ranked by AICc, was chosen. Moreover, in order to estimate the variance

260 accounted by each model, adjusted R^2, R^2_{D,γ_P} (which behaves satisfactorily when overdispersion is

261 present; Heinzl & Mittlböck 2003), and the adjusted likelihood-ratio based pseudo-R^2 (Magee 1990)

262 were calculated for linear models, generalised linear models and general linear mixed models,

263 respectively. All data used for the analyses can be found in Bagaria *et al.* (2017).

264

265 **Results**

266 The model for *Aphyllanthes monspeliensis* frequency at each site accounted for 40% of the variance

267 ($R^2 = 0.4$). The only significant predictor for the selected model was the historical grassland cover

268 in the landscape, which was positively correlated with plant frequency (0.66 ± 0.18; standardised

269 coefficient ± SD) (Fig. 3a). In contrast, current grassland cover was not correlated with species

270 frequency (Fig. 3b). Hence, sites with higher grassland proportion in the landscape 50 years ago still

271 harbour larger populations of *Aphyllanthes monspeliensis*. As for flowering frequency, the selected

272 model accounted for 34% of the variance ($R^2_{D, Y_P} = 0.34$), and it only included soil pH as significant

273 variable, that showed a positive association with flowering frequency (0.03 ± 0.01).

274   The model for individual size (area) accounted for a small proportion of the variance (6%), and

275 only included current tree cover as significant variable, with a negative effect (Table 1), besides a

276 significant site effect (random factor). The model for stem number accounted for 64% of the

277 variance, but it only included individual size (with positive association) and none of the

278 environmental factors as predictors, although site was significant again. The model for flowers per

279 stem accounted, again, for a small proportion of the variation (9%), and included current

280 connectivity, which was negatively correlated, and soil pH, which showed a positive effect. The

281 model for seeds per flower accounted for 21% of the variance and was positively associated with

282 current tree cover and historical connectivity, in addition to a significant site effect (random factor).

283   None of the tree cover measures predicted either branching intensity or internode length of

284 *Aphyllanthes monspeliensis* rhizomes (Table 2). Nevertheless, the aboveground/belowground

285 biomass ratio was predicted by tree cover change (with 16% of the variance explained): plants that

286 experienced a greater increase in tree cover show lower stem biomass in relation to rhizome

287 biomass.

288   All models were checked for autocorrelation of residuals using Moran's tests, and all of them

289 met spatial independence. For details on model results and autocorrelation tests see Appendix S1 in

290 Supporting Information.

291

Discussion

Our study provides evidence of an extinction debt for the long-lived common plant *Aphyllanthes monspeliensis* in Mediterranean grasslands following more than 50 years of forest encroachment, using historical and current landscape characteristics, one of the approaches suggested by Kuussaari et al. (2009): the species frequency in the study sites is significantly and positively associated to historical grassland cover in the landscape, but not with that of current grasslands (Fig. 3a,b). Similarly, previous studies found an extinction debt for grassland specialists at the community level in these encroached Mediterranean grasslands (Guardiola *et al.* 2013; Bagaria *et al.* 2015). The main contribution of our study is the focus on how vegetative and reproductive indicators of species fitness might respond to habitat and landscape factors associated to forest encroachment. Changes in these indicators, in turn, potentially affect the species demography and the likelihood of species persistence. Thus, changes in fitness associated to forest encroachment confirm the extinction risk of remnant populations showing an extinction debt (Adriaens *et al.* 2009; Takkis *et al.* 2013).

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

316   what has been found in other rhizomatous species (De Kroon & Hutchings 1995), although high

317   plasticity between genets within a species can occur (Skálová *et al.* 1997). Tree cover and a forested

318   landscape, however, enhance seed set (mean number of seeds per flower) and flowering

319   respectively, probably because shadow provides a moister environment than open grassland habitat

320   (Williams, Caldwell & Richards 1993; Payne & Norton 2011), and likely promotes flower

321   production (Akhalkatsi & Lösch 2005) and prevents the abortion of flowers and seeds in drought

322   conditions (Stephenson 1981; Lee & Bazzaz 1982), especially in dry years. High forest cover in the

323   landscape might also be related with generally moist conditions responsible for high flower

324   frequency. As for other local habitat factors, no effects of grazing or soil texture or depth were

325   found for any of the vegetative or reproductive performance traits. Grazing differences between

326   sites are probably small and not consistent through time, because few livestock grazing occurs

327   nowadays. Regarding soil variables, only pH appeared as an important factor. Clay proportion

328   presents little variation between sites (0.25 ± 0.07; mean $\pm$ SE), while soil depth shows high local

329   variability and the site mean may not correlate to water availability for the sampled individual

330   plants.

331   As for landscape effects, seed set is promoted by historical grassland connectivity, which is

332   likely to be linked to genetic diversity in this long-lived species, since population size is still

333   dependent on historical landscape and because genetic drift acts more slowly in plants with long

334   generation times (Young *et al.* 1996). Thus, large population size and probably high genetic

335   diversity would enhance reproductive fitness (Leimu *et al.* 2006; Takkis *et al.* 2013). Although

336   positive effects of current connectivity on pollinator activity and seed set have been reported

337   (Ghazoul 2005; Schmucki & de Blois 2009), *Aphyllanthes monspeliensis* populations in currently

338   isolated grasslands would still benefit from maintained population size to attract pollinators (Lienert

339   2004; but see Adriaens et al. 2009).

340   In this study we show a negative impact of local habitat deterioration (i.e. increased tree cover

341 and soil acidification) on plant vegetative performance and flowering, indicating population decay

342 and confirming that extinction debt really occurs for *Aphyllanthes monspeliensis* after forest

343 encroachment. Moreover, the positive historical connectivity effect on current seed set suggests that

344 genetic diversity and entomophilous pollination are still maintained thanks to large population sizes

345 in habitats that showed high connectivity in the past. However, forestation of the current landscape

346 also promotes flowering, and tree cover positively influences seed set. Therefore, forest

347 encroachment into these Mediterranean grasslands might exert contrasting effects on the

348 demography of grassland specialists. On the one hand, connectivity loss, light competition and soil

349 acidification threaten, respectively, seed production, vegetative performance and flowering of

350 *Aphyllanthes monspeliensis*. On the other hand, a forested landscape and increased tree cover

351 probably reduce summer water stress for understory plants and avoid seed and flower abortion, to

352 some extent, in very dry years. These contrasting consequences of habitat loss and deterioration

353 suggest that open forests might benefit plant fitness more than grasslands in the study area in a

354 context of climate change, with increased temperature and summer drought in the Mediterranean

355 Basin (IPCC 2014). In a recent study across Europe and North America, De Frenne *et al.* (2013)

356 found that forest canopy closure, which promotes a cooler and moister microclimate, is responsible

357 for the persistence of plants adapted to cooler conditions after recent global warming. In a climate

358 change context, *Aphyllanthes monspeliensis* populations might show lowered flowering and seed

359 production in open grasslands, and they would perform better in open forests. This is reinforced by

360 the climatic space where the species occurs. While the climatic late-spring and summer

361 precipitation of the study area matches the optimum for the species, the precipitation value of the

362 sampling year for the same season falls in the percentile 1.6 of the climatic records for the species

363 (Fig. 4a). Moreover, the precipitation in the study area for this season shows a tendency to decrease

364 in the last 38 years, with the four lowest values found between 2005 and 2012 (Fig. 4b). Hence, if

365 very dry years become more common, open grasslands in the study region would probably no

366 longer be optimum habitats. However, further forest encroachment would continue decreasing both

367 solar radiation under the tree canopy and soil pH, thus threatening even more the survival and

368 reproduction of grassland specialists as *Aphyllanthes monspeliensis*. Moreover, habitat deterioration

369 probably limits recruitment, since the species forms a transient seed bank germinating in the first

370 favourable season after dispersal (Paula & Pausas 2009) and seed germination rate was found to be

371 low in similar grasslands (Ninot, Petit & Casas 2008). Other studies previously found comparable

372 contrasting effects of habitat deterioration on different indicators of population fitness for other

373 clonal grassland plants. Adriaens et al. (2009) detected a positive effect of shrub cover on the

374 number of flowers per plant, while shrub removal (and production of wood debris) favoured seed

375 set and recruitment. Moreover, de Vere et al. (2009) found that bare soil promoted plant

376 establishment, while flowering increased in unmanaged sites with tall vegetation.

377   In the case of *Aphyllanthes monspeliensis* in semi-natural Mediterranean grasslands, we suggest

378 that extinction debt does not rely on a rescue effect, but on biological inertia, owing to the low

379 dispersal ability promoted by relatively large (~2mm) seed size (Rico 2014) and the lack of specific

380 dispersal mechanisms (Bagaria *et al.* 2012). In addition, the proposed rhizome necrosis would

381 indicate slow individual decay, although assessing plant recruitment and studying landscape

382 genetics (Holderegger *et al.* 2010) would be needed to fully disentangle the role of both

383 mechanisms, i.e., rescue effect and biological inertia. Thus, the death of individuals as a

384 consequence of increased light competition and the likely limited recruitment would lead to a

385 decrease in population size, and the chance of stochastic processes threatening population

386 persistence (environmental stochasticity, demographic stochasticity and genetic drift) would

387 increase (Shaffer 1987). Inbreeding depression, the reduction in fitness caused by the mating of

388 related individuals, is another threat for small populations, which negatively affects flower and seed

389 production, seed size and seed germination potential (Lienert 2004, and references therein).

390 However, ecological factors might be as important as inbreeding affecting fitness in the short term

391 (Ellstrand & Elam 1993). Thus, if isolation increases and plant density diminishes, pollinator

392 efficiency might decrease (Rathcke & Jules 1993; Ashworth *et al.* 2004; Lienert 2004), hampering

393 reproductive success (Aguilar *et al.* 2006; Aizen & Harder 2007). Moreover, in a fragmented

394 landscape, colonisation of new favourable habitats that could eventually appear would be limited by

395 the low dispersal ability of *Aphyllanthes monspeliensis*. Although regressive populations of long-

396 lived plants may persist for a long period in unsuitable habitats (Eriksson 1996), extinction debt is

397 expected to be slowly paid if no habitat improvement occurs.

398   Conservation measures to allow the long-term persistence of this and likely other grassland

399 specialists of semi-natural Mediterranean grasslands before extinction debt is paid would need to

400 guarantee high habitat heterogeneity, by the preservation of open forests with grassland areas. This

401 would promote light availability and high pH in gaps, but also the preservation of moisture by open

402 forest conditions. However, since plant abundance and seed production of *Aphyllanthes*

403 *monspeliensis* are likely to decrease in the future even if no further forestation occurs because they

404 depend on landscape properties that no longer exist, grassland connectivity should be enhanced by

405 opening gaps in forested areas near persistent grassland habitat and managing them by means of

406 livestock grazing, to promote restoration.

407

408

409 **Author's Contributions**

410 GB, JP and FR conceived the ideas and designed methodology; GB and MC collected the data on

411 the field; MC and SM processed samples at the laboratory; GB and MC analysed the data; GB led

412 the writing of the manuscript. All authors contributed critically to the drafts and gave final approval

413 for publication.

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424   **Data accessibility**

425   Available data deposited in the Dryad Digital Repository (Bagaria *et al.* 2017) at

426   http://dx.doi.org/10.5061/dryad.d83md.

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428

429   **References**

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625

626 **Supporting Information**

627 Details of electronic Supporting Information are provided below.

628 **Appendix S1.** Additional information on model results.

Tables

Table 1. General linear mixed models (LMMs) for size, stem number, flowers/stem and seed set

(seeds/flower) at individual level and site as random factor. Standardised coefficients and their

standard error for all the selected variables and total adjusted likelihood-ratio based pseudo-R^2 of

the model are presented.

	Size (area) (n=329)	Stem number (n=329)	Flowers/stem (n=329)	Seeds/flower (n=320)
Historical tree cover				
Current tree cover	-0.19±0.07			0.15±0.07
Proportion of historical grassland in the landscape				0.31±0.11
Proportion of current grassland in the landscape			-0.25±0.07	
Soil depth				
Soil pH			0.17±0.07	
Clay proportion				
Grazing				
Individual size[1]	-	0.75±0.03	-	-
Total pseudo-R^2	0.06	0.64	0.09	0.21

[1]Only tested for stem number, to control for area.

638 **Table 2.** General linear mixed models (LMMs) for aboveground/belowgroud biomass ratio,

639 branching intensity and internode length at individual level and site as random factor. Standardised

640 coefficients and their standard error for all the selected variables and total adjusted likelihood-ratio

641 based pseudo-R^2 of the model are presented.

	Aboveground/belowground biomass ratio (n=30)	Branching intensity (n=30)	Internode length (n=30)
Current tree cover			
Tree cover change	-0.37±0.18		
Total pseudo-R^2	0.16	0	0

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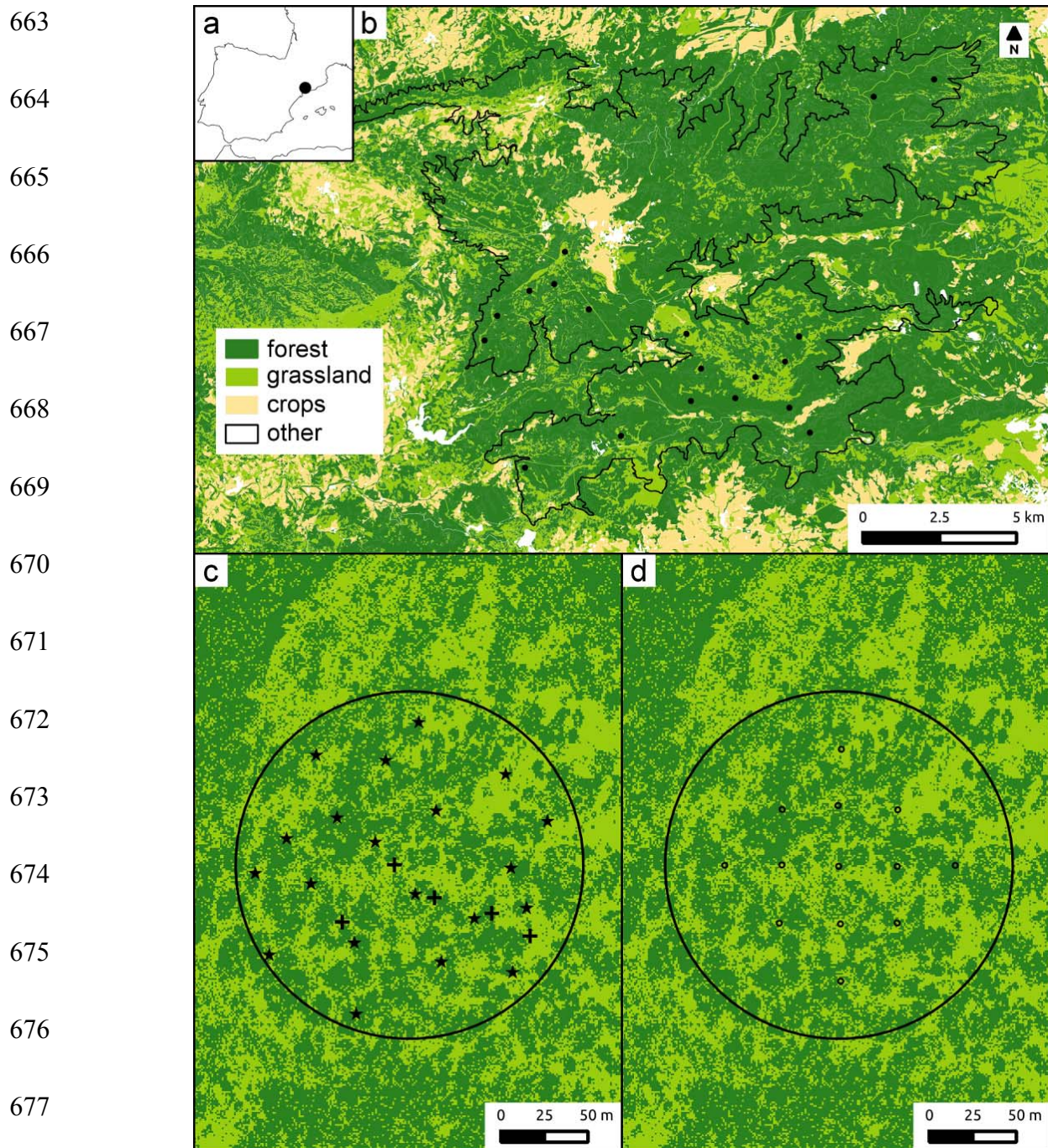647

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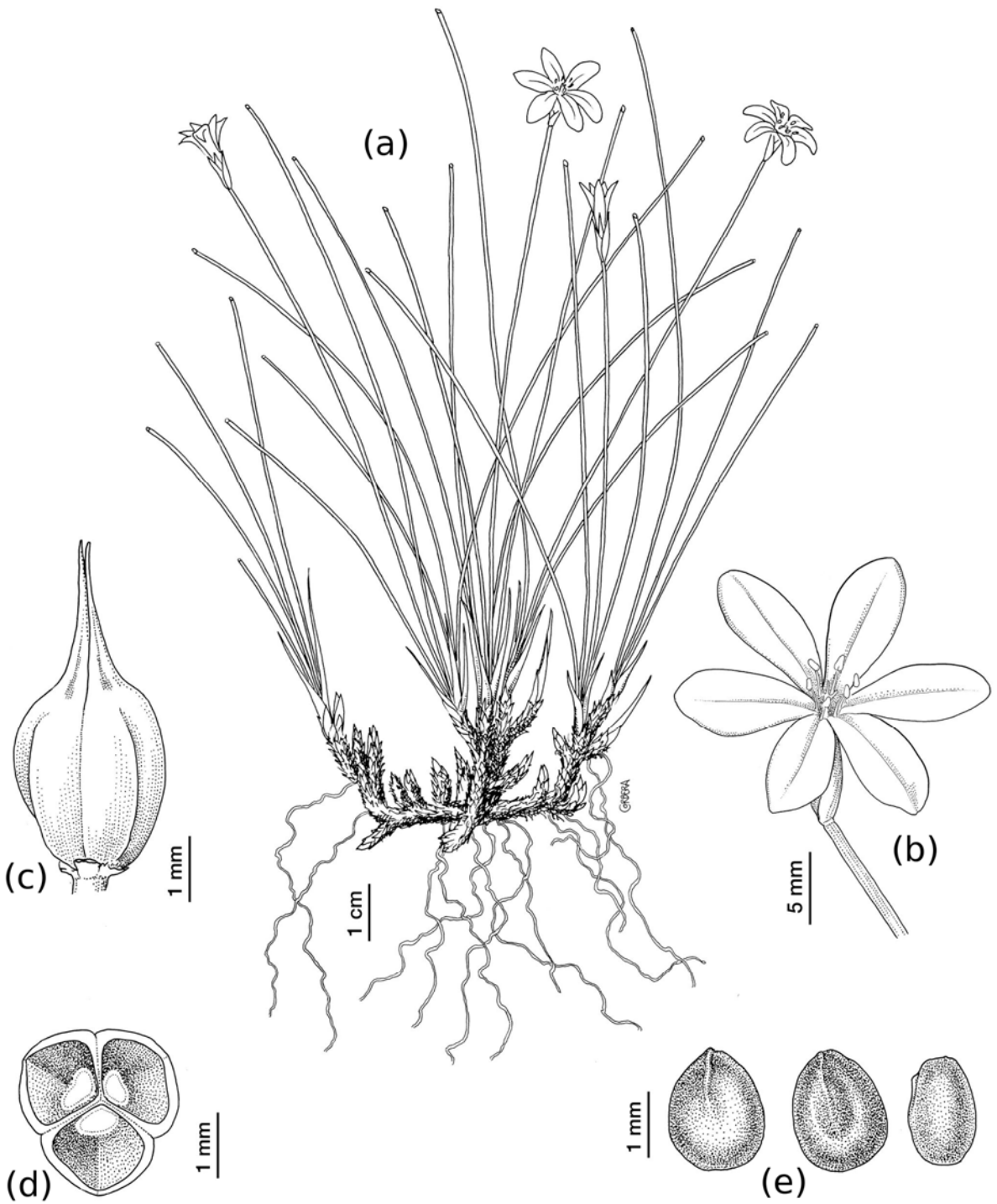
660 **Fig. 1.** *Aphyllanthes monspeliensis* morphology; a) part of an individual, showing junciform stems

661 arising in groups from its horizontal, branched rhizome; b) flower; c) capsule; d) cross-section of a

662 capsule, showing its three locules; e) seeds.

Fig. 2. Map of the study sites and sampling methodology; a) study area location in the Iberian

Peninsula; b) distribution of the 19 sites in the Prades Mountains (contour line 800 m) over the

2009 land-cover map; c) location of the 20 individuals sampled for vegetative and reproductive

plant performance (stars), and the 5 individuals collected for belowground measurements (crosses),

within a site; d) location of the 13 plots of 3 m of diameter in a regular grid within a site, where

Aphyllanthes monspeliensis frequency was assessed.

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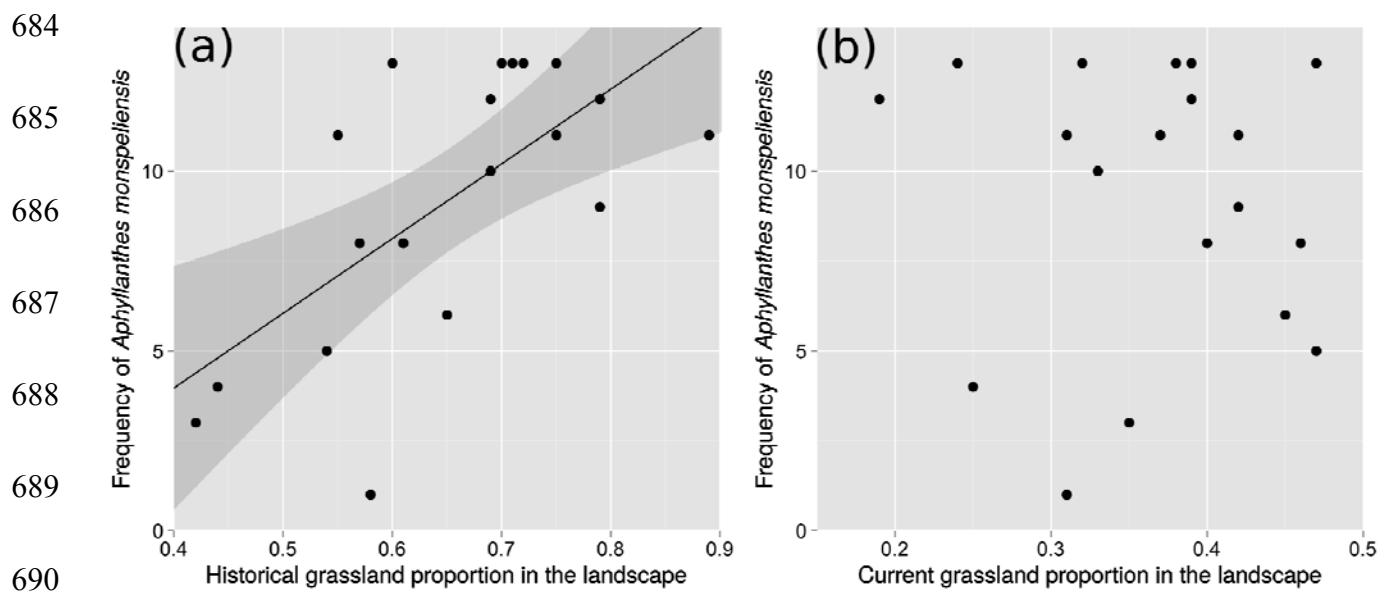
Fig. 3. Relationship between *Aphyllanthes monspeliensis* frequency at each of the 19 sites and

historical (a) and current (b) grassland connectivity. Historical connectivity explains 40% of the

variance in current plant frequency, while current connectivity shows no relationship with it.

Regression line and confidence intervals at 95% are shown for the significant model.
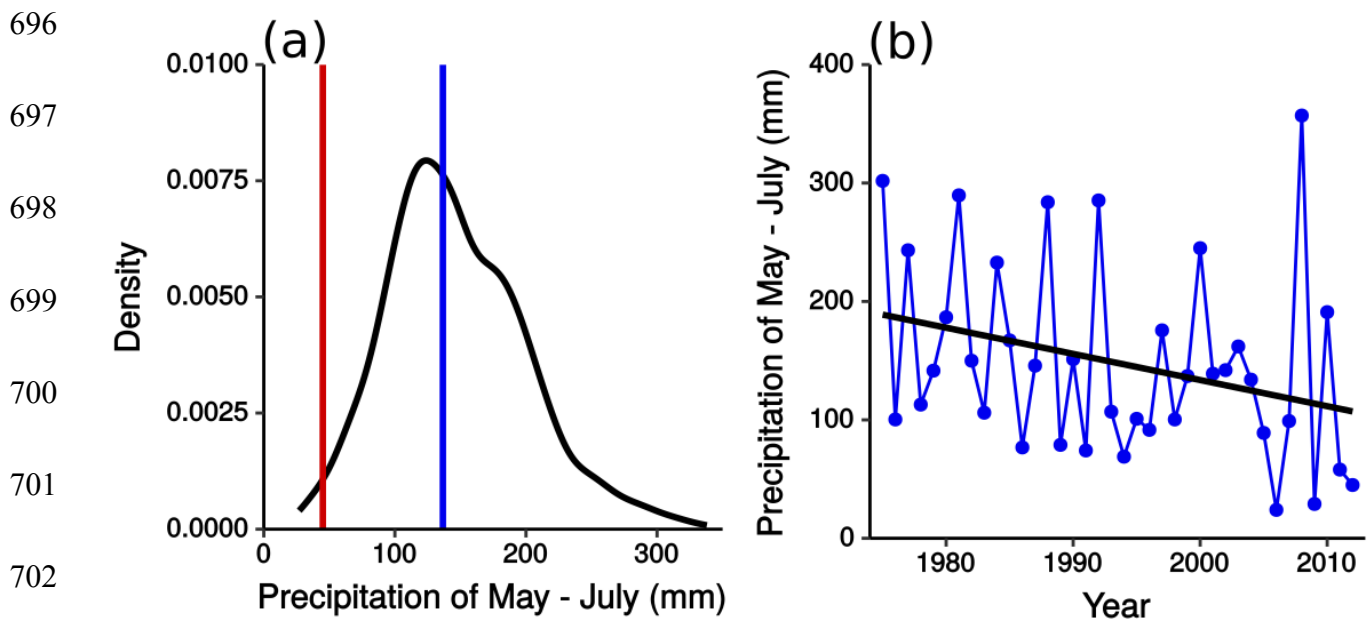
Fig. 4. (a) Climatic (blue line; 136.6 mm) and sampling year's (red line; 45 mm) late-spring and summer precipitation values on the species climatic precipitation density plot for the same season. The climatic density plot for the species was obtained extracting the WorldClim (www.worldclim.org) values of the GBIF (www.gbif.org) locations of *Aphyllanthes monspeliensis*, after reducing the density of the records to a maximum of a point every 7.5 km approximately. (b) Late-spring and summer precipitation (May - July) at the study area between 1975 and 2012. The black regression line shows a tendency to precipitation decrease over the last 38 years, with the lowest four values within the last eight years.