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1 **Dissecting the hydrological niche: soil moisture, space and lifespan**

2 **Running title:** Dissecting the hydrological niche

3

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18 summary)

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20 **Summary**

- 21 1. Niche segregation on fine-scale soil-moisture gradients is found in many plant
22 communities, but the contribution of this to community structure has yet to be analysed
23 in a fully spatial manner. We introduce a univariate and multivariate analytical
24 approach that, taking spatial autocorrelation into account, decomposes the spatial
25 structure of species composition into components that correlate with linear and non-
26 linear soil-moisture gradients plus a residual.
- 27 2. The analysis is applied to two contrasting plant communities in Western and Central-
28 Northern Spain, a seasonally dry *dehesa* meadow that is rich in both annual and
29 perennial herbs and an alpine meadow community. In each we: (i) determined whether
30 the community was structured on a soil moisture gradient, (ii) tested whether there was
31 spatial segregation into hydrological niches, (iii) Characterised the hydrological niches
32 of individual species, and (iv) controlling for spatial autocorrelation, determined how
33 much of the spatial structure in the community was due to variation in hydrology. In the
34 *dehesa* community we also compared annuals and perennials with respect to questions
35 1-4.
- 36 3. We found that both plant communities were primarily structured along hydrological
37 gradients and that spatial segregation into hydrological niches occurred among
38 perennial species, though not among the annuals in the *dehesa* community. *Dehesa*
39 annuals were spatially aggregated in the driest niches. Hydrological variation shaped
40 the responses of 60% of the annual and about 70% of the perennial species in both the
41 *dehesa* meadow and the alpine community. Most responses were either monotonic or
42 hump-shaped. Finally, spatially structured hydrological variation proved to be the main

43 driver of spatially structured species composition in all three cases.

44 4. *Synthesis* Consistent univariate and multivariate results showed that linearly (gradient
45 of slope) and topographically (at a fine scale) structured variation in hydrology is the
46 main driver of spatially structured species composition in both test communities. Our
47 results support the hypothesis that spatial niche segregation on soil-moisture gradients
48 is an important mechanism of coexistence for perennials in both test communities,
49 though not for the species-rich sub-community of annuals in the *dehesa* meadow.

50

51 **Key words:** *dehesa*, RDA models, hydrological niche, Iberian Peninsula, GAMM regression,
52 Pianka's index, plant coexistence, Water Table Depth, MEM spatial variables

53

54 **Introduction**

55 Most plant communities contain mixtures of species that compete for the same essential
56 resources. How competing plants manage to coexist with one another is a long-standing
57 conundrum (Silvertown 2004), but we may at last be nearing a solution. The various
58 mechanisms of coexistence that have been proposed can be divided into two types (Chesson
59 2000): stabilizing mechanisms such as niche segregation, in which the effects of interspecific
60 competition are frequency-dependent, thus protecting species from local extinction when they
61 become rare, and equalizing mechanisms such as the neutral theory (Hubbell 2001), that limit
62 or delay the monopolization of resources by potentially dominant species.

63 For a decade after the publication of Hubbell's (2001) book, *The unified neutral theory of*
64 *biodiversity and biogeography*, it was often argued that plant communities must be assembled
65 by equalizing mechanisms because the plant niches that would stabilize communities had not
66 been demonstrated (Rosindell, Hubbell & Etienne 2011). Silvertown (2004) pointed out that an
67 absence of evidence for niche-based coexistence was not evidence of its absence. Purves &
68 Turnbull (2010), showed that the central assumption of neutral theory, which is that species
69 that are different in phenotype will have equal fitness, is only likely to be true in the rarest of
70 circumstances.

71 Examples of stable coexistence achieved through niche segregation and tested in competition
72 models have now begun to accumulate (Adler *et al.* 2006; Angert *et al.* 2009; Levine &
73 HilleRisLambers 2009; Adler, Ellner & Levine 2010; Clark *et al.* 2010). Nonetheless, the
74 ultimate solution to the conundrum of coexistence is likely to be pluralistic because it is widely
75 recognised that the composition of plant communities can be influenced by both stabilizing and
76 equalizing mechanisms to varying degrees (Adler, HilleRisLambers & Levine 2007; Stokes &

77 Archer 2010; Chase & Myers 2011; Rosindell *et al.* 2012; Chase 2014), although stabilizing
78 mechanisms of some kind are essential for indefinite coexistence (Chesson 2000). Wilson
79 (2011) evaluated the 12 theories that he believed contain the only distinct mechanisms of plant
80 coexistence and concluded that 5 stabilizing mechanisms and 2 equalizing ones had at least
81 some empirical support. Niche segregation is the best-supported stabilizing mechanism and
82 there is growing field and experimental evidence that soil-moisture gradients are an important
83 niche dimension in many plant communities (Silvertown *et al.* 1999; Araya *et al.* 2011;
84 Markham 2014), reviewed by Silvertown, Araya & Gowing (2014).

85 While segregation on soil-moisture gradients appears to be ubiquitous across the gamut of
86 plant communities from arid environments through to wetlands (Silvertown, Araya & Gowing
87 2014), we still do not know what contribution this makes to plant community structure or
88 coexistence. In this paper we introduce a new methodology that makes it possible to answer the
89 first of these questions. We use this methodology to dissect the hydrological niche in two
90 different plant communities and to estimate how much of the spatial variance in plant
91 community structure is due to segregation on a soil-moisture gradient and how much is due to
92 other processes including spatial autocorrelation. Both plant communities are in Spain, one in a
93 wet, sub-alpine environment containing only perennial herbs and the other is a lowland,
94 seasonally dry *dehesa* grassland with a high diversity of both annuals and perennials.

95 Annuals and perennials have different regeneration biology, with possible consequences for
96 coexistence (Grubb 1977). Many annuals have life cycles that contain a persistent seed bank,
97 which lends itself to coexistence mediated by temporal niche segregation and the storage effect
98 (Warner & Chesson 1985; Pake & Venable 1996; Angert *et al.* 2009). This might mean that
99 spatial niche segregation is weaker in annuals than in perennials and so we also test for this.

100 Our analysis enables us to answer five questions:

- 101 1. Are the communities structured on a hydrological (soil moisture) gradient?
- 102 2. Is there spatial segregation into hydrological niches?
- 103 3. What is the shape of the hydrological niches of individual species?
- 104 4. Controlling for spatial autocorrelation, how much of the spatial structure in the
105 community is due to variation in hydrology?
- 106 5. Do annuals and perennials behave alike with respect to questions 1 - 4?

107 ***Materials and methods***

108 ***Sampling design***

109 An Iberian *dehesa* meadow (Eunis habitat type 6310) (European Commission 2007) on granite
110 soils was sampled at La Mina in Moscosa Farm (41° 8' 21.88" N, 6° 6' 52.33" W; 780 m a.s.l.),
111 Salamanca province, Western Spain. A 50 x 50 m study plot with a 1.8% gradient was sampled
112 (Appendix S1). The presence of plant species was recorded (Spring 2007) in 196 1-m² quadrats
113 placed on a 14 x 14 grid. Similarly, an Iberian alpine meadow on sandstone/conglomerate soils,
114 with a 2.1% gradient, was sampled at Laguna Larga in the Urbión Peaks (42° 0' 19.50" N, 2°
115 52' 2.26" W; 2080 m a.s.l.), Soria province, Central-Northern Spain, using 172 1-m² quadrats
116 placed regularly. Plant nomenclature followed standard Floras (Tutin *et al.* 1964-1980;
117 Castroviejo 1986-2011), except for the species included in Appendix S1. The spatial variables
118 northing, easting and elevation were measured using a total station machine (Leica Geosystems
119 TPS800).

120 ***Quantification of the hydrological gradient***

121 We made fortnightly measurements of water-table depth from nine dipwells over a two-year
122 period. A fine-scale topographic map (constructed from the surveyed points) was used to
123 construct a field-scale hydrological models for each of the sites (Gowing & Youngs 1997).
124 This model quantified by interpolation the average water-table depth (AWTD) in each quadrat
125 during the growing season (30 weeks for Moscosa, from mid February to end of September; 20
126 weeks for Urbión, from mid May to mid September). Since water-table depth measurements
127 are made from an origin at ground level, low values of AWTD correspond to high levels of
128 oxygen-deficit stress (due to waterlogging); high values of AWTD correspond to high levels of
129 water-deficit stress (due to soil drying) over the growing season.

130 ***Data analysis***

131 Our dissection of the spatial distribution of species in relation to soil-moisture gradients had
132 four steps. First, we used unconstrained non-metric multidimensional scaling (NMDS) to
133 reduce the dimensionality of the plant distribution data without any reference to environmental
134 gradients of any kind. We then tested whether the principal dimensions that result from the
135 NMDS analysis align with the soil-moisture gradient. In the second step, we tested for niche
136 segregation against a null model; in the third step we characterised the hydrological niche of
137 each species using Generalised Additive Mixed Models (GAMMs) while taking spatial
138 autocorrelation into account. Finally, in the fourth step, we partitioned the spatial variance in
139 species distribution into three components, (i) an hydrological component, (ii), a linear trend
140 component, and (iii) and a spatial component defined through sets of independent spatial
141 variables constructed using the Moran's eigenvector maps method.

142 At both sites, we used a two-matrix dataset. For Moscosa, it included a species composition
143 matrix of $n = 196$ sample units \times $p = 123$ species, where each element represented the
144 presence-absence of a species in a sample unit. An environment and spatial matrix of $n = 196$
145 sample units \times $q = 3$ represented the values of AWTD, northing, and easting. The same
146 matrices were used for Urbi3n, with $n = 172$ sample units and $p = 52$ species.

147 **Step 1: reducing dimensionality**

148 We explored the main underlying gradients in the species composition of the communities by
149 means of unconstrained ordination, i.e. by species ordination determined only by the species
150 data and not by any other external (environmental) variables. For this purpose, non-metric
151 multidimensional scaling (NMDS) (Kruskal 1964; McCune & Grace 2002) was used because
152 this technique preserves the ordering among objects and may be used on the basis of any
153 distance matrix (Legendre & Legendre 2012). NMDS was here performed on the basis of the
154 Jaccard dissimilarity measure (Legendre & Legendre 2012), which is directly interpretable as a
155 (dis)similarity percentage and does not take joint absences into account (Field, Clarke &
156 Warwick 1982). Prior to NMDS itself, in order to reduce the data dimensionality (McCune &
157 Grace 2002) and hence facilitate a simplified ordination in two dimensions, 42 species with
158 relative frequency ≤ 0.05 were deleted from the Moscosa species matrix and 24 from the
159 Urbi3n species matrix. Then the ordination was performed through the function `metaMDS()` of
160 the package `vegan` (Oksanen *et al.* 2013) in R software (R Core Team 2013). The correct
161 preservation of the ordering relationships among objects was assessed by means of a Shepard
162 diagram (Borcard, Gillet & Legendre 2011); the non-metric goodness-of-fit of the ordination
163 were $R^2 = 0.967$ (Moscosa) and $R^2 = 0.989$ (Urbi3n). We then used the function `ordisurf()` in
164 `vegan` to fit a quadratic trend surface corresponding to the hydrological descriptor AWTD onto

165 the ordination that had been defined. As a result, the AWTB surface thus overlaid onto the
166 NMDS ordination did not influence the ordination itself.

167 **Step 2: testing for niche segregation**

168 We computed pairwise values of *Pianka's index* of niche overlap (Pianka 1973) for all species
169 combinations to test the null hypothesis of random overlap between species. Indices were
170 computed by dividing the available niche space into sections of AWTB width equal to 0.05 m.
171 Hence we defined thirteen hydrological niches: AWTB (m) = 0.200-0.249 (first niche), 0.250-
172 0.299 (second niche), ..., 0.800-0.849 (thirteenth niche) for the Moscosa site, but only seven
173 niches (0.200-0.249, 0.250-0.299, ..., 0.500-0.549) for the Urbión site because its hydrological
174 gradient is shorter. AWTB was measured in a positive downwards direction with origin at
175 ground level. Hence the severity in conditions of oxygen-deficit stress in the first niche is at a
176 maximum (due to soil flooding), whereas the severity in conditions of water-deficit stress is at
177 a minimum. Conversely, the severity in conditions of water-deficit stress in the thirteenth niche
178 is at a maximum (due to soil drying), whereas the severity in conditions of oxygen-deficit
179 stress is at a minimum. The availability of the different resource states was introduced into the
180 analysis (Hurlbert 1978). Departures of mean niche overlap from random expectation were
181 determined by a randomization test that used 10,000 simulated matrices ("RA4 randomization
182 algorithms") in *EcoSim 7.0* software (Gotelli & Entsminger 2009); the *p*-values reported in the
183 results section correspond to the probability that the simulated index \leq the observed index.

184 **Step 3: characterising hydrological niches**

185 Generalised Additive Mixed Modelling (GAMM regression) was used to model the
186 relationships between the presence of individual species and the hydrological descriptor
187 AWTB, while controlling for spatial autocorrelation to improve estimation and inference

188 (Zuur *et al.* 2010). Tests were restricted to 81 (Moscosa) and 28 (Urbión) species with relative
189 frequency > 0.05 , for which the null hypothesis that AWTD has no effect on the probability of
190 presence for species was tested. This non-parametric model is, in summary, the additive
191 modelling equivalent of a logistic regression model (Zuur *et al.* 2010). Additive modelling fits
192 smoothers (smoothing curves) through the data without assuming linearity and is thus able to
193 describe a wide variety of relationships (Wood 2006). Additive modelling was implemented
194 here via the function `gamm()` of the package `mgcv` (Wood 2011). This function was chosen
195 because it automatically determines the right amount of smoothing and it allows mixed models
196 to be used for including spatial correlation structure (Pinheiro & Bates 2000), as may be
197 required when autocorrelation is present in model residuals.

198 Additive modelling assumes independence, but there are at least two reasons for expecting
199 spatial autocorrelation (SAC) (Griffith 1992) in our species data. First, SAC will occur if the
200 habitat is spatially structured, i.e. if the habitat variables that drive species are themselves
201 spatially correlated. Second, SAC may occur because of spatially constrained species dispersal.
202 In the first case, we expect environmental explanatory variables to partially account for this
203 SAC; however, since it is unlikely that the presence of species is driven by hydrology alone,
204 we may also expect some remaining SAC in model residuals. SAC due to species dispersal will
205 not be accounted for by environmental variables. In summary, the presence of SAC must be
206 assessed for each species model by means, for example, of spline correlograms (Bjornstad &
207 Falck 2001) that are here estimated using the function `spline.correlog()` of the package `ncf`
208 (Bjornstad 2013). Therefore, for each species, if no SAC was found in the residuals of the basic
209 model (as defined above), we proceeded to validate the fitted model as usual (Zuur, Ieno &
210 Smith 2007) and then employ it for statistical inference. If, on the contrary, SAC was found in
211 the model residuals (i.e. if the assumption of independence did not hold), we identified an

212 appropriate spatial autocorrelation structure (Pinheiro & Bates 2000) to account for SAC and
213 then re-fitted the model. Appropriate structures were identified using semivariograms (Cressie
214 1993), which was implemented here with the function Variogram() of the package nlme
215 (Pinheiro *et al.* 2014). Once identified, we introduced the appropriate spatial autocorrelation
216 structure into the basic model (Zuur *et al.* 2010) and re-fitted it using the above function
217 gamm(). Though, as stressed above, the effect of AWTD on the presence-absence of species
218 was fitted while controlling for SAC, the results presented in Tables 1-3 and Figures 2-4 focus
219 on the models' fixed parts (the smoothers) because this work deals with such (hydrological)
220 effects.

221 **Step 4: partitioning spatial variance**

222 Redundancy analysis, or RDA (Wollenberg 1977; Legendre & Legendre 2012), was used to
223 test for relationships between species composition (response) and spatial or hydrological
224 predictors. RDA can be described as a technique for multivariate regression (Zuur, Ieno &
225 Smith 2007). The response was a Hellinger-transformed (Legendre & Gallagher 2001)
226 presence-absence species matrix. We used three kinds of predictors: (i) MEM spatial variables
227 (see below); (ii) a linear trend; and (iii) the hydrological predictor AWTD. The linear trend is a
228 surface specified by the X-Y coordinates and is here used to describe linear spatial structures.
229 In order to model non-linear hydrological relationships (Borcard, Gillet & Legendre 2011), the
230 hydrological component included not only an AWTD first-degree term, but also second- and
231 third-degree terms; these were computed using the function poly() of the package stats in R
232 software (R Core Team 2013), so they are orthogonal. The spatial component was modelled
233 using sets of independent spatial variables that were specifically constructed for each species
234 assemblage using the MEM (Moran's eigenvector maps) method (Dray, Legendre & Peres-
235 Neto 2006; Legendre & Legendre 2012). These MEM spatial variables, which can model

236 positive and negative spatial autocorrelation, were constructed using weighted connectivity
237 matrices (Borcard, Gillet & Legendre 2011) via the R package spacemakeR (Dray 2013).

238 In addition to RDA marginal tests, we carried out a partitioning of variation in species
239 composition (Borcard, Legendre & Drapeau 1992; Legendre & Legendre 2012) into
240 components of unexplained variation, non-spatial hydrological variation and non-hydrological
241 spatial variation (either as a linear surface or as modelled by MEM variables); besides,
242 fractions corresponding to spatial structuring shared by hydrological conditions (induced
243 spatial variation), and others, were also estimated. This partitioning supports models of causal
244 relationship (Legendre & Legendre 2012) and here was used mainly in order to estimate the
245 extent of induced spatial variation, i.e. spatially structured species composition that is
246 explained by spatially structured hydrological variation. Induced spatial variation has two
247 fractions. The first (fraction [g] in the partition diagram) corresponds to spatially structured
248 variation in species composition that is associated with linear gradients, i.e. with the sites'
249 gradients of slope. The second (fraction [f] in the partition diagram) corresponds to spatially
250 structured variation in species composition that is associated with the sites' local topographies.
251 The unique contribution of each component, as well as the extent of overlap among them, was
252 estimated by varying the order of fit using the functions `rda()` and `varpart()` of the R package
253 `vegan` (Oksanen *et al.* 2013).

254 Supporting information provides data (Appendices S4 and S5) and R coding (Appendix S3)
255 sufficient to replicate the analysis described above.

256 **Results**

257 At Moscosa, we recorded 71 species of annual and 52 perennials (Appendix S1), but only 81 of
258 the 123 species had a relative frequency greater than 0.05; in Urbión, 52 species were found,

259 but only 28 had frequency greater than 0.05 (Appendix S1). Unconstrained ordination (Fig. 1),
260 which here shows the two main species gradients in the meadows without external reference to
261 any environmental variables, shows that the first main species gradient (NMDS1) is, in both
262 cases, strongly associated with the hydrological gradient (as measured by AWTD in m).

263 For Moscosa, the null hypothesis of random overlap across the hydrological space at a fine
264 scale (thirteen niches) was rejected for the whole community (observed mean = 0.399 <
265 simulated index = 0.410; $p = 0.000$) and for perennials as a group (observed mean = 0.369 <
266 simulated index = 0.383; $p = 0.001$). Hence perennials segregate along the hydrological
267 gradient. Significant GAMM models were fitted for 23 (70% of species with frequency > 5%)
268 perennials (Table S1 in Appendix S2). The average model fit was R^2 (adj.) = 21.3%. Consistent
269 with the observed segregation pattern, monotonic perennial responses were either increasing or
270 decreasing with increasing soil drying (Fig. 2), thus contributing to segregation (compare, for
271 example, *Poa bulbosa* with *Poa trivialis* or *Senecio jacobaea* with *Thapsia villosa* in Fig. 2).
272 The various hump-shaped, or similar, responses (35% of the fitted models) also contribute to
273 segregation (compare, for example, *Briza media*, *Galium verum* and *Echium plantagineum* in
274 Fig. 2). In contrast and as suggested by the unconstrained ordination (Fig. 1), the null
275 hypothesis of random overlap was not rejected for annuals (observed mean = 0.463 > simulated
276 index = 0.448; $p = 0.995$). Hence annuals do not segregate along the hydrological gradient, but
277 rather tend to aggregate at the dry end. Significant GAMM models were fitted for 26 annuals
278 (54%) (Table S2 in Appendix S2), with average model fit R^2 (adj.) = 18.6%. No significant
279 relationships were found for invasive annuals (e.g. *Trifolium dubium*) with relative frequency
280 greater than c.0.9. Consistent with the observed aggregation pattern, most annual responses
281 (60% of the fitted models) are both monotonic (either sigmoid or curvilinear) and increasing
282 with increasing soil drying (Table S4; Fig. 3). This contributes greatly to generate this pattern

283 of species aggregation (compare *Aphanes arvensis*, *Bellardia trixago*, *Brassica barrelieri*,
284 *Galium parisiense*, *Jasione montana*, *Ornithopus perpusillus*, *Trifolium glomeratum* and
285 *Xolantha guttata* in Fig. 3), in spite of 24% of fitted models for annuals being found to display
286 hump-shaped relationships.

287 For Urbión, the null hypothesis of random overlap across the hydrological space at a fine scale
288 (seven niches) was rejected for the whole community (observed mean = 0.579 < simulated
289 index = 0.595; $p = 0.010$). Hence species segregate along the hydrological gradient.

290 Significant GAMM models were fitted for 20 (71% of species with frequency > 5%) species
291 (Table S3 in Appendix S2). The average model fit was R^2 (adj.) = 17.2%. Consistent with the
292 observed segregation pattern, monotonic species responses were either increasing or decreasing
293 with increasing soil drying (Fig. 4), thus contributing to segregation. Hump-shaped responses
294 (25% of the fitted models) also contribute to segregation.

295 Marginal tests (Table 1) show that, for Moscosa, AWTD explains c.18% of multivariate
296 variation in perennials composition and c.16% in annuals composition. Likewise, species
297 composition is strongly spatially structured (c.30% for perennials; c.27% for annuals, as
298 described by MEM spatial variables), with a linear gradient (Table 1; Fig. 5) being responsible
299 for a relatively important part of these spatial structures. Variation partitioning (Fig. 5),
300 however, shows that the unique contribution of the hydrological descriptors to explain
301 composition (fraction [a]) is less than 2% in all three cases, i.e. species composition explained
302 by non-spatially structured hydrological variation is minor. In other words, induced spatial
303 variation (fractions [f] and [g]), which corresponds to spatially structured species composition
304 that is explained by spatially structured hydrological variation, is the strongest element in all
305 three cases. The sum of fractions [f] and [g] amounts to 16.2% of the variance explained in the
306 perennials assemblage and 14.3% in the annuals assemblage. In both cases fraction [g],

307 corresponding to variation in species composition that is associated with the (linear) gradient of
308 slope, is the most important (9.6%). Fraction [f] corresponds to spatially structured variation in
309 species composition that is not associated with linear gradients, but with local topography; this
310 fraction is stronger for perennials (6.6%) than for annuals (4.7%).

311 For the Urbión meadow, AWTD explains c.16% of multivariate variation in species
312 composition (Table 1). Likewise, species composition is strongly spatially structured (38.7%),
313 as described by MEM spatial variables). Variance partitioning (Fig. 5) shows that species
314 composition explained by non-spatially structured hydrological variation (fraction [a]) is minor
315 (2.4%). Hence, as in the Moscosa site, induced spatial variation (fractions [f] and [g]), which
316 together add up to 11.9%, represents a strong component). However, in the Urbión site, the
317 spatially structured variation in species composition that is associated with the local
318 topography (fraction [f]) explains 8.1% of adjusted variance and is, therefore, greater than the
319 variation in species composition that is associated with the (linear) gradient of slope (fraction
320 [g]), which represents only 3.8% of adjusted variance

321 Finally, in both meadows, the unique contributions of the MEM spatial variables (fraction [c]),
322 which correspond to spatially structured species composition that is not explained by the
323 hydrological descriptor, but by latent processes, suggests the existence of spatially structured
324 ecological factor(s) other than AWTD driving species composition. This component is stronger
325 in the Urbión site (14.8% of total variance) than for the annuals (8.3%) or the perennials
326 (10.1%) in the Moscosa site.

327 **Discussion**

328 Although soil moisture and local topography are well known influences on plant distribution
329 (Moeslund *et al.* 2013), we believe that this is the first study to formally decompose plant

330 distribution into spatial components that include the important effect of hydrology. The
331 methods developed by Borcard (1992) and Borcard & Legendre (1994) have been widely used,
332 for example to test competing theories regarding dispersal limitation, environmental
333 determinism and neutral models in an American temperate forest (Gilbert & Lechowicz 2004).
334 In Amazonian forests, Tuomisto et al (2003) found that spatially-structured environmental
335 variation was the most important ecological factor explaining plant composition at a regional
336 scale, with dispersal having also some ecological effect though neutrality was not supported.

337 By analysing the spatial variance in plant community structure, we have been able to answer 5
338 questions about the hydrological niche, including whether annuals and perennials behave alike.
339 First, we found that both the plant communities that we investigated were structured along
340 hydrological gradients and that their primary axes of variation aligned with Average Water
341 Table Depth (Fig. 1a, b). Average Water Table Depth (AWTD) is measured as a distance
342 below the surface so this measure of hydrological conditions is necessarily highly correlated
343 with fine scale topography. Second, we found that spatial segregation occurred among
344 perennial species, though not among the annuals in the *dehesa* community (Fig.1a). *Dehesa*
345 annuals as a group were aggregated at the dry end of the hydrological gradient where most fell
346 into just three or four niches (niches 0.50-0.70) out of the 17 that were present.

347 Third, we investigated the shape of species' hydrological niches by fitting GAMS, which
348 showed that species responses were, with very few exceptions, either monotonic (increasing or
349 decreasing along the hydrological gradient) or hump-shaped (between 24-35% of species
350 responses). The important conclusion here is that, contrary to the assumptions of the neutral
351 model, co-occurring species show different responses along soil-moisture gradients. Similar
352 results have now been found in many plant communities (Silvertown, Araya & Gowing 2014).

353 A comparison of the shapes of the species' hydrological niches illuminates the difference in
354 community structure found between perennials and annuals. Since most perennial responses
355 were either monotonic increasing or monotonic decreasing with increasing soil drying (65-
356 75%), this difference created segregation, with hump-shaped responses also contributing to
357 segregation. In contrast, most annuals responses were monotonic increasing with increasing
358 soil drying (62%), thus creating a pattern of overlap and species aggregation. These results
359 suggest that, at least in the *dehesa* community we studied, spatial niche segregation on soil
360 moisture gradients may not be an important mechanism of coexistence in the species-rich sub-
361 community of annuals. Given that rainfall in the area is highly variable from year-to-year
362 (Ceballos, Morán & López 2013), temporal niche segregation (the storage effect), as found
363 among Sonoran desert annuals by Angert *et al.*(2009), is an alternative possibility.

364 Fourth, we partitioned the components of spatial structure in the two plant communities, with
365 complex results (Table 1 and Fig. 5). Among the perennials at Moscosa, 18% of the variance in
366 species composition was ascribed to variation in hydrology. For the annuals in Moscosa and
367 for perennials in Urbión, the variance explained was about 16% in both (16.2% and 15.6%,
368 respectively). Nearly all the variance in hydrology that drove species composition was spatially
369 structured, but about 2% of the adjusted variance explaining change in species composition
370 was not. This may simply reflect error in the hydrological models fitted. This spatial structure
371 can be further subdivided into components that correspond to the linear gradients of slope
372 (fraction [g]) and to local topographic variation (fraction [f]). In Moscosa, the gradient of slope
373 (9.6% for both annuals and perennials) was more important than local topography (6.6% for
374 perennials and 4.7% for annuals). In contrast, local topography (8.1%) was more important in
375 Urbión than the gradient of slope (3.8%).

376 Overall, the contributions of hydrologically-correlated spatial variation may appear rather
377 small (16.2% for Moscosa perennials; 14.3% for Moscosa annuals; 11.9% for Urbi3n), but this
378 was nevertheless the most important driver of spatially structured species composition in the
379 Moscosa data. Spatial structure not correlated with hydrology ([b] + [c] + [e] in Fig.5),
380 amounted to 13.4% for perennials and 12.4% for annuals. By comparison, species composition
381 in the Urbi3n data was even more strongly spatially structured than in the Moscosa data, and
382 more than a quarter of its spatial variance (26.7%) was not accounted for by hydrology.
383 Nonetheless, we can conclude that hydrology was at least as important as any other single
384 cause of spatial structure because it correlates with the primary axis of variation in Fig.1. Other
385 causes of spatial structure could have included such ecological factors as vegetation pattern
386 created by clonal growth, local variation in soil nutrients, or population processes such as
387 dispersal (Legendre & Legendre 2012).

388 Our fifth question was whether annuals behaved differently from perennials and we found that
389 indeed they did, occupying a distinct zone of niche space at the drier end of the hydrological
390 gradient at Moscosa farm. Elsewhere, annuals have been found to partition a hydrological
391 gradient in vernal pools in California (Bauder 2000), where even different genotypes of a
392 single species occupy different zones of water depth (Linhart & Baker 1973). Niche
393 segregation has also been experimentally demonstrated in several annual communities, though
394 without always identifying the precise nature of the niche axes that are important for this
395 (Sharitz & McCormick 1973; Turnbull, Manley & Rees 2005; Levine & HilleRisLambers
396 2009).

397 Our analysis has demonstrated that niche segregation along soil-moisture gradients contributes
398 significantly to community structure, but the results are based in observational data and
399 therefore offer only circumstantial evidence of the importance of the hydrological niche to

400 coexistence (Silvertown 2004). Complementary experimental and theoretical studies are
401 required to quantify what contribution hydrological niche segregation makes to coexistence.
402 We must also be cautious about how the soil-moisture gradient influences plant distribution,
403 since soil moisture has direct and indirect effects upon the soil environment for plants. It not
404 only controls water availability, but also when present in excess it affects oxygen availability,
405 microbial community composition and function, and nutrient availability (Araya, Gowing &
406 Dise 2012). That said, our dissection of the hydrological niche offers a firm statistical
407 justification for exploring the underlying mechanisms and their consequences.

408

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1. Sites photographs, full species names and species frequencies.

Appendix S2. Generalised Additive Mixed Modelling summaries.

Appendix S3. R code

Appendix S4. Moscosa dataset

Appendix S5. Lifespan

Table 1. Marginal tests results from direct RDAs (Redundancy Analysis) fitting groups of spatial MEM variables, linear trends, and the hydrological descriptor (AWTD) to explain species composition in a *dehesa* meadow at Moscosa Farm and in an alpine meadow at Urbión Peaks, Spain. The response is a Hellinger-transformed presence-absence species matrix in both cases. The hydrological component includes first-, second- and third-degree AWTD terms. The linear trend component is a surface described by the X-Y coordinates. The spatial component comprises sets of MEM spatial variables created specifically for each test (14 variables for the whole community and annuals in Moscosa; 15 for perennials in Moscosa; 18 for the whole community in Urbión); these MEM spatial variables describe spatial structuring. In complex models the amount of variation explained by each component depends on the other components (see Figure 5). *p*-values were obtained by means of 1000 permutations

Source of variation	Model var. (d.f.)	Resid var. (d.f.)	<i>F</i>	<i>p</i>	<i>R</i> ² (adj.)
Whole community (Moscosa)					
Hydrological descriptor	0.0806 (3)	0.3447 (192)	15.0	0.001	0.177
Linear trend	0.0631 (2)	0.3622 (193)	16.8	0.001	0.140
Spatial MEM variables	0.1397 (14)	0.2856 (181)	6.3	0.001	0.277
Perennials (Moscosa)					

Hydrological descriptor	0.0843 (3)	0.3560 (192)	15.1	0.001	0.179
Linear trend	0.0615 (2)	0.3788 (193)	15.7	0.001	0.131
Spatial MEM variables	0.1518 (15)	0.2885 (180)	6.3	0.001	0.290

Annuals (Moscosa)

Hydrological descriptor	0.0712 (3)	0.3358 (192)	13.6	0.001	0.162
Linear trend	0.0596 (2)	0.3474 (193)	16.6	0.001	0.138
Spatial MEM variables	0.1296 (14)	0.2774 (181)	6.0	0.001	0.266

Whole community (Urbión)

Hydrological descriptor	0.0997 (3)	0.4834 (168)	11.6	0.001	0.156
Linear trend	0.1057 (2)	0.4775 (169)	18.7	0.001	0.171
Spatial MEM variables	0.2631 (18)	0.3201 (153)	7.0	0.001	0.387

Figure 1. Main species gradients for La Mina meadow in Moscosa Farm (a) and Laguna Larga meadow in the Urbión Peaks (b), as described by non-metric multidimensional scaling (NMDS), in multivariate space (Jaccard measure). Species scores are weighted averages scores. Species with relative frequency ≤ 0.05 were not included. Moscosa annuals are indicated in red and perennials in green. Non-metric goodness-of-fit of the ordination: $R^2 = 0.967$ (Moscosa) and $R^2 = 0.989$ (Urbión). NMDS is a technique for unconstrained ordination, so the trend surface for AWTD in m (blue lines) was overlaid onto the species ordination only after the NMDS procedure was concluded. Significance of trend surface: $F = 24.3$, e.d.f. = 2.94, p -value < 0.000 (Moscosa) and $F = 34.1$, e.d.f. = 2.85, p -value < 0.000 (Urbión). In both cases, the hydrological gradient accounts for most of the variation observed in the first ordination axis (NMDS1); the levels of the contours depict the hydrological niches used in this work. Key to species: see Appendix 1

Figure 2. Fitted Generalised Additive Mixed Models (GAMM) for 16 perennial species (Moscosa). The smooth terms (smoothers) represent the estimated probability of presence (occurrence) with increasing Average Water Table Depth (AWTD in m) at La Mina site, Moscosa Farm, Western Spain. See Table S1 in Appendix S2, where significance (F - and p -values together with the estimated degrees of freedom) and adjusted R^2 are detailed. Note consistency with NMDS ordination

Figure 3. Fitted Generalised Additive Mixed Models (GAMM) for 16 annual species (Moscosa). The smoothers represent the probability of presence with increasing Average Water

Table Depth (AWTD in m) at the field site. See Table S2 in Appendix S2. Note consistency with NMDS ordination

Figure 4. Fitted Generalised Additive Mixed Models (GAMM) for 16 species (Urbión). The smoothers represent the probability of presence with increasing Average Water Table Depth (AWTD in m) at the field site. See Table S3 in Appendix S2. Note consistency with NMDS ordination

Figure 5. Venn diagrams showing how variation in the composition of the two plant communities was partitioned among a hydrological component, a linear trend component and a spatial component described by MEM variables. Numbers are adjusted R^2 values (%). The hydrological component includes AWTD and its second- and third-degree terms. The linear trend represents a surface described by the X-Y coordinates. The spatial component comprises sets of MEM spatial variables, selected for each partition specifically (14 MEM variables for the annuals community in Moscosa; 15 for the perennials community in Moscosa; 18 for the whole community in Urbión). The unique contributions of the hydrological, trend and spatial components are denoted by [a], [b] and [c], respectively. The fractions [f] and [g] correspond to spatially structured biological variation that is explained by the hydrological component, which is also spatially structured (induced spatial variation); fraction [f] is related to local topography at a fine scale; fraction [g] is related to local linear gradients (mainly local gradients of slope). Fraction [d] corresponds to linearly structured hydrological variation. Fraction [e] corresponds to linear variation that is shared by the spatial MEM variables (the MEM variables model both purely linear variation and any complex structures present in the data)

Fig. 1a

(a) NMDS ordination with AWTD

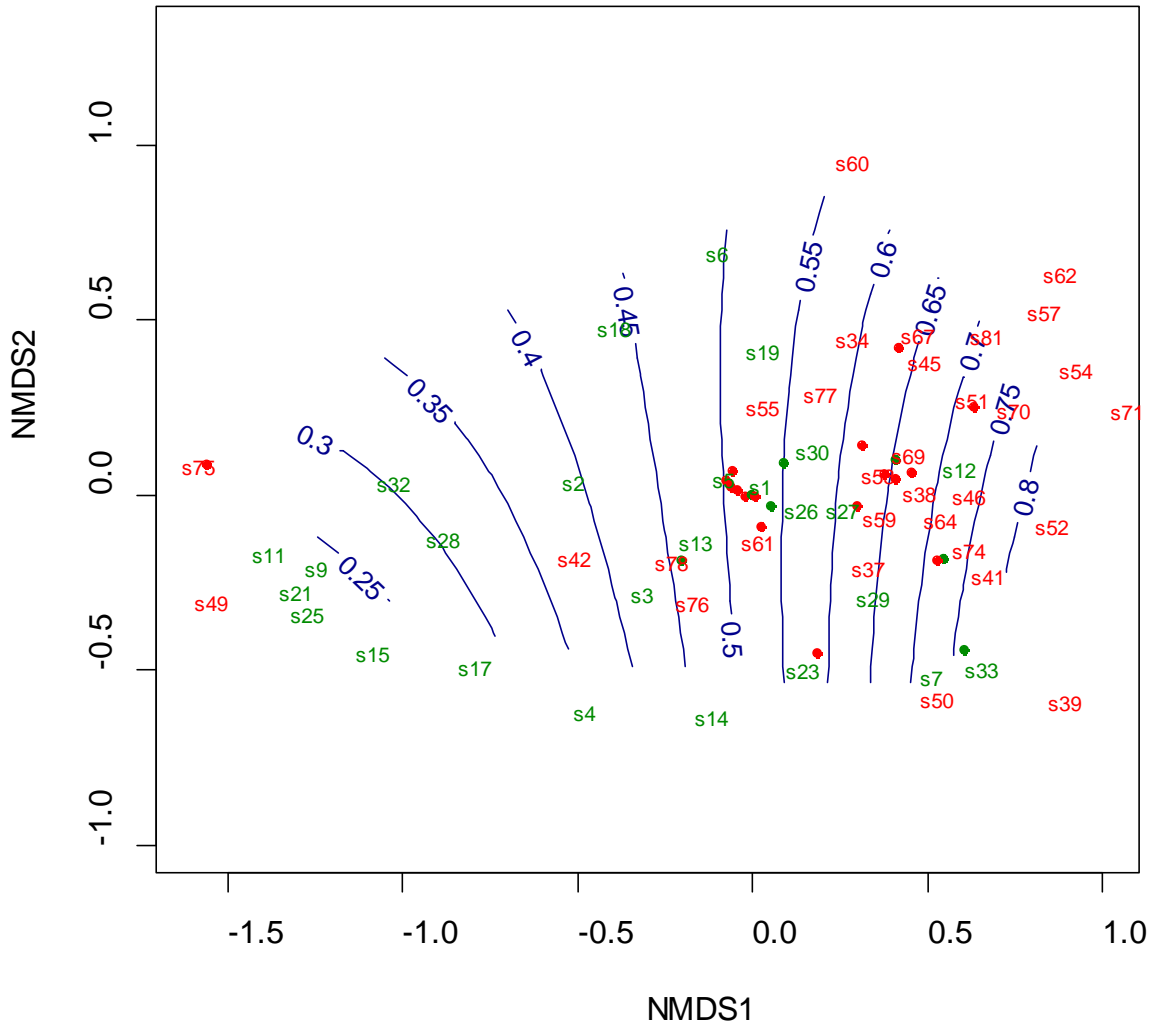


Fig. 1b

(b) NMDS ordination with AWTD

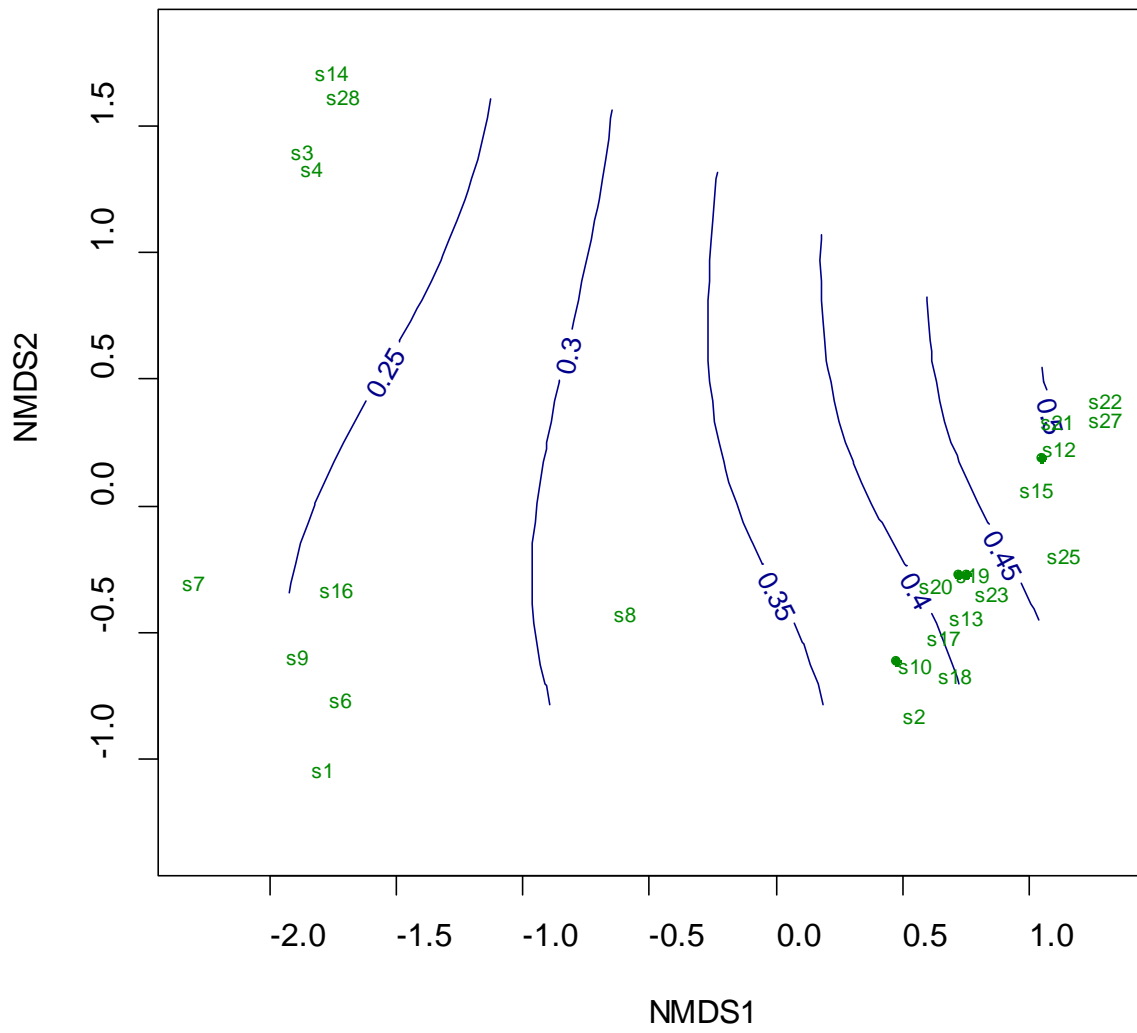


Fig. 2

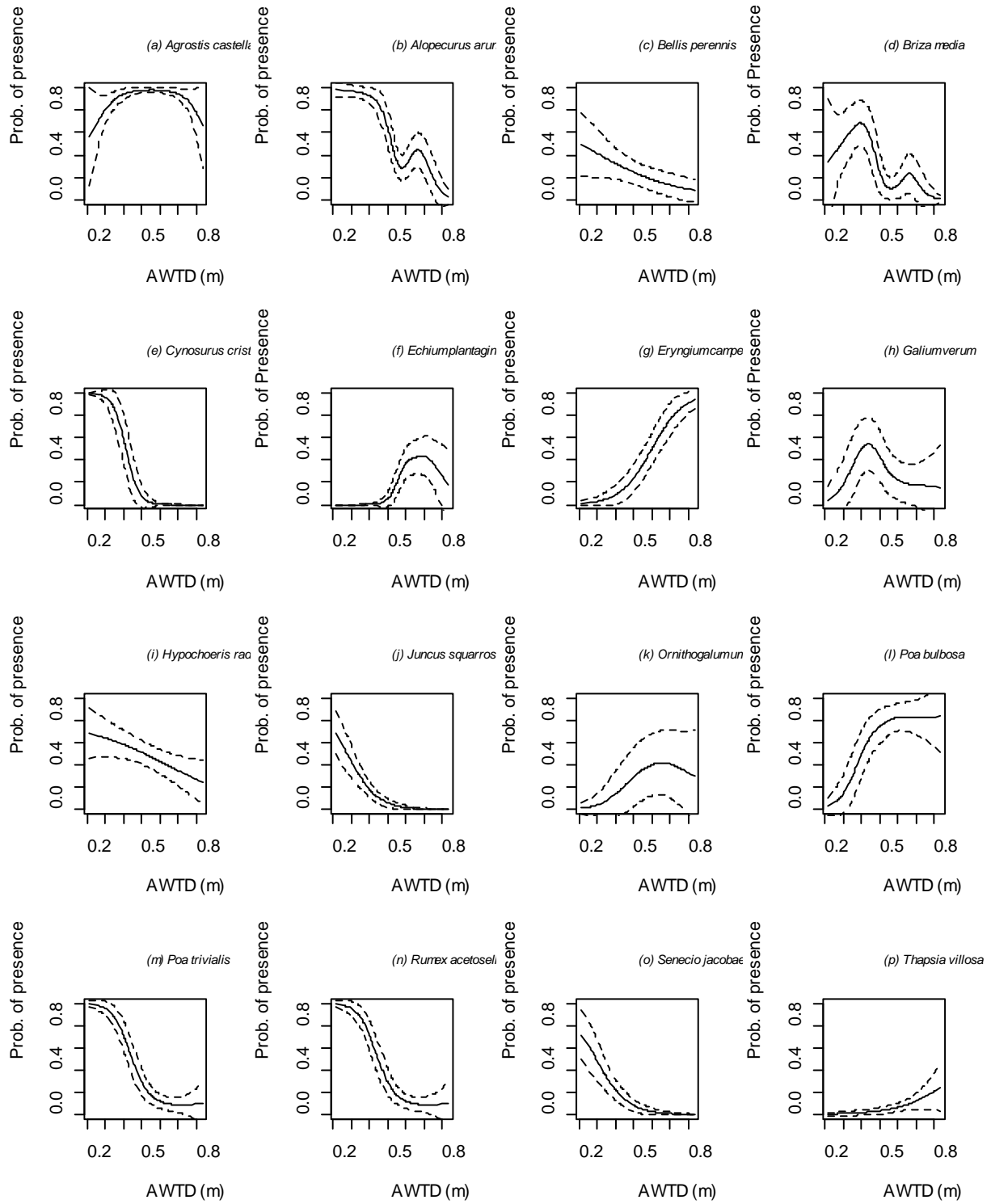


Fig. 3

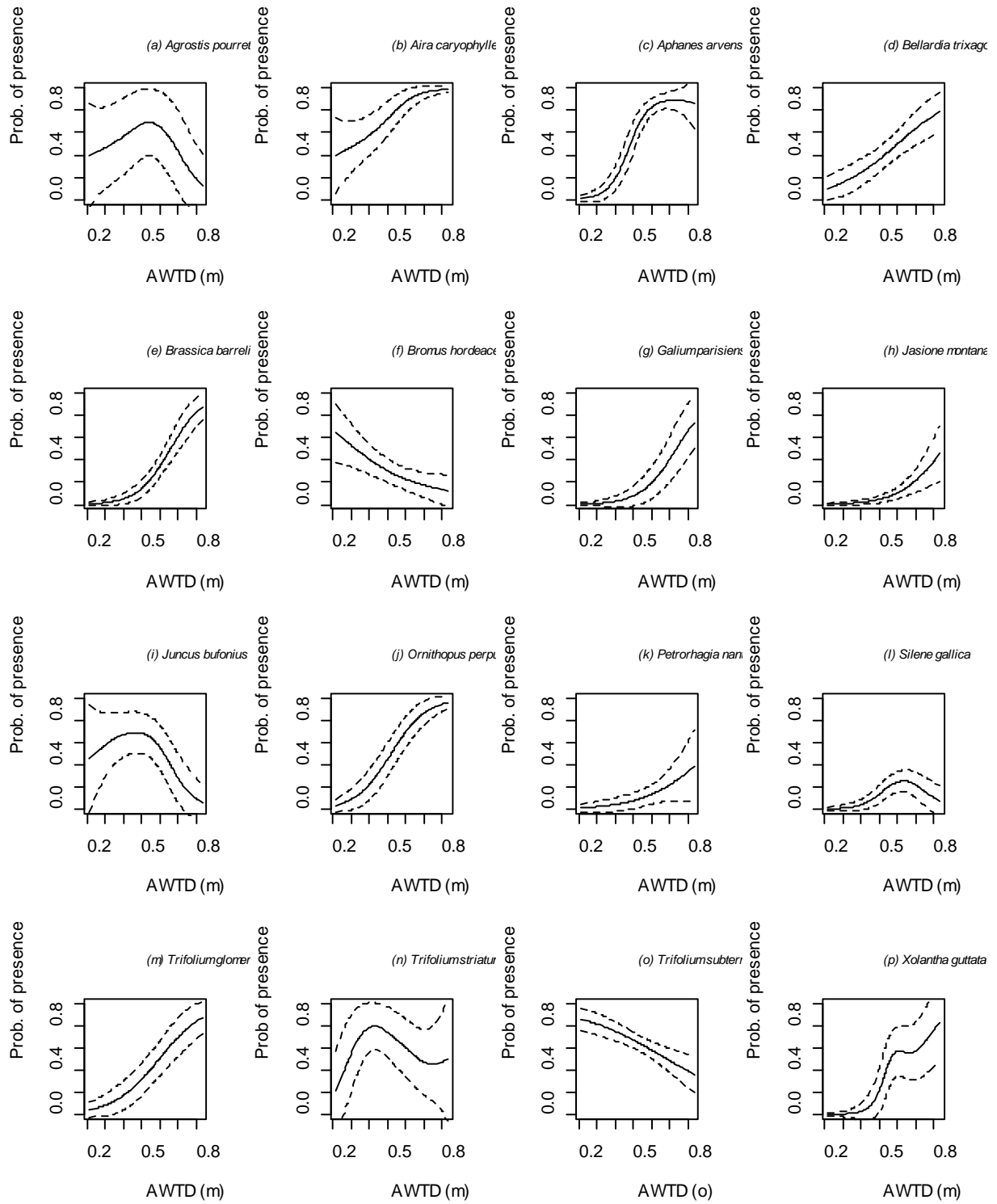


Fig. 4

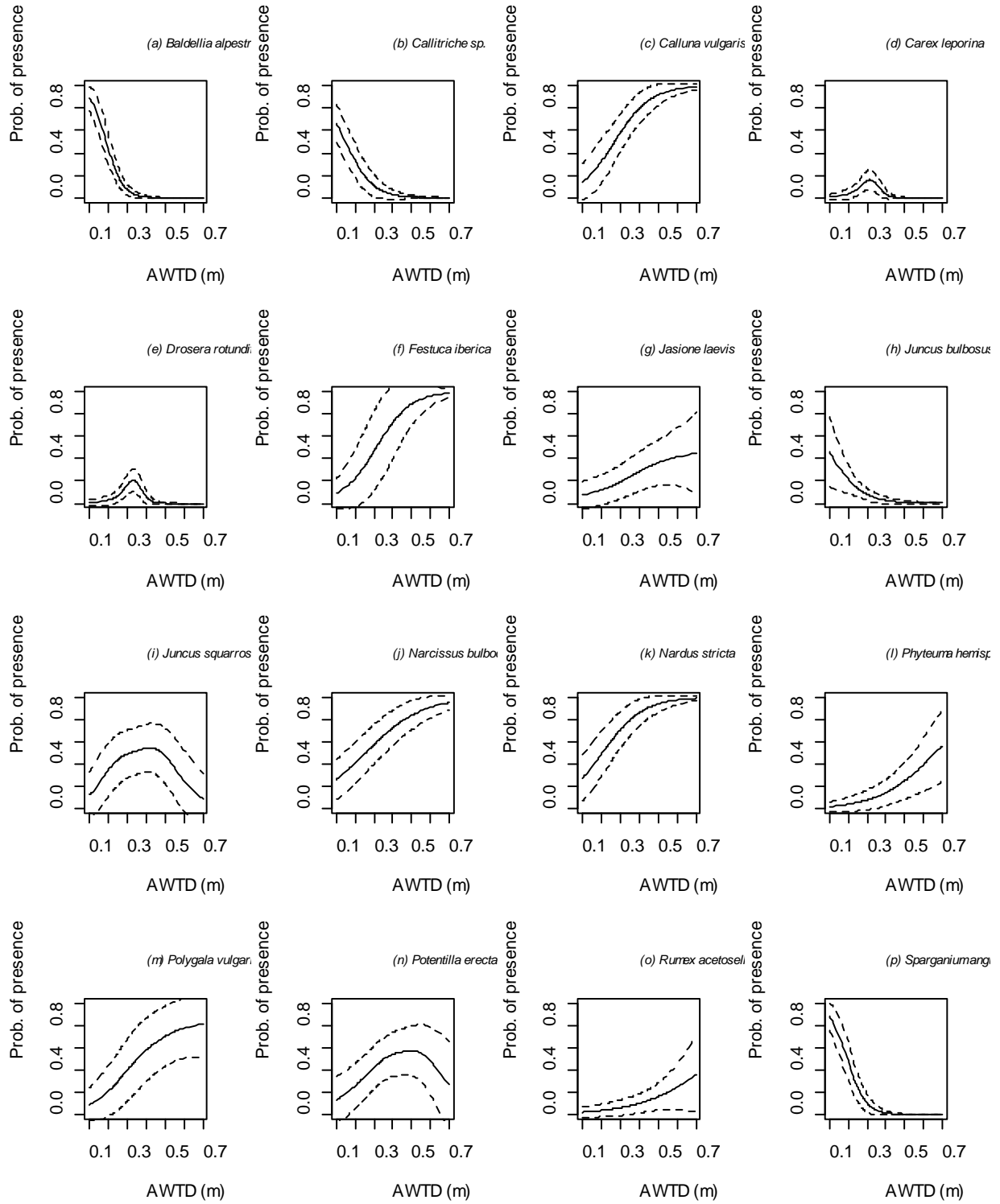


Fig. 5

