

1 **Interspecific competition induces asymmetrical rooting profile adjustments in shrub encroached open**  
2 **oak woodlands.**

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6 Rolo V. and Moreno G.\*

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9 School of Forestry, Universidad de Extremadura, Plasencia 10600, Spain

10 \*Corresponding author; Tel: +34 927427000 Fax: +34 927425209

11 E-mail: gmoreno@unex.es

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26 **Abstract**

27 It is recognized that the extent of woody encroachment is increasing worldwide, but less is known concerning  
28 the likely altered below-ground structure of co-occurring plants. The aim of this study was to analyze the root  
29 system plasticity of three layers of vegetation growing together (trees, shrubs and pasture) and identify potential  
30 mechanisms of competition avoidance.

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32 Root morphology and distribution of trees, pasture and two contrasting shrub species (deep and shallow rooted)  
33 were studied in open oak woodlands of South-Western Spain. Soil samples were obtained from paired adjacent  
34 plots, with and without shrubs, by taking soil cores to a depth of 3 m.

35

36 Analysis of tree and pasture root systems in the presence of shrubs revealed significant reductions in root  
37 biomass and consistent increases in specific root length. Additionally, root profiles changed substantially,  
38 becoming significantly deeper for trees and shallower for pasture. In contrast, both types of shrub increased their  
39 root biomass when growing in competition, but without significantly modifying their rooting profile.

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41 Shrubs seem to be stronger competitors for below-ground resources than neighboring species. However, trees  
42 and pasture show clear plastic responses to shrub presence, irrespective of their rooting patterns, probably to  
43 minimize competitive interactions and maximize resource acquisition.

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46 **Keywords:** *Quercus ilex*, *Retama sphaerocarpa*, *Cistus ladanifer*, Dehesa, Root plasticity, Niche separation.

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## 52 **Introduction**

53

54 Abiotic conditions and interactions among plants are key factors determining plant growth and survival at the  
55 community level. In semi-arid systems, abiotic conditions, such as water or nutrient availability, are able to  
56 shape vegetation and control plant physiological activity (Rambal 2001; Zavala et al. 2000), and consequently  
57 they have been considered determinant in plant ecology (Prentice et al. 1992; Stephenson 1990). In systems with  
58 low plant density, where interactions between plants usually take place below ground (Casper and Jackson  
59 1997), the consequences of plant-to-plant interactions on plant physiological activity and on regulation of the  
60 composition of plant communities can be as strong a determinant as abiotic conditions (Brooker 2006). For  
61 instance, several studies have shown an improvement in plant nutritional status, physiological activity and  
62 productivity as plant density decreases (Gouveia and Freitas 2008; Joffre et al. 1999; Moreno and Cubera 2008).  
63 Nevertheless, the importance of plant-to-plant interactions in these systems, especially of those mediated by  
64 below-ground resources, remains unclear (Casper et al. 2003).

65 Tree thinning is a well-documented forestry practice (Aussenac and Granier 1988; Absjornsen et al. 2007; Breda  
66 et al. 1995; White et al. 2009) and it could become very useful to enable forests to face predicted aridification in  
67 many regions (Millar et al. 2007). Indeed, tree clearance, a management practice intended to optimize the  
68 availability of soil resources for the remaining plants, has been the origin of well-established cultural  
69 landscapes, such as European wood-pasture (Bergmeier et al. 2010; Eichhorn et al. 2006), Mediterranean open  
70 woodlands (e.g. Iberian dehesas; Vicente and Ales 2006) and some scattered oak systems in North America  
71 (McEwan and McCarthy 2008), which have been sustained over a long period of time (Manning et al. 2006).  
72 However, usually, these systems are an unstable vegetation type and require continuous human intervention to  
73 prevent woody encroachment. For instance, in Iberian open oak woodlands, the reduction of grazing intensity  
74 has resulted in a rapid shrub encroachment and a three-layered structure (Ramírez and Díaz 2008). Worldwide,  
75 the extent of woody encroachment and its consequences are increasingly apparent (van Auken 2000). Together  
76 with deforestation and desertification, woody encroachment has been pointed to as one of the major aspects of  
77 global land-cover and land-use change (Asner et al. 2004). However, little attention has been paid to a less  
78 apparent consequence in woody encroachment systems, namely, the altered structure of plants below ground  
79 (Jackson et al. 2000).

80 Vertical root distribution is commonly described with the greatest density of fine roots in the top soil layers,  
81 where nutrient levels are highest, decreasing exponentially with depth (Jackson et al. 1996). However, root  
82 systems are continuously adapting to changes in their environment, such as an uneven availability of nutrient or  
83 plant competition (Hodge 2004; Messier et al. 2009). Indeed, plants can avoid nutrient-poor areas or those with  
84 competitors and opt to explore and exploit soil patches more fully (Semchenko et al. 2008). Therefore, root  
85 plasticity may determine the below-ground success of plants to cope with environmental variations (Hodge  
86 2004; Poot and Lambers 2008).

87 For instance, vertical niche differentiation has been suggested as a morphological mechanism by which plants  
88 reduce competition for resources due to a complementary use of soil layers (Casper and Jackson 1997). Mulia &  
89 Dupraz (2006) reported that trees growing in agroforestry systems significantly modified their rooting profile,  
90 showing a higher root density in depth than when growing without crop competition. Moreno et al. (2005) also  
91 reported a limited vertical overlap of root systems between trees and herbaceous species in open oak woodlands.  
92 However, both studies involved contrasting plant growth forms (tree-pasture) with different abilities to adjust to  
93 a dynamically heterogeneous soil environment. In shrub encroached systems, co-occurring woody species, with  
94 similar abilities to explore soil resources, may be able to tolerate greater competition between root systems.  
95 Indeed, in a semi-arid savanna, Meyer et al (2008) found evidence that overlapping root systems mediate  
96 competitive interactions between woody species. Barnes and Archer (1999) described strongly asymmetric  
97 interactions between shallow-rooted understory shrubs and deeply-rooted overstory plants. Indeed, knowledge  
98 of below-ground properties of co-occurring plants is required to better understand interactions between plant  
99 species (van Noordwijk et al. 1996).

100 Root systems of Mediterranean type vegetation have differentiated into various morphologically different root  
101 types (Paula and Pausas 2011; Silva et al. 2002) mainly because of the heavy restrictions, both spatial and  
102 temporal, on water availability. *Quercus ilex* L., one of the main tree species of the Iberian open oak woodlands,  
103 has been shown to explore a very large volume of soil to meet its water needs during the dry Mediterranean  
104 summers (Moreno et al. 2005). On the other hand, other species have enhanced their water saving mechanisms,  
105 rather than develop a deep rooted system, to withstand summer drought. Therefore, the influence of shrubs on  
106 the systems they encroach may vary greatly with the functional and morphological characteristics of the resident  
107 plants as well as of the shrubs themselves. Thus, exploring root distribution in the vicinity of contrasting shrub  
108 species, differing markedly in their ecological characteristics but both growing in the same system, could be  
109 expected to provide interesting information on shrub impact on the system functioning.

110 The present study focuses on root morphological and distributional changes in tree, shrub and pasture growing  
111 in encroached open oak woodlands in the South-Western Iberian Peninsula. We selected two shrub species  
112 widely represented in the Mediterranean basin, *Retama sphaerocarpa* (L.) Boiss. and *Cistus ladanifer* L.  
113 (hereafter *Retama* and *Cistus*, respectively) with contrasting root traits, namely, deep rooted (Haase et al. 1996)  
114 and shallow rooted (Silva and Rego 2004), respectively. The specific aims were: (i) to assess the rooting profile  
115 of trees, shrubs and pasture growing in competition in Mediterranean open oak woodlands; (ii) to analyze  
116 whether the presence of shrubs modifies the rooting pattern of trees and pasture, leading either to a  
117 complementary use of soil between species or, by contrast, to a constraint on tree and pasture root distribution;  
118 and, finally, (iii) to assess whether tree and pasture rooting patterns react differently to each type of shrub (deep  
119 or shallow rooted). Given the similar rooting strategies of trees and *Retama*, we expected *Retama* would modify  
120 pasture rooting patterns in a similar way to trees, and overlap the root system of trees to a greater extent than  
121 *Cistus*. By contrast, we expected *Cistus* would have a more substantial effect on the pasture understory and  
122 would allow, to a greater extent than *Retama*, a complementary use of soil with trees.

123

## 124 **Material and Methods**

### 125 *Study site and system*

126 The study was carried out in the North of the region of Extremadura (Western Iberian Peninsula, 40°01'N,  
127 06°05'W). The climate of the area is Mediterranean with hot, dry summers and mild, rainy winters. Mean annual  
128 precipitation and temperature are 568 mm and 15.7 °C (Aldehuela del Jerte station records, 40°00'N, 6°13'W).  
129 Soils of the area are classified as Distric Cambisols (IUSS Working Group WRB, 2006). These soils are  
130 moderately deep (80 – 150 cm), with a slightly acid pH (5.0-5.5) and low cation-exchange capacity.

131 The site vegetation is savanna-like open woodland, called Iberian “dehesa”, which is dominated by scattered oak  
132 overstory (*Quercus ilex*; 10 - 40 trees ha<sup>-1</sup>) with native pastures as understory, and occasionally a sparse-to-  
133 dense shrub layer, mostly represented by *Retama* and *Cistus*. *Q. ilex* is a small- to medium-sized tree (3-20 m in  
134 height, usually 5-10 m in Iberian dehesas), that is commonly found in the dry subhumid climate of the  
135 Mediterranean basin. This tree species develops a deep (> 5 m depth) and extended (7 times the projection of the  
136 canopy) root system (Moreno et al. 2005). *Retama* is a resprouting, N-fixing leafless shrub, which often forms  
137 monospecific scattered patches and develops a dimorphic root system, with both shallow lateral roots and a  
138 main tap root up to 16 m deep (Haase et al. 1996). On the other hand, *Cistus* is a non-resprouting, drought-

139 tolerant semideciduous shrub with a dense shallow-rooted system, mostly in the first meter, often forming  
140 extensive clusters of pure stands (Silva and Rego 2004). Pasture understory is dominated by annual native  
141 Mediterranean herbaceous species, specifically the grasses *Agrostis curtisii*, *Agrostis castellana* and *Gaudinia*  
142 *fragilis*, the legumes *Ornithopus compressus*, *Trifolium resupinatum* and *Trifolium angustifolium*, and the forbs  
143 *Bellis perennis*, *Helianthemum guttatum* and *Raphanus raphanistrum*. The root biomass of these species is  
144 mostly located in the first 30 cm of soil (Joffre et al. 1987; Moreno et al. 2005).

145

#### 146 *Experimental layout*

147 For the experiment, we selected six sites (Table 1) characterized by a *Q. ilex* overstory with an average tree  
148 density of 18 tree ha<sup>-1</sup>, and average tree diameter at breast height of 44.8 ± 4.8 (± SE) cm. On each site, we  
149 identified two adjacent plots, similar in terms of tree cover, soil type and slope, but differing in the presence of  
150 shrubs: a control plot (CT) without shrubs, herbaceous plants being the only understory, and an encroached plot  
151 (SE) where the understory was a mix of shrubs and pasture. Thus, four different habitats were defined: two from  
152 control plots, beneath the tree canopy (hereafter tree) and away from the tree canopy (> 20 m from nearest tree;  
153 hereafter pasture); and two from encroached plots, beneath trees (hereafter tree+shrub) and away from the tree  
154 canopy (again > 20 m from nearest tree; hereafter shrub). There was herbaceous understory in all the habitats  
155 studied. In half of the experimental sites (n = 3), *Retama* was the only shrub species in the encroached plots, in  
156 the other half of the experimental sites (n = 3) the only shrub species present was *Cistus* (hereafter named  
157 *Retama* sites and *Cistus* sites, respectively). Thus, in *Retama* sites the four habitats studied were: tree, pasture,  
158 *Retama* and tree+*Retama*; likewise in *Cistus* sites they were: tree, pasture, *Cistus* and tree+*Cistus*. In plots  
159 encroached with *Retama* an average of 40 ± 10 % of the area was covered by shrubs, with an average shrub  
160 height of 2.3 ± 0.17 m. On the other hand, in plots encroached with *Cistus* the average shrub cover was 70.0 ±  
161 1.6 % and average shrub height was 2.1 ± 0.1 m. These values reflect the typical growth pattern of these shrub  
162 species.

163

#### 164 *Soil core*

165 We used a stainless steel auger (10 x 100 cm) to extract unaltered soil coring from each habitat. The auger was  
166 driven into the soil with a heavy electrically powered percussion hammer (Makita HM 1800, provided by  
167 Eijkelkamp, Giesbeek, The Netherlands) down to hard bedrock (including weathered bedrock) using,

168 progressively, 100 cm length extensions. Soil core depth was up to 150 cm in *Cistus* sites and up to 300 cm  
169 depth in *Retama* sites. Under trees, soil cores were positioned half way between the tree trunk and the canopy  
170 edge. At each site, three soil cores were taken from each habitat (three replicated soil cores per habitat x three  
171 sites; n = 9). Hence, in total 72 soil cores were collected in May 2008.

172 Soil columns were divided into 10 cm length samples and stored at 6 °C to keep the roots fresh until analysis.  
173 Samples were washed using different filters (mesh size between 2 and 0.125 mm) in order to avoid losing fine  
174 roots. Each plant species was identified visually and separated manually according to root morphology and  
175 color. Compared to woody species, herbaceous plants have finer and more flexible roots and, in addition, the  
176 roots are mostly white. Specifically, *Q. ilex* roots have a dark-brown bark, *Cistus* a dark-red bark and *Retama* a  
177 pale-brown bark. Root samples were scanned (Epson Expression 10000XL) and images analyzed with  
178 WinRhizo™ software. The samples were oven dried (65°C for 48 h) to measure root dry weight.

179

#### 180 *Data analysis*

181 Data analysis was conducted separately for *Cistus* sites (sites 1-3) and *Retama* sites (sites 4-6). Differences in  
182 root vertical distribution of each growth form (tree, shrub and pasture) growing in the different habitats were  
183 assessed by a two-way ANOVA, considering habitat and depth as factors and root length density (RLD, cm of  
184 root per dm<sup>3</sup> of soil) and root dry mass (RDM, g of root per m<sup>2</sup> of soil) as response variables. Additionally, the  
185 depth containing cumulative root fractions of 50% and 95% ( $d_{50}$  and  $d_{95}$ , respectively) were calculated using the  
186 Gale and Grigal (1987) model to describe the vertical root distribution pattern. Specifically, a non-linear  
187 regression was used to fit the function  $f_c = 1 - \beta^d$  to the profile of cumulative root fraction ( $f_c$ ), from the soil  
188 surface to depth  $d$  (cm), where  $\beta$  is the fitted “extinction coefficient”. Values of cumulative rooting depth,  $d_{50}$   
189 and  $d_{95}$  were then calculated from  $d_{50} = \text{Ln}(0.5) / \text{Ln}(\beta)$  and from  $d_{95} = \text{Ln}(0.05) / \text{Ln}(\beta)$ , respectively. The  
190 model was able to explain between 90 and 98% of the variance. We compared both  $\beta$ ,  $d_{50}$  and  $d_{95}$  values of each  
191 growth form between habitats with a nonparametric test (Kruskal-Wallis) as the data did not follow a normal  
192 distribution but the variances were similar. Additionally, root morphological parameters, namely specific root  
193 length (SRL, m of root per g of root), root diameter ( $\emptyset$ , mm) and maximum rooting depth (MRD, cm) of trees,  
194 shrubs (*Cistus* or *Retama*) and pasture were compared between habitats by one-way ANOVA. Data were  
195 transformed, when necessary, prior to analysis to comply with ANOVA assumptions. The significance level

196 was established at  $P \leq 0.05$ , but when  $P$  was between 0.05 and 0.10, differences were considered marginally  
197 significant. All analyses were performed using Statistica 7.0.

198

## 199 **Results**

200 Herbaceous species showed the shallowest rooting distribution, with the highest RLD values in the top-soil  
201 decreasing steeply with depth (Fig. 1). In all cases, the 50% cumulative rooting fraction ( $d_{50}$ ) was within the first  
202 15 cm of soil, and a 95% fraction was reached ( $d_{95}$ ) at a depth of less than 60 cm, irrespective of the habitat  
203 studied (Table 2). Pasture RLD distribution was slightly different between habitats. The lowest values of  $d_{95}$   
204 were found when growing under the two types of shrubs, *Cistus* and *Retama*, although differences were  
205 significant only under *Retama* (Table 2). Maximum rooting depth values (MRD) were consistent with this  
206 general trend, having lower values beneath the two types of shrubs than in open areas (Table 3). Pasture species  
207 showed a significant lower RLD in the top soil beneath the two types of shrubs ( $P = 0.008$  and  $P = 0.006$  for  
208 pasture growing under tree+*Retama* and under *Retama* respectively;  $P < 0.001$  for pasture growing under  
209 tree+*Cistus* and under *Cistus*) (Fig. 1). By contrast, beneath trees, pasture species just exhibited a weak effect on  
210 RLD in the top soil in *Retama* sites ( $P = 0.07$ ), while in *Cistus* sites there was not any effect ( $P = 0.14$ ).

211 Similarly to pasture, trees showed a significant reduction in RLD in the top soil when growing either with *Cistus*  
212 ( $P = 0.01$  at 20 and 40 cm) or with *Retama* ( $P < 0.001$  and  $P = 0.06$  at 20 and 40 cm; Fig. 1). By contrast, at  
213 deeper soil layers, trees growing with shrubs tended to show higher RLD and RDM than when growing alone  
214 (Fig. 1 and Fig.2). This effect was more evident in sites encroached with *Retama* ( $P = 0.013$  habitat and depth  
215 interaction) than with *Cistus* ( $P = 0.084$  habitat and depth interaction) (Table 4). In addition, trees growing with  
216 *Retama* also exhibited a marginally significantly higher MRD than those growing alone ( $P = 0.059$ ).  
217 Nevertheless, not only trees growing with *Retama* ( $P < 0.05$ ) but also with *Cistus* ( $P < 0.02$ ) exhibited  
218 significant higher values of  $d_{50}$  and  $d_{95}$ .

219 As expected, each shrub specie exhibited a markedly different root system. Firstly, the value of RLD in the first  
220 20 cm was about twice as high for *Cistus* (up to 600 cm/dm<sup>3</sup>) as for *Retama* (300 cm/dm<sup>3</sup>). In addition,  $d_{95}$  for  
221 *Cistus* ( $d_{95}$ ) was lower than 1 m, while for *Retama* was lower than 2 m (Table 2). Secondly, *Retama* growing  
222 with trees showed a significant increase in RLD in the top soil ( $P = 0.032$  at 20 cm, Fig. 1b) and also some  
223 degree of increase in depth ( $P = 0.044$  at 120 cm) compared to *Retama* growing alone. By contrast, *Cistus*



224 growing with trees had a lower RLD along the whole profile, reaching significance in the top soil ( $P = 0.019$   
225 and  $P = 0.061$  at 20 and 40 cm respectively, Fig. 1b).

226 In tree+*Cistus* or tree+*Retama* habitats, almost 50% of all the roots analyzed in the top soil belonged to shrubs  
227 (Fig. 3). The percentage of *Retama* roots increased progressively up to 80% at a depth of 140 cm, whereas the  
228 *Cistus* roots showed the opposite trend, decreasing across the whole profile reaching 10% of roots at a depth of  
229 120 cm. Accordingly, the percentage of tree roots followed an opposite trend to that of the shrubs on both sites,  
230 representing less than 20% of all the roots analyzed in the uppermost soil layer and increasing in deeper soil  
231 layers. This effect was most evident in sites encroached with *Cistus* where tree roots represented up to 90% of  
232 all the roots analyzed at deeper soil layers. Pasture roots were mostly concentrated in the top soil where they  
233 represented more than 20% of all the roots analyzed.

234 Trees showed a reduction in root dry mass per surface unit (RDM) growing with shrubs compared to the value  
235 measured in control plots ( $P = 0.002$  and  $P = 0.018$ ; with *Cistus* or *Retama* respectively). Similarly, pasture in  
236 shrub or tree+shrub habitats also showed a significant reduction of RDM (Fig 2). By contrast, pasture growing  
237 under trees showed a positive, in *Cistus* sites, to neutral, in *Retama* sites, effect ( $P = 0.001$  and  $P > 0.10$ , in  
238 *Cistus* and *Retama* sites, respectively). Both types of shrubs showed the same pattern on increasing RDM  
239 beneath trees. The slight increase in *Cistus* RDM beneath trees is very likely to be related to the significant  
240 decrease in SRL (Table 3), which, together with the significant increase in root diameter, suggest that *Cistus*  
241 growing under trees develops a thicker root system and thus with lower values of RLD (Fig.1). *Retama* also  
242 showed the same pattern of increasing root diameter with a slightly lower SRL. However, this shrub also  
243 exhibited certain increases in RLD (Fig. 1) which evidence the lower extent of this effect. By contrast, trees and  
244 pasture exhibited the opposite effect with a significant increase in SRL when growing with shrubs, which was  
245 accompanied by a reduction in the average diameter of roots (Table 3).

246

## 247 **Discussion**

### 248 *Rooting profile in two-layered, tree-pasture, systems*

249 Previous studies in open oak woodlands have indicated that tree and pasture co-existence relies on a certain  
250 vertical partitioning between root systems (Moreno et al. 2005). Our results are in accordance with these  
251 previous findings. Indeed, although the tree root profiles observed in our study, with a value of  $d_{95}$  of 120 cm,  
252 was shallower than that reported by Moreno et al. (2005), with a value of  $d_{95}$  of 416 cm, both studies found a

253 consistently higher  $\beta$  value for trees than for pasture. These results imply a greater accumulation of pasture roots  
254 in the top soil with a sharp reduction in depth, whereas the root system of trees decreases less markedly, thus  
255 reaching deeper soil layers. Nevertheless, rooting depth and the extent of the root segregation can be influenced  
256 by factors such as local soil conditions (Mulia and Dupraz 2006; van Wijk 2011). For instance,  $\beta$  values of  
257 pasture were notably different between sites, root density decreasing more sharply with depth in *Cistus* sites  
258 (finer textured and shallower soil) than in *Retama* ones. Other factors, as the differences in pasture species  
259 composition between these habitats, could also modulate the outcome of these parameters. It has been shown  
260 that the increase of the micro-scale spatial heterogeneity under trees affect pasture species composition and  
261 diversity (Marañón et al. 2009). For instance, tolerant pasture species to low light availability are abundant  
262 under tree canopy. However, Fernández-Moya et al. (2011) reported that only a few species of pasture respond  
263 directly to the gradient generated by trees in abiotic resources, suggesting that other factors should be involved.  
264 One of these factors could be the ability of these species to segregate in a higher extent their root system with  
265 trees, although evidence of this is scarce and further research is needed.

266 The spatial separation of root systems would reduce inter-specific competition between plant forms and allow  
267 plant co-existence (Casper and Jackson, 1997). This mechanism, although it was first proposed in a subtropical  
268 savanna, is not exclusively applicable in scattered tree systems (Walter and Mueller-Dombois, 1971). Indeed,  
269 examples of a vertical segregation between competing plants is often reported in the literature, e.g., in  
270 agroforestry systems (Mulia and Dupraz 2006), between shrub understory and tree overstory (Achat et al. 2008)  
271 and between tree species (Bolte and Villanueva 2006).

272

### 273 *Shrubs root systems*

274 In the Mediterranean ecosystem, plants follow two main strategies to survive after disturbance: resprouting and  
275 non-resprouting. Each one is strongly associated with marked ecological differences (Paula and Pausas 2011). In  
276 general terms, we found evidence that the two shrubs species followed this pattern: *Retama*, a resprouter  
277 species, was found to have a deeper rooting profile than *Cistus*. Haase et al. (1996) reported that *Retama* root  
278 systems explore soil layers as deep as 16 – 28 m. As a phreatophyte, *Retama* penetrates the soil deeply to satisfy  
279 its water requirements, maintaining a low water potential throughout the drought period (Pugnaire et al. 1996).  
280 However, in the uppermost soil layers, *Cistus* had a denser and thinner root system than *Retama*, probably in  
281 order to maximize soil exploration. Indeed, *Cistus*, as a non-resprouter species, is strongly dependent on soil

282 water and nutrients because it relies on seedling recruitment and fast growth to reach maturation (Paula and  
283 Pausas 2011).

#### 284 *Tree and pasture response to shrub competition*

285 The general rooting pattern followed by trees and pasture growing without competition was strongly modify  
286 when growing with shrubs, confirming our hypothesis. Trees growing with either *Cistus* or *Retama* had a  
287 significantly deeper rooting profile than growing without competition. By contrast, herbaceous species showed  
288 the opposite trend when growing with shrubs, shifting most of their roots upwards to shallower soil horizons.  
289 These results suggest a vertical stratification of root systems, tree-pasture-shrub, in the use of soil between the  
290 plant growth forms studied. However, despite this attempt at vertical segregation, trees and pasture growing  
291 both with *Cistus* or *Retama* showed a significant reduction in root biomass, suggesting the two shrub species  
292 have a stronger competitive ability. Indeed, both *Cistus* and *Retama* showed the opposite trend to the trees and  
293 pasture, with an increase in root biomass when growing with, compared to without, competition (Fig. 2). These  
294 findings would explain the detrimental effect that *Cistus* exert on tree physiological status (Rolo et al. 2011) and  
295 on pasture and tree production (Rivest et al. 2011). If the competitive ability of shrubs exceeds the benefit of  
296 niche differentiation, our results might represent just an intermediate state which potentially may end up in the  
297 exclusion of neighboring plants (Mayfield and Levine 2010). However, such scenario is difficult to predict and  
298 further research containing gradients of niche and competitive ability differences is needed. In addition, other  
299 processes as facilitation may modulate the final outcome (Maestre et al. 2009).

300 Spatial segregation of root systems between competing species has been shown to be due to both below-ground  
301 resource and non-resource competition (Messier et al. 2009). Roots appear to avoid root depletion zones near  
302 other roots (Schenk et al. 1999) and occupied soil by other species or even conspecifics (Novoplansky 2009).  
303 Bolte & Villanueva (2006) showed how co-occurring tree species can adjust their root morphology and  
304 architecture in response to competition, suggesting SRL as a sensitive morphological indicator. Indeed, SRL is  
305 one of the root traits most relevant to soil exploration and resource uptake (Smit et al. 2000). Our results show a  
306 significant increase in tree and pasture SRL when growing with shrubs, suggesting that their root systems were  
307 more elongated, and probably more ramified, compared to when growing without competition. The adaptive  
308 rooting and soil exploitation strategies of trees could be inherent, as a late-successional species, in order to  
309 withstand the invasion of other species (Grime 1979). Mediterranean woody species with finer roots (less mass  
310 per unit length, and hence higher SRL) have been shown to enhance their hydraulic conductance (Hernández et

311 al. 2010) and also resource acquisition (Paula and Pausas 2011). Hence, the clear plastic response showed by  
312 trees and herbaceous species in the presence of shrubs could be interpreted as an attempt to minimize  
313 competitive interaction and at the same time maximize resource acquisition (Ryser 2006). However, at the  
314 whole-plant level, the costs of investing in transport infrastructure could exceed the benefits from spreading fine  
315 roots over a large soil volume (van Noordwijk and Mulia 2002). Recently, Mulia et al. (2010) have proposed,  
316 with a successful model, that structural root growth is a consequence of fine root function, rather than a driver.  
317 Hence, our results give new insights to contribute to the development of future models of how root systems of  
318 competing plants grow in mixed systems, and allow a better understanding and prediction of the consequences  
319 of plant adaptive strategies on ecosystem functioning.

320

### 321 **Conclusion**

322 The vertical segregation of root systems seems to be the co-existence mechanism employed by trees and  
323 pastures in open oak woodlands in the South-Western Iberian peninsula. However, the presence of shrubs  
324 markedly modifies the root distribution pattern and morphology of co-existing trees and herbaceous species. The  
325 two types of shrubs studied (*Cistus ladanifer* and *Retama sphaerocarpa*) both seemed to be stronger competitors  
326 than trees and pasture as growing in competition they achieved higher root dry mass, while this mass fell in trees  
327 and pasture. Nevertheless, tree and pasture root systems showed a certain degree of plastic response to avoid  
328 direct competition with shrubs.

329

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335

### 336 **References**

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460 Figure 1 Absolute and relative (%) root length density (RLD, cm / dm<sup>3</sup>) distribution profile for (A) pasture, (B)  
461 trees and (C) the two types of shrubs, *Cistus* or *Retama*, growing with and without competition. Significant  
462 differences between habitats per depth: \*\*  $P < 0.05$  and \*  $0.10 < P < 0.05$ . Habitats with different letters are  
463 significantly different ( $P < 0.05$ ).

464  
465 Figure 2 Root dry mass (RDM, g / m<sup>2</sup>) averaged per profile (top) and depth (middle and bottom) of pasture,  
466 trees and the two types of shrubs, *Cistus* or *Retama*, growing with and without competition. Significant  
467 differences between habitats: \*\*\*  $P < 0.001$ , \*\*  $P < 0.05$  and \*  $0.1 < P < 0.05$ . Habitats with different letters are  
468 significantly different ( $P < 0.05$ ).

469  
470 Figure 3 Relative percentages of tree, shrub and pasture RLD per depth growing in competition (habitat:  
471 tree+shrub) in sites encroached with *Cistus* or *Retama*.

472  
473 Table 1 Main site characteristics of tree and shrub layer of paired adjacent plots without (control, CT) and with  
474 (shrub encroached, SE) presence of shrubs, either *Cistus ladanifer* or *Retama sphaerocarpa*.

475  
476 Table 2 Comparison of the shape of rooting systems of trees, shrubs and pasture growing with and without  
477 competition in sites encroached with *Cistus* or *Retama*. Values with different letters are significantly different ( $P$   
478  $< 0.05$ )

479  
480 Table 3 Mean values ( $\pm$  S.E.) of tree, shrub and pasture specific root length (SRL, cm/g), root average diameter  
481 ( $\emptyset$ , mm) and maximum rooting depth (MRD, cm) growing with and without competition in sites encroached  
482 with *Cistus* or *Retama*. Significant differences between habitats: \* $P < 0.05$ . Values with different letters are  
483 significantly different ( $P < 0.05$ ).

484  
485 Table 4 Results from a two-way ANOVA testing the effect of habitat (with and without competition) and depth  
486 as factors and RLD (cm / dm<sup>3</sup>) as response variable per growth form (tree, shrub and pasture) and type of stand  
487 (encroached with *Cistus* or *Retama*).

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490