1 **Shrubs affect soil nutrients availability with contrasting consequences for pasture**

2 **understory and tree overstory production and nutrient status in Mediterranean grazed**

3 **open woodlands**

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6 Rolo V[a]., López-Díaz M.L.[a] and Moreno G.[ab]

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9 [a]Forestry School, Universidad de Extremadura, Plasencia 10600, Spain

10 [b]Corresponding author; Phone: +34 927427000 Fax: +34 927425209

11 E-mail: gmoreno@unex.es

12   **Abstract**

13   Increases of woody plant cover in former savannas and managed savanna-like systems are

14   currently considered one of the major components of global environmental change. Besides,

15   shrub encroachment is frequently recommended as a practical tool to ensure tree regeneration

16   in these systems. We investigated if the appearance of a shrub layer in Mediterranean open

17   woodlands can affect neighboring plants -tree and pasture- production and nutrient status,

18   through the modification of soil properties. The effect of two contrasting, in terms relevant to

19   nutrient cycling, shrub species, *Cistus ladanifer* and *Retama sphaerocarpa,* were

20   investigated. *Cistus*, a shallow rooted shrub, enhanced soil P and K but depleted N and Mg

21   availability under its canopy. Its effects both on tree overstory and pasture understory were

22   consistently detrimental. By contrast, *Retama*, a deep-rooted shrub, ameliorated widely top

23   soil fertility, improving soil nutrient availability, but affecting distinctively to pasture

24   (facilitation) and trees (competition) biomass production. Overall, vector analysis revealed

25   that pasture production, irrespective of habitat, was mainly limited by P, whereas trees

26   growing with *Cistus* were limited by Ca and N and trees growing with *Retama* by P. Our

27   results showed the capability of shrub to affect consistently both pasture and tree production

28   and nutrient status through the modification of soil nutrient availability. However, these

29   effects are specific to shrub-understory and shrub-overstory combinations, which must be

30   born in mind when using shrub encroachment as a management practice in degraded grazed

31   open woodlands.

32   *Keywords:* plant-to-plant interaction, ion-exchange resin membrane, vector analysis, dehesa, shrub

33   encroachment.

Introduction

Landscapes with scattered trees represent complex systems where pasture, shrubs and trees coexist in dynamic equilibrium. Currently, the density and cover of shrubs in former pasturelands and savannas, including man-made savannas, as Iberian dehesas (Fernández-Ales et al. 1992; García del Barrio et al. 2004; Pinto-Correia and Mascarenhas 1999) and other Mediterranean open woodlands (Papanastasis 2004), have increased markedly worldwide (Montané et al. 2007; Parizek et al. 2002; Price and Morgan 2008). Not only there are a variety of causes, ranging from climate change to fire and grazing suppression, responsible of the increases of shrub cover (van Auken 2000); but also there are a variety of consequences of shrub encroachment at population, community and landscape level (Cushman et al. 2010). The extent of this phenomenon has led to consider woody encroachment, together with deforestation and desertification, as one of the major components of global environmental change (Asner et al. 2004).

However, a practical side of this phenomenon has arisen recently. Restoration ecology is considering increasingly spontaneous vegetation succession as a tool to recover degraded environments (Prach et al. 2001). For instance, in Mediterranean degraded pasturelands, shrub encroachment has been shown as an important step in the reversal of desertification processes (Maestre et al. 2009) and also in promoting natural regeneration in open woodlands (Ramírez and Díaz 2008). These recommendations are partly based on the fact that shrubs can cause shifts in the components of the hydrological cycle and improve patterns and rates of biogeochemical turnover (Huxman et al. 2005). In turn, these processes can lead to the modification of the distribution patterns of soil resources (Maestre et al. 2009). Marzaioli et al. (2010) showed how the abandonment of cultivated lands, with consequent development of shrubs, can improve soil quality. Therefore, the allowance of woody presence in grazed systems that are limited in their productivity potential, as the silvopastoral systems of the

59 Iberian Peninsula (Gómez-Gutierrez and Pérez-Fernández 1996), can be seen as a way to

60 improve their soil properties (Paudel et al. 2011).

61   On the other hand, it is often assumed that shrub encroachment may reduce the

62 profitability of grazing systems (Scholes and Archer 1997). Several studies have described

63 how productivity and diversity of pastures decline as shrubs encroach into grasslands or

64 savannas (Briggs et al. 2005; Peco et al. 2006; Price and Morgan 2008; Rivest et al. 2011). In

65 addition, rather detrimental effects of shrubs on the nutritional status of tree overstory have

66 also been shown (Barnes and Archer 1999; Cubera and Moreno 2007). For instance, in a

67 semi-arid Savanna, Meyer et al. (2008) found a prevalence of competitive interaction

68 between woody species mediated by overlapping rooting systems.

69   In this way, it is crucial to better understand nutrient relations of shrub-encroached

70 Mediterranean grazing systems before considering woody species as a tool to ameliorate soil

71 properties. However, the fact that shrubs can modify nutrient, light and water availability

72 simultaneously hinders the understanding of the mechanisms which determine their net effect

73 on neighboring plants. For example, neighboring species may profit from the presence of

74 plants that can increase nutrient availability, as N fixing species (Raynaud et al. 2008) and

75 through the provision of shading in systems with excessive radiation (Brooker et al. 2008).

76 By contrast, shrubs can affect nutrient supplies to neighboring vegetation directly by

77 competition for soil resources, or indirectly by altering the availability of a limiting resource

78 through the differential use of a second resource (Everard et al. 2010). For instance, the

79 mineral nutrient relations with neighboring plants can be altered through the modification of

80 soil water availability by shrubs. Overall, the prevalence of positive or negative modifications

81 of both above- and below-ground resources would determine the net effect of shrubs on

82 neighboring species (Holmgren et al. 1997), which can vary between pasture understory and

83   tree overstory because of different nutrient-uptake strategies (Marañón et al. 2009; Schroth

84   1995).

85   The aim of this study was to examine soil and plant nutrient relations between vegetation

86   layers (tree-shrub-pasture) in shrub encroached open oak woodlands of Central-Western

87   Spain (named Iberian dehesa). The effect of shrubs on the nutritional status of herbaceous

88   understory (pasture) and woody overstory (tree) productivity was investigated. We employed

89   ion-exchange resin membranes to evaluate the effect of plants on soil nutrient availability,

90   and used vector analysis (Haase and Rose 1995) to assess the response of understory and

91   overstory species. We studied the effect of two shrub species widely represented in the

92   Mediterranean basin, *Retama sphaerocarpa* (L.) Boiss. and *Cistus ladanifer* L. (hereafter

93   *Retama* and *Cistus*, respectively), with contrasting traits relevant to nutrient cycling (e.g.

94   rooting depth and N acquisition strategy) to asses species-specific relationships. Specifically,

95   we answered the following questions (i) can the presence of shrubs modify soil nutrient

96   availability?; (ii) can these changes affect the nutritional status of under- and overstory

97   plants?; (iii) can different shrub species exert a differential response? We hypothesized that

98   shrubs may affect the nutritional status of neighboring plants, both the herbage understory

99   and tree overstory, due to the modification of soil nutrient availability. Given the similar

100   rooting strategies of trees and *Retama,* i.e., both with deep and sparse rooting systems (Rolo

101   and Moreno 2012), we expected that both of them can modify herbage nutrient status

102   positively. By contrast, *Cistus,* with a dense and shallower rooting profile (Rolo and Moreno

103   2012), can affect more considerably the nutritional status of herbage understory. However,

104   *Retama* can affect the nutritional status of trees more markedly than *Cistus*.

105

106

107   **Material and Methods**

Study site and system

109   The study was carried out in the North of the Extremadura region (Western Iberian

110   Peninsula, 39º54' N, 06º30' W), located at an average 400-500 m asl. The climate of the area

111   is Mediterranean with hot, dry summers & mild, rainy winters. Mean annual precipitation and

112   temperature are 568 mm and 15.7 ºC (Aldehuela del Jerte station records, 40º00' N, 6º13'W).

113   Soils of the area are classified as Distric Cambisol (IUSS Working Group 2006). These soils

114   are moderately deep (80 – 150 cm), with pH slightly acid (5.0-5.5) and low cation exchange

115   capacity.

116   Site vegetation is a savanna-like open woodland, named Iberian dehesa. This extensive

117   silvopastoral system is used by cattle, pig, sheep and horse rearing. The vegetation is

118   dominated by scattered oak overstory (*Quercus ilex*; 10 - 40 trees ha^{-1}) with native pastures as

119   understory, and occasionally a sparse to dense shrub layer, mostly represented by *Retama* and

120   *Cistus*. Pasture is the main fodder source for livestock in this system, but acorns and tree

121   foliage are also important fodder sources (Olea y San Miguel 2006). *Q. ilex* is a small- to

122   medium-sized tree (3-20 m in height, usually 5-10 m in Iberian dehesas), that grows in dry

123   subhumid climate in the Mediterranean basin. This species is characterized with deep and

124   extended rooting profile (Moreno et al. 2005), which allows *Q. ilex* to cope with seasonal

125   drought and to reduce competition with herbaceous plants for belowground resources trough

126   a certain spatial separation of roots. In addition, *Q. ilex* has been shown to contribute

127   significantly to increased soil fertility in Iberian dehesas (Gallardo 2003; Gea-Izquierdo et al.

128   2010; Moreno and Obrador 2007). *Retama* is a N fixing leafless shrub often forming

129   monospecific scattered patches and developing a dimorphic rooting system, with both lateral

130   roots and a main taproot (Prieto et al. 2010). This species has been shown to enhance pasture

131   diversity and productivity and to promote herbage succession under its canopy (Moro et al.

132   1997; Pugnaire et al. 1996). In addition, soil and pasture N content has been shown to be

higher under *Retama* than in open spaces of pastures (Rodríguez-Echeverría and Pérez-Fernández 2003). *Cistus* is a drought-tolerant semideciduous shrub with a dense shallow-rooted system often forming extensive clumped pure stands (Silva and Rego 2004). Its leaves and stems secret a large amount of exudates with allelopathic agents that have been shown to inhibit germination and growth to competing herb seedlings (Sosa et al. 2010). Furthermore, a positive effect of *Cistus* on soil P availability has been shown indicating its potential to improve soil fertility in degraded areas (Simões et al. 2009). Pasture understorey is dominated by annual native Mediterranean herbaceous species, specifically the grasses *Agrostis curtisii*, *Agrostis castellana* and *Gaudinia fragilis*, the legumes *Ornithopus compressus*, *Trifolium resupinatum* and *Trifolium angustifolium*, and the forbs *Bellis perennis, Helianthemum guttatum* and *Raphanus raphanistrum*.

Sampling protocol

We selected 16 sites characterized by a pasture/shrub mosaic with a *Q. ilex* overstory with an average tree density of 26 tree ha^{-1}, an average tree diameter at breast height of 44.8 ± 1.3 (SE) cm and an average canopy width of 10.9 ± 0.1 (SE) m. Prior and during the study, all sites were regularly grazed by cattle, sheep and horses. Although stocking rates were not assessed, it was verified visually that grazing intensity was similar in all sites. Each site included two adjacent plots (0.5 - 1 ha), similar in tree cover, soil type and slope, but differing in the presence of shrubs: one plot without shrubs and one with the presence of shrubs. Both plots have pastures as understory (Fig. 1). Shrubs had developed because of a recent reduction in grazing pressure (last 10–15 years; farmer's communication). In half of the sites (n = 8), *Retama* was present as shrub species, in the other half of the sites (n = 8) the shrub species present was *Cistus*. *Retama* plots exhibited an average shrubby cover of 32.3 ± 1.6 % and an average shrub height of 1.9 ± 0.04 m. *Cistus* plots exhibited an average shrubby

158 cover of 76.1 ± 1.6 % and an average shrub height of 2.0 ± 0.05 m. These values reflect the

159 typical growth pattern of these shrub species (Devesa Alcaraz 1995).

160

161 *Plant Sampling*

162   The effect of shrubs on tree overstory, leaf nutrient concentration and production, was

163 assessed during two consecutive years (2007 and 2008) in 16 sites, eight per shrub species. In

164 each site 12 trees were selected, six with shrub understory and six as control (without shrub

165 layer). 40 current-year twigs (10 per aspect) were harvested per tree by the end of July (at leaf

166 maturity) each year. Twigs were collected from the upper part of the canopy to avoid grazing

167 damages (ca. 4-6 m). Leaves were removed from twigs and oven dried (65° C for 48 h) to

168 assess leaf dry matter.

169   Pasture samples were collected by early June (end of the growing season) same years.

170 Samples were collected from 0.5 x 0.5 m quadrats that were protected by exclusion cages to

171 prevent grazing (1 x 1 m at the base x 0.5 m in height). The exclusion cages were installed

172 before the growing season of 2007 in three different habitats: beneath tree, beneath shrub (>

173 20 m from nearest tree), and in open pasture (> 20 m from nearest tree, control) (Fig. 1).

174 Pasture sampling was limited to 12 sites, six per shrub species, and four replicate quadrats per

175 site and habitat because of exclusion cages availability. Pasture samples were oven dried (65

176 °C for 48 h) to assess pasture dry matter.

177   In order to estimate tree leaf and pasture N, P, Mg, Ca and K concentration, samples

178 were grounded to pass a 250 µm mesh and 0.5 g subsamples were wet-digested with

179 H_2SO_4/H_2O_2 (Kalra y Maynard 1991). N concentration was analyzed by Kjeldhal procedure

180 and P, Mg, K and Ca concentration by means of inductively coupled plasma (ELAN[®] 9000

181 ICP-MS; PerkinElmer Inc).

182

183   *Soil nutrient availability and properties.*

184   Soil availability of Ammonium, Nitrate, Phosphate, Mg^{2+}, Ca^{2+} and K^+ (hereafter: N =

185    $N-NO_3$ + $N-NH_4$, P, Mg, Ca and K) was determined in same 16 sites selected for tree

186   sampling by means of ion-exchange resin membranes (Plant Root Simulator, PRS^{TM}-probes;

187   Western Ag Innovations Inc., Saskatoon, Canada). PRS^{TM}-probes were installed in the three

188   habitats defined for pasture sampling, with four replicate per site and habitat. PRS^{TM}-probes

189   were located in points regularly grazed, using small (5x5 cm) protection. Each sampling point

190   consisted of two pairs of PRS^{TM}-probes, one at 0 – 10 cm and the other at 20 - 30 cm depth.

191   Each pair was composed of a cation and an anion resin exchange membrane. In total 768

192   PRS^{TM}-probes were installed (16 sites x three habitats x two depths x four replicates x two

193   PRS^{TM}-probes). PRS^{TM}-probes were placed during 40 days from April to May of 2009,

194   coinciding with the vegetation growing season of our study site. PRS^{TM}-probes were installed

195   as recommended by the manufacturer (http://www.westernag.ca/innov/index.php). PRS™-

196   probes per depth within habitat and site were combined for elution and chemical analysis.

197   PRS™-probes were returned to Western Ag Innovations for analysis. The probes were

198   extracted with 0.5 N HCl. $N-NO_3$ and $N-NH_4$ were analyzed by means of colorimetry

199   (automated flow injection analysis system) and P, Mg, Ca and K, by means of ICP

200   (Inductively Coupled Plasma).

201   In addition, soil characteristics were assessed by collecting a composite soil sample

202   (approximately 100 g per sample) to a depth of 0-30 cm in the same sampling points as

203   nutrient availability measurements at the time of probe installation. Soil samples were air-

204   dried and sieved to pass 2 mm. The parameters analyzed were: soil particle size distribution

205   by the pipette method, soil pH by a pH-meter (soil-water 1:2.5) and soil organic matter by

206   Walkley and Black method. Soil water content (SWC) and temperature were measured at 0-

207   10 and 20-30 cm depth before the installation and after the removal of PRS™-probes by

208   means of a portable TDR and a soil thermometer, respectively.

209

210   *Data analysis*

211   Normality of data was checked through the Kolmogorov–Smirnov test, and

212   independence of soil properties and nutrients availability at different depths was assessed

213   through the Mauchley's sphericity test. Mauchley's test revealed dependence among values

214   of different depths in all cases. Hence, soil properties and nutrient availability (variables

215   response) were analyzed by means of multivariate analysis of variance (MANOVA)

216   considering together both soil layers studied (0 – 10 and 20 – 30 cm) per variable response. In

217   order to assess separately the effect of trees and both type of shrubs (*Cistus* and *Retama*),

218   three independent MANOVAs were used, comparing mean values beneath tree (n=16 sites)

219   or shrub (n=8 sites per shrub type) versus their respective control habitats (open pasture). The

220   effects of the presence of trees or shrubs on pasture production and nutrient status were also

221   analyzed independently using repeated-measured ANOVAs, with one between-subject

222   factors (habitat: open pasture vs tree or shrub) and one within-subject factor (year). The effect

223   on tree leaf production and nutrient status of shrub presence was analyzed similarly with one

224   between-subject factor (habitat: tree and tree+shrub) and one within-subject factor (year).

225   Data were transformed, when necessary, prior to analysis to comply with ANOVA

226   assumptions. We did not find any significant effect or interaction between years neither in

227   tree nor pasture production and nutrient status analysis, thus we show mean values of both

228   years. All analyses were performed with Statistica 7.0.

229   Vector analysis (Haase and Rose 1995; Mead et al. 2010) was used to assess the

230   nutritional response of pasture and tree to the different habitats. This diagram provide a

231   graphical comparison of simultaneous changes in nutrient concentration (vertical axis), dry

232   mass (top horizontal axis) and nutrient content which is expressed as the function of nutrient

233   concentration multiplied by dry mass (bottom horizontal axis). Habitat-induced deviations of

234   this variables relates to two key process driving plant growth: nutrient uptake and dry matter

235   production. The magnitude and direction of vectors allow for diagnosis of habitat-induced

236   responses of nutrient status as deficiency, sufficiency, dilution and antagonism. Responses

237   are expressed relative to the control (open pasture or tree), that is normalized to 100,

238   respectively.

239

240   **Results**

241   *Soil properties and nutrient availability*

242   Soil organic matter (SOM) was significantly higher under trees than that of open pasture,

243   and neither *Cistus* nor *Retama* showed a significant effect (Table 1). Soil moisture was

244   significantly higher under *Retama* compared to pasture in top 10 cm. By contrast, tree and

245   *Cistus* showed the opposite trend, reaching significant differences under *Citus*. The presence

246   of a woody cover lowered the soil temperature in 0-20 cm soil layer compared with open

247   pasture, reaching a significant reduction under trees and *Cistus* (Table 1).

248   Compared to open pasture, soil under trees exhibited a significantly higher availability of

249   all nutrients analyzed (N, P, Ca, Mg and K) in the uppermost soil layer. At depth, just K

250   availability was significantly higher under tree than that of open pasture, while N and Ca

251   availability diminished, being significant only for Ca (Fig. 2). Soil under *Retama* showed a

252   significantly higher availability of N and Mg in the uppermost soil layer compared to zones

253   of open pasture, which were also consistent in depth, but there was only a significant

254   difference for Mg. Ca and K followed a similar pattern as N and Mg under *Retama* but

255   without significant differences. Additionally, it is noteworthy a slight reduction in P

256   availability under *Retama* compared to open pasture in the uppermost soil layer, although it

257   was just marginally significant ($p = 0.07$). Soil under *Cistus* showed a higher availability of P

258   and K, these differences being limited to the uppermost soil layer for P whereas consistent

259   over the depth for K. In addition, there was a significantly lower availability of Mg and N,

260   although restricted to the second soil layer (Fig. 2). Ca followed the same declining pattern as

261   Mg and N in depth but without significant differences.

262

263   *Plant production and nutrition*

264   Pasture yield was significantly lower under trees and *Cistus* compared to control zones (p

265   < 0.001), achieving just 63 and 30 % respectively. *Retama*, by contrast, exhibited a positive

266   effect on pasture yield increasing it significantly compared to control zones ($p < 0.001$).

267   Pasture nutrient concentrations were dependent on the type of woody overstory present.

268   Pasture growing under trees showed a significantly higher concentration of K ($p < 0.001$) and

269   a significantly lower concentration of P ($p = 0.047$) (Table 2). Pasture growing under *Retama*

270   had a lower concentration of Ca ($p = 0.019$), while growing under *Cistus* showed a

271   significantly lower concentration of N ($p = 0.04$), P ($p = 0.01$), Ca ($p = 0.002$), and Mg ($p =$

272   0.01) than that growing in control zones (Table 2). Consequently, nutrient uptake by pasture

273   growing under trees and under *Cistus* was significantly lower for all nutrients analyzed ($p <$

274   0.003 for all nutrients). While pasture growing under *Retama* exhibited a significantly higher

275   N ($p = 0.013$), Mg ($p = 0.032$) and K ($p = 0.038$) uptake compared to control zones (Table 2).

276   Nutrient interactions of pasture growing under tree, *Cistus* and *Retama* are summarized

277   in Fig. 3, where the relative production, nutrient concentration and nutrient content are

278   compared in a single nomogram with pasture growing in open zones normalized to 100.

279   Pasture growing in association with trees showed a significant deficiency of P (reduction in

280   pasture production, nutrient concentration and content, $p < 0.047$) and an excess of K

281   (increase in nutrient concentration and content, $p < 0.002$). Pasture growing under *Cistus*

282   showed a deficiency in N, P, Ca and Mg, and, similar to trees, an excess of K, although the

283   effect was less clear due to the lack of a significant increase in K concentration (Fig. 3, Table

284   2). Pasture growing under *Retama*, by contrast, showed a steady accumulation of N, Mg and

285   K (increase of pasture production and nutrient content, $p < 0.038$) and a likely P and Ca

286   dilution (increase in pasture production, and nutrient content and a decrease in nutrient

287   concentration) (Table 2).

288   Regarding the effect of the presence of shrubs on tree overstory, both *Cistus* and *Retama*

289   reduced significantly mean tree leaf weight per annual twig ($p < 0.001$ and $p = 0.017$;

290   respectively) (Table 3). However, their effect on tree nutrient status was function of the type

291   of shrub present. Trees growing with *Cistus* exhibited a significantly lower leaf N ($p < 0.001$)

292   and Ca ($p = 0.047$) concentration and a significant increase in K ($p = 0.005$) compared to

293   control trees (Table 3), whilst trees growing with *Retam*a exhibited a slight, marginally

294   significant decline in P ($p = 0.072$). The significant reduction in mean leaf weight per annual

295   twig in trees with a shrub understory led to a significantly lower leaf nutrient content of all

296   nutrients analyzed in trees growing with *Cistus* and with *Retama* except for Mg and N (only

297   marginal differences) with *Retama* (Table 3).

298   Leaf nutrient interactions of trees growing with shrubs are shown in Fig. 4. Again, there

299   was contrasting tree leaf nutrient interactions in function of the type of shrub understory

300   present. Trees growing with *Cistus* as understory exhibited a deficiency pattern of Ca and N

301   (reduction in leaf weight, nutrient concentration and content, $p < 0.047$ and $p < 0.001$

302   respectively) and an excessive K accumulation ($p < 0.005$), whilst trees growing with *Retama*

303   as understory showed a slight deficiency of P ($p < 0.072$).

304

Discussion

Woody plants affect soil nutrients availability differentially

305

306

307   The presence of woody plants in the study sites exerted a significant effect on soil

308 nutrient availability under their canopy, with contrasting effects between species. *Quercus*

309 *ilex* trees showed a marked effect on topsoil fertility with a higher availability of most of the

310 nutrient analyzed compared to that of open pasture zones (Fig. 2). The marked differences

311 between depths under tree canopy are probably linked to a stepper decrease with depth of

312 SOM content than in open pasture (Moreno and Obrador 2007). In the literature on scattered

313 tree systems, improvement of soil fertility under tree canopy usually is attributed to litter

314 accumulation, interception of nutrients, movement of nutrients upwards from deeper soil

315 layers or even to animal dung (Gallardo 2003; Ludwig et al. 2004; Moreno et al. 2007). Thus,

316 besides other ecological functions, trees have been considered keystone components of these

317 systems due to their contribution to ecosystem functioning (Manning et al. 2006).

318   In accordance with our hypothesis, shrubs modified soil nutrient availability and,

319 moreover, different shrub species exerted marked contrasting effects on particular soil

320 nutrients. The concentration of mineral nutrients in the soil solution depends on factors such

321 as soil cation exchange capacity, redox potential, soil moisture and temperature, pH, quantity

322 of soil organic matter and microbial activity (Marschner 1995). Shrubs can modify some of

323 these factors through abiotic, e.g. sediment transport and moisture availability, and biotic, e.g.

324 root activity, mechanism resulting in the modification of soil nutrient availability

325 (Schlesinger y Pilmanis 1998). Yet, the net effect of each shrub species on a particular

326 nutrient availability may be modulated by its specific traits. For instance, the higher N

327 availability under *Retama* could be related to its N_2-fixing capacity (Pugnaire et al. 1996),

328 and its deep rooting profile (Haase et al. 1996) could allow this shrub to bring up nutrients as

329 Mg. Regarding *Cistus*, the higher leaf area index than *Retama* (Garcia-Estringana et al.

14

330   2010), could allow a higher litterfall and a higher leaching of certain nutrients as K; a mineral

331   nutrient that is not constituent of organic structures in plants and that has a high mobility in

332   plants at all levels (Marschner 1995). This effect would be consistent with the enrichment of

333   K ions in net throughfall that has been described under *Cistus* (Simões et al. 2009).

334   Nevertheless, differences among these woody species could be modulated by specific abiotic

335   conditions (soil temperature and moisture) and the quality of litter produced under each

336   species which largely determine the composition and activity of soil organisms and

337   consequently soil nutrient availability (BassiriRad 2005).

338

339   *Trees compromise pasture performance despite their ameliorative effect on soil properties*

340   The net effect of trees on pasture understory in this system is controversial, with

341   examples showing positive (Gea-Izquierdo et al. 2009) to negative (Moreno et al. 2007)

342   effects. The interplay between the provision of shade and the modification of soil resources

343   availability (water and nutrients) define the final outcome of trees. A positive effect of

344   canopy shade on pasture production has been commonly reported in this system (Moreno

345   2008). It has been argued that nutrient availability is the main influencing factor for pasture

346   production (Cubera et al. 2009). Yet water availability may play a crucial role for

347   determining the final effect of trees on pasture production (McPherson 1997; Moreno 2008;

348   Gea-Izquierdo et al. 2009). Our results are in agreement with the latter notion, showing that

349   the increase in the availability of all nutrients analyzed in the top soil under tree canopy (Fig.

350   2), did not consequently lead to an improvement of pasture production and nutrient status

351   (concentration and content). Maestre et al. (2005) argued that, in semi-arid environments,

352   microsite benefits are only apparent when enough water is available to profit from other

353   factors such as nutrient enrichment. The slight decrease of SWC observed under the influence

354   of trees could have outweighed the profit of nutrient enrichment. Nevertheless, we caution

355   that the SWC measurements were made in the top-soil in a single time and that a continuous

356   monitoring of this parameter accounting higher soil depths may have been more conclusive to

357   effectively assess this effect.

358   Mead et al. (2010) successfully used vector analysis techniques to differentiate between

359   moisture and nutrients limitations to plant growth. Accordingly, the direction of the vectors

360   observed suggests that water stress may be involved in the reduction of pasture growth. The

361   vector followed by P suggests that pasture growth was likely limited by this nutrient, despite

362   its higher availability under tree canopy. Trees may promote P availability to enhance its

363   uptake by means of an active role of their roots, but, interestingly, pasture could not profit

364   from it. The temporal mismatch between plant sampling and soil availability measurement

365   (different years) could have led to this discrepancy between soil availability and pasture

366   uptake. Nevertheless, different utilizations of P between trees and pasture have been reported

367   in other tree-pasture systems (Chen et al. 2004) and linked to distinct types of mycorrhizal

368   associations. In addition, the positive effect of trees on K availability was well mirrored by

369   pasture uptake. This result suggests that the different nature of each nutrient could also be

370   involved in the differential response on pasture nutrition. The lower mobility of P than K in

371   the soil, along with the slight reduction in SWC beneath tree canopies, could have limited in a

372   greater extent P than K uptake by pasture. Nevertheless, factors such as plant age or botanical

373   composition could have also determined pasture nutrient concentration (Marschner 1995).

374

375   *Retama affects distinctively pasture and tree nutrient status and production*

376   An increase of soil N under *Retama* has been commonly reported and attributed to

377   decomposing litter of this N_2-fixing shrub (Moro et al. 1997; Pugnaire et al. 1996; Rodríguez-

378   Echeverría and Pérez-Fernández 2003), and our results were in accordance with these

379   findings. Additionally, we found also a positive effect on Mg availability too, what could be

380   related to the concentration of Mg in root nodules (Kiss et al. 2004). This effect was well

381   mirrored by neighboring plants. In this way, *Retama* exhibited neutral to positive effects on

382   pasture understory and tree overstory Mg and N nutrition. On the other hand, there was a

383   detrimental effect on P availability under *Retama* and both pastures and trees showed the

384   same diminishing pattern on P concentration when growing with *Retama*. Shaben & Myers

385   (2010), in a study of oak savannas threatened by the encroachment of a similar species than

386   *Retama* (*Cytisus scoparius*), concluded that leguminous shrubs can deplete soil phosphorous

387   availability. P, among other physiological functions, is related with N-fixation (Vance et al.

388   2003), thus *Retama* may exert a certain competition for this nutrient. In our study, Ca

389   exhibited a similar pattern as P, what could be related with a concomitant dilution effect of

390   both nutrients caused by the increased pasture growth under *Retama*. Alternatively, this

391   pattern could have been favored by a higher solubility of Ca phosphates with decreasing pH

392   in the *Retama* rhizosphere (Hinsinger 2001). However, in our study the decrease pH with the

393   presence of *Retama* was not significant, and this issue deserves further investigation.

394   According to our expectations, the presence of a deep rooted shrub such as *Retama*,

395   exhibited contrasting effects on trees and pastures. While tree leaf dry mass was significantly

396   reduced growing with *Retama*, a strong facilitative effect was observed on pasture

397   production. Nevertheless, tree overstory and pasture understory vector analysis showed

398   almost similar nutrient patterns which mirrored the effect of this shrub on soil nutrient

399   availability. Thus, the different effects of *Retama* on pasture and tree production suggest that

400   this shrub is affecting contrastingly a common resource, which plays a great role on biomass

401   production for both vegetation layers. Rivest et al. (2011) found a significant reduction on

402   acorn mean weight in trees growing with *Retama,* which was interpreted as a likely

403   competition for soil water between these plants, whereas they found that pasture production

404   was facilitated under *Retama* even in extremely dry years. The higher SWC in the topsoil

405   under *Retama* could have acted synergically with nutrient availability hence facilitating

406   growth of pasture biomass. However, the argued competition for deep water between *Retama*

407   and trees (Rolo and Moreno 2011; Rivest et al. 2011) could have outweighed the profit of soil

408   nutrient enrichment and lead to a leaf biomass reduction. Indeed, in water limited systems

409   trees adapt leaf area to minimize water stress (Limousin et al. 2009).

410

411   *Cistus severely diminish tree and pasture nutrient status and production*

412   *Cistus* exhibited contrasting effects on the availability of the different nutrients analyzed.

413   Similarly to trees, *Cistus* reinforce the availability of P and K under its canopy. However,

414   *Cistus* exhibited a higher effect on P than trees (2.3 times higher P availability than control

415   compared to 1.5 times higher under trees), while trees showed a higher effect on K than

416   *Cistus* (3.9 times higher K availability than control compared to 2.5 times higher under

417   *Cistus*). These results suggest that, although both species could share similar mechanism to

418   induce changes in P and K availability (active root action or leaching, respectively), their

419   specific differences in traits relevant to nutrient cycling (e.g. shallower rooting profile of

420   *Cistus* or higher aboveground biomass of trees) lead to contrasting specific effects in nutrient

421   availability.

422   The positive effect on soil K availability induced by *Cistus* was better mirrored in

423   neighboring trees than pasture. By contrast, again the increase on soil available P was not

424   observed in the nutritional status of neighboring plants. Moreover, the effect of *Cistus* on

425   pasture P concentration was very negative, while this shrub did not affect trees P

426   concentration. The strong negative influence of *Cistus* on pasture might also be related to its

427   allelophatic behavior. Sosa et al. (2010) confirmed the presence of exudates, belonging to the

428   family of polyphenols, with long residence time in the soil under *Cistus*. Polyphenols are

429   known to affect decomposition rates, pools and chemical forms of nutrients (Hattenschwiler

430   and Vitousek 2000). For instance, these components have been shown to reduce net N

431   availability. Indeed, our results showed that N and Mg availability was significantly reduced

432   beneath *Cistus,* contrary to trees, and this reduction was observed in the nutritional status of

433   both tree and pasture.

434   Unlike to *Retama*, *Cistus* exhibited a detrimental effect both on pasture understory and

435   tree overstory production (Table 2 and 3). Rolo and Moreno (2011) reported a significant

436   reduction of predawn leaf water potential on trees growing with *Cistus* than that growing

437   without competition, arguing that this shrub can compete strongly for soil water. This finding

438   is in agreement with our results of a significant reduction of SWC in the topsoil under *Cistus*.

439   Thus, the detrimental effect of *Cistus* on neighboring plant production seems to be also

440   driven by a high competition for soil water. Water shortage could explain the differences

441   between the increment of soil P availability under *Cistus* and the deficiencies in neighboring

442   plant P nutrition, especially in pasture, which is more sensitive to any soil water diminution

443   in the uppermost soil layer. Indeed, in terms of nutrient, *Cistus* seemed to affect more

444   negatively the P nutrition of pasture, whereas trees seemed to be more affected by N

445   nutrition. Overall, the nutrient patterns showed in vector analysis suggest, according to Mead

446   et al. (2010), that nutrients and water limited both understory and overstory production.

447

448   **Conclusions**

449   Woody plants have the potential to alter abiotic factors, such as mineral nutrients and soil

450   water availability, that can determine the growth response of neighboring plants, but the sign

451   and magnitude of these responses are species-specific. The three woody species exhibited

452   differential effects on the array of nutrients analyzed with contrasting consequences on

453   neighboring plants. *Retama* enhanced the availability of N and Mg showing opposing effects

454   on pasture (facilitation) and tree (competition) biomass production. Thus management

455 practices of this silvopastoral system oriented to promote *Retama* formations may have

456 positive effects on pasture productivity and quality, but with potential detrimental

457 consequences for long-term productivity on trees. On the other hand, the positive effect of

458 *Cistus* on the availability of K and P suggests that a rotational thinning of *Cistus* formations

459 in dehesas could be a profitable way of optimizing soil nutrient amelioration, especially P,

460 while minimizing competitive effects, but this approach needs further research.

461 Despite the positive effects of woody plants on the availability of certain limiting nutrients

462 and their potential implication for management practices, our results suggest that water

463 availability play a crucial role for nutrient relations in plant-to-plant interactions in semi-arid

464 systems. Overall, the use of shrubs as a management tool must be considered carefully, in

465 terms of species involve and the studied system, due to the contrasting effects induced by

466 different species and their strong influence on neighboring vegetation.

467

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650 **Table 1** Mean values and standard error of soil organic matter (SOM, %), pH, clay, silt and
651 sand (%) of a composite sample of 0-30 cm and volumetric soil water content (SWC, %) and
652 soil temperature (ºC) at two depths (0-10 cm and 20-30 cm) measured in April 2009 under
653 tree (*Quercus ilex*) (n = 64) and two types of shrubs, *Cistus ladanifer* (n = 32) or *Retama*
654 *sphaerocarpa* (n = 32), compared to their respective areas of open pastures (Control).
655 Significant differences between woody habitat (tree, *Retama* and *Cistus*) and control zones
656 are shown: ***$P < 0.001$, **$P < 0.05$ and *$0.1 < P < 0.05$

657

	Soil Properties					
	Tree	Control	*Retama*	Control	*Cistus*	Control
SOM	3.6 ± 0.6***	2.0 ± 0.2	2.0 ± 0.4	2.0 ± 0.3	2.5 ± 0.5	2.0 ± 0.2
pH	5.2 ± 0.2	5.3 ± 0.1	5.3 ± 0.1	5.5 ± 0.1	5.1 ± 0.1	5.2 ± 0.1
% Clay	12.2 ± 1.8	10.5 ± 0.6	10.4 ± 1.2	10.3 ± 0.9	10.2 ± 1.1	10.6 ± 0.7
% Sand	49.8 ± 3.0	51.6 ± 1.6	53.2 ± 1.1	55.7 ± 1.9	46.1 ± 2.3	46.9 ± 1.6
% Silt	38.0 ± 1.6	37.9 ± 1.5	36.4 ± 1.2	33.9 ± 1.6	43.7 ± 1.5	42.5 ± 1.4
SWC 0-10	20.7 ± 1.6	21.3 ± 0.7	27.8 ± 2.3***	20.1 ± 0.8	17.3 ± 0.5**	22.9 ± 0.5
SWC 20-30	22.1 ± 1.5	23.8 ± 1.1	23.3 ± 1.9	21.7± 0.6	22.2 ± 1.2*	25.9 ± 1.5
Temp. 0-10	13.0 ± 0.5***	16.8 ± 0.8	16.7 ± 1.4	17.3 ± 0.6	13.3 ± 0.9**	16.3 ± 1.5
Temp. 20-30	12.7 ± 0.4	14.0 ± 0.6	14.3 ± 0.6	15.5 ± 0.9	11.9 ± 0.4	12.5 ± 0.6

658 **Table 2** Mean values ± standard error of pasture production (g / m^2), N, P, Ca, Mg and K concentration (mg / g) and content (mg / m^2) measured

659 beneath tree (*Quercus ilex*) (n = 48) and two types of shrubs, *Cistus ladanifer* (n = 24) or *Retama sphaerocarpa* (n = 24), compared to their

660 respective areas of open pastures (Control). Values were averaged over the two years of measurements (2007 and 2008).

661

				Pasture nutrient status					
	Tree	Control	*P-value*	Retama	Control	*P-value*	Cistus	Control	*P-value*
Production (g/m^2)	175.7 ± 16.6	279.0 ± 32.5	**< 0.001**	352.0 ± 21.7	246.6 ± 20.1	**< 0.001**	94.2 ± 21.9	311.4 ± 24.5	**< 0.001**
Concentration (mg/g)									
N	14.3 ± 0.7	14.1 ± 0.6	0.565	12.8 ± 0.4	13.3 ± 0.6	0.329	13.7 ± 0.5	14.8 ± 0.5	**0.040**
P	2.0 ± 0.2	2.5 ± 0.2	**0.047**	1.6 ± 0.3	2.0 ± 0.3	0.299	2.0 ± 0.3	3.1 ± 0.4	**0.011**
Ca	5.1 ± 0.2	5.4 ± 0.2	0.237	3.9 ± 0.3	4.8 ± 0.4	**0.019**	4.6 ± 0.8	6.0 ± 0.6	**0.002**
Mg	0.8 ± 0.06	0.9 ± 0.04	0.805	1.0 ± 0.05	0.9 ± 0.05	0.210	0.6 ± 0.04	0.8 ± 0.04	**0.011**
K	20.6 ± 0.7	17.3 ± 0.7	**< 0.001**	14.4 ± 0.5	14.9 ± 0.7	0.524	20.2 ± 0.8	19.7 ± 0.9	0.617
Content (g /m^2)									
N	2.5 ± 0.1	3.9 ± 0.2	**< 0.001**	4.5 ± 0.4	3.3 ± 0.3	**0.013**	1.3 ± 0.35	4.6 ± 0.4	**< 0.001**
P	0.4 ± 0.01	0.7 ± 0.02	**< 0.001**	0.6 ± 0.06	0.5 ± 0.08	0.526	0.2 ± 0.09	1.0 ± 0.1	**< 0.001**
Ca	0.9 ± 0.03	1.5 ± 0.08	**< 0.001**	1.4 ± 0.1	1.2 ± 0.1	0.394	0.4 ± 0.09	1.9 ± 0.2	**< 0.001**
Mg	0.1 ± 0.03	0.2 ± 0.02	**< 0.001**	0.3 ± 0.01	0.2 ± 0.02	**0.032**	0.1 ± 0.01	0.2 ± 0.03	**< 0.001**
K	3.6 ± 0.1	4.8 ± 0.3	**0.002**	5.1 ± 0.1	3.7 ± 0.2	**0.038**	1.9 ± 0.1	6.1 ± 0.4	**< 0.001**

662   **Table 3** Mean values and standard error of leaf dry mass (g/twig) and N, P, Ca, Mg and K

663   concentration (mg / g) and content (mg / twig) of trees (*Quercus ilex*) with and without the

664   presence of two types of shrub, *Cistus ladanifer* (n = 48) or *Retama sphaerocarpa* (n = 48).

665   Values were averaged over the two years of measurements (2007 and 2008).

666
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	Tree leaf nutrient status					
	Retama	*Control*	*P-value*	*Cistus*	*Control*	*P-value*
Leaf dry mass (g/twig)	0.77 ± 0.03	0.83 ± 0.04	**0.017**	0.61 ± 0.03	0.79 ± 0.04	**< 0.001**
Concentration (mg/g)						
N	13.6 ± 0.1	13.4 ± 0.1	0.222	12.9 ± 0.1	13.8 ± 0.1	**< 0.001**
P	0.9 ± 0.02	1.0 ± 0.02	*0.072*	1.0 ± 0.03	1.0 ± 0.03	0.656
Ca	3.0 ± 0.2	3.2 ± 0.1	0.212	3.1 ± 0.2	3.4 ± 0.2	**0.047**
Mg	1.2 ± 0.03	1.2 ± 0.03	0.393	1.3 ± 0.1	1.3 ± 0.1	0.364
K	5.5 ± 0.1	5.6 ± 0.1	0.278	6.3 ± 0.1	5.9 ± 0.1	**0.005**
Content (mg /twig)						
N	10.5 ± 0.4	11.1 ± 0.6	*0.054*	7.9 ± 0.5	10.9 ± 0.5	**< 0.001**
P	0.7 ± 0.04	0.8 ± 0.05	**0.003**	0.6 ± 0.04	0.8 ± 0.05	**< 0.001**
Ca	2.3 ± 0.1	2.7 ± 0.1	**0.018**	1.9 ± 0.1	2.7 ± 0.1	**< 0.001**
Mg	0.9 ± 0.1	1.0 ± 0.1	0.366	0.8 ± 0.05	1.0 ± 0.1	**0.001**
K	4.2 ± 0.2	4.6 ± 0.3	**0.005**	3.8 ± 0.2	4.7 ± 0.3	**< 0.001**

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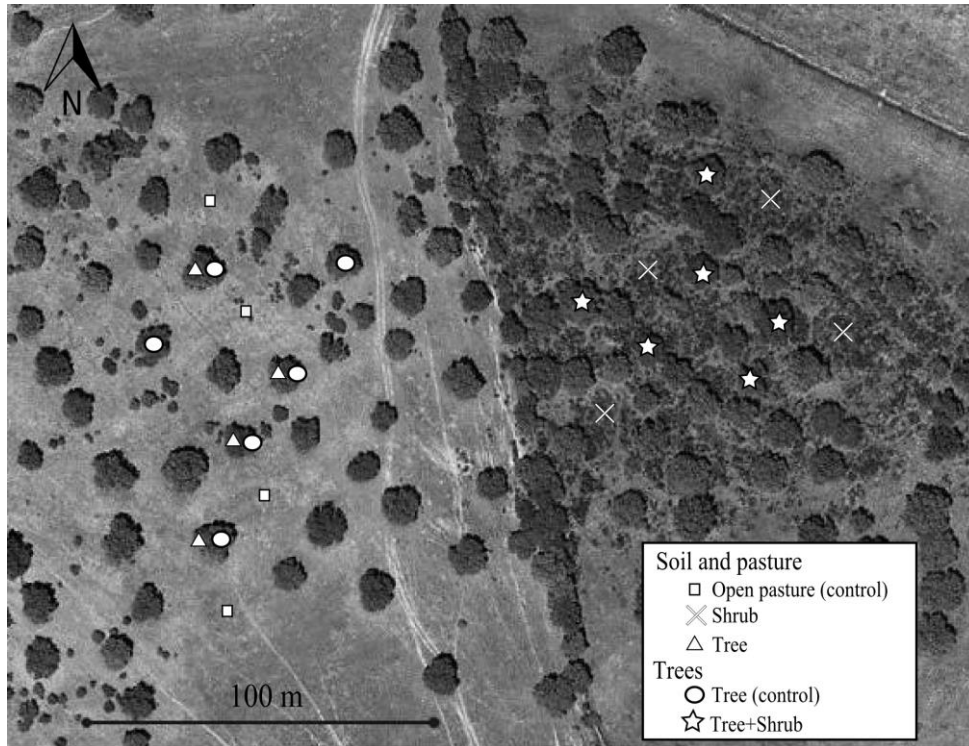
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671
672 **Fig. 1** Schematic view of a site encroached with *Cistus* depicting the sampling points selected
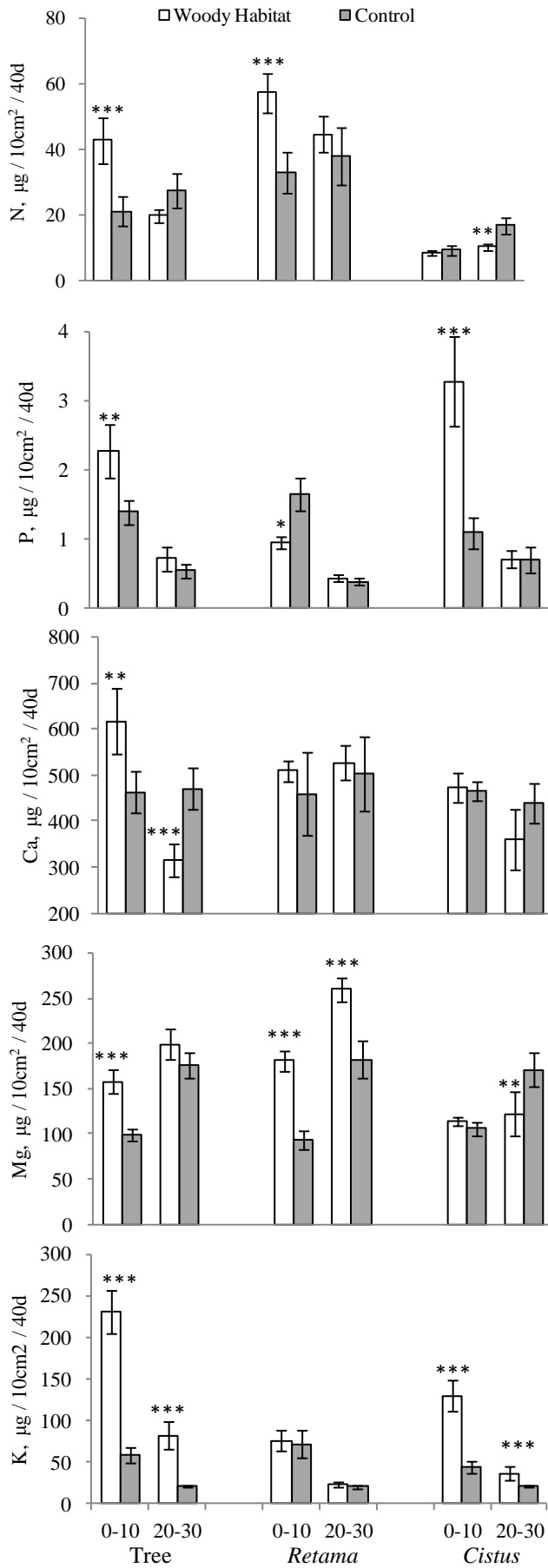673 for soil nutrient availability and pasture and tree production and nutrient status analysis
674

Fig. 2 Mean values and standard error of soil availability of N, P, Ca, Mg and K (µg/10cm^2/40d) measured in 2009 under tree (*Quercus ilex*) and two types of shrubs, *Cistus ladanifer* or *Retama sphaerocarpa*, and their respective areas of open pastures at two depths (0 - 10 and 20 - 30 cm). Significant differences between habitat and control: ***P < 0.001, **P < 0.05 and *0.1 < *P* < 0.05

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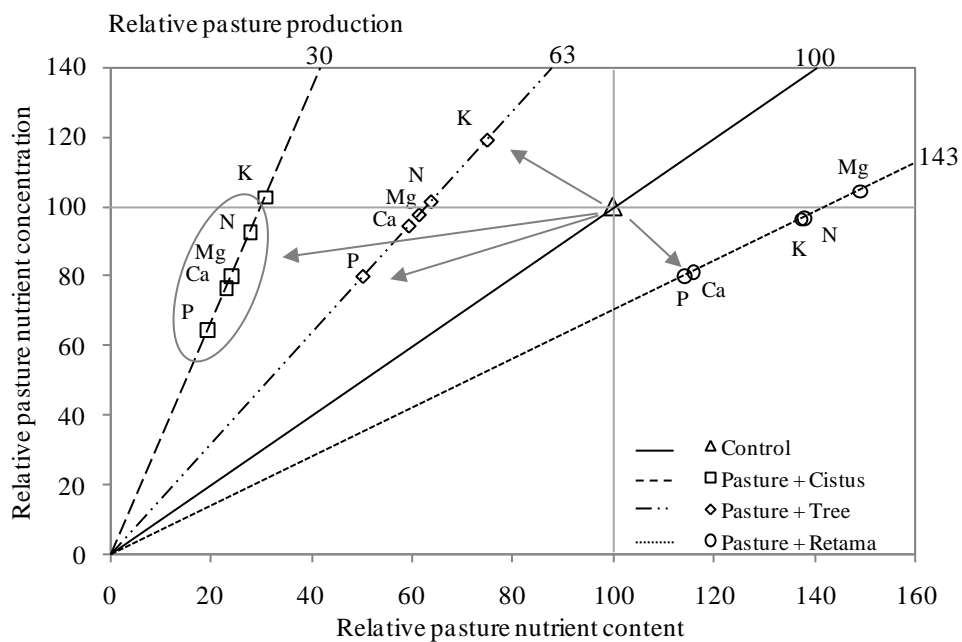
Relative pasture production

679 **Fig. 3** Graphical vector analysis of relative response in pasture production, nutrient
680 concentration and content (N, P, K, Mg and Ca) growing under tree (*Quercus ilex*), *Retama*
681 or *Cistus*. Data for pasture growing in open areas were used as reference (100 value for
682 pasture production, nutrient content and concentration). Diagonal lines indicate pasture
683 production (g / m^2). Arrows and circles depict significant vector shifts
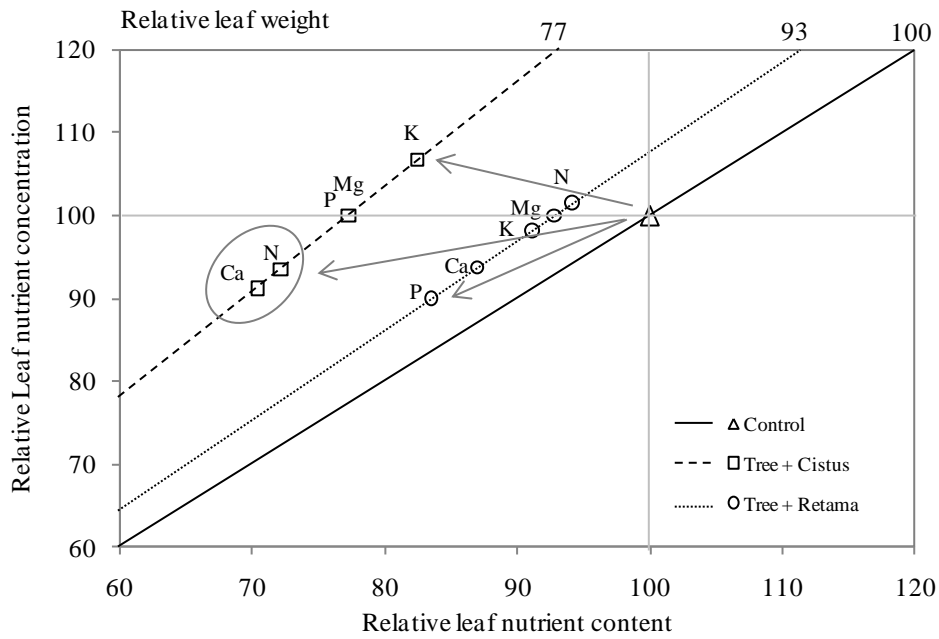
Fig. 4 Graphical vector analysis of relative response in leaf dry mass, nutrient concentration and content (N, P, K, Mg and Ca) per annual twig in trees (*Quercus ilex*) growing with the presence of *Retama* or *Cistus*. Data for trees growing without shrubs were used as reference (100 value for leaf dry mass, nutrient content and concentration per twig). Diagonal lines indicate biomass production (mg / twig). Arrows and circles depict significant vector shifts