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1 Long-term drought decreases ecosystem C and nutrient storage in a

2 Mediterranean holm oak forest.

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16 Running title: Nutrient stocks decrease under drought

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32   **Abstract**

33   Aridity has increased in recent decades in the Mediterranean Basin and is projected to continue

34   to increase in the coming decades. We studied the consequences of drought on the

35   concentrations, stoichiometries and stocks of carbon (C), nitrogen (N), phosphorus (P) and

36   potassium (K) in leaves, foliar litter of a three dominant woody species and soil of a

37   Mediterranean montane holm oak forest where soil-water content was experimentally reduced

38   (15% lower than the control plots) for 15 years. Nitrogen stocks were lower in the drought plots

39   than in the control plots (8.81±1.01 kg ha^{-1} in the forest canopy and 856±120 kg ha^{-1} in the 0-15

40   cm soil layer), thus representing 7 and 18% lower N stocks in the canopy and soil respectively.

41   δ^{15}N was consistently higher under drought conditions in all samples, indicating a general loss

42   of N. Foliar C and K stocks were also lower but to a lesser extent than N. Decreases in biomass

43   and C and N stocks due to drought were smallest for the most dominant tall shrub, *Phillyrea*

44   *latifolia*, so our results suggest a lower capacity of this forest to store C and nutrients but also

45   substantial resulting changes in forest structure with increasing drought.

46   **Key words**: Climate change, aridity, nitrogen, phosphorus, potassium, stoichiometry, carbon

47   stocks.

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1.Introduction

Mediterranean climates occur in five occidental coastal regions of the continents between latitudes 34 and 45° in both hemispheres and are characterized by moderately wet and cold winters and dry and hot summers. The intensity of the drier periods, increasing from high to low latitudes in these areas, can vary widely (Di Castri and Mooney, 1973; Di Castri, 1981). Woody plants with conservative traits, such as slow growth and high sclerophylly associated with low availabilities of water and nutrients, typically dominate the Mediterranean communities (Valiente-Baunet et al., 1998; Ching et al., 2000; Edwards et al., 2000; Barchuk and Valiente-Banuet, 2006; Sardans et al., 2006). Most studies suggest that the impacts of global change promote degradation and desertification in current Mediterranean areas (Sardans and Peñuelas, 2013; Salvati et al., 2014; Loizidou et al., 2016; Peñuelas et al., 2017a; 2018). Precipitation has exhibited either a long-term downward trend, principally in the dry season (Kutiel et al., 1996; Esteban-Parra et al., 1998), or absence of any significant change, even though accompanied by an increase in the potential evapotranspiration that has led to increased aridity (Piñol et al., 1998; Peñuelas et al., 2002). Furthermore, there has been a rise in climate extreme events such as severe droughts and heat waves (Goldreich, 2003; Sánchez et al., 2004; Hernández-Ceballos et al., 2016).The decline in total rainfall and/or availability of soil water expected for the coming decades (IPCC, 2013) may be even more severe under warmer conditions, with a CO_2-rich atmosphere and a higher demand for water (Zhao and Running, 2010). Defoliation has substantially increased in the Mediterranean forests of southern Europe (Carnicer et al., 2011; De la Cruz et al., 2014; De Marco et al., 2014).

Several specific structural and functional characteristics of Mediterranean plants are associated with conservative mechanisms linked to the avoidance of water stress but also frequently to the scarcity of soil nutrients (Bussotti et al., 2000; Verdú et al., 2003; Kooilman et al., 2005; Galmés et al., 2007).. Nutrient supplies are often an important factor in the growth, structure and distribution of these ecosystems (Specht, 1979; Henkin et al. ,1998; Sardans and Peñuelas, 2013), and Mediterranean plants frequently change and adapt foliar chemical and physical traits in response to both water deficits and nutrient stress (Gratani et al., 2003; Sardans et al., 2006, 2008a,b,c). Drought conditions can both shorten the growing period in spring and increase water stress during the summer dry season (Peñuelas et al., 2013). Mediterranean soils are often deficient in nutrients (Specht, 1979; Kruger, 1979; Sardans and Peñuelas, 2013), which can be exacerbated by climate change. Moreover, drought can decrease the capacity to retain nutrients in Mediterranean forests (Kreuzwieser and Gessler, 2010; Peñuelas et al., 2018). Drought may negatively interact with nutrient limitation by favoring soil degradation an*d*

95   nutrient losses thus impairing ecosystem water-use efficiency (WUE), which could lead to a point

96   of no return toward desertification (García-Fayos and Bochet, 2009; Matías et al., 2011; Sardans

97   and Peñuelas, 2013; Peñuelas et al., 2013).

98   Long-term field experiments, though, have not provided consistent evidence that

99   moderate drought can also imply a change in nutrient concentrations, stoichiometries and

100   stocks. The recent implementation of mechanistic nitrogen (N) and phosphorus (P) cycling in

101   models of the terrestrial carbon (C) cycle underscores the importance of nutrient feedbacks,

102   indicating potential reductions in the productivity of terrestrial ecosystems (Goll *et al*., 2012;

103   Peñuelas et al., 2013; Fernández-Martínez et al., 2017; Peñuelas et al., 2017b; Sun et al., 2017;

104   Peñuelas et al., 2020). The recent increase in aridity at a global continental scale together with

105   nutrient imbalances have demonstrated decreases in the continental capacity to store C

106   (Peñuelas et al., 2017b). Drought linked to increased nutrient limitation can thus feedback on C

107   balances, because limited P and N availability are likely to jointly reduce future C storage by

108   natural ecosystems during this century (Peñuelas et al., 2013). This feedback can be especially

109   important in semiarid regions such as the Mediterranean Basin and the Sahel where water

110   limitations can have strong negative feedbacks with increased nutrient deficits and thus with a

111   strongly diminished productive capacity and ecosystem biomass (IPCC 2007, Sardans et al.,

112   2012; Sanaullah et al., 2012; Sardans and Peñuelas, 2013; Gessler et al., 2016).

113   Long-term experimental climatic-manipulation studies are rare in the Mediterranean

114   biome. C, N, P and potassium (K) cycles clearly changed after five years of continuous drought

115   in a field experiment in an old holm oak forest (*Quercus ilex* L. ssp *ilex*) established in 1999. C, N

116   and P mineralization tended to be slower and N accumulation was lower in some dominant plant

117   species in response to five years of continuous drought (Sardans et al., 2008a,b; Sardans and

118   Peñuelas 2007, 2010). The results after the five years of continuous drought, however, were not

119   conclusive, because the stand biomass stocks only decreased in some (*Quercus ilex* and *Arbutus*

120   *unedo* L.) but not all species, whereas C and N accumulation increased in the soil (Sardans et al.,

121   2008a,b; Sardans and Peñuelas, 2007, 2010). We now aimed to identify the mid- to long-term

122   links of ten more years of prolonged moderate drought with the concentrations, stocks and

123   stoichiometries of C, N, P and K in this Mediterranean holm oak forest.

124   The potential shifts in plant elemental concentrations and stoichiometries can provide

125   information of the capacity of different species to adapt or disappear due to some biotic and

126   abiotic stresses (Peñuelas et al., 2019). The biogeochemical niche (BN) hypothesis is an emerging

127   tool to test the shifts in plant communities along spatial and temporal scales as functions of the

128   main environmental abiotic and biotic conditions (Peñuelas et al., 2019). The BN hypothesis is

defined as the multidimensional space defined by the concentrations of bio-elements in individuals of a given species (Peñuelas et al., 2008, 2019). The hypothesis is thus based on the species-specific needs and uses of bio-elements in different amounts and proportions from the organismic structure and function in abiotic and biotic environmental spaces. BN distances among species should thus be a function of taxonomic difference, sympatric coexistence and homeostasis/plasticity. The hypothesis thus also suggests stoichiometric flexibility (the capacity to change the BN) of species, being an important trait, because it involves their capacity to cope with changes in environmental conditions. Each species conserved its own foliar elemental composition, consistent with the BN hypothesis, after five years of continuous drought, despite tending toward changing its overall foliar elemental composition in response to drought (Peñuelas et al., 2019). We now aimed to determine if more intense changes under long-term (15 years) continuous drought could affect the BNs of the dominant species.

We thus studied the effects of 15 years of continuous drought on ecosystem concentrations and stocks of C, N, P and K. We also assessed the isotopic signals of C and N cycles in this long-term field climatic-manipulation experiment in the old Mediterranean holm oak forest. We hypothesized a loss of C and nutrient stocks in foliar biomass. We also hypothesized a loss of capacity to retain C in plant, soil and litter and a decrease in soil stocks of some bio-elements due to the lower capacity of plants to retain and store them under drought. Reductions in plant uptake and activities of soil enzymes should imply a decrease in the rates of cycling of bio-elements in the plant-soil system (Sardans and Peñuelas, 2005) and thus increasing the probability of leaching and erosion in this climatic zone characterized by a high recurrence of intense torrential rain (Llasat et al., 2016; Ziv et al., 2016). Finally, we hypothesized a general shift in elemental composition and stoichiometry of the dominant woody species, but with maintenance of their BNs as expected by the BN hypothesis. We tested these hypotheses by investigating the effects of 15 continuous years of drought on: (i) the foliar, litter and soil concentrations of C, N, P and K and their pairwise ratios in the three dominant woody species, (ii) the effects on C and N cycles by analyzing foliar, litter and soil δ^{13}C and δ^{15}N, (iii) the shifts in the BNs of the three species due to drought and (iv) the shifts in the C, N, P and K stocks in leaves, litter and soil.

2.Materials and Methods

2.1.Study site

161   The study was carried out on a south-facing slope (25%) in a natural holm oak forest in

162   the Prades Mountains in southern Catalonia (northeastern Iberian Peninsula, 41°13'N, 0°55' E).

163   The soil is a stony Xerochrept on a bedrock of metamorphic sandstone ranging between 35 and

164   90 cm in depth. The average annual temperature is 12 °C, and the average annual rainfall is 658

165   mm. The summer drought is pronounced and usually lasts for three months. The vegetation

166   consists of a dense forest dominated by *Quercus ilex* L. with abundant *Phillyrea latifolia* L.,

167   *Arbutus unedo* L. and other evergreen species well-adapted to drought conditions (*Erica arborea*

168   L., *Juniperus oxycedrus* L. and *Cistus albidus* L.) and the occasional occurrence of deciduous

169   species (*Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.).

170   *2.2.Experimental design*

171   Eight 15 × 10 m plots were delimited at the same altitude (950m) along the slope (25º)

172   (Ogaya et al. 2020). Half of the plots received the drought treatment, and the other half were

173   designated as control plots. The drought treatment consisted of the partial exclusion of rain by

174   suspending PVC strips and funnels 0.5–0.8 m above the soil surface. The strips and funnels

175   covered approximately 30% of the total plot surface. A ditch ca. 0.8 m deep was excavated along

176   the entire top edge of the treatment plots to intercept runoff, and all water intercepted by the

177   strips, funnels and ditches was conducted below the bottom edge of the plots. This drought

178   treatment has been applied since 1999.

179   *2.3.Sampling*

180   We sampled biomass (including leaves and foliar litter) and soil at the beginning of the

181   experiment (December 1999) and after six years of applying the drought treatment (January

182   2005) to estimate the total contents (Sardans et al., 2008a,b). Tereafter, in the present study,

183   we sampled leaves, foliar litter and soil in 2014 in mid-May (spring), the end of July (summer)

184   and mid-November (autumn) to determinate the changes in C, N, P and K stocks after 15 years

185   of constant chronic drought. Litterfall has been collected every three months during the two

186   pass campaigns (1999 and 2004) and the present campaign (2014) in 20 circular baskets (27 cm

187   in diameter with 1.5-mm mesh diameter) randomly distributed on the ground in each one of the

188   eight plots. Total litterfall was estimated by the proportion of the surface area of the plots

189   covered by the collection baskets. We have measured the sample biomass since the beginning

190   of the experiment several times, the methodology is described in (Ogaya and Peñuelas, 2007).

191   Briefly, the stem circumference was measured annually since 1999 each winter throughout the

192   study. The measurements were conducted with a metric tape at 50 cm height in all living stems

193   of all the species with a diameter larger than 2 cm at 50 cm height. Aboveground biomass in the

194   plots was estimated by allometric relationships between tree aboveground biomass (AB) and

195   the stem diameter at 50 cm height (D50). Outside the plots, 12 Q. ilex and 13 *P. latifolia* trees

196   were harvested, their perimeter at 50 cm height was measured and all their aboveground

197   biomass was weighed after drying them in an oven to reach constant weight. To estimate the

198   aboveground biomass of *Q. ilex* and *P. latifolia* we used the calculated allometric relationships

199   ($\ln AB = 4.9 + 2.3 \ln D50$; R2 = 0.98; n = 12: P < 0.001 for *Q. ilex*, and $\ln AB = 4.3 + 2.5 \ln D50$; R2 =

200   0.97; n = 13; P < 0.001 for *P. latifolia*), and to estimate the aboveground biomass of *A. unedo* we

201   used the allometric relationship calculated few years ago on the same area by Lledo´ (1990)

202   ($\ln AB = 3.8 + 2.6 \ln D50$; R2 = 0.99; n = 10; P < 0.001) (Ogaya & Peñuelas, 2007). The foliar

203   biomasses of the three studied species in 1999, 2004 and 2014 in control and drought plots are

204   shown in the Table S1.

205   We sampled leaves of the three dominant species of this ecosystem: *Quercus ilex*,

206   *Phillyrea latifolia* and *Arbutus unedo* (that represents on average the 97% of total aboveground

207   biomass) (Ogaya and Peñuelas, 2007). The sample collection was always standardized to avoid

208   biased results due to different tissue ages and orientation to sunlight, the sampled leaves were

209   sun-lit leaves oriented toward the south. The mean life span of the leaves of these species is

210   approximately 18 months (Ogaya and Peñuelas, 2006a), so for example most sampled foliar

211   biomass during 2014 corresponded to the leaves of the 2013 cohort. We randomly sampled five

212   trees of each species on each seasonal sampling day in each plot. Leaves were sampled at a

213   height of 2-3 m. We only sampled trees and shrubs with diameters between 2 and 12 cm (at 5

214   cm), which represented the majority (approximately 70%) of the community biomass (Ogaya

215   and Peñuelas, 2003, 2006b). We randomly collected five soil cores to a depth of 15 cm of horizon

216   A in each plot on each seasonal sampling day.

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218   *2.4.Chemical and physical analyses*

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220   All samples were taken to the laboratory and stored at 4 °C until analysis. The soil

221   samples were sieved, and the 2-mm fraction was analyzed. The leaves and foliar litter were

222   washed with distilled water as described by Porter (1986) for analyzing C, N, P and K. The washed

223   samples were dried in an oven at 60 °C to a constant weight and then ground in a CYCLOTEC

224   1093 (Foss Tecator, Höganäs, Sweden) for analyzing the biomasses or in a FRITSCH Pulverisette

225   (Rudolstadt, Germany) for analyzing the soils. The concentrations of C and N in the foliar, foliar

226   litter and soil samples were quantified by placing 1.4 mg of powder of each sample in a tin

227   microcapsule and using combustion coupled to gas chromatography in a CHNS-O Elemental

228 Analyser (EuroVector, Milan, Italy). The concentrations of P and K in the samples were

229 determined by first grinding the samples and then digesting them in acid in a MARSXpress

230 microwave (CEM, Mattheus, USA) at high pressure and temperature (see Peñuelas et al., 2010).

231 A total of 250 mg of leaf powder, 5 mL of nitric acid and 2 mL of H_2O_2 were placed into Teflon

232 tubes. The products of the digestions were placed in 50-mL flasks and diluted with Milli-Q water

233 to a volume of 50 mL. The concentrations of P and K were determined using Optic Emission

234 Spectrometry with Inductively Coupled Plasma. The accuracy of the digestions and the analytical

235 biomass procedures were assessed using a certified standard biomass (NIST 1573a, tomato leaf;

236 NIST, Gaitherburg, MD) as a reference.

237   The isotopic analyses of δ^{13}C and δ^{15}N in the foliar and soil samples (sampled in 2014)

238 were conducted in the Stable Isotope Facility at the University of California, Davis using using a

239 PDZ Europa ANCA-GSL elemental analyzer connected to a PDZ Europa 20-20 isotope ratio mass

240 spectrometer (Sercon Ltd., Cheshire, UK).

241   Soil moisture has been measured each month throughout the experiment by time

242 domain reflectometry (Tektronix 1502C, Tektronix, Beaverton, OR, USA) connecting the time

243 domain reflectometer to the ends of three stainless steel cylindrical rods, 25 cm long, fully driven

244 into the soil (Zegelin et al. , 1989). Four sites per plot were randomly selected to install the steel

245 cylindrical rods for soil moisture measurements (Ogaya et al., 2014). During these 15 years of

246 drought treatment soil moisture of drought plots compared to control plots (P < 0.01), but this

247 reduction was larger during rainy seasons and lower during dry seasons (Ogaya et al., 2014).

248 *2.5.Statistical analyses*

249   Significant differences in foliar, foliar litter and soil C, N, P and K concentrations and

250 ratios, δ^{13}C, δ^{15}N and C, N, P and K stocks in 2014 among treatments and seasons were tested

251 by general mixed models, with treatment and season as fixed factors and plot as a random

252 factor. We also used mixed models to analyze the treatment effects within each season, with

253 treatment as a fixed factor and plot as a random factor. We used the "lme" function of the

254 "nlme" R package (Pinheiro et al., 2016). Non-normally distributed variables were normalized

255 by log-transformation. We chose the best model for each dependent variable using the Akaike

256 information criterion (AIC). We used the MuMIn (Barton, 2012) R package in the mixed models

257 to estimate the percentage of the variance explained by the model. We conducted Tukey's post

258 hoc tests to detect significant differences in the analyses for more than two communities using

259 the "glht" function of the "multcomp" (Hothorn et al., 2013) R package.

260   We also performed multivariate statistical analyses. We used a general discriminant

261   analysis (GDA) to determine the overall differences of the foliar variables: C, N, P and K

262   concentrations and their pairwise ratios among the three dominant species sampled at different

263   times during the experiment: Desember 1999 (before the start of the experiment), November

264   2005 (after six years of continuous drought) and November 2014 (after 15 years of continuous

265   drought). GDA uses squared Mahalanobis distances that depend on the Euclidean distance in

266   the model between two sets of samples. The squared Mahalanobis distance is low when the

267   samples are similar, indicating little difference between the two sets. In contrast, the distance is

268   higher and the difference between the sets increases the more the samples differ (De

269   Maesschalk et al., 2000). We conducted one-way ANOVAs with Bonferroni post hoc tests of the

270   scores of the first two GDA roots to identify differences among the dominant species sampled

271   over time. We used Statistica 8.0 (StatSoft, Inc. Tulsa, USA) for these analyses.

272

273   **3.Results**

274   *3.1.Foliar, litter and soil C, N, P and K concentrations and ratios*

275   The effects of the drought treatment on foliar and foliar litter C, N, P and K

276   concentrations depended strongly on season and species (Tables S2 and S3). A decrease in foliar

277   K concentrations in summer in the three species and a decrease in foliar P concentrations in *P.*

278   *latifolia* and *A. unedo* (Table S2) were the most general effects of the treatment.

279   The drought treatment increased foliar C and N concentrations and decreased foliar K

280   concentrations in summer and increased foliar N concentrations in autumn in *Q. ilex*, the

281   dominant species (Table S2). The treatment also decreased foliar C:N ratios in summer and

282   autumn in *Q. ilex*. Foliar N and P concentrations were higher and the N:P ratio was lower in

283   spring, and the foliar N and P concentrations were lowest in summer, in all species in both the

284   drought and control plots. Drought was associated with decreases in C and K concentrations and

285   increases in N and P concentrations in the *Q. ilex* foliar litter in summer (Table S3).

286   *Phillyrea latifolia* foliar P and K concentrations decreased and foliar C:P, C:K, N:P and N:K

287   ratios increased under drought in summer (Table S2). Foliar C, N and P concentrations were

288   lowest in summer. Drought was associated with a decrease in K concentrations and increases in

289   C:K, N:K and P:K ratios in the foliar litter in summer (Table S3). *Arbutus unedo* foliar N and K

290   concentrations and the foliar N:P ratio decreased under drought in summer (Table S2). Foliar C,

291   N and P concentrations were lowest in summer for both *A. unedo* and *P. latifolia*. Drought was

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292   associated with decreases in C concentrations and C:N and C:P ratios and an increase in N
293   concentrations of the foliar litter in spring (Table S3).

294   Drought was associated with lower total soil C and N concentrations, especially in spring
295   and summer (Table S3). Total soil C:N and C:P ratios were lower in the drought plots, particularly
296   in summer and autumn, whereas total soil C:K, N:K and P:K ratios were also lower in the drought
297   plots, particularly in spring and summer.

298   *3.2.δ^{13}C and δ^{15}N*

299   Drought had moderate effects on δ^{13}C of leaves and soil. δ^{13}C was lower in *Q. ilex* leaves
300   under drought, mainly in summer, whereas soil δ^{13}C was higher under drought in summer (Table
301   S4). Drought was associated with higher δ^{15}N in the leaves and foliar litter of *Q. ilex*, *P. latifolia*
302   (not in autumn) and *A. unedo* and in the soil in all seasons (Figures 1 and 2, Table S4).

303   *3.3.Overall temporal shifts in foliar composition in the species under drought*

304   The GDA hypervolume shifted in the sample sets of the three species sampled at
305   different times. All elemental concentrations and their pair-wise ratios contributes significantly
306   in the model (Table S4). The controls occupied a hypervolume distinct from that of the samples
307   of the three species sampled in 2005 and in *P. latifolia* sampled in 2014 under drought (Table
308   S5, Figure 3). This shift between the control and drought samples in *A. unedo* and *Q. ilex* was
309   along Root 1, with a displacement of the drought plots toward lower N concentrations and
310   N:Mg, N:P and N:Ca ratios and higher Mg concentrations and C:N ratios. The multivariate
311   analyses indicated that the effect of drought on foliar elemental concentrations was not
312   homogeneous. The squared Mahalanobis distances differed significantly between the species,
313   except for *P. latifolia* and *Q. ilex* between the control and drought plots sampled in 1999 (Table
314   S5).

315   *3.4.Foliar, litter and soil C, N, P and K stocks during the 15 years of drought*

316   Drought decreased the accumulation of foliar C stocks in *Q. ilex* (significantly only for
317   1999-2004) (Figure 4A), *A. unedo* (Figure 4B) and the sum of all three species (Figure 4d), mainly
318   associated with the smaller increase in foliar biomass under drought. Drought decreased the
319   accumulation of foliar N stocks in *A. unedo* and the sum of all three species (significantly only
320   for 1999-2014) (Figure 5), mainly associated with the lower N concentrations and smaller
321   increase in foliar biomass under drought. Drought did not affect the accumulation of foliar P
322   stocks relative to the control plots in the three species and the study periods (Figure 6). Drought
323   decreased the accumulation of foliar K stocks in *A. unedo* (significantly only for 1999-2014)

324 (Figure 7). The overall results indicated a significant decrease in foliar C, N and K stocks in *A.*
325 *unedo* and in C and N stocks in the three dominant species (representing >90% of the total
326 ecosystem aboveground biomass) after 15 years of moderate drought (a mean reduction in soil-
327 water content of 15%). C stocks decreased significantly in the 0-15 cm soil profile for 2004-2014
328 in the drought but not the control plots (Figure 8), but not significantly (P=0.87). The difference
329 in total soil N stock between the drought and control plots for 2005-2014 in the upper 15 cm of
330 soil was 856 ± 120 kg ha^{-1}. Total soil P stocks in the upper 15 cm did not change significantly in
331 either the control or drought plots. Total soil K stocks for 2005-2014 generally decreased in the
332 upper 15 cm (3476 ± 444 and 3541 ± 200 kg ha^{-1} in the control and drought plots, respectively),
333 but not significantly. C, N, P and K stocks of leaf-litter did not change in 2015 relative to those
334 in 1999 (Table S6).

335

336 **4.Discussion**

337 This study demonstrated that a moderate increase in drought, in this case a mean
338 reduction in soil-water content of 15%, for 15 years strongly affected C and some nutrient stocks
339 at the ecosystem level. Drought decreased the stocks of N, the most limiting nutrient, in the
340 entire canopy and the upper soil layer. However, the drought effects on C and nutrient stocks
341 varied depending on the species. This asymmetrical effect of drought on species stocks agrees
342 with changes in other variables such as growth and mortality and thus species biomass: the
343 dominant evergreen shrub *P. latifolia* maintained its foliar C and nutrient stocks, whereas C and
344 nutrient stocks in the dominant evergreen tree *Q. ilex* and mainly the shrub *A. unedo* decreased.
345 These results indicate that a moderate increase in drought in the coming decades would
346 accelerate shifts in species composition and dominance, from a forest to a shrubland ecosystem
347 dominated by evergreen shrubs (Peñuelas et al., 2018).

348 *4.1.Foliar C, N, P and K concentrations after 15 years of drought*

349 The foliar elemental concentrations and ratios had a strong seasonal component
350 independent of the treatments, e.g. the concentrations of N, P and K were higher in spring,
351 coinciding with the period of growth in this Mediterranean ecosystem. The foliar N:P ratio in the
352 three species was noticeably lower in spring, thus coinciding with the higher foliar N and P
353 concentrations in the growing season, consistent with the growth-rate hypothesis, which states
354 that the period of growth coincides with a higher capacity for fast protein synthesis (Sterner and
355 Elser, 2002). In fact, this link between higher metabolic capacity associated with higher foliar N
356 and P concentrations and a lower N:P ratio was experimentally demonstrated in a previous study

357   (Rivas-Ubach et al., 2012), indicating that the plasticity of this phenological component was not

358   affected by the drought treatment. The maximum productive capacity, from a nutritional

359   perspective, thus coincides with the optimum annual climatic conditions in both the control and

360   drought plots.

361   Foliar concentrations tended to decrease in the drought plots or did not change relative

362   to the control plots. The differences between the foliar concentrations in the control and

363   drought plots were largest in summer, thus coinciding with the most arid conditions with water

364   deficits and stress. The effects of drought on the foliar concentrations also depended on the

365   species and element, similar to the elemental stocks. Global changes in foliar concentrations

366   associated with drought, however, were rare. Differences between the control and drought

367   treatments generally occurred in summer; leaves in the drought plots had lower concentrations

368   of C and N in *Q. ilex*, P and K in *P. latifolia* and N and K in *A. unedo*. Studies of gradients and/or

369   field experiments frequently find that N foliar concentrations in Mediterranean forest species

370   are very homeostatic in response to abiotic shifts (Martin-StPaul et al., 2013). From an ecological

371   stoichiometric perspective (Sterner and Elser, 2002), elemental composition is more

372   homeostatic in stress-tolerant plant species than competitor and ruderal species in response to

373   environmental changes (Peñuelas et al., 2019). Our results showing only small changes in foliar

374   C, N, P and K concentrations support the idea of the stress tolerance strategy in Mediterranean

375   plants. Mediterranean forest tree species such as *Quercus ilex* or *Pinus halepensis* have less

376   variability in their elemental foliar composition than wet temperate species such as *Quercus*

377   *robur* or *Quercus petraea* adapted to wetter climate and nutrient richer soils (Sardans et al.

378   2020). We had previously observed in 2011 that *Q. ilex* foliar K concentrations were higher in

379   the drought than the control plots (Sardans et al., 2013; Rivas-Ubach et al., 2014), whereas *Q.*

380   *ilex* leaves analyzed in 2014 had the opposite pattern, specifically in summer, perhaps due to

381   the loss of K in the upper 15 cm of soil in both the control and drought plots from 2005 to the

382   present. Some studies have reported that some elemental concentrations can increase and

383   others decrease, depending on the site and species (Luo et al., 2018; Urbina et al., 2015). We

384   thus cannot draw a clear conclusion about the impact of experimental drought on distinct foliar

385   elemental compositions in Mediterranean environments under field conditions.

386   The level of resistance to drought stress in Mediterranean plant species is highly

387   variable, so fitness decreases more in some species than others (Sardans and Peñuelas, 2013).

388   For example, foliar N concentrations were lower in summer and litter N concentrations were

389   higher in spring in *A. unedo* in the drought than the control plots. These results are consistent

390   with previous results that drought affects fitness and all the variables involved in growth (Ogaya

391   and Peñuelas, 2007; Sardans et al., 2008a,b; Barbeta et al., 2013), photosynthetic capacity

392   (Ogaya and Peñuelas, 2006) and flowering (Ogaya and Peñuelas, 2004; Barbeta et al., 2013) that

393   we measured more in *A. unedo* than the other dominant species. Some studies, however, have

394   found that *P. latifolia* was the most stress-tolerant species of our three dominant species, where

395   growth and mortality (Ogaya and Peñuelas, 2007; Barbeta et al., 2013) or ecophysiology (Ogaya

396   et al., 2011) were little or not affected. Our results are consistent with these studies, because

397   bio-elemental concentrations and foliar stocks were less affected by drought in *P. latifolia* than

398   the other species Our results are consistent with these studies, because bio-elemental

399   concentrations, foliar stocks and growth were less affected by drought in *P. latifolia* than the

400   other species, specially than *A. unedo* (Ogaya and Peñuelas, 2007; Barbeta et al., 2013;2015).

401   Our results therefore reinforce previous findings in this same study and in other studies, with

402   evidence of species specific responses to drought in Mediterranean ecosystems, which may

403   eventually lead to a partial community shift favoring more drought-resistant species (Sardans

404   and Peñuelas, 2013; Peñuelas et al., 2013; 2017a; 2018; Gazol et al., 2017).The adaptation of

405   Mediterranean plants to drought generally frequently varies with species; some species cope

406   with moderate drought without significant losses in fitness, but others are strongly affected

407   (Sardans and Peñuelas, 2013). Our experiment demonstrated that the three dominant species

408   responded to this persistent moderate drought, but to different extents. As observed in previous

409   studies in this research site, the studied species, despite at different extend, all they the plants

410   subjected to the long-term experimental drought shifted water uptake toward deeper (10–35

411   cm) soil layers during the wet season and reduced groundwater uptake in summer, indicating

412   plasticity in their functional distribution of fine roots that dampened the effect of our

413   experimental drought in the long term (Barbeta el at., 2015).

414   The reduction of foliage biomass under drought relative to the control plots, and thus

415   plant cover, may decrease soil quality, as in several Mediterranean areas (Gallardo et al., 2000;

416   Garcia et al., 2002), decreasing the capacity of soil to retain moisture and thus fertility (Sadaka

417   and Ponge 2003; Ruiz-Sinoga et al., 2011). In fact, reductions in canopy cover and N

418   concentrations have been reported in previous studies in various Mediterranean communities,

419   including forests (Goldstein et al., 2000; Inclan et al., 2005; Pérez-Ramos et al., 2010; Cotrufo et

420   al., 2011), consistent with the decrease in soil N stocks in the drought versus the control plots

421   for 2005-2014.

422   *4.2.δ^{13}C and δ^{15}N in the soil, foliar litter and leaves after 15 years of drought*

423   δ^{13}C has frequently been used as an alternative measurable variable to WUE, especially

424   in drought conditions (Jucker et al., 2017). Plants with limited capacity for water uptake

425   preferentially have higher δ^{13}C (Jucker et al., 2017). Soil δ^{13}C in our study was higher in the

426   drought than the control plots, potentially associated with a high rate of loss of C from the soil

427   and thus with a more open soil C cycle. In contrast, δ^{13}C in the summer leaves of the dominant

428   species *Q. ilex* was lower in the drought than the control plots, indicating better conditions for

429   fixing C in the drought plots. This apparently contradictory results are probably linked to the

430   large loss of biomass in the *Q. ilex* canopy during the period 1999-2014 in the drought plots (-

431   5000 ± 1600 kg ha^{-1}), which did not occur in the control plots, and thus the drop in biomass due

432   to 15 consecutive years of chronic drought has decreased the competition for available water

433   counteracting the decrease in its availability. Less canopy biomass in *Q. ilex*, nearly 7% in the

434   drought plots, together with the general decrease in ecosystem aboveground biomass in the

435   drought than the control plots for this period (Barbeta et al., 2013), can improve WUE in the

436   remaining canopy leaves, thus decreasing δ^{13}C. Soil δ^{13}C was higher in the drought plots. Soil C

437   concentrations are negative correlated with δ^{13}C, such as demonstrated in several studies

438   (Farquar et al., 1989; Balesdent et al., 1993; Garten et al., 2000), indicating a decay of soil C cycle

439   rates, thus consistent with the slower soil enzyme activity observed in previous studies in

440   drought plots (Sardans and Peñuelas, 2005; Sardans et al., 2008a,b), and thus with slower

441   mineralization rates under drought conditions.

442   δ^{15}N in all foliar, foliar-litter and soil samples was higher in the drought than the control

443   plots, highlighting the increase in N-cycle openness in the drought relative to the control plots,

444   consistent with the decreases in the accumulation of N stocks for 1999-2014 in leaves and for

445   2005-2014 in soil. Drought has thus been correlated with a loss of N in these ecosystems, which

446   is especially important due to the limiting role of N in this mountainous area (Sabaté and Gracia,

447   1994). The decreases in plant N uptake and activities of soil enzymes involved in N mineralization

448   reported in previous studies (Sardans et al., 2008a,b) could account for this reduction in N stocks

449   under drought. Lower rates of the plant-soil N cycle, with lower proportional stocks of N stored

450   in plants relative to the soil, can increase the loss of soil N associated with the large capacity for

451   leaching and erosion due to the frequent and intense torrential rains typical of Mediterranean

452   areas. Drier soil conditions can favor desertification by several processes affecting the sequential

453   loss of plant cover (Sardans and Peñuelas, 2013).

454

455   *4.3.* BN shifts along 15 year of drought

456   The GDA suggested the existence of species-specific BNs, consistent with the BN

457   hypothesis (Peñuelas et al., 2009, 2019); each species had a unique foliar elemental composition

458   but also an overall foliar elemental plasticity to shifts, depending on the season and

459   environmental circumstances but within its species-specific space in the hypervolume. The

460   spaces of multivariant analyses also shifted over time in response to drought (in some cases)

461   within the BN space of each species. *Phillyrea latifolia* and *A. unedo* have evolved over time

462   toward higher foliar K concentrations, which should be correlated with the decrease in soil K

463   concentrations and stocks during the study period as a mechanism to conserve K as much as

464   possible. The large differences in K concentration between the leaves and foliar litter, with 8.5,

465   7.3 and 4.0 (mg g^{-1} DW) less K in the litter than the leaves in *Q. ilex, P. latifolia* and *A. unedo*,

466   respectively, suggests a large effort to resorb K in this ecosystem (in both the control and

467   drought plots). Our data thus generally indicated that at least *P. latifolia* and *A. unedo* have

468   increased their foliar K concentrations, whereas the upper soil layer has lost K.

469

470   *4.4.Changes in C, N, P and K stocks in leaves, foliar litter and soil*

471   Drought decreased total soil N stocks in the upper 15 cm. Interestingly, C and K were

472   notably lost in both the control and drought plots for 1999-2015. Previous studies have reported

473   that past recent extreme droughts in the study area (such as in summer 2011) could have

474   decreased the reserves of ground water and decreased weathering from rocks and thus the

475   uptake by plants of ground water and minerals (Barbeta et al., 2015) such as K, which may have

476   contributed to the decrease in K concentrations in the upper soil layer and the leaves. We thus

477   observed how a long-term moderate reduction in the availability of soil water (averaging 15%)

478   could be sufficient to cause a general and continuous loss of nutrient stocks in a Mediterranean

479   forest. The effects of droughts are element-dependent and affect nutrient stoichiometry at the

480   ecosystem level, with the potential to generate nutrient imbalances. The most abundant shrub,

481   *P. latifolia* was able to conserve more the C and nutrient stocks than the dominant tree species

482   Q. ilex. Thus these new results are consistent with those of previous studies in this long-term

483   experiment of chronic drought in field conditions observing an slow but continuous increase in

484   dominance of the most drought-tolerant species, the shrub *P. latifolia* (Ogaya and Peñuelas,

485   2006,2007; Barbeta et al., 2015,2017; Ogaya et al., 2020).

486

487   **Final remarks and conclusions**

488   (i) The variation in foliar elemental composition was strongly seasonal but notably homeostatic

489   in response to the drought treatment. This result is consistent with the notable homeostatic

490   elemental composition expected for stress tolerant species biogeochemical niche (Peñuelas et

al., 2019). Moreover, N, P and K concentrations were higher and the N:P ratio was lower in spring, coinciding with the period of growth in this Mediterranean ecosystem, consistent with the growth-rate hypothesis.

(ii) The foliar concentrations of C and N in *Q. ilex*, P and K in P. latifolia and N and K in *A. unedo* in summer were lower in the drought plots .The results thus showed that drier conditions have a detrimental effect on nutrient capture mainly during the already dry periods in this Mediterranean system. The largest differences in foliar concentrations between the control and drought plots were found in summer, thus coinciding with the most arid annual conditions.

(iii) Soil δ^{13}C was higher in the drought plots, indicating a decay of soil C cycle and consistent with lower soil C and could be related with slow soil enzyme activity observed in previous studies and thus with slower mineralization rates. δ^{15}N was higher in the drought than the control plots in all samples, highlighting the increase in openness of the N cycle in the drought relative to the control plots. This difference is especially important given the limiting role of N in this mountainous area in previous studies. It indicates a decrease in the capacity of the global ecosystem to store C and an increase in the soil N losses associated with leaching and erosion due to the frequent and intense torrential rains typical of Mediterranean areas. δ^{13}C in the summer leaves of the dominant *Q. ilex*, however, was lower in the drought than the control plots, indicating better conditions for fixing C in the drought plots, probably due to the large loss of aboveground biomass in the drought plots, which could improve WUE in the remaining leaves of the canopy.

(iv) Each species had its own foliar elemental composition, consistent with the BN hypothesis. The foliar elemental composition, however, shifted in response to drought in the BN space of each species. *Phillyrea latifolia* and *A. unedo* have evolved over time toward higher foliar K concentrations associated with the decrease in soil K concentrations and stocks during the study period. The species were thus able to vary over time due to environmental changes but maintaining their own "multi-variant space".

(v) A long-term moderate reduction in the availability of soil water (averaging 15%) can cause a general and continuous loss of nutrient stocks in this Mediterranean forest. This reduction would have a negative synergistic effect of less water together with lower nutrient availability on the capacity of the forest to resist these conditions. A gradual substitution of forest by shrubland is thus expected. The most drought-tolerant species in this study, the shrub *P. latifolia*, which retained more C and nutrients in its foliar stocks under drought, is slowly but continuously increasing its dominance over time.

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Figure Captions

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783 Figure 1. Seasonal δ^{15}N (mean ± S.E.) in *Q. ilex* leaves (A) and foliar litter (B), *P. latifolia* leaves
784   (C) and foliar litter (D) and *A. unedo* leaves (E) and foliar litter (G) in the control and drought
785   plots 15 years after the beginning of the experiment. Different letters indicate significant
786   differences (*P*<0.05).
787 Figure 2. Seasonal soil δ^{15}N (mean ± S.E.) in the control and drought plots 15 years after the
788   beginning of the experiment. Different letters indicate significant differences (*P*<0.05).
789 Figure 3. GDA analysis of the foliar concentrations in the three dominant species and pairwise
790   ratios as variables. The samples of each species in the control and drought plots were
791   collected in 1999, 2004 and 2014 as grouping factors.
792 Figure 4. Differences in foliar carbon stocks (mean ± S.E.) for 1999-2004 and 1999-2014
793   between the control and drought plots for *Q. ilex* (A), *P. latifolia* (B), *A. unedo* (C) and all
794   three species (D). Different letters indicate significant differences (*P*<0.05). Positive and
795   negative values indicate increases and decreases, respectively, of the carbon stocks during
796   the corresponding time period.
797 Figure 5. Differences in foliar nitrogen stocks (mean ± S.E.) for 1999-2004 and 1999-2014
798   between the control and drought plots for *Q. ilex* (A), *P. latifolia* (B), *A. unedo* (C) and all
799   three species (D). Different letters indicate significant differences (*P*<0.005). Positive and
800   negative values represents increases and decreases, respectively, of the nitrogen stocks
801   during the corresponding time period.
802 Figure 6. Differences in foliar phosphorus stocks (mean ± S.E.) for 1999-2004 and 1999-2014
803   between the control and drought plots for *Q. ilex* (A), *P. latifolia* (B), *A. unedo* (C) and all
804   three species (D). Different letters indicate significant differences (*P*<0.005). Positive and
805   negative values represents increases and decreases, respectively, ofthe phosphorus stocks
806   during the corresponding time period.
807 Figure 7. Differences in foliar potassium stocks (mean ± S.E.) for 1999-2004 and 1999-2014
808   between the control and drought plots for *Q. ilex* (A), *P. latifolia* (B), *A. unedo* (C) and all
809   three species (D). Different letters indicate significant differences (*P*<0.005). Positive and
810   negative values represents increases and decreases, respectively, of potassium stocks
811   during the corresponding time period.
812 Figure 8. Differences in soil stocks (upper 15 cm, kg ha^{-1}) for 2005-2014 between the control and
813   drought plots. Positive and negative values represents increases and decreases, respectively,
814   of the carbon, nitrogen, phosphorus stocks in soils during the corresponding time period.
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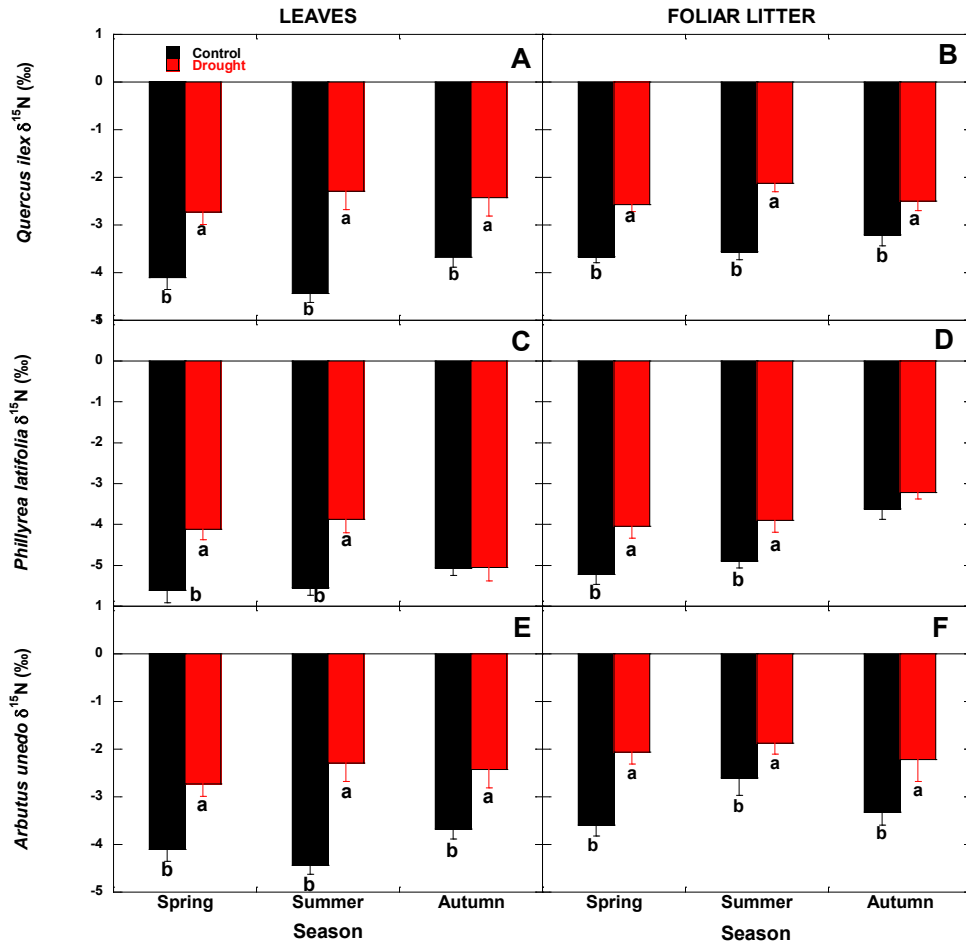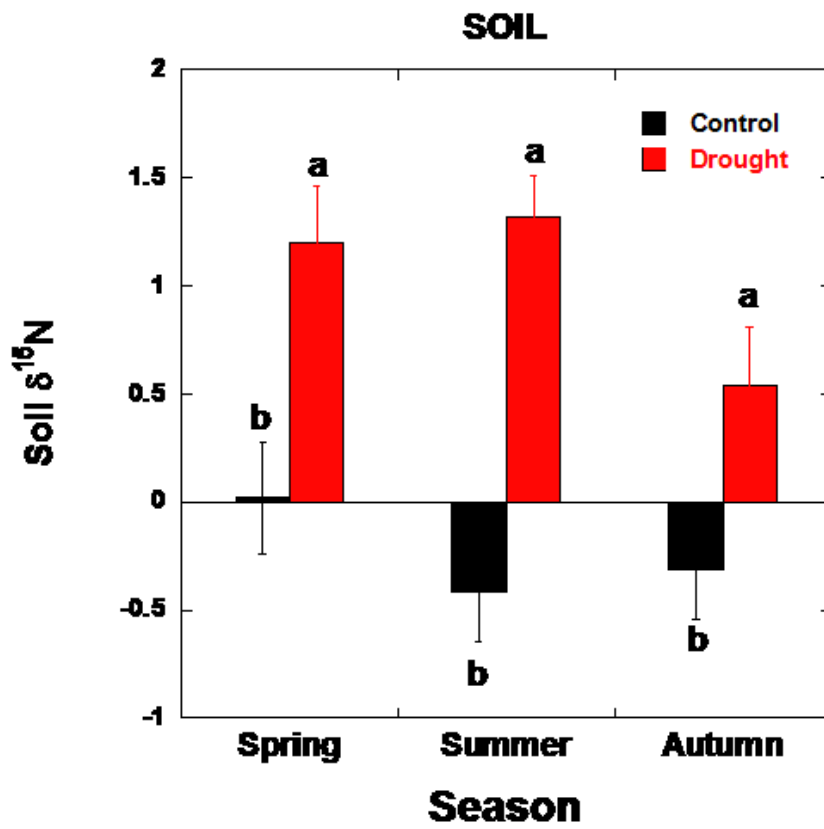
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Figure 1

Figure 2

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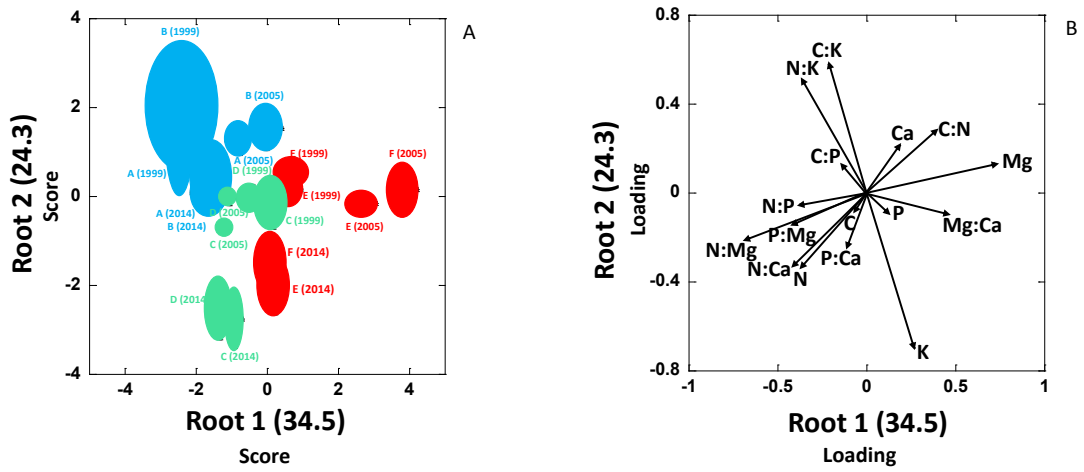
Figure 3

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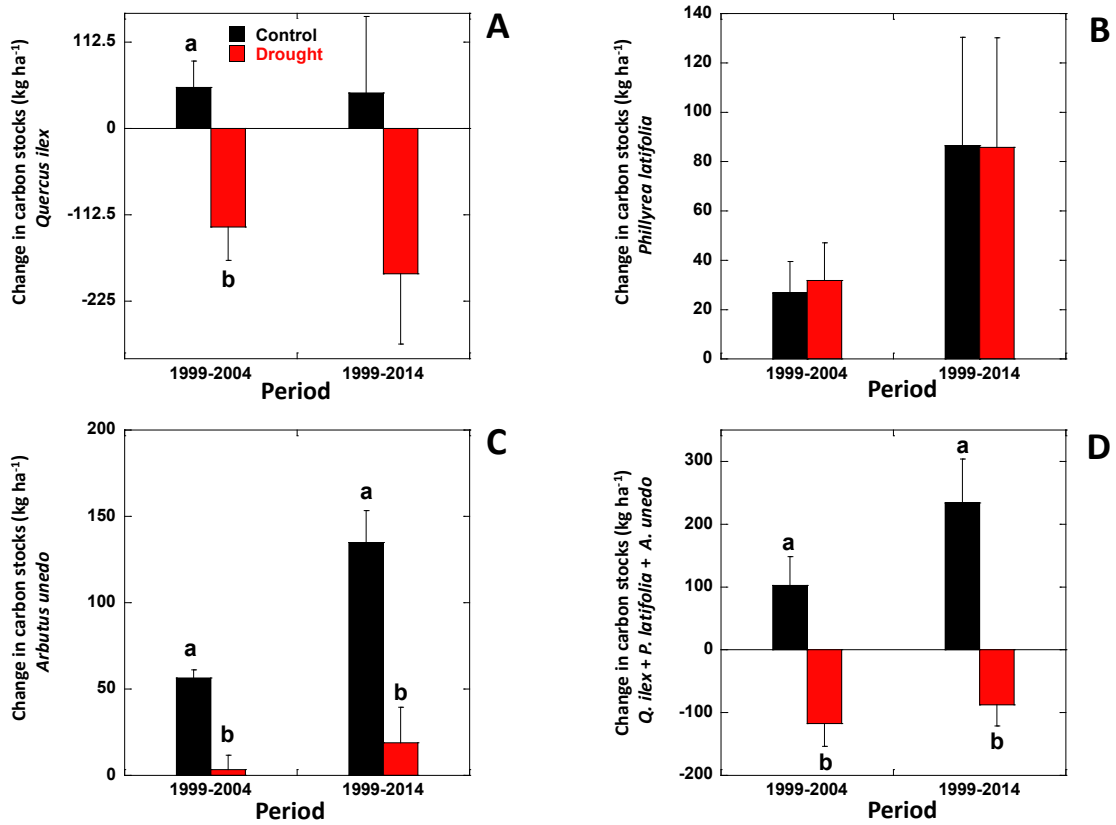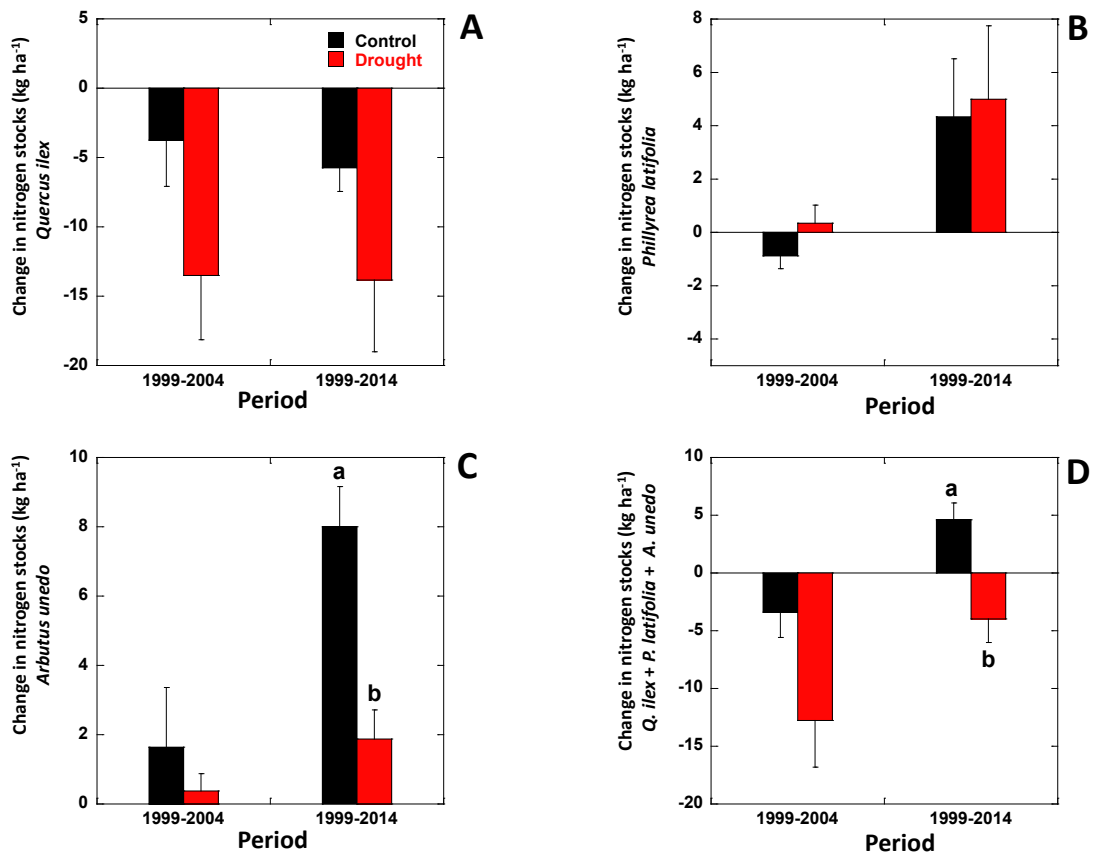897

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913 **Figure 4**

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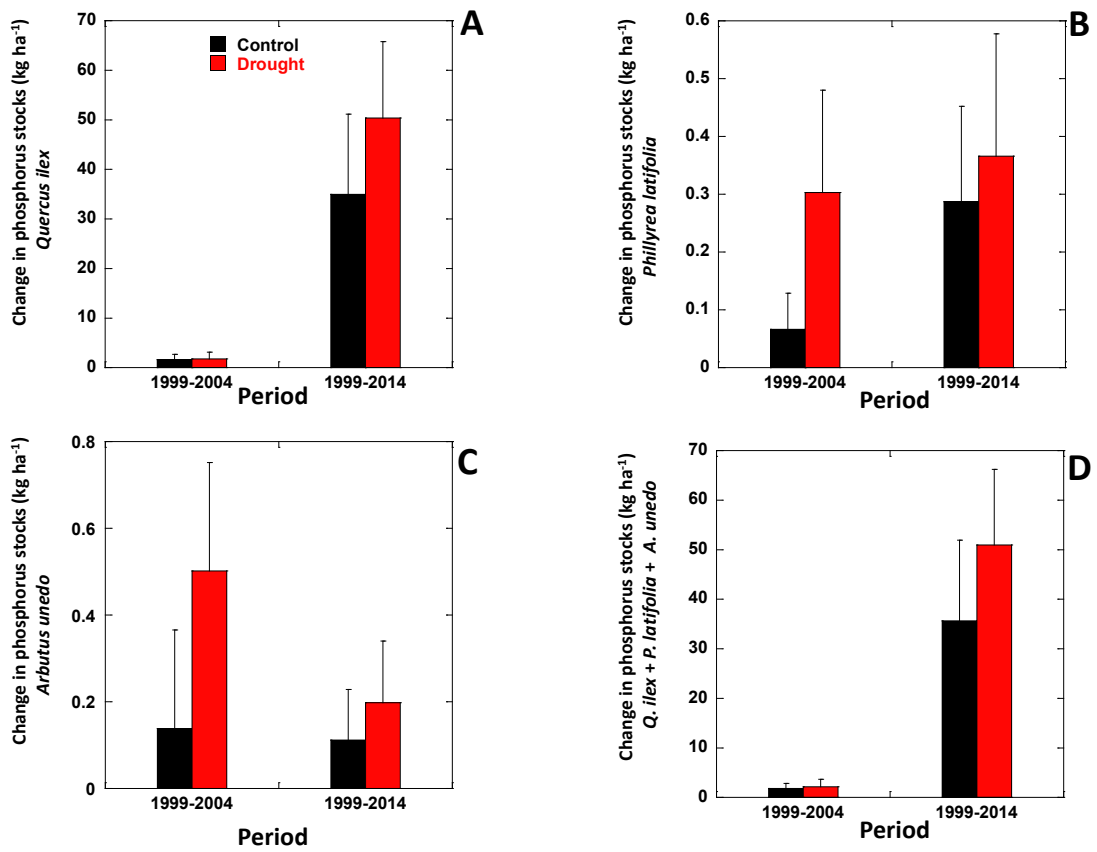950

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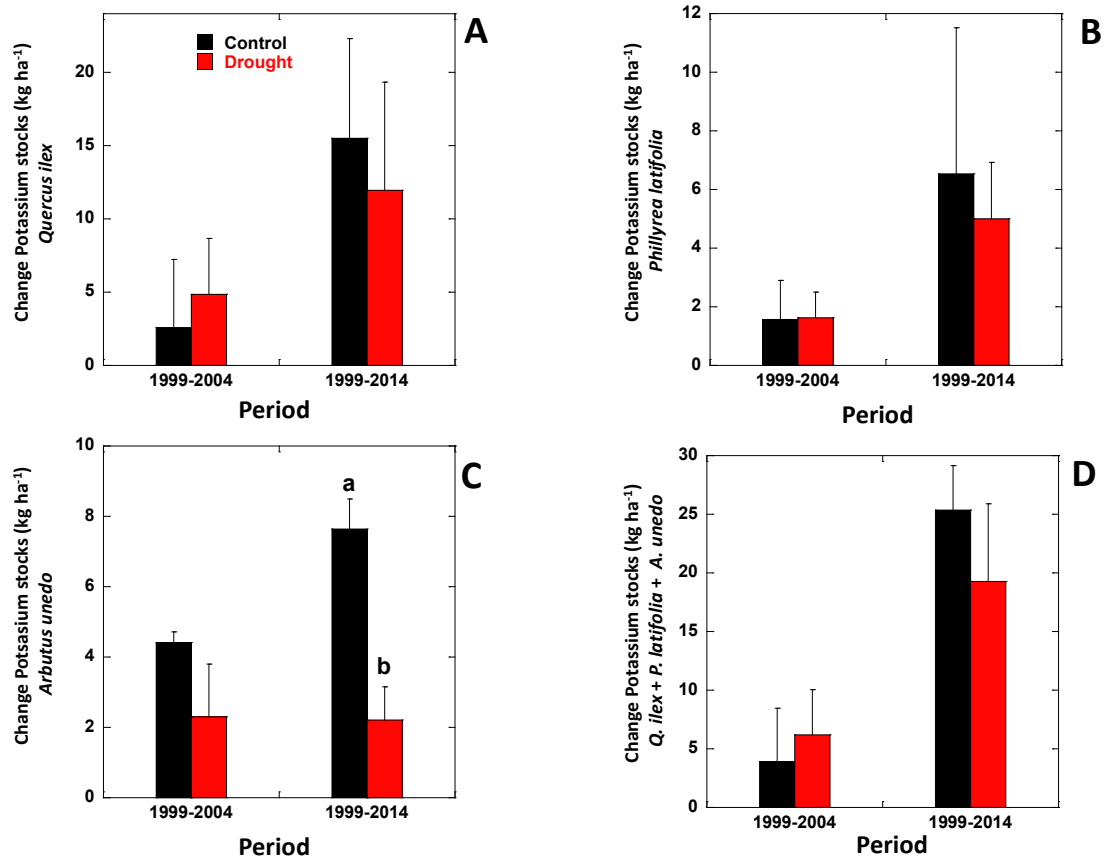954
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967 **Figure 6**
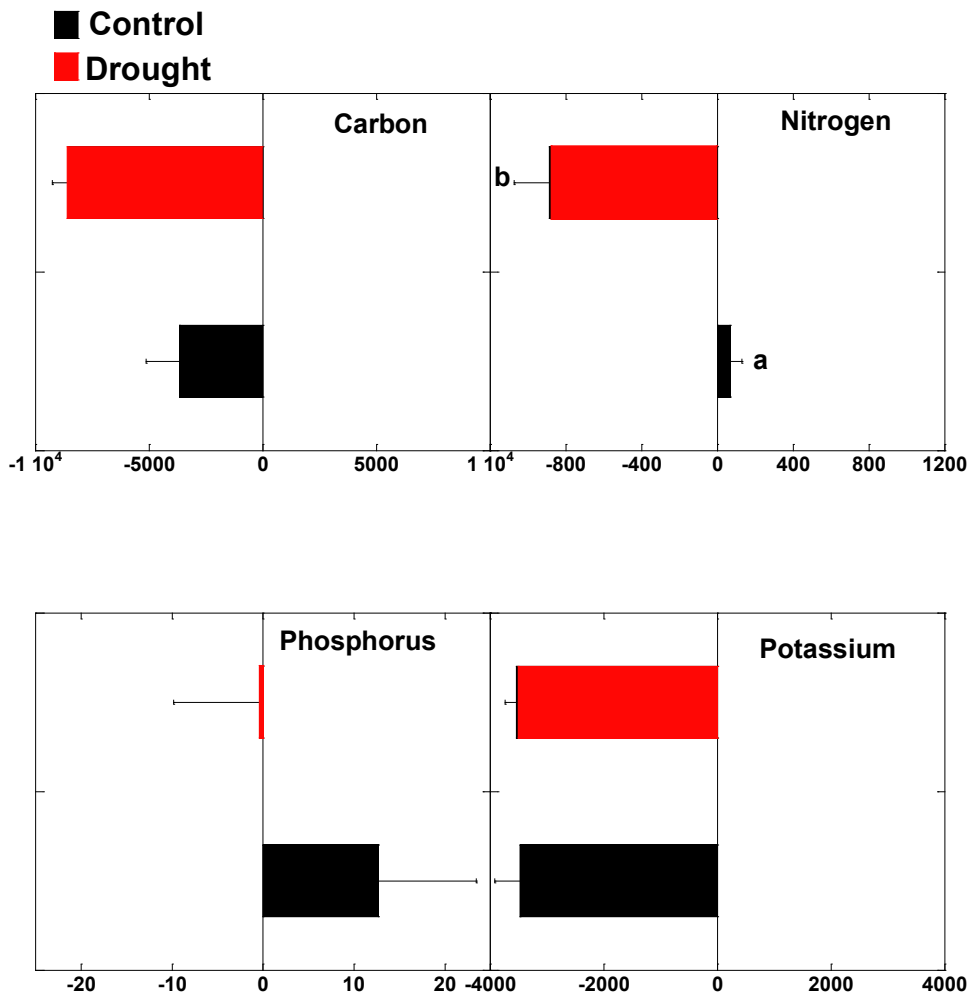968
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1000 **Figure 7**
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Increase of stocks (kg ha^{-1} in the first 15 cm of soil) in the period 2005-2014

1013   **Figure 8**