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Long-term drought decreases ecosystem C and nutrient storage in a Mediterranean holm oak forest.

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Running title: Nutrient stocks decrease under drought
Abstract

Aridity has increased in recent decades in the Mediterranean Basin and is projected to continue to increase in the coming decades. We studied the consequences of drought on the concentrations, stoichiometries and stocks of carbon (C), nitrogen (N), phosphorus (P) and potassium (K) in leaves, foliar litter of a three dominant woody species and soil of a Mediterranean montane holm oak forest where soil-water content was experimentally reduced (15% lower than the control plots) for 15 years. Nitrogen stocks were lower in the drought plots 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 cm soil layer), thus representing 7 and 18% lower N stocks in the canopy and soil respectively. \(\delta^{15}\)N was consistently higher under drought conditions in all samples, indicating a general loss of N. Foliar C and K stocks were also lower but to a lesser extent than N. Decreases in biomass and C and N stocks due to drought were smallest for the most dominant tall shrub, *Phillyrea latifolia*, so our results suggest a lower capacity of this forest to store C and nutrients but also substantial resulting changes in forest structure with increasing drought.

**Key words:** Climate change, aridity, nitrogen, phosphorus, potassium, stoichiometry, carbon stocks.
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 sclerophyll 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 CO₂-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 and
nutrient losses thus impairing ecosystem water-use efficiency (WUE), which could lead to a point
of no return toward desertification (García-Fayos and Bochet, 2009; Matías et al., 2011; Sardans
and Peñuelas, 2013; Peñuelas et al., 2013).

Long-term field experiments, though, have not provided consistent evidence that
moderate drought can also imply a change in nutrient concentrations, stoichiometries and
stocks. The recent implementation of mechanistic nitrogen (N) and phosphorus (P) cycling in
models of the terrestrial carbon (C) cycle underscores the importance of nutrient feedbacks,
indicating potential reductions in the productivity of terrestrial ecosystems (Goll et al., 2012;
Peñuelas et al., 2013; Fernández-Martínez et al., 2017; Peñuelas et al., 2017b; Sun et al., 2017;
Peñuelas et al., 2020). The recent increase in aridity at a global continental scale together with
nutrient imbalances have demonstrated decreases in the continental capacity to store C
(Peñuelas et al., 2017b). Drought linked to increased nutrient limitation can thus feedback on C
balances, because limited P and N availability are likely to jointly reduce future C storage by
natural ecosystems during this century (Peñuelas et al., 2013). This feedback can be especially
important in semiarid regions such as the Mediterranean Basin and the Sahel where water
limitations can have strong negative feedbacks with increased nutrient deficits and thus with a
strongly diminished productive capacity and ecosystem biomass (IPCC 2007, Sardans et al.,
2012; Sanaullah et al., 2012; Sardans and Peñuelas, 2013; Gessler et al., 2016).

Long-term experimental climatic-manipulation studies are rare in the Mediterranean
biome. C, N, P and potassium (K) cycles clearly changed after five years of continuous drought
in a field experiment in an old holm oak forest (Quercus ilex L. ssp ilex) established in 1999. C, N
and P mineralization tended to be slower and N accumulation was lower in some dominant plant
species in response to five years of continuous drought (Sardans et al., 2008a,b; Sardans and
Peñuelas 2007, 2010). The results after the five years of continuous drought, however, were not
conclusive, because the stand biomass stocks only decreased in some (Quercus ilex and Arbutus
unedo L.) but not all species, whereas C and N accumulation increased in the soil (Sardans et al.,
2008a,b; Sardans and Peñuelas, 2007, 2010). We now aimed to identify the mid- to long-term
links of ten more years of prolonged moderate drought with the concentrations, stocks and
stoichiometries of C, N, P and K in this Mediterranean holm oak forest.

The potential shifts in plant elemental concentrations and stoichiometries can provide
information of the capacity of different species to adapt or disappear due to some biotic and
abiotic stresses (Peñuelas et al., 2019). The biogeochemical niche (BN) hypothesis is an emerging
tool to test the shifts in plant communities along spatial and temporal scales as functions of the
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 $\delta^{13}$C and $\delta^{15}$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
The study was carried out on a south-facing slope (25%) in a natural holm oak forest in the Prades Mountains in southern Catalonia (northeastern Iberian Peninsula, 41°13′N, 0°55′E). The soil is a stony Xerochrept on a bedrock of metamorphic sandstone ranging between 35 and 90 cm in depth. The average annual temperature is 12 °C, and the average annual rainfall is 658 mm. The summer drought is pronounced and usually lasts for three months. The vegetation consists of a dense forest dominated by Quercus ilex L. with abundant Phillyrea latifolia L., Arbutus unedo L. and other evergreen species well-adapted to drought conditions (Erica arborea L., Juniperus oxycedrus L. and Cistus albidus L.) and the occasional occurrence of deciduous species (Sorbus torminalis L. Crantz and Acer monspessulanum L.).

2.2. Experimental design

Eight 15 × 10 m plots were delimited at the same altitude (950m) along the slope (25°) (Ogaya et al. 2020). Half of the plots received the drought treatment, and the other half were designated as control plots. The drought treatment consisted of the partial exclusion of rain by suspending PVC strips and funnels 0.5–0.8 m above the soil surface. The strips and funnels covered approximately 30% of the total plot surface. A ditch ca. 0.8 m deep was excavated along the entire top edge of the treatment plots to intercept runoff, and all water intercepted by the strips, funnels and ditches was conducted below the bottom edge of the plots. This drought treatment has been applied since 1999.

2.3. Sampling

We sampled biomass (including leaves and foliar litter) and soil at the beginning of the experiment (December 1999) and after six years of applying the drought treatment (January 2005) to estimate the total contents (Sardans et al., 2008a,b). Tereafter, in the present study, we sampled leaves, foliar litter and soil in 2014 in mid-May (spring), the end of July (summer) and mid-November (autumn) to determinate the changes in C, N, P and K stocks after 15 years of constant chronic drought. Litterfall has been collected every three months during the two pass campaigns (1999 and 2004) and the present campaign (2014) in 20 circular baskets (27 cm in diameter with 1.5-mm mesh diameter) randomly distributed on the ground in each one of the eight plots. Total litterfall was estimated by the proportion of the surface area of the plots covered by the collection baskets. We have measured the sample biomass since the beginning of the experiment several times, the methodology is described in (Ogaya and Peñuelas, 2007). Briefly, the stem circumference was measured annually since 1999 each winter throughout the study. The measurements were conducted with a metric tape at 50 cm height in all living stems of all the species with a diameter larger than 2 cm at 50 cm height. Aboveground biomass in the
plots was estimated by allometric relationships between tree aboveground biomass (AB) and the stem diameter at 50 cm height (D50). Outside the plots, 12 Q. ilex and 13 P. latifolia trees were harvested, their perimeter at 50 cm height was measured and all their aboveground biomass was weighed after drying them in an oven to reach constant weight. To estimate the aboveground biomass of Q. ilex and P. latifolia we used the calculated allometric relationships (\(\ln{AB} = 4.9 + 2.3 \ln{D50}; R^2 = 0.98; n = 12; P < 0.001\) for Q. ilex, and \(\ln{AB} = 4.3 + 2.5 \ln{D50}; R^2 = 0.97; n = 13; P < 0.001\) for P. latifolia), and to estimate the aboveground biomass of A. unedo we used the allometric relationship calculated few years ago on the same area by Lledó (1990) (\(\ln{AB} = 3.8 + 2.6 \ln{D50}; R^2 = 0.99; n = 10; P < 0.001\) (Ogaya & Peñuelas, 2007). The foliar biomasses of the three studied species in 1999, 2004 and 2014 in control and drought plots are shown in the Table S1.

We sampled leaves of the three dominant species of this ecosystem: Quercus ilex, Phillyrea latifolia and Arbutus unedo (that represents on average the 97% of total aboveground biomass) (Ogaya and Peñuelas, 2007). The sample collection was always standardized to avoid biased results due to different tissue ages and orientation to sunlight, the sampled leaves were sun-lit leaves oriented toward the south. The mean life span of the leaves of these species is approximately 18 months (Ogaya and Peñuelas, 2006a), so for example most sampled foliar biomass during 2014 corresponded to the leaves of the 2013 cohort. We randomly sampled five trees of each species on each seasonal sampling day in each plot. Leaves were sampled at a height of 2-3 m. We only sampled trees and shrubs with diameters between 2 and 12 cm (at 5 cm), which represented the majority (approximately 70%) of the community biomass (Ogaya and Peñuelas, 2003, 2006b). We randomly collected five soil cores to a depth of 15 cm of horizon A in each plot on each seasonal sampling day.

2.4. Chemical and physical analyses

All samples were taken to the laboratory and stored at 4 °C until analysis. The soil samples were sieved, and the 2-mm fraction was analyzed. The leaves and foliar litter were washed with distilled water as described by Porter (1986) for analyzing C, N, P and K. The washed samples were dried in an oven at 60 °C to a constant weight and then ground in a CYCLOTEC 1093 (Foss Tecator, Höganäs, Sweden) for analyzing the biomasses or in a FRITSCH Pulverisette (Rudolstadt, Germany) for analyzing the soils. The concentrations of C and N in the foliar, foliar litter and soil samples were quantified by placing 1.4 mg of powder of each sample in a tin microcapsule and using combustion coupled to gas chromatography in a CHNS-O Elemental
The concentrations of P and K in the samples were determined by first grinding the samples and then digesting them in acid in a MARSXpress microwave (CEM, Mattheus, USA) at high pressure and temperature (see Peñuelas et al., 2010). A total of 250 mg of leaf powder, 5 mL of nitric acid and 2 mL of H₂O₂ were placed into Teflon tubes. The products of the digestions were placed in 50-mL flasks and diluted with Milli-Q water to a volume of 50 mL. The concentrations of P and K were determined using Optic Emission Spectrometry with Inductively Coupled Plasma. The accuracy of the digestions and the analytical biomass procedures were assessed using a certified standard biomass (NIST 1573a, tomato leaf; NIST, Gaitherburg, MD) as a reference.

The isotopic analyses of δ¹³C and δ¹⁵N in the foliar and soil samples (sampled in 2014) were conducted in the Stable Isotope Facility at the University of California, Davis using using a PDZ Europa ANCA-GSL elemental analyzer connected to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Soil moisture has been measured each month throughout the experiment by time domain reflectometry (Tektronix 1502C, Tektronix, Beaverton, OR, USA) connecting the time domain reflectometer to the ends of three stainless steel cylindrical rods, 25 cm long, fully driven into the soil (Zegelin et al., 1989). Four sites per plot were randomly selected to install the steel cylindrical rods for soil moisture measurements (Ogaya et al., 2014). During these 15 years of drought treatment soil moisture of drought plots compared to control plots (P < 0.01), but this reduction was larger during rainy seasons and lower during dry seasons (Ogaya et al., 2014).

### 2.5. Statistical analyses

Significant differences in foliar, foliar litter and soil C, N, P and K concentrations and ratios, δ¹³C, δ¹⁵N and C, N, P and K stocks in 2014 among treatments and seasons were tested by general mixed models, with treatment and season as fixed factors and plot as a random factor. We also used mixed models to analyze the treatment effects within each season, with treatment as a fixed factor and plot as a random factor. We used the “lme” function of the “nlme” R package (Pinheiro et al., 2016). Non-normally distributed variables were normalized by log-transformation. We chose the best model for each dependent variable using the Akaike information criterion (AIC). We used the MuMln (Barton, 2012) R package in the mixed models to estimate the percentage of the variance explained by the model. We conducted Tukey’s post hoc tests to detect significant differences in the analyses for more than two communities using the “glht” function of the “multcomp” (Hothorn et al., 2013) R package.
We also performed multivariate statistical analyses. We used a general discriminant analysis (GDA) to determine the overall differences of the foliar variables: C, N, P and K concentrations and their pairwise ratios among the three dominant species sampled at different times during the experiment: December 1999 (before the start of the experiment), November 2005 (after six years of continuous drought) and November 2014 (after 15 years of continuous drought). GDA uses squared Mahalanobis distances that depend on the Euclidean distance in the model between two sets of samples. The squared Mahalanobis distance is low when the samples are similar, indicating little difference between the two sets. In contrast, the distance is higher and the difference between the sets increases the more the samples differ (De Maesschalk et al., 2000). We conducted one-way ANOVAs with Bonferroni post hoc tests of the scores of the first two GDA roots to identify differences among the dominant species sampled over time. We used Statistica 8.0 (StatSoft, Inc. Tulsa, USA) for these analyses.

3. Results

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

The effects of the drought treatment on foliar and foliar litter C, N, P and K concentrations depended strongly on season and species (Tables S2 and S3). A decrease in foliar K concentrations in summer in the three species and a decrease in foliar P concentrations in P. latifolia and A. unedo (Table S2) were the most general effects of the treatment.

The drought treatment increased foliar C and N concentrations and decreased foliar K concentrations in summer and increased foliar N concentrations in autumn in Q. ilex, the dominant species (Table S2). The treatment also decreased foliar C:N ratios in summer and autumn in Q. ilex. Foliar N and P concentrations were higher and the N:P ratio was lower in spring, and the foliar N and P concentrations were lowest in summer, in all species in both the drought and control plots. Drought was associated with decreases in C and K concentrations and increases in N and P concentrations in the Q. ilex foliar litter in summer (Table S3).

Phillyrea latifolia foliar P and K concentrations decreased and foliar C:P, C:K, N:P and N:K ratios increased under drought in summer (Table S2). Foliar C, N and P concentrations were lowest in summer. Drought was associated with a decrease in K concentrations and increases in C:K, N:K and P:K ratios in the foliar litter in summer (Table S3). Arbutus unedo foliar N and K concentrations and the foliar N:P ratio decreased under drought in summer (Table S2). Foliar C, N and P concentrations were lowest in summer for both A. unedo and P. latifolia. Drought was
Drought decreased the accumulation of foliar litter in spring (Table S3).

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

3.2. $\delta^{13}C$ and $\delta^{15}N$

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

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

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

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

Drought decreased the accumulation of foliar C stocks in Q. ilex (significantly only for 1999-2004) (Figure 4A), A. unedo (Figure 4B) and the sum of all three species (Figure 4d), mainly associated with the smaller increase in foliar biomass under drought. Drought decreased the accumulation of foliar N stocks in A. unedo and the sum of all three species (significantly only for 1999-2014) (Figure 5), mainly associated with the lower N concentrations and smaller increase in foliar biomass under drought. Drought did not affect the accumulation of foliar P stocks relative to the control plots in the three species and the study periods (Figure 6). Drought decreased the accumulation of foliar K stocks in A. unedo (significantly only for 1999-2014)
The overall results indicated a significant decrease in foliar C, N and K stocks in \textit{A. unedo} and in C and N stocks in the three dominant species (representing >90% of the total ecosystem aboveground biomass) after 15 years of moderate drought (a mean reduction in soil-water content of 15%). C stocks decreased significantly in the 0-15 cm soil profile for 2004-2014 in the drought but not the control plots (Figure 8), but not significantly ($P=0.87$). The difference in total soil N stock between the drought and control plots for 2005-2014 in the upper 15 cm of soil was 856 ± 120 kg ha\(^{-1}\). Total soil P stocks in the upper 15 cm did not change significantly in either the control or drought plots. Total soil K stocks for 2005-2014 generally decreased in the upper 15 cm (3476 ± 444 and 3541 ± 200 kg ha\(^{-1}\) in the control and drought plots, respectively), but not significantly. C, N, P and K stocks of leaf-litter did not change in 2015 relative to those in 1999 (Table S6).

### 4. Discussion

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

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

The foliar elemental concentrations and ratios had a strong seasonal component independent of the treatments, e.g. the concentrations of N, P and K were higher in spring, coinciding with the period of growth in this Mediterranean ecosystem. The foliar N:P ratio in the three species was noticeably lower in spring, thus coinciding with the higher foliar N and P concentrations in the growing season, consistent with the growth-rate hypothesis, which states that the period of growth coincides with a higher capacity for fast protein synthesis (Sterner and Elser, 2002). In fact, this link between higher metabolic capacity associated with higher foliar N and P concentrations and a lower N:P ratio was experimentally demonstrated in a previous study.
(Rivas-Ubach et al., 2012), indicating that the plasticity of this phenological component was not affected by the drought treatment. The maximum productive capacity, from a nutritional perspective, thus coincides with the optimum annual climatic conditions in both the control and drought plots.

Foliar concentrations tended to decrease in the drought plots or did not change relative to the control plots. The differences between the foliar concentrations in the control and drought plots were largest in summer, thus coinciding with the most arid conditions with water deficits and stress. The effects of drought on the foliar concentrations also depended on the species and element, similar to the elemental stocks. Global changes in foliar concentrations associated with drought, however, were rare. Differences between the control and drought treatments generally occurred in summer; leaves in the drought plots had lower concentrations 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 field experiments frequently find that N foliar concentrations in Mediterranean forest species are very homeostatic in response to abiotic shifts (Martin-StPaul et al., 2013). From an ecological stoichiometric perspective (Sterner and Elser, 2002), elemental composition is more homeostatic in stress-tolerant plant species than competitor and ruderal species in response to environmental changes (Peñuelas et al., 2019). Our results showing only small changes in foliar C, N, P and K concentrations support the idea of the stress tolerance strategy in Mediterranean plants. Mediterranean forest tree species such as *Quercus ilex* or *Pinus halepensis* have less variability in their elemental foliar composition than wet temperate species such as *Quercus robur* or *Quercus petraea* adapted to wetter climate and nutrient richer soils (Sardans et al. 2020). We had previously observed in 2011 that *Q. ilex* foliar K concentrations were higher in the drought than the control plots (Sardans et al., 2013; Rivas-Ubach et al., 2014), whereas *Q. ilex* leaves analyzed in 2014 had the opposite pattern, specifically in summer, perhaps due to the loss of K in the upper 15 cm of soil in both the control and drought plots from 2005 to the present. Some studies have reported that some elemental concentrations can increase and others decrease, depending on the site and species (Luo et al., 2018; Urbina et al., 2015). We thus cannot draw a clear conclusion about the impact of experimental drought on distinct foliar elemental compositions in Mediterranean environments under field conditions.

The level of resistance to drought stress in Mediterranean plant species is highly variable, so fitness decreases more in some species than others (Sardans and Peñuelas, 2013). For example, foliar N concentrations were lower in summer and litter N concentrations were higher in spring in *A. unedo* in the drought than the control plots. These results are consistent with previous results that drought affects fitness and all the variables involved in growth (Ogaya
and Peñuelas, 2007; Sardans et al., 2008a,b; Barbeta et al., 2013), photosynthetic capacity
(Ogaya and Peñuelas, 2006) and flowering (Ogaya and Peñuelas, 2004; Barbeta et al., 2013) that
we measured more in A. unedo than the other dominant species. Some studies, however, have
found that P. latifolia was the most stress-tolerant species of our three dominant species, where
growth and mortality (Ogaya and Peñuelas, 2007; Barbeta et al., 2013) or ecophysiology (Ogaya
et al., 2011) were little or not affected. Our results are consistent with these studies, because
bio-elemental concentrations and foliar stocks were less affected by drought in P. latifolia than
the other species Our results are consistent with these studies, because bio-elemental
concentrations, foliar stocks and growth were less affected by drought in P. latifolia than the
other species, specially than A. unedo (Ogaya and Peñuelas, 2007; Barbeta et al., 2013;2015).
Our results therefore reinforce previous findings in this same study and in other studies, with
evidence of species specific responses to drought in Mediterranean ecosystems, which may
eventually lead to a partial community shift favoring more drought-resistant species (Sardans
and Peñuelas, 2013; Peñuelas et al., 2013; 2017a; 2018; Gazol et al., 2017). The adaptation of
Mediterranean plants to drought generally frequently varies with species; some species cope
with moderate drought without significant losses in fitness, but others are strongly affected
(Sardans and Peñuelas, 2013). Our experiment demonstrated that the three dominant species
responded to this persistent moderate drought, but to different extents. As observed in previous
studies in this research site, the studied species, despite at different extend, all they the plants
subjected to the long-term experimental drought shifted water uptake toward deeper (10–35
cm) soil layers during the wet season and reduced groundwater uptake in summer, indicating
plasticity in their functional distribution of fine roots that dampened the effect of our
experimental drought in the long term (Barbeta et al., 2015).

The reduction of foliage biomass under drought relative to the control plots, and thus
plant cover, may decrease soil quality, as in several Mediterranean areas (Gallardo et al., 2000;
Garcia et al., 2002), decreasing the capacity of soil to retain moisture and thus fertility (Sadaka
and Ponge 2003; Ruiz-Sinoga et al., 2011). In fact, reductions in canopy cover and N
concentrations have been reported in previous studies in various Mediterranean communities,
including forests (Goldstein et al., 2000; Inclan et al., 2005; Pérez-Ramos et al., 2010; Cotrufo et
al., 2011), consistent with the decrease in soil N stocks in the drought versus the control plots

4.2. $\delta^{13}C$ and $\delta^{15}N$ in the soil, foliar litter and leaves after 15 years of drought

$\delta^{13}C$ has frequently been used as an alternative measurable variable to WUE, especially
in drought conditions (Jucker et al., 2017). Plants with limited capacity for water uptake
preferentially have higher $\delta^{13}C$ (Jucker et al., 2017). Soil $\delta^{13}C$ in our study was higher in the
drought than the control plots, potentially associated with a high rate of loss of C from the soil
and thus with a more open soil C cycle. In contrast, $\delta^{13}C$ in the summer leaves of the dominant
species Q. ilex was lower in the drought than the control plots, indicating better conditions for
fixing C in the drought plots. This apparently contradictory results are probably linked to the
large loss of biomass in the Q. ilex canopy during the period 1999-2014 in the drought plots (-
5000 ± 1600 kg ha$^{-1}$), which did not occur in the control plots, and thus the drop in biomass due
to 15 consecutive years of chronic drought has decreased the competition for available water
counteracting the decrease in its availability. Less canopy biomass in Q. ilex, nearly 7% in the
drought plots, together with the general decrease in ecosystem aboveground biomass in the
drought than the control plots for this period (Barbeta et al., 2013), can improve WUE in the
remaining canopy leaves, thus decreasing $\delta^{13}C$. Soil $\delta^{13}C$ was higher in the drought plots. Soil C
concentrations are negative correlated with $\delta^{13}C$, such as demonstrated in several studies
(Farquar et al., 1989; Balesdent et al., 1993; Garten et al., 2000), indicating a decay of soil C cycle
rates, thus consistent with the slower soil enzyme activity observed in previous studies in
drought plots (Sardans and Peñuelas, 2005; Sardans et al., 2008a,b), and thus with slower
mineralization rates under drought conditions.

$\delta^{15}N$ in all foliar, foliar-litter and soil samples was higher in the drought than the control
plots, highlighting the increase in N-cycle openness in the drought relative to the control plots,
consistent with the decreases in the accumulation of N stocks for 1999-2014 in leaves and for
2005-2014 in soil. Drought has thus been correlated with a loss of N in these ecosystems, which
is especially important due to the limiting role of N in this mountainous area (Sabaté and Gracia,
1994). The decreases in plant N uptake and activities of soil enzymes involved in N mineralization
reported in previous studies (Sardans et al., 2008a,b) could account for this reduction in N stocks
under drought. Lower rates of the plant-soil N cycle, with lower proportional stocks of N stored
in plants relative to the soil, can increase the loss of soil N associated with the large capacity for
leaching and erosion due to the frequent and intense torrential rains typical of Mediterranean
areas. Drier soil conditions can favor desertification by several processes affecting the sequential
loss of plant cover (Sardans and Peñuelas, 2013).

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

The GDA suggested the existence of species-specific BNs, consistent with the BN
hypothesis (Peñuelas et al., 2009, 2019); each species had a unique foliar elemental composition
but also an overall foliar elemental plasticity to shifts, depending on the season and environmental circumstances but within its species-specific space in the hypervolume. The spaces of multivariate analyses also shifted over time in response to drought (in some cases) within the BN space of each species. *Phillyrea latifolia* and *A. unedo* have evolved over time toward higher foliar K concentrations, which should be correlated with the decrease in soil K concentrations and stocks during the study period as a mechanism to conserve K as much as possible. The large differences in K concentration between the leaves and foliar litter, with 8.5, 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*, respectively, suggests a large effort to resorb K in this ecosystem (in both the control and drought plots). Our data thus generally indicated that at least *P. latifolia* and *A. unedo* have increased their foliar K concentrations, whereas the upper soil layer has lost K.

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

Drought decreased total soil N stocks in the upper 15 cm. Interestingly, C and K were notably lost in both the control and drought plots for 1999-2015. Previous studies have reported that past recent extreme droughts in the study area (such as in summer 2011) could have decreased the reserves of ground water and decreased weathering from rocks and thus the uptake by plants of ground water and minerals (Barbeta et al., 2015) such as K, which may have contributed to the decrease in K concentrations in the upper soil layer and the leaves. We thus observed how a long-term moderate reduction in the availability of soil water (averaging 15%) could be sufficient to cause a general and continuous loss of nutrient stocks in a Mediterranean forest. The effects of droughts are element-dependent and affect nutrient stoichiometry at the ecosystem level, with the potential to generate nutrient imbalances. The most abundant shrub, *P. latifolia* was able to conserve more the C and nutrient stocks than the dominant tree species *Q. ilex*. Thus these new results are consistent with those of previous studies in this long-term experiment of chronic drought in field conditions observing an slow but continuous increase in dominance of the most drought-tolerant species, the shrub *P. latifolia* (Ogaya and Peñuelas, 2006, 2007; Barbeta et al., 2015, 2017; Ogaya et al., 2020).

**Final remarks and conclusions**

(i) The variation in foliar elemental composition was strongly seasonal but notably homeostatic in response to the drought treatment. This result is consistent with the notable homeostatic elemental composition expected for stress tolerant species biogeochemical niche (Peñuelas et
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 $\delta^{13}C$ 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. $\delta^{15}N$ 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. $\delta^{13}C$ 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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Chemometrics Intelligent Laboratory Systems 50, 1–18.


Figure Captions

Figure 1. Seasonal $\delta^{15}$N (mean ± S.E.) in Q. ilex leaves (A) and foliar litter (B), P. latifolia leaves (C) and foliar litter (D) and A. unedo leaves (E) and foliar litter (G) in the control and drought plots 15 years after the beginning of the experiment. Different letters indicate significant differences ($P<0.05$).

Figure 2. Seasonal soil $\delta^{15}$N (mean ± S.E.) in the control and drought plots 15 years after the beginning of the experiment. Different letters indicate significant differences ($P<0.05$).

Figure 3. GDA analysis of the foliar concentrations in the three dominant species and pairwise ratios as variables. The samples of each species in the control and drought plots were collected in 1999, 2004 and 2014 as grouping factors.

Figure 4. Differences in foliar carbon stocks (mean ± S.E.) for 1999-2004 and 1999-2014 between the control and drought plots for Q. ilex (A), P. latifolia (B), A. unedo (C) and all three species (D). Different letters indicate significant differences ($P<0.05$). Positive and negative values indicate increases and decreases, respectively, of the carbon stocks during the corresponding time period.

Figure 5. Differences in foliar nitrogen stocks (mean ± S.E.) for 1999-2004 and 1999-2014 between the control and drought plots for Q. ilex (A), P. latifolia (B), A. unedo (C) and all three species (D). Different letters indicate significant differences ($P<0.005$). Positive and negative values represent increases and decreases, respectively, of the nitrogen stocks during the corresponding time period.

Figure 6. Differences in foliar phosphorus stocks (mean ± S.E.) for 1999-2004 and 1999-2014 between the control and drought plots for Q. ilex (A), P. latifolia (B), A. unedo (C) and all three species (D). Different letters indicate significant differences ($P<0.005$). Positive and negative values represent increases and decreases, respectively, of the phosphorus stocks during the corresponding time period.

Figure 7. Differences in foliar potassium stocks (mean ± S.E.) for 1999-2004 and 1999-2014 between the control and drought plots for Q. ilex (A), P. latifolia (B), A. unedo (C) and all three species (D). Different letters indicate significant differences ($P<0.005$). Positive and negative values represent increases and decreases, respectively, of potassium stocks during the corresponding time period.

Figure 8. Differences in soil stocks (upper 15 cm, kg ha$^{-1}$) for 2005-2014 between the control and drought plots. Positive and negative values represent increases and decreases, respectively, of the carbon, nitrogen, phosphorus stocks in soils during the corresponding time period.
Figure 1
Figure 2
Figure 3
Figure 4

A) Change in carbon stocks (kg ha\(^{-1}\)) for Quercus ilex during the period 1999-2004 and 1999-2014 under control and drought conditions.

B) Change in carbon stocks (kg ha\(^{-1}\)) for Phillyrea latifolia during the period 1999-2004 and 1999-2014.

C) Change in carbon stocks (kg ha\(^{-1}\)) for Arbutus unedo during the period 1999-2004 and 1999-2014.

D) Change in carbon stocks (kg ha\(^{-1}\)) for Quercus ilex + Phillyrea latifolia + Arbutus unedo during the period 1999-2004 and 1999-2014.
Figure 5

Change in nitrogen stocks (kg ha\(^{-1}\))

A. Quercus ilex

B. Phillyrea latifolia

C. Arbutus unedo

D. Q. ilex + P. latifolia + A. unedo

Control vs. Drought

Figure 6

A) Change in phosphorus stocks (kg ha$^{-1}$) for Quercus ilex from 1999-2004 and 1999-2014 for Control and Drought treatments.

B) Change in phosphorus stocks (kg ha$^{-1}$) for Phillyrea latifolia from 1999-2004 and 1999-2014 for Control and Drought treatments.

C) Change in phosphorus stocks (kg ha$^{-1}$) for Arbutus unedo from 1999-2004 and 1999-2014.

D) Change in phosphorus stocks (kg ha$^{-1}$) for Quercus ilex + Phillyrea latifolia + Arbutus unedo from 1999-2004 and 1999-2014 for Control and Drought treatments.
Figure 7

A. Change Potassium stocks (kg ha\(^{-1}\)) for Quercus ilex

B. Change Potassium stocks (kg ha\(^{-1}\)) for Phillyrea latifolia

C. Change Potassium stocks (kg ha\(^{-1}\)) for Arbutus unedo

D. Change Potassium stocks (kg ha\(^{-1}\)) for Quercus ilex + Phillyrea latifolia + Arbutus unedo
Increase of stocks (kg ha\(^{-1}\) in the first 15 cm of soil) in the period 2005-2014