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Shrub understorey clearing and drought affects water status and growth of juvenile *Quercus suber* trees



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ABSTRACT

Shrub encroachment is a global phenomenon occurring in a wide range of ecosystems, including in tree plantations, which is known to affect ecosystem functioning and productivity. Forest management in tree plantations (e.g. shrub clearing, tree thinning and pruning) often aims to increase wood and non-woody products yield while optimizing other ecosystems services such as carbon sequestration. However, little is known on the effect of shrub clearing on tree growth and tree water status in juvenile tree stands. Under climate change scenarios that predict increased frequency of extreme events such as droughts, management during the early stages of stand plantations may be crucial for its sustainability. This study aimed to explore the combined effect of shrub understorey removal and drought in two cork oak (Quercus suber L.) juvenile plantations, with similar stand age but distinct soil types and forest management, on tree water status, assessed through leaf water potential measurement, and on tree growth. The study was conducted during two consecutive years, including a drought year. Results revealed that the shrub understorey removal positively affected the physiological response of trees in both plantations but only affected tree growth (i.e. total height and leaf area) in the plantation with a higher tree density and shrub biomass. Shrub biomass and species composition most probably had a determinant effect on tree growth due to soil water competition. Our results emphasize the need for site-specific forest management during the early stages of cork oak plantation for maintaining its future sustainability and provision of ecosystem services.

1. Introduction

The increase in density, cover and biomass of shrubs, i.e. shrub encroachment, is a global phenomenon in grasslands (D'Odorico et al., 2012, Archer et al., 2017), open woodlands and other types of ecosystems (Sala and Maestre, 2014; Stevens et al., 2017). Shrub encroachment is part of the natural succession dynamics in many regions of the world (Swanson et al., 2011). However, this phenomenon has been increasing due to changes in climate (e.g. precipitation and temperature regimes) and in land-use (e.g. grazing intensity and wildfire regimes) (Blois et al., 2013, O'Connor et al., 2014, Archer et al., 2017). Shrub encroachment affects the ecosystem structure and functioning and the provision of ecosystem services (Jackson et al., 2002, Eldridge et al., 2011, Vila et al., 2011). For example, it increases ecosystem carbon sequestration and storage (Pan et al., 2011, Li et al., 2016, Lecomte et al., 2019) or herbivory fodder availability (Bugalho and Milne, 2003, Azorit et al., 2012, López-Díaz et al., 2015). However, negative impacts on ecosystem functioning such as increased fire hazard (Lecomte et al., 2019, Damianidis et al., 2021) or decreased water yield are also observed (Li et al., 2012). Specifically, shrub encroachment through higher levels of transpiration and reduction of soil water availability may alter the ecosystem water balance, particularly in water limited ecosystems (Rascher et al., 2011, Caldeira et al., 2015, Le Maitre et al., 2015). The potential reduction of soil water availability by shrubs (Rolo and Moreno, 2019) can be exacerbated by seasonal droughts with direct negative implications on tree functioning and growth (Niinemets, 2010, Lempereur et al., 2017, Lobo-do-Vale et al., 2019). This is particularly relevant under the ongoing climatic changes and increased frequency of drought occurrence (IPCC, 2014), particularly in water-limited ecosystems such as in Mediterranean regions.

Understorey management of forests frequently includes periodic clearing of the vegetation layer to promote tree growth and carbon sequestration or reduce wildfire hazard (e.g. Garcia-Gonzalo et al., 2012). Understorey vegetation clearing, for example, had an overall positive effect on seedling survival and tree growth in *Pinus taeda* stands (Zhao et al., 2016, Blazier et al., 2017, Restrepo et al., 2019) and

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Received 28 July 2021; Received in revised form 29 September 2021; Accepted 1 October 2021 Available online 19 October 2021 0378-1127/© 2021 Elsevier B.V. All rights reserved. Eucalyptus spp. stands (Vargas et al., 2018, Zhou et al., 2018). However, the direction and magnitude of these effects may depend on several factors such as climate, shrub species, soil properties and fertility (Li et al., 2012) or stand age (Little et al., 2018). Liles et al. (2019) showed that periodic shrub understorey clearing, along a 20 years period, significantly increased tree growth rates and stand-level productivity in Pinus ponderosa stands in California, USA. Nonetheless, there were significant interactions between clearing treatment and site conditions, defined by soil productivity (i.e. water holding capacity, soil depth and soil organic matter content) and climatic data (e.g. mean annual precipitation, temperature). In a cork oak (Quercus suber L.) stand, in Portugal, with an understorey dominated by Cistus salvifolius, Rosmarinus officinalis and Ulex airensis, shrub clearing did not significantly affect wood or cork growth in adult trees (Faias et al., 2018, Faias et al., 2019a). However, shrub encroachment, by Cistus ladanifer, in a cork oak stand, combined with an extreme drought, reduced diameter growth (Haberstroh et al., 2021), transpiration and resilience of trees to drought (Caldeira et al., 2015). This is in agreement with the high levels of cork oak tree mortality observed in C. ladanifer encroached cork oak forests, particularly in areas with shallow soils (Costa et al., 2010).

Cork oak woodlands occupy ca. 2.2 million ha in southern and northern Western Mediterranean Basin (Tomé et al., 2020). These savannah-type ecosystems provide high socio-economic benefits, mainly by cork production, support high biodiversity and provide important ecosystem services such as carbon storage and water regulation (Bugalho et al., 2011, Bugalho et al., 2018). In Portugal, they account for 34% of the world cork oak forest cover and 60.9% of world cork production, which generated 975 Million euros in export in 2019 (ICT, 2021). Due to their important ecological role, these ecosystems are listed in the European Habitats Directive (i.e. Habitat 9330: Quercus suber forests, EEC, 1992) and were also highly protected and supported in Portugal, through financial afforestation initiatives (Mendes and Silva Dias, 2002). However, these forests are threatened due to different causes, such as inadequate understorey and tree management (Paulo and Tomé, 2017), pests and diseases (Branco et al., 2014, Tiberi et al., 2016) or severe droughts (Acácio et al., 2017). This has been reflected in an overall decline from 28.0 to 26.5% of the tree crown cover between 1996 and 2005 (Paulo et al., 2016) and of ca. 12% of cork oak forest area between 2005 and 2015 (Aubard et al., 2019). Cork oak forests are human-shaped ecosystems that have to be managed to be sustainable (e. g. Bugalho et al., 2011), particularly to maintain an open structure and diverse mosaic of shrub and grassland understorey species. As such, and even more in the context of climate change, it is critical to understand how the management of the shrub layer affects the water status and growth of cork oak trees.

Most studies addressing forest management effects were conducted on mature cork oak stands and few on juvenile cork oak stands. During this early stage, thinning may be performed in dense stands to reduce initial density to 400-600 trees ha⁻¹ (Pereira and Tomé, 2004, Cañellas et al., 2017). Thinning is conducted to reduce intra-specific competition and increase tree survival and growth (see also Fonseca et al., 2017, on self-thinning). Nevertheless, Faias et al. (2020) observed no intraspecific competition, except a slight effect on tree diameter growth, on juvenile stands with densities between 103 and 943 trees ha⁻¹. However, inter-specific competition with understorey shrubs may have a critical role in the survival and growth of juvenile cork oak trees. Juvenile trees may be susceptible to shrub competition for soil water which may be further aggravated by the occurrence of droughts. Nevertheless, to our knowledge, there are no studies on the effect of shrub encroachment on juvenile cork oak functioning and growth. The main objective of this study was to investigate the effect of shrub clearing on juvenile tree water relations and growth in two contrasting meteorological years. We studied two juvenile cork oak stands with different tree densities, in two different locations, and, along two years, including a drought year. More specifically, we compared the understorey vegetation (i.e., species composition, shrub cover and biomass) and the tree

water status and growth biometrics before and after shrub clearing in each location. We hypothesized that shrub clearing would positively affect tree water status and growth, independently of the location and tree densities.

2. Materials and methods

2.1. Study areas

We conducted this study in cork oak stands on two distinct private owned properties, 60 km apart from each other, in South-Center Portugal that were planted in 1998 and 1999 with seedlings from local nurseries. The first site (Estremoz site), located near Estremoz (38°50.510′ N 7°35.532′ W), was characterized by a site index of 14.9 m for a tree base age of 80 years, calculated using a dominant height growth model (Sánchez-González et al., 2005). Soils, 1.20 m deep, were classified as Luvisols (WRB, 2015), characterized by a silty clay loam texture above a schist horizon (Paulo et al., 2015). Trees were planted in 1999, with a tree density of 680 trees ha⁻¹ and spacing of 2.5 m × 4 m. No thinning or pruning was performed. Understorey vegetation consisted of a mix of shrub species dominated by *Cistus ladanifer* L., *Cistus salviifolius* L. and *Retama sphaerocarpa* (L.) Boiss., interspersed with annual herbaceous plant species.

The second site (Montargil site), located near Montargil (39°3.242′ N, 8°10.588′ W), was characterized by a site index of 17.7 m for a tree base age of 80 years (Sánchez-González et al., 2005), reflecting higher site productivity than the Estremoz site. Soils, 1.92 m deep, were classified as Arenosols, characterized by a sandy texture, with high permeability (Paulo et al., 2015). Tree plantation was established in 1998, with an initial tree density of 690 trees ha⁻¹. Thinning, performed in 2014, reduced tree density to 420 trees ha⁻¹, with a spacing of 3 m × 7 m. Selective tree formation pruning, which did not affect tree growth (Paulo and Faias, 2020), was also carried out in 2014. Understorey vegetation consisted of a mix of shrub species (e.g. *Cistus crispus* L., *Cistus salviifolius* L., *Stauracanthus genistoides* (Brot.) Samp., *Ulex airensis* Esp. Santo, Cubas, Lousã, C.Pardo & J.C.Costa and Halimium ocymoides L. Willk), interspersed with annual herbaceous plant species.

2.2. Climate data

Precipitation and temperature data for the study period were gathered from the nearest meteorological stations (Vila Viçosa (21 M/01UG) and Évora for Estremoz site, and Chouto and Santarém (18G/01G) for Montargil site, available at the National Water Resources Network -SNIRH; https://snirh.apambiente.pt/, assessed on January 2020 - and Portuguese Institute for Sea and Atmosphere - https://ipma.pt, assessed on November 2020, respectively).

2.3. Experimental design and sampling

In April 2017, at each site (Montargil and Estremoz), two blocks, each with two plots, were randomly established. The two plots were approximately 1000 m^2 and 1500 m^2 in Estremoz and Montargil, respectively. Within each plot a total of eight trees were randomly selected for conducting the tree measurements described in the next sections, totalizing 32 trees per site. Two treatments were considered: shrub-removal and control (no shrubs were removed). Prior to the establishment of the shrub-removal treatment, we conducted baseline measurements in the selected trees (see below). In April/May 2018, we cut all shrubs to the ground level in one of the two plots, in each block and in each site.

2.4. Shrub layer

For characterizing the understorey shrub vegetation, we sampled 16 quadrats of 1 m \times 1 m (Higgins et al., 2012), systematically distributed

within the plots, where shrub vegetation was left. We visually estimated total shrub cover (%) and counted all shrubs within each quadrat for estimating the relative frequency of all species.

Due to the complex branching architecture of some shrub species (e. g. *Ulex airensis, Retama sphaerocarpa*), that did not allow a clear stem diameter measurement, total plant height was used for estimating biomass of all shrub species using biomass–height allometric equations (Supporting Information Appendices S1). First, we measured the maximum height of all shrubs within each of the 16 quadrats. Secondly, we randomly selected 10 plants of each species (four and six species, in Estremoz and Montargil, respectively) from areas adjacent to the plots according to maximum height classes. These shrubs were cut to ground level, oven-dried at 60 °C to constant mass and weighed.

2.5. Tree water status

Predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential were measured in two to three leaves per tree with a Scholander pressure chamber (PMS 1000, PMS Instrument Co., Corvallis, Oregon, USA). Leaf water potentials were measured on-site, immediately after leaves being cut from the top south canopy of the tree. Predawn leaf water potential was measured before sunrise and midday leaf water potential at solar noon. In December 2017 and January 2018, before clearing the shrubs (baseline measurement), we measured the predawn leaf water potential in a subset of 3–5 trees per plot. In June, August and November of 2018 and March, May and July 2019 both predawn and midday leaf water potential were measured in all eight trees per plot.

2.6. Tree biometry

Total tree height (h) and diameter at breast height (d) were measured in September/October 2017 (baseline measurement). Total tree height of the trees was again measured in July 2018 and July 2019. Leaf area index (LAI) was calculated for each tree, after 2D tree crown image acquisition, using a digital camera Nikon Coolpix P7800 with a 12.2megapixel resolution full-frame image sensor. Images were analyzed with WinsCanopy software (Régent Instruments Inc., Quebec City, QC), following the method from Lindsey and Bassuk (1992) and modified by McPherson and Peper (1998). LAI was measured in July 2018 and July 2019.

The relative growth rate (RGR) is an efficient measure of the tree responses to abiotic factors such as water or nutrient availability, and biotic factors such as intra- or inter-specific competition (Poorter, 1989). RGR is the plant increase in size for a given time interval which allows to compare individuals that differ in size (Pérez-Harguindeguy et al., 2013).

The RGR was annually computed for tree height between 2017 and 2019 (i.e., RGR₂₀₁₇₋₂₀₁₈ and RGR₂₀₁₈₋₂₀₁₉). RGR in LAI was only computed between 2018 and 2019:

$$RGR = \frac{(lnX2 - lnX1)}{(t2 - t1)}$$

X2 is tree height or LAI at time t2, and X1 is tree height or LAI at time t1. t2-t1 is the number of days between measurements.

2.7. Soil nutrient and moisture contents

In January 2017, in each plot, we randomly sampled five soil samples of 10 cm depth, one meter away from the tree lines, totalizing 20 samples per site. In April 2019, we repeated the soil sampling in the same locations. Samples were air-dried and analyzed for organic carbon (C) (Wet Combustion Method), total nitrogen (N) (Kjeldahl method), phosphorus (P) and potassium (K) (Egner-Riehm method) contents (Sparks et al., 1996).

Moreover, at each date of leaf water potential measurements, we also

estimated gravimetric soil moisture at 0–10 cm and 10–20 cm. We collected six soil samples at each depth (i.e. total of 12 samples) per plot. Samples were weighted and oven-dried at 60 $^{\circ}$ C to constant weight and weighted again to calculate soil moisture.

2.8. Data analysis

All statistical analyses were conducted in SPSS® software package (PASW Statistics, v. 24.0.00, 2019).

2.8.1. Total shrub cover and biomass

The relationship between biomass (i.e. dry weight) and height of shrub species was modeled using non-linear regression analyses, applying a power function, as it is widely used for woody plants (e.g., Paul et al., 2019). For comparing total shrub cover and biomass between sites, we used Generalized Linear Mixed Models (GLMMs, Jiang 2007), specifying a normal error and identity-link function. Site was set as fixed effect while plot was set as random factor.

2.8.2. Assessing pre-treatment differences between plots and site

GLMM procedure, specifying a normal error and identity-link function, was used for analyzing the following data. For comparing tree height and diameter, leaf water potential and soil nutrient contents between plots before shrub removal in each site in 2017, and also for RGR₂₀₁₇₋₂₀₁₈, the plot was set as fixed effects, while block was set as random. For comparing the sites, we specified site as fixed effect and block (nested within site) as random factor.

2.8.3. Assessing treatment effects

GLMM procedure, specifying a normal error and identity-link function, was also used for assessing treatment effects. For leaf water potentials and soil moisture content during the study period (along seasons), which were analyzed with repeated measurements, we specified treatment (i.e. with or without shrub) as a fixed effect, as well as interaction between site and treatment. Site and plot (nested within site) were set as a random factor. A significant interaction between site and treatment would indicate site-specific inconsistency in the effect of shrub removal on above-mentioned parameters. For comparing the sites, we set site as fixed effect and site and plot (nested within block) as random factor.

As we were especially interested in assessing how shrub removal affected tree growth and soil nutrient contents in each site, we performed the following analyses per site. For analyzing RGR₂₀₁₈₋₂₀₁₉, both in height and in LAI, and soil nutrient contents in 2019 in each site, we specified treatment (i.e. with or without shrub) as fixed effects and block as random factor. We also compared overall RGR₂₀₁₇₋₂₀₁₈ and RGR₂₀₁₈₋₂₀₁₉ in height in each site specifying year as fixed effect. Finally, for analyzing RGR₂₀₁₈₋₂₀₁₉ in height and in LAI, between sites, we specified site as fixed effect.

The latter model (i.e. average effect of shrub removal on water potential and soil moisture content) is

$$Y_{ijk} = X\alpha + (X^*J)\beta + K\gamma + W\zeta + \varepsilon_{ijk}$$

where Y_{ijk} is the studied variable for the tree i within the block j and site k; X is the predictor variable (i.e. fixed effect: shrub removal treatment), α the coefficient of the fixed effect, X*J is the interaction effect (between fixed effect X and block K) and β is the coefficient of the interaction effect. K and W (plot (nested within block)) are the random effects with corresponding coefficient γ and ζ . ϵ the residual.

3. Results

3.1. Climate data

Long-term mean annual precipitation (1989-2010) for the

hydrological year (Oct-Set) was 628.03 mm, with a coefficient of variation of 38.5%, and 654.11 mm, with a coefficient of variation of 31.2% in Estremoz and Montargil sites, respectively.

In the hydrological year of 2017–18, the annual precipitation was 92.81% (i.e., 582.9 mm) and 92.23% (i.e., 603.3 mm) of the long-term mean annual precipitation in Estremoz and Montargil sites, respectively (Fig. 1). Maximum precipitation was observed in March 2018 in both sites. However, a strong rain deficit was observed in Montargil during the autumn (19.85% of the long-term cumulative precipitation, between October and December), and again in May (47.52% of the long-term precipitation).

In the hydrological year of 2018-19, the precipitation was much below the long-term precipitation (1989-2010), with only 26.27% (171.2 mm) in Estremoz and 78.56% (513.9 mm) of the long-term precipitation, in Montargil. In Estremoz, the overall strong precipitation deficit reflected the dramatic rain deficit during the winter (16.29% of the long-term cumulative precipitation from January to March) and during the spring (7.47% of the long-term cumulative precipitation from April to June). Additionally, a very strong deficit was observed between May and July (1.76% of the long-term cumulative precipitation), a crucial period for tree growth. In Montargil, the precipitation pattern was similar to the long-term distribution, with a mean of 76.43% of the long-term cumulative precipitation for the three main seasons (i.e. autumn, winter and spring). The maximum precipitation was observed in November 2018 (i.e. 193.5 mm) and a deficit, albeit lower than in Estremoz site, was observed between May and July (i.e. 48.49% of the long-term cumulative precipitation) (Fig. 1).

In Estremoz, mean annual temperature was 15.9 $^{\circ}$ C, with a maximum of 31.1 $^{\circ}$ C in July and a minimum of 5.8 $^{\circ}$ C in January. In Montargil, mean annual temperature was 17.2 $^{\circ}$ C, with a maximum of 31.1 $^{\circ}$ C in August and a minimum of 5.8 $^{\circ}$ C in January (period 1981–2010).

In 2017–18, the temperature deviation from the long-term mean was similar in both sites for annual mean temperature (i.e. < 0.5 °C). Mean maximum annual temperature deviation was higher in Estremoz, reflecting higher deviation during autumn and summer (i.e. deviation > 3.5 °C). Minimum temperature deviation was higher during all seasons in Estremoz, with minimum annual temperature deviation of -1.53 °C, while in Montargil such annual deviation was marginal (i.e.-0.08 °C).

(Fig. 2).

In 2018–19, the mean annual temperature deviation was very low in both sites (i.e. +0.21 and +0.43 °C in Estremoz and Montargil, respectively). However, in Estremoz, a high deviation was observed for minimum and maximum temperatures during all seasons (i.e. annual deviation of -2.07 °C and +2.27 °C for minimum and maximum temperatures respectively), while in Montargil annual deviation of minimum temperature was only -0.22 °C and annual deviation of maximum temperature was +1.18 °C (Fig. 2).

3.2. Shrub layer

In Estremoz, *C. ladanifer* dominated the shrub community cover (>70%), while in Montargil the shrub community was more species-rich and even (Table 1). Overall, mean shrub cover, before the application of the shrub removal treatment, was not significantly different between sites (P > 0.5), with 41.72 ± 5.83 % and 45.06 ± 5.43 % in Montargil and Estremoz, respectively. However, shrub biomass was significantly higher in Estremoz (8720.25 \pm 1534.08 kg/ha) than in Montargil (2692.72 \pm 411.50 kg/ha) ($F_{1,62} = 14.401$, P < 0.001).

3.3. Leaf water potential

In December 2017/January 2018 (pre-treatment), there were no significant differences in the predawn leaf water potential between trees from different plots (P > 0.05; data not shown) in each site. However, predawn leaf water potential was already significantly lower in Estremoz than in Montargil (Estremoz: mean \pm SE: -0.46 ± 0.02 MPa; Montargil: -0.20 ± 0.02 MPa; F_{1,38} = 22.013, P < 0.001), indicating the lower soil water availability in Estremoz.

During 2018–2019, a seasonal trend of leaf water potential occurred. In winter, leaf water potential was high, indicating the higher tree water status. During spring, leaf water potentials decreased, reaching a minimum during the summer, corresponding to the lowest soil water availability and to severe tree hydric stress (Fig. 3).

Overall, the shrub removal treatment significantly affected the water status of the trees. Shrub removal significantly and positively affected both predawn ($F_{1,378} = 11.95$, P = 0.001) and midday ($F_{1,370} = 5.34$, P = 0.021) leaf water potential. This was consistent among sites, as



Fig. 1. Monthly precipitation and long-term (1989–2010) mean monthly precipitation for Estremoz and Montargil sites (data from the nearest stations, https://snirh.apambiente.pt).



Fig. 2. Seasonal deviations from the long-term (1981–2010) for minimum (Tmin), mean (Tmean) and maximum (Tmax) average temperature in Estremoz and Montargil sites (data from the nearest stations, https://ipma.pt).

Table 1

Total shrub layer cover (%, Mean \pm SE) and biomass (Kg/ha, Mean \pm SE) and species composition (relative frequency, %) in Estremoz and Montargil sites.

Estremoz	block 1	block 2
Cover (%)	45.44 (±8.62)	44.69 (±6.88)
Biomass (kg/ha)	8705.66	8734.85
	(± 2438.19)	(±1944.90)
Relative species frequency (%)		
Cistus ladanifer (L.)	78.57	66.67
Retama sphaerocarpa (L.) Boiss.	21.43	9.52
Cistus salvifolius (L.)	0	19.05
Cistus crispus (L.)	0	4.76
Montargil	block 1	block 2
Cover (%)	37.88 (±8.23)	45.56 (±8.42)
Cover (%) Biomass (kg/ha)	37.88 (±8.23) 2509.28	45.56 (±8.42) 2876.16
Cover (%) Biomass (kg/ha)	37.88 (±8.23) 2509.28 (±619.11)	45.56 (±8.42) 2876.16 (±558.69)
Cover (%) Biomass (kg/ha) Relative species frequency (%)	37.88 (±8.23) 2509.28 (±619.11)	45.56 (±8.42) 2876.16 (±558.69)
Cover (%) Biomass (kg/ha) Relative species frequency (%) Stauracanthus genistoides (Brot) Samp.	37.88 (±8.23) 2509.28 (±619.11) 30.43	45.56 (±8.42) 2876.16 (±558.69) 36.00
Cover (%) Biomass (kg/ha) Relative species frequency (%) Stauracanthus genistoides (Brot) Samp. Cistus salvifolius (L.)	37.88 (±8.23) 2509.28 (±619.11) 30.43 30.43	$\begin{array}{c} 45.56 \ (\pm 8.42) \\ 2876.16 \\ (\pm 558.69) \\ 36.00 \\ 16.00 \end{array}$
Cover (%) Biomass (kg/ha) Relative species frequency (%) Stauracanthus genistoides (Brot) Samp. Cistus salvifolius (L.) Cistus crispus (L.)	$\begin{array}{c} 37.88 \ (\pm 8.23) \\ 2509.28 \\ (\pm 619.11) \\ 30.43 \\ 30.43 \\ 21.74 \end{array}$	$\begin{array}{c} 45.56 (\pm 8.42) \\ 2876.16 \\ (\pm 558.69) \\ 36.00 \\ 16.00 \\ 20.00 \end{array}$
Cover (%) Biomass (kg/ha) Relative species frequency (%) Stauracanthus genistoides (Brot) Samp. Cistus salvifolius (L.) Cistus crispus (L.) Ulex arvensis Esp.Santo, Cubas, Lousã, C.	$\begin{array}{c} 37.88 \ (\pm 8.23) \\ 2509.28 \\ (\pm 619.11) \\ 30.43 \\ 30.43 \\ 21.74 \\ 8.70 \end{array}$	$\begin{array}{c} 45.56 (\pm 8.42) \\ 2876.16 \\ (\pm 558.69) \\ \hline \\ 36.00 \\ 16.00 \\ 20.00 \\ 16.00 \\ \end{array}$
Cover (%) Biomass (kg/ha) Relative species frequency (%) Stauracanthus genistoides (Brot) Samp. Cistus salvifolius (L.) Cistus crispus (L.) Ulex arvensis Esp.Santo, Cubas, Lousã, C. Pardo & J.C. Costa	$\begin{array}{c} 37.88 \ (\pm 8.23) \\ 2509.28 \\ (\pm 619.11) \\ 30.43 \\ 30.43 \\ 21.74 \\ 8.70 \end{array}$	$\begin{array}{c} 45.56 (\pm 8.42) \\ 2876.16 \\ (\pm 558.69) \\ 36.00 \\ 16.00 \\ 20.00 \\ 16.00 \end{array}$
Cover (%) Biomass (kg/ha) Relative species frequency (%) Stauracanthus genistoides (Brot) Samp. Cistus salvifolius (L.) Cistus crispus (L.) Ulex arvensis Esp.Santo, Cubas, Lousã, C. Pardo & J.C. Costa Pterospartum tridentatum (L.) Willk.	$\begin{array}{c} 37.88 \ (\pm 8.23) \\ 2509.28 \\ (\pm 619.11) \\ 30.43 \\ 30.43 \\ 21.74 \\ 8.70 \\ 4.35 \end{array}$	$\begin{array}{c} 45.56 (\pm 8.42) \\ 2876.16 \\ (\pm 558.69) \\ 36.00 \\ 16.00 \\ 20.00 \\ 16.00 \\ 12.00 \end{array}$

indicated by the non-significant interaction between site and treatment (P > 0.1) (Fig. 3).

Significant differences in predawn leaf water potential could be already observed in June 2018 in Estremoz, one month after the shrubs were cut (-2.37 ± 0.06 vs. -2.16 ± 0.06 MPa, in plots with shrubs and plots where shrubs were removed, respectively), while in Montargil such differences were only observed in August 2018. Overall, leaf water potentials were lower in July 2019 than in August 2018 in both sites, attesting to the drier 2018–19 hydrological year. In accordance, predawn leaf water potentials in the plots where shrubs were present were also significantly lower in July 2019 in both sites (and reaching the lowest values measured along the study period). Actually, in Estremoz the differences between treatments were already clear and significant in

May 2019. The midday leaf water potential had similar responses between treatments, sites and hydrological years.

Leaf water potentials were significantly lower, throughout the study period, in Estremoz than in Montargil (Predawn: $F_{1,380} = 41.13$, P < 1000.001; Midday: $F_{1,372} = 9.62$, P = 0.002). In Estremoz, predawn leaf water potential varied from -0.14 ± 0.01 MPa in June 2018 (i.e. without shrubs) to -2.07 ± 0.03 MPa in July 2019 (i.e. with shrubs), while in Montargil such large variations were not observed, with the highest value of -0.14 ± 0.01 MPa in November 2018 (i.e. without shrubs) and the lowest of only -0.55 ± 0.04 MPa in July 2019 (i.e. with shrubs). Overall, predawn leaf water potential of trees in Estremoz was 23% lower than Montargil in March 2019 (–0.21 \pm 0.01 MPa vs. –0.17 \pm 0.01 MPa in Estremoz and Montargil, respectively), almost 500 % in August 2018 (-1.51 ± 0.09 MPa vs. -0.25 ± 0.02 MPa in Estremoz and Montargil, respectively) and 270% in July 2019 (-1.75 ± 0.07 MPa vs. -0.47 ± 0.03 MPa in Estremoz and Montargil, respectively). For midday leaf water potential these variations were less marked with a maximum variation of 28% in November 2018 (–1.15 \pm 0.04 MPa vs. –0.89 \pm 0.02 MPa in Estremoz and Montargil, respectively) and a minimum of 4.6% in July 2019 (-2.36 \pm 0.05 MPa vs. -2.26 \pm 0.05 MPa in Estremoz and Montargil, respectively).

3.4. Tree biometry

In 2017, prior to the shrub removal, there were no significant differences in tree height and diameter between plots, in both sites (P >0.05). However, tree height and diameter were significantly higher in Montargil than in Estremoz (Table 2), as expected, due to their site productivity characteristics.

Tree height relative growth rates between 2017 and 2018 (RGR₂₀₁₇. 2018) were not significantly different between plots in both sites (P > 0.05), even after two months of shrub removal treatment. However, RGR₂₀₁₇₋₂₀₁₈ in height was significantly higher in Estremoz than in Montargil ($F_{1,62} = 81.10$, P < 0.001) (Fig. 4).

After shrub clearing, in Estremoz, tree $RGR_{2018-2019}$ in height and in LAI were significantly higher (height: $F_{1,30} = 6.098$, P = 0.019; LAI: $F_{1,30} = 18.27$, P < 0.001) in the plots where shrub understorey was removed (Figs. 4 and 5). However, in Montargil, shrub understorey



Fig. 3. Tree leaf water potential (MPa) from June 2018 to July 2019 at predawn (Ψ_{pd}) and midday (Ψ_{md}) in Estremoz and Montargil sites (trees with shrub understorey (black line) and trees where shrubs were cut (dotted line), Mean \pm SE).

Table 2

Tree height and diameter in Estremoz and Montargil sites at the experiment installation in 2017 (mean \pm SE).

	Height (m)	Diameter (cm)
Estremoz Montargil Statistics	$\begin{array}{l} 3.60 \pm 0.09 \\ 6.60 \pm 0.18 \\ F_{1,62} = 115.63, \mbox{$P < 0.001$} \end{array}$	$\begin{array}{l} 8.97 \pm 0.60 \\ 14.70 \pm 0.57 \\ F_{1,52} = 28.19, \textit{P} < 0.001 \end{array}$

removal did not have a significant effect on tree RGR₂₀₁₈₋₂₀₁₉ in height ($F_{1,28} = 0.12$, P = 0.73) or in LAI ($F_{1,28} = 0.058$, P = 0.81) (Figs. 4 and 5). Overall, in Estremoz, RGR₂₀₁₈₋₂₀₁₉ in height was significantly lower than RGR₂₀₁₇₋₂₀₁₈ ($F_{1,62} = 14.298$, P < 0.001) while in Montargil, RGR₂₀₁₈₋₂₀₁₉ in height was significantly higher than RGR₂₀₁₇₋₂₀₁₈ ($F_{1,62} = 32.295$, P < 0.001). Moreover, there were no significant differences between sites in overall RGR₂₀₁₈₋₂₀₁₉ in height ($F_{1.62} = 0.018$, P = 0.894).

3.5. Soil moisture and nutrient contents

At the start of the experiment, in 2017, there were no significant differences in soil nutrient concentrations between plots in each site (P > 0.05). However, P concentrations were significantly higher and K concentration lower in Montargil than in Estremoz (Table 3). No differences in C and N concentrations were found between sites (Table 3).

In 2019, shrub understorey removal only had a significant negative effect on P concentration in Montargil (Mean \pm SE: 9.77 \pm 1.00 vs. 6.76



Fig. 4. Relative Growth Rate (RGR) in height (mm/day) in A) Estremoz and in B) Montargil, for 2017–2018 and 2018–2019 periods, in trees with shrub understorey and in trees where shrubs were cut (Mean \pm SE; *: P < 0.05 and ns: not significant [P > 0.05]).



Fig. 5. Relative Growth Rate (RGR) in LAI $(m^2/m^2/day)$ in Estremoz and Montargil sites, between 2018 and 2019 in trees with shrub understorey and in trees where shrubs were cut (Mean \pm SE; *: *P* < 0.05 and ns: not significant [P > 0.05]).

 \pm 0.36 g P / kg dry soil in plots with and without shrubs, respectively, F_{1,18} = 9.38, P = 0.007) while it had no significant effect on C, N and K soil concentrations. In Estremoz, there were no significant differences in the nutrient concentrations between treatments (data not shown). Differences between sites remained the same as in 2017 except for C content which was higher in Montargil site than in Estremoz site (Table 3).

Table 3

Soil nutrient contents: organic carbon (C), total nitrogen (N), phosphorus (P) and potassium (K) in 2017 and 2019 (Overall mean \pm SE). Statistics are reported for comparing Montargil and Estremoz sites (GLMM).

Year	site	C (g/kg dry soil)	N (g/kg dry soil)	P (g/kg dry soil)	K (g/kg dry soil)
2017	Montargil	$\begin{array}{c} 15.64 \pm \\ 1.34 \end{array}$	$\begin{array}{c} \textbf{0.88} \pm \\ \textbf{0.06} \end{array}$	$\textbf{9.84} \pm \textbf{0.90}$	52.54 ± 2.74
	Estremoz	$\begin{array}{c} 10.83 \pm \\ 0.94 \end{array}$	$\begin{array}{c} \textbf{0.93} \pm \\ \textbf{0.07} \end{array}$	$\textbf{2.61} \pm \textbf{0.23}$	$\begin{array}{c} 102.10 \pm \\ 6.28 \end{array}$
	Statictics	$F_{1,38} =$ 3.15, <i>P</i> = 0.084	$F_{1,38} = 0.23, P = 0.635$	$F_{1,38} =$ 18.42, <i>P</i> < 0.001	F _{1.38} = 52.30, <i>P</i> < 0.001
2019	Montargil	$\begin{array}{c} 15.53 \pm \\ 0.88 \end{array}$	$\begin{array}{c} \textbf{0.85} \pm \\ \textbf{0.06} \end{array}$	$\textbf{8.27} \pm \textbf{0.62}$	42.39 ± 1.50
	Estremoz	$\begin{array}{c} 11.57 \pm \\ 0.65 \end{array}$	$\begin{array}{c} 0.92 \pm \\ 0.03 \end{array}$	$\textbf{1.84} \pm \textbf{0.14}$	$\begin{array}{c} 110.01 \pm \\ 8.86 \end{array}$
	Statictics	$F_{1,38} =$ 7.21, <i>P</i> = 0.011	$F_{1,38} = 0.99, P = 0.324$	F _{1,38} = 41.64, <i>P</i> < 0.001	F _{1,38} = 52.24, <i>P</i> < 0.001

Shrub understorey removal did not have a significant effect on soil moisture at both depths (P > 0.05), which remained very low over the study period (Supporting Information Appendices S2). This was consistent in both sites as indicated by a non-significant interaction between treatment and sites. No differences were found between sites. However overall soil moisture at 20 cm depth was higher than in 10 cm depth ($F_{1,448} = 101.14$, P < 0.001). The maximum soil moisture was measured in November 2018 (11.75 ± 0.38 % and 10.75 ± 0.38 % at 10 cm and 20 cm respectively) while the minimum was found in July 2019 (0.67 ± 0.06 % and 1.54 ± 0.07 % at 10 cm and 20 cm respectively).

4. Discussion

In this study, we showed that shrub understorey clearing had a positive effect on the tree leaf water potential in both sites, regardless of tree stand density. However, during this study, the shrub understorey removal only had a positive effect on the relative growth rates in height and in LAI of juvenile cork oak trees in Estremoz.

At the start of the experiment, tree height and diameter were already higher in Montargil than in Estremoz in 2017, as a result of distinct growing conditions as attested by the higher site index in Montargil site. The difference in tree growth between the two sites may be further explained by several factors such as abiotic factors (e.g. soil attributes and precipitation), stand management and understorey composition. In our study, the deeper soil depth in Montargil, as compared to Estremoz (i.e. 1.92 vs. 1.20 m, respectively) may have favored tree root development and possibly access to deeper soil water resources. Indeed, Paulo et al. (2015), showed a positive correlation between soil depth and site index, reflecting a higher productivity of cork oaks. These authors also showed that cork oaks had higher productivity in arenosols, the type of soil that exists in Montargil, that presents a higher soil hydraulic conductivity and soil water retention capacity than in luvisols, present in Estremoz. Also, the higher P concentrations observed in Montargil, may have also contributed to the higher relative growth rates of the trees in this site, as it has been shown for holm oak (Quercus ilex subsp. rotundifolia) (Sardans et al., 2004). Overall, these differences in soil attributes may play a crucial role in Mediterranean ecosystems where soil water availability is one of the most limiting resources for tree growth, especially in the context of severe drought events. In Estremoz, a high mean annual precipitation deficit was observed since the beginning of the stand plantation, with a mean annual deviation from the long-term mean of -102.3 mm (i.e. 16 %, for the period 1998-2017). In Montargil, such mean annual deviation was only -60.9 mm (i.e. 9%, for the period 1997-2017). However, the relative growth rate of the trees in 2017-2018 in Montargil was lower than in Estremoz. This difference may be explained by the strong rain deficit observed in the autumn, with

only 19.85 % of the long-term cumulative precipitation and in May with a reduction of 52.48 % relative to the long-term precipitation in Montargil. Such precipitation deficits were not observed in Estremoz.

Furthermore, while tree density was similar in both sites at the time of planting (i.e. 680 and 690 tree/ha in Estremoz and Montargil, respectively), thinning, which occurred only in the Montargil site in 2014, reduced tree density by 40%, up to 420 trees ha⁻¹. This density reduction may have favored tree growth, by reducing intra-specific competition. Several studies showed that thinning mitigates water limitation effects on trees, increasing leaf production (Gavinet et al., 2019, Gavinet et al., 2020) and tree growth (Wang et al., 2019). del Campo et al. (2019) showed, during a 4-year study period, dominated by dry years, that LAI and tree transpiration were positively affected by tree thinning in a water-limited holm oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.) Mediterranean forest. Also, Cabon et al. (2018) showed that thinning in a holm oak forest increased tree growth by delaying growth cessation due to water limitation.

The high shrub biomass and dominance by *C. ladanifer* in Estremoz most probably have also contributed to the overall lower tree height and diameter in Estremoz as compared to Montargil. In Estremoz, shrub biomass was more than three times higher than in Montargil, which may have led to a higher inter-specific competition with trees for shallow water resources. Moreover, *C. ladanifer*, which contributed to 73% of shrub cover in Estremoz, is highly competitive for water and has been shown to decrease adult cork oak water availability in a similar system (Caldeira et al., 2015).

Shrub encroachment into tree stands may have a high competitive effect on trees by decreasing their water availability and use. Rascher et al. (2011), for example, showed that Acacia longifolia, invading a pine forest (Pinus pinaster), significantly contributed to the transpiration of the ecosystem and drastically reduced water availability for trees and consequent tree transpiration. Water absorption by the roots and tree transpiration is critical to sustain photosynthesis and tree growth (Otieno et al., 2006, Lempereur et al., 2015). Measures, such as predawn leaf water potential that reflect soil water availability for trees, are thus essential. Indeed, Pereira et al (1987) considered the predawn leaf water potential of -1.0 MPa, which corresponds to severe water stress, as a threshold that arrests growth in cork oak. Lempereur et al. (2015) suggested a threshold of -1.1 MPa for holm oak trees for growth cessation. Also, in juvenile cork oak trees, reduced water availability led to a 48% reduction of the growing season length, with consequent reduction of the total tree growth (Besson et al., 2014, Lobo-do-Vale et al., 2019). In our study, trees with lower predawn leaf water potentials were the ones with a higher reduction of height and LAI growth. Indeed, in Estremoz, predawn leaf water potential was lower than -1.0 MPa during both summers (2018 and 2019), particularly in the trees growing in the plots with the shrub understorey, which simultaneously had the lowest relative growth rates. Trees growing in competition with shrubs had lower predawn leaf water potentials over long periods that reduced height and LAI growth comparing to trees growing with no shrubs. However, in Montargil the lowest predawn leaf water potential did not surpass -0.55 MPa (July 2019, trees in the plot with understorey), not influencing tree growth after one year of treatment. Still, although no clear significant differences could be observed in tree relative growth rates in Montargil, there was a trend for higher RGR in height, during 2018-2019, in trees released from shrub competition, probably reflecting the better water status of these trees. These findings are of particular interest in the context of climate change scenarios which predict more intense and frequent drought events (Dai, 2013, Lionello and Scarascia, 2018). Droughts severely affect cork oak tree water status (Caldeira et al., 2015, Lobo-do-Vale et al., 2019, Haberstroh et al., 2021) and cork growth (Caritat et al., 1996; Oliveira et al., 2016). In 2018–19, the precipitation deficit was very high in Estremoz, with only 27.26% of the long-term mean precipitation and a dramatic rain deficit during the winter (i.e. 30.9 mm from January to March), while in Montargil, such precipitation deficit was not observed, with, nonetheless, only 78.56%

of the long-term mean precipitation but without a seasonal trend. Indeed, Costa-e-Silva et al (2015) showed a 63% reduction of annual tree diameter growth and a 9% reduction of leaf area in cork oaks after a very dry winter. In our study, adding to the winter drought, spring precipitation (April-June) was only 9.3 mm in Estremoz, which may have certainly further aggravated tree water stress. Also, in Estremoz, the high temperature deviation from the long-term mean, observed for minimum and maximum temperatures throughout the year (i.e., annual deviation of -2.07 °C and +2.27 °C for minimum and maximum temperatures, respectively), may have contribute to lower growth during colder months (Aranda et al., 2005) and during hotter months by increasing water demand through evapotranspiration (Caldeira et al., 2015). Although, just 60 km away, the Montargil site suffered a mild drought which affected tree water potential but did not allow us to clearly understand if a synergy between drought and shrub encroachment could affect tree growth. Nevertheless, the low water availability for tree growth during the drought year in Estremoz was further reduced by shrub competition as shown by the much lower predawn leaf water potential values reached by these trees in July 2019.

5. Conclusion

The results of this study showed that shrub clearing directly increased tree water status of cork oaks. They also show that this management practice, in the short term, results in different contributions for tree growth according to site characteristics. While clearing directly contributed to increased tree growth in the site characterized by lower productivity and in a drought year, it did not have the same clear effect on the site with higher productivity. These results are of critical importance for the management and resilience of juvenile plantations under increasing drought conditions. Particularly, they demonstrate the importance of knowing local forest conditions for adjustment of the management objectives. Nevertheless, further research is needed to understand how complementary management strategies, such as thinning may increase tree resistance and resilience to drought and maintain forest sustainability.

CRediT authorship contribution statement

X. Lecomte: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. J.A. Paulo: Conceptualization, Writing – review & editing. M. Tomé: Conceptualization, Supervision, Funding acquisition, Writing – review & editing. S. Veloso: Investigation, Writing – review & editing. P.N. Firmino: Investigation, Writing – review & editing. S.P. Faias: Investigation, Writing – review & editing. M.C. Caldeira: Conceptualization, Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Margarida Tome reports a relationship with Forest Ecology and management journal Editorial Board that includes: board membership.

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