



Seasonal adjustment of primary and secondary growth in maritime pine under simulated climatic changes

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Abstract

- **Key message** Reducing irrigation by up to 75% in spring decreased primary and secondary growth in *Pinus pinaster* Aiton saplings, whereas an extra irrigation in September prompted secondary growth, confirming the high plasticity of xylogenesis in this species.
- **Context** Water availability is the main limiting factor for plant growth and forest productivity in drought-prone environments, such as the Mediterranean region. Future scenarios for this region predict an increase in spring drought and in autumn precipitation. Understanding how tree growth responds to these conditions is imperative to anticipate forest productivity shifts under future climate change scenarios.
- **Aims** We simulated the expected climatic conditions for the Mediterranean region in order to study the response of primary and secondary growth in maritime pine (*Pinus pinaster* Aiton) saplings.
- **Methods** In February 2017, 2-year-old maritime pine saplings ($n = 512$) were subjected to a water manipulation experiment in a tunnel greenhouse. Saplings were randomly divided into four groups: control, exclusion, irrigation and exclusion—irrigation. Between May and August, saplings in the exclusion and exclusion—irrigation groups received 50 to 75% less water than saplings in the control and irrigation groups. In September, saplings in the irrigation groups received an extra irrigation. From February 2017 to February 2018, primary (height) and secondary growth (diameter and wood formation) were monitored. Seasonal dynamics of cambial activity and wood formation were monitored monthly through destructive sampling.
- **Results** Primary and secondary growth were reduced in exclusion saplings. The extra irrigation in September triggered the production of tracheids with wider lumen diameter at the end of the tree ring.
- **Conclusion** Maritime pine saplings survived an intense spring drought, despite primary and secondary growth reduction. The extra irrigation in September triggered a rapid increase in cambial activity, demonstrating the high plasticity of xylogenesis in this species.

Keywords Cambial activity · Climate change · Drought · Wood anatomy · Xylogenesis

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Contribution of the co-authors FC and JV designed the study and proposed the hypothesis tested; MM and JV analyzed the samples; FC analyzed the data; JV and FC wrote the manuscript; all authors read and revised the final manuscript.

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1 Introduction

In the last decades, the Mediterranean basin has experienced climate change. The average mean temperature in the region has increased approximately 2 °C (Lelieveld et al. 2012) and the precipitation regime has changed (IPCC 2007). An analysis of the Portuguese rainfall regime for the last 50 years (1941–2007) has revealed that springs are getting dry and autumns wet (Espírito Santo et al. 2014). Moreover, although the general trend is for a reduction in annual precipitation, the frequency of rain spells has increased since the 1960s (Kutiel and Trigo 2014). Climate change is expected to continue to affect the Mediterranean region, by increasing the frequency and intensity of extreme events, especially drought (Trenberth

2012; Neelin et al. 2017). Climatic projections for the region predict an increase of up to 30 more dry days per year by the end of this century (Polade et al. 2014), and more frequent rain spells.

Drought has a direct impact in forests by increasing water stress, and an indirect one by increasing the susceptibility of trees to other biotic or abiotic declining factors (Allen et al. 2015; Cailleret et al. 2016). Studying trees response to drought is, therefore, crucial to predict how Mediterranean forests will respond to climate change scenarios, and to help adjusting forest management practices. There are several experimental studies focused on the physiological response of trees to drought (Nguyen-Queyrens and Bouchet-Lannat 2003; Anderegg and Anderegg 2013; Bachofen et al. 2018), but only a few have studied its impact on wood production (de Luis et al. 2011; Balducci et al. 2013; Fonti et al. 2013; Balducci et al. 2015; Balducci et al. 2016), and even fewer have been performed under Mediterranean climate (de Luis et al. 2011; Vieira et al. 2017). In an experimental study in Spain, de Luis et al. (2011) showed that the cambial activity of *Pinus halepensis* Miller saplings was strongly regulated by water availability throughout the growing season. They also noted that, although the cambium is typically quiescent in the summer, a wet summer after a dry spring could trigger cambial division, and that this post-summer cambial reactivation was also dependent on early growing season conditions. In another experimental study, Vieira et al. (2017) found that adult *Pinus pinaster* Aiton trees subjected to rain exclusion formed fewer tracheids and that the lumen diameter of latewood tracheids was narrower than in control trees. All trees exhibited a second period of cambial activity and wood formation after the summer; however, in trees under exclusion, the latewood tracheids presenting wider lumen diameters were observed at the end of tree ring whereas in control trees, it was observed in mid latewood. This delayed response corresponds to the end of the exclusion treatment. These studies revealed that cambial activity and wood formation in *P. halepensis* and *P. pinaster*, two typical Mediterranean species, present a plastic response to climatic conditions.

Studies on Mediterranean tree species have reported that the timing and duration of drought events are essential to predict tree productivity (Camarero et al. 2015; Forner et al. 2018) and survival (McDowell et al. 2008). Mediterranean tree species are adapted to summer drought by presenting a bimodal growth pattern, which is characterized by two periods of activity, in spring and autumn (Camarero et al. 2010; de Luis et al. 2011; Campelo et al. 2018). Drought events outside the summer period are expected to be more harmful to trees growing in this environment. In fact, several studies have found evidence of a negative impact of increasing drought

stress on growth and resilience of several Mediterranean tree species (Pasho et al. 2012; Granda et al. 2013). Although there are experimental studies performed in Mediterranean tree species, few climate change scenarios and even less tree species have been investigated. In this study, we followed primary and secondary growth in 2-year-old maritime pine saplings subjected to spring drought and autumn irrigation, as predicted by the climatic scenario projections for the Mediterranean region. We hypothesize that spring drought decreases primary and secondary growth, while increased water availability in autumn only promotes secondary growth. Knowing that *P. pinaster* is highly responsive to fluctuations in soil water availability during the growing season, we tested whether the climatic changes scenarios reduce or enhance (i) primary (ii) and secondary growth, and (iii) tracheid dimensions.

2 Material and methods

2.1 Experimental design

The water manipulation experiment was conducted in a polytunnel greenhouse in InProPlant, Coimbra, Portugal (40° 10' 58.7" N 8° 24' 52.8" W). Two-year-old maritime pine saplings (*Pinus pinaster* Aiton) from the same population were acquired in a forestry nursery (Veiga & Silva Lda., Anadia, Portugal) in August 2016 and immediately transplanted to plastic pots of 30 cm deep and a volume of 6 L. The soil substrate was a mixture of blonde peat (0–40 mm), pine bark (0–15 mm), and up to 9 months slow-release fertilizer (Siro® Ácido). The soil substrate presented a field capacity of 25–30% of water per volume of soil. After the transplant, saplings were watered regularly to overcome the impact of transplanting.

In February 2017, 512 saplings of uniform size (27.9 ± 8.3 cm in height and 4.0 ± 0.8 cm diameter at the collar) were randomly assigned to the four water manipulation treatment levels: control, exclusion, irrigation, and exclusion–irrigation. The reference rainfall used in the water manipulation experiment was the average rainfall for the grid point closest to Coimbra (annual averages calculated for the period 1987–2016 from monthly data downloaded from the Royal Netherlands Meteorological Institute, <http://www.climexp.knmi.nl/>; Figs. 1 and Annex Fig. 7). In the acclimation period, saplings were irrigated according to the rainfall pattern. From April to August, saplings in the control and irrigation groups were irrigated following the reference rainfall pattern, whereas the saplings in the exclusion groups were subjected to an irrigation reduction corresponding to 50% in April and 75% from May to August (Figs. 1 and Annex Fig. 7). In September, the saplings in the irrigation groups received an extra

irrigation whereas saplings in the control groups received the water volume corresponding to the reference autumn rainfall pattern (Figs. 1 and Annex Fig. 7). Irrigation saplings were intensely watered until soil water content reached the levels observed during spring. From September onward, all saplings were irrigated following the reference rainfall pattern (Fig. 1).

2.2 Greenhouse environmental conditions

Air temperature and humidity in the greenhouse were monitored hourly using 8 iButton® loggers (model DS1923-F5; Annex Fig. 8) placed at sapling height (*ca.* 50 cm) scattered in the greenhouse. The air temperature in the greenhouse presented the characteristic seasonal variation, with an average temperature of 19.6 °C (Annex Fig. 8). The lowest temperatures were registered in December 2017 and February 2018 (0 °C) and the warmest absolute temperatures, close to 50 °C, were registered in three separate occasions in May, June, and August. The air relative humidity in the greenhouse also presented a seasonal pattern, with minimum values during the summer months and maximum values in the winter (Annex Fig. 8). The minimum relative humidity detected in the greenhouse was of 15% in the end of May, beginning of June. The maximum air relative humidity was of 100% and was observed in December 2017 and January 2018.

2.3 Plant monitoring

Plant growth was monitored monthly by measuring increment in height (primary growth), and diameter at the collar (secondary growth) before the destructive sampling of 32 saplings (8 from each treatment), randomly selected. Diameter at the collar was measured using a digital Vernier caliper (MacFer,

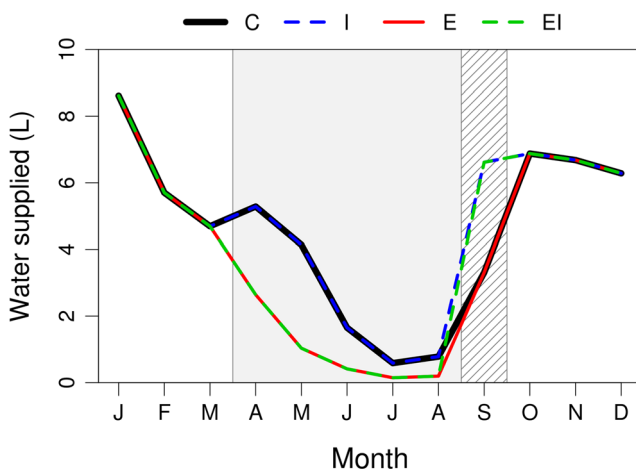


Fig. 1 Diagram of the monthly water supplied to the four levels of the water manipulation experiment. Treatment levels: C, control; E, exclusion; I, irrigation; EI, exclusion-irrigation. Gray-shaded area corresponds to the water exclusion period and the oblique lines area to the extra irrigation treatment

0.01 mm resolution) and sapling height was measured using a measuring tape.

Soil moisture (volumetric water content) was monitored every 10–20 days in a group of 32 saplings (8 from each treatment level) randomly selected for each treatment levels, using a time domain reflectometer sensor (model SM300, coupled with a HH2 meter, both from Delta-T Devices). Soil moisture content was measured at 10 cm from the pot base (root depth), using holes especially drilled for that purpose. Each pot was measured 3 times.

2.4 Wood formation dynamics

Cambial activity and wood formation were monitored from February 2017 to February 2018. Each month, a destructive sampling was performed on 32 saplings, 8 per treatment, randomly selected. A wood disc was collected from each sapling 10 cm above the collar. The wood disc was then placed in FAA solution (formaldehyde-acetic-acid-ethanol solution) and processed following the protocol by Rossi et al. (2006a). After embedding in paraffin, the samples were cut using a rotary microtome, stained with an Astra Blue (0.15%) and Safranin (0.04%) water solution and permanently mounted using Eukitt, a histological mounting medium. The histological wood sections were then observed under a light microscope with polarized light in order to distinguish each xylem differentiation phase. Cambial, enlarging, cell wall thickening, and mature cells were counted along three radial files in order to monitor cambial activity and wood formation. Microscopic observations were performed under $\times 1000$ magnification using a Leica DM4000B microscope.

2.5 Tracheid dimensions

The samples collected in February 2018 were photographed using a camera fixed on a microscope (Leica, model DCF295), at $\times 50$ magnification. Three radial rows were selected in each image to measure the radial lumen diameter (LD) and cell wall thickness (CWT) of each tracheid. Images were processed using ImageJ (<https://imagej.nih.gov/ij/>). The tree ring formed in 2017 presented several density fluctuations, with no clear transition between earlywood and latewood (Fig. 2). For this reason, we divided each tree ring into three equal sections. Maritime pine latewood typically corresponds to 30% of the tree ring (Denne 1988; Campelo et al. 2016); thus, by dividing the ring in three equal parts, the two first would correspond to earlywood and the last one to latewood (Fig. 2).

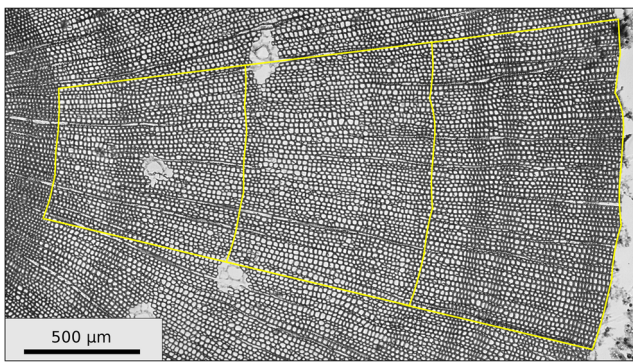


Fig. 2 Photograph of the 2017 tree ring from the control treatment level illustrating the three identical sections in which the tree rings were divided in order to compare tracheid dimensions between treatment levels

2.6 Statistical analysis

The variation throughout the growing season in the number of cambial cells (C) and in the sum of enlarging, cell wall thickening, and mature cells (ELM) was investigated with generalized additive models (GAMs), using the *mgcv* package (Wood 2006) in the R computing environment (R Development Core Team, 2016). A GAM is a generalized linear model (GLM) in which the linear predictor depends linearly on some unknown smooth functions of some predictor variables, i.e., extends the linear model to allow for non-linear relationships (Wood 2006). The strength of GAMs is their ability to deal with non-linear and non-monotonic relationships between the dependent variable (e.g., number of cells) and the set of independent variables or explanatory variables (e.g., day and treatment). By doing so, it was possible to compare the effect of treatment (4 levels) throughout the growing season on xylogenesis. Differences between groups were considered significant when the pointwise confidence intervals of the fitted curves did not overlap.

Primary and secondary growth (height and stem diameter increment, respectively) were also investigated with GAMs to study the effect of treatment and time.

The total number of cells and tree-ring width formed in 2017 were compared between treatment levels using fixed-effects ANOVAs, with cell number and tree-ring width as dependent variables, and water regime as independent variable.

Lumen diameter and cell wall thickness were investigated in the three sections of the tree ring using multivariate ANOVAs (MANOVAs). The MANOVA was used to explore the effect of water manipulation (independent variable with four levels) on cell dimensions (LD and CWT) in three sections of the tree ring (start, middle, and end; 3 dependent variables). All MANOVA assumptions were verified and checked before running the test. When significant (using the Pillai criterion to adjust *F* tests), the MANOVA was followed by ANOVAs for each tree-ring section and paired comparisons were corrected using the Bonferroni adjustment.

3 Results

3.1 Soil water status

Soil water content was fitted with a GAM in order to identify the periods when it differed between treatment levels (Fig. 3, Annex Table 2). In February 2017, before the start of the water manipulation experiment, the soil water content was similar between treatments representing approximately 20 parts per volume of soil. Soil water content increased equally in all treatments, reaching a maximum of 22% in March, immediately before the start of the exclusion treatment. Starting in April, soil water content decreased in all treatments until July. From June to August, soil water content was significantly lower in the exclusion groups. Soil water content continued to decrease in the exclusion saplings, reaching its lowest in August (*ca.* 1.1%). Soil water content increased rapidly once the exclusion treatment ended, in September. The fastest recovery was observed in irrigation saplings, reaching 20% soil water content in 15 days (17.6% in irrigation and 18.6% in exclusion–irrigation). Irrigation saplings presented significantly more soil water content than control saplings (6.8% in control and 10.0% in exclusion; Fig. 3, Annex Table 2). In November, soil water content was similar in all treatment levels.

3.2 Primary and secondary growth

Sapling height and diameter at collar were fitted with GAMs in order to identify differences between treatment groups (Fig. 4, Table 3). At the start of the experiment, in February 2017, height and diameter showed no differences between groups (Fig. 4; Annex Table 3). By the end of the exclusion treatment (end of August), control saplings were significantly taller than exclusion saplings (63.1 ± 15 vs. 58.75 ± 5.8 cm). The differences in diameter at collar were also significant (7.16 ± 0.63 cm vs. 6.81 ± 0.87 cm in control and exclusion, respectively). After irrigation, diameter increased rapidly in all treatment groups (control 7.21 ± 0.79 cm; exclusion 6.83 ± 0.8 cm; irrigation 7.36 ± 0.54 cm; exclusion–irrigation 6.68 ± 0.67 cm). But sapling height, however, did not respond to irrigation (control 61.7 ± 16.1 ; exclusion 59.4 ± 10.6 ; irrigation 71.4 ± 9.6 ; exclusion–irrigation 54.8 ± 7 cm). From the timing of irrigation until December, sapling height remained constant (Fig. 4). In December, sapling height increased in all treatment groups, corresponding to the start of primary growth in the 2018 growing season. In the end of monitoring (February 2018), saplings in control and irrigation were significantly taller (control 65.3 ± 13.8 and irrigation 66.5 ± 10.8 cm) than saplings in exclusion and exclusion–irrigation water regimes (exclusion 56.7 ± 16.3 and exclusion–irrigation 56.7 ± 10.1 cm). Diameter at collar increased constantly until the end of the monitoring. The differences between treatment groups became significant in October, similar to what was

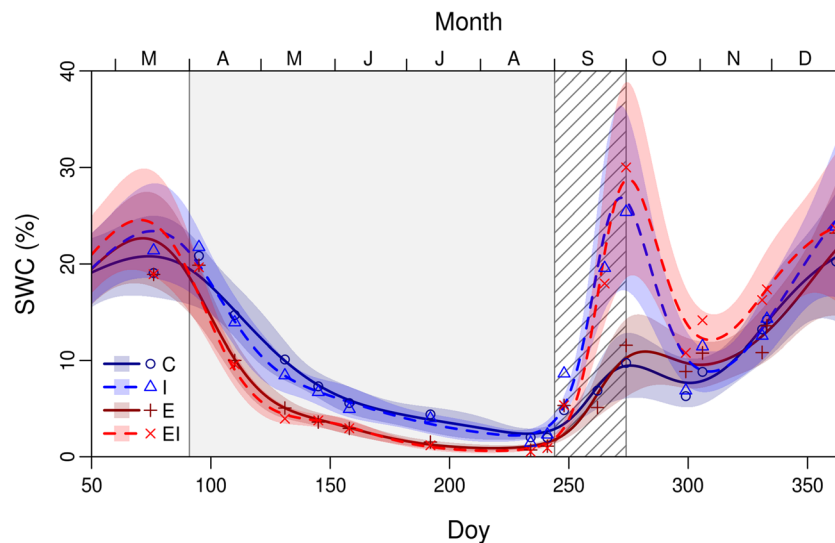


Fig. 3 Seasonal variation of the soil water content (volume/volume) in the sapling pots fitted with a generalized mixed model. Dots represent the average measures per treatment level at each date. Lines represent the soil water content in the different treatment levels and the colored shaded areas denote the 95% pointwise confidence interval. Treatment levels:

observed in sapling height, control and irrigation saplings presented a diameter significantly wider (control 65.3 ± 13.8 and irrigation 66.5 ± 10.8 cm) than exclusion and exclusion-irrigation saplings (exclusion 56.7 ± 16.3 and exclusion-irrigation 56.7 ± 10.1 cm; Fig. 4, Annex Table 3).

During the experimental trial, five saplings died, in August, representing a mortality rate of less than 1%. One sapling was

in the control group and the other four in the exclusion-irrigation group.

3.3 Xylem differentiation

Seasonal cambial activity and xylem differentiation were similar between groups, as seen by the pointwise overlap of the confidence interval (Figs. 5 and Annex Fig. 9, Table 4). The number of cambial cells started to increase in the beginning of the sampling season (March), reaching a maximum of 5 ± 0.8 cells in April–May, afterwards it started to decrease, reaching a minimum of 3 ± 0.4 cells in mid-July. By the end of July, the cambium presented a second period of division, increasing the average number of cambial cells until after the irrigation period in September. Afterwards, the number of cambial cells started to slowly decrease until a minimum of 2 ± 0.5 cells was observed in Jan–Feb 2018. There were no significant differences between groups (Fig. 5, Annex Table 4).

Regarding the differentiating xylem cells (ELM, Figs. 5 and 9), its distribution presented a bimodal pattern, with two periods of xylem differentiation, the first from March to September, and the second from October to February 2018. Between July and October, the number of total xylem cells remained almost constant. At the end of the water manipulation experiment (February 2018), there were 123.71 ± 22.58 cells in control; 116.46 ± 15.62 in irrigation; 107.00 ± 17.26 in exclusion and 103.04 ± 22.30 cells in the exclusion-irrigation group (Fig. 5 and Annex Table 4). Although the number of tracheids produced in 2017 was slightly

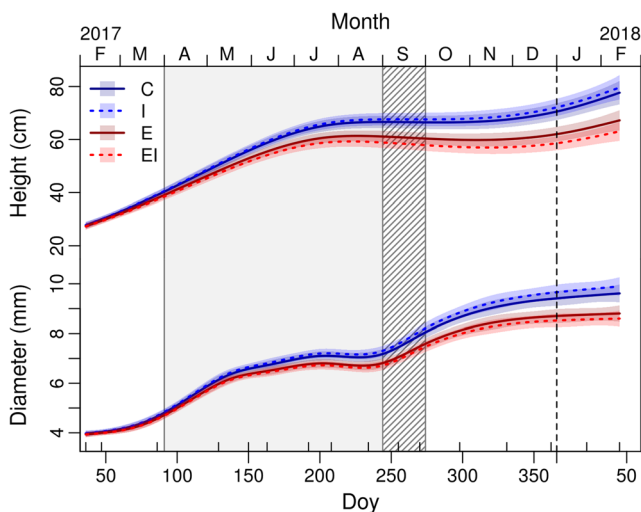


Fig. 4 Sapling height and diameter in 2017 fitted with generalized additive models. Solid and dashed lines represent the average diameter or height of the saplings and the shaded colored areas denote the 90% pointwise confidence interval. Treatment levels: C, control; E, exclusion; I, irrigation; EI, exclusion-irrigation. Significant differences between treatment levels were found whenever the confidence interval of the models did not overlap. Gray-shaded area corresponds to the water exclusion period and the oblique lines area to the extra irrigation treatment

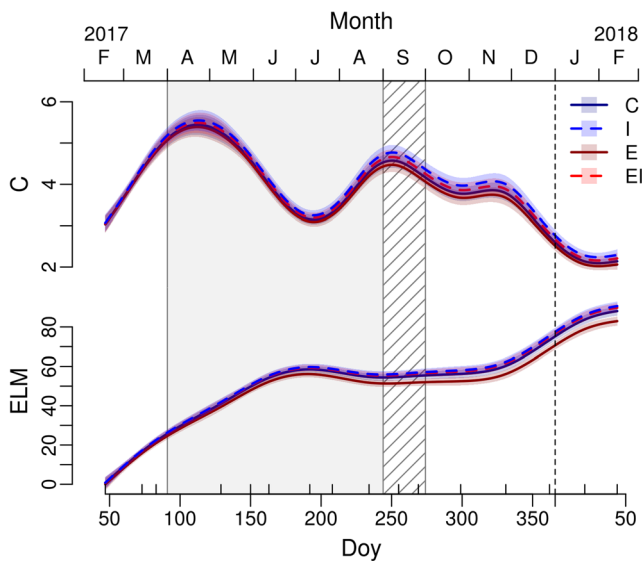


Fig. 5 Number of cambial cells (C) and total differentiating xylem cells [ELM, cells in enlargement (E), lignification (L), and mature (M)]; in the four water manipulation treatment levels during the 2017 growing season fitted with generalized additive models. Treatment levels: C, control; E, exclusion; I, irrigation; EI, exclusion–irrigation. Solid and dashed lines represent the average number of cambial or total xylem cells and the shaded colored areas denote the 90% pointwise confidence interval. Significant differences between treatment levels were found whenever the confidence interval of the models did not overlap. Gray-shaded area corresponds to the water exclusion period and the oblique lines area to the extra irrigation treatment

higher in the control group, no significant differences occurred between treatment groups ($F_{3;28} = 1.8$; $p = 0.171$), as well as the tree-ring width ($F_{3;28} = 1.920$; $p = 0.149$). Tree-ring width mirrored the differences observed in the number of tracheids produced, with saplings from the control group presenting a wider tree ring than the remaining saplings. At the end of the growing season, the average tree-ring width of the saplings was $2.45 \pm 0.42 \mu\text{m}$ in control; $2.36 \pm 0.39 \mu\text{m}$ in irrigation; $2.07 \pm 0.34 \mu\text{m}$ in exclusion; and $2.05 \pm 0.51 \mu\text{m}$ in exclusion–irrigation.

3.4 Tracheid features

Regarding tracheid properties (Fig. 6), MANOVA detected significant differences in the lumen diameter but not in cell wall thickness (Table 1). Subsequent univariate ANOVAs showed that differences were only significant on the lumen diameter of the third part of the ring (Table 1, Fig. 6). Lumen diameter in the third section of the tree ring was significantly wider in saplings receiving the extra irrigation (irrigation and exclusion–irrigation groups).

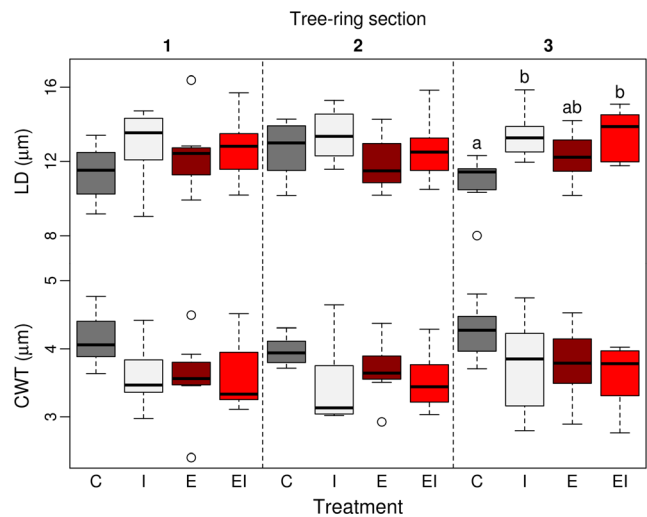


Fig. 6 Lumen diameter (LD) and cell wall thickness (CWT) of the tracheids from the 2017 tree ring in three equal sections (sections are described in Fig. 2). Treatment levels: C, control; E, exclusion; I, irrigation; EI, exclusion–irrigation. Differences between treatment levels are indicated by the letters

4 Discussion

The water manipulation experiment shows that maritime pine saplings responded to water variation during the growing season. Primary growth was reduced in the drought-treated saplings, as well as the total number of tracheids produced—secondary growth. The extra irrigation in September did not promote primary growth, but triggered the formation of tracheids with wider lumen diameter in the final third of the tree ring. The results confirmed the initial hypotheses that primary and secondary growth is limited by drought and that tracheid lumen diameter increases in response to September irrigation.

Increments in height (primary) and diameter (secondary growth) presented greater differences between treatments than the total number of cells produced during the 2017 growing season. Saplings in the control and irrigation groups were significantly taller than saplings in the exclusion groups. Grossiord et al. (2017) compared shoot elongation in control and rain exclusion Piñon pine (*Pinus edulis* Eng.) trees and observed that shoot elongation decreased in rain exclusion trees. In another rain exclusion study, performed on *Pinus sylvestris* L. and *Quercus pyrenaica* Willd. growing in central Spain, Fernández-de-Uña et al. (2017) reported that rainfall exclusion had no effect on leaf phenology but cambial activity stopped earlier in rain exclusion trees. Primary growth allows the development of both aerial and underground organs, providing plants with efficient photosynthetic activity and functional

Table 1 Multivariate MANOVA comparing the effect of water manipulation treatment (independent variable, with four levels) on lumen diameter (LD) and cell wall thickness (CWT) tree-ring sections; followed by univariate ANOVAs, when significant. *df*, degrees of freedom; Pillai's criterion which verifies the assumptions of the MANOVA; F_{apro} , approximated

F ; df_{num} , degrees of freedom in the numerator; df_{den} , degrees of freedom denominator; LL and UL represent the lower-limit and upper-limit of the partial η^2 confidence interval, respectively; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

MANOVA		<i>df</i>	Pillai	F_{apro}	df_{num}	df_{den}	<i>p</i>
LD	Intercept	1	0.99205	1081.33	3	26.84	< 0.001***
	Treatment	3	0.63246	2.49	9		0.014*
	Residuals	28					
CWT	Intercept	1	0.9887	758.02	3	26	< 0.001***
	Treatment	3	0.4538	1.66	9	84	0.111
	Residuals	28					
ANOVA		Sum of squares	<i>df</i>	Mean square	<i>F</i>	<i>p</i>	Partial η^2 95%CI [LL, UL]
LD1	(Intercept)	2257.93	1	227.93	337.53	0	[0, 0.28]
	Treatment	24.95	3	8.32	1.24	0.313	
	Error	187.31	28	6.69			
LD2	(Intercept)	2793.05	1	2793.05	582.63	0	[0, 0.30]
	Treatment	20.35	3	6.78	1.42	0.259	
	Error	134.23	28	4.79			
LD3	(Intercept)	2075.81	1	2075.81	561.78	0	[0.09, 0.57]
	Treatment	74.28	3	24.76	6.7	0.02*	
	Error	103.46	28	3.69			

translocation of water, nutrients, and signaling molecules (Baucher et al. 2007). In their early life stages, trees invest most of their resources in establishing in a forest (Andivia et al. 2018; Vieira et al. 2018); thus, it is expected that under favorable conditions, saplings will present higher increments in primary growth.

Regarding the increment in diameter, control and irrigation saplings also presented larger diameter than exclusion and exclusion-irrigation saplings. After the irrigation in September, there was a fast increment in diameter in all groups, corresponding to a recovery period. Previous studies in the Mediterranean region, using automatic dendrometers, have shown that after summer drought, and associated with autumn rain, stem diameter presents a re-hydration period when diameter rapidly increases (Vieira et al. 2013). The stem diameter increments observed here are in agreement with those previous findings and indicate that saplings were recovering from water stress.

The differences in diameter increment observed between groups receiving less water (exclusion and exclusion-irrigation) and groups receiving more water (control and irrigation) did not correspond to differences in the number of cells or tree-ring width in 2017. In fact, only the exclusion group showed fewer tracheids than the remaining groups. The diameter of a tree is the sum of wood, bark, and the living tissues of the inner bark—phloem and parenchyma (Downes et al. 1999;

Deslauriers et al. 2003). In 2-year-old saplings, the ratio of total diameter to wood diameter is lower than in adult trees. Thus, it is possible that the differences observed in diameter between control and exclusion saplings were due to the proportion of the living tissues of the inner bark and to the hydration level of these tissues.

The water manipulation experiment did not trigger differences in the dynamics of cambial activity in the 2-year-old maritime pine saplings. Regarding the seasonal trend in the total number of differentiating xylem cells (ELM), saplings in the exclusion group formed significantly less xylem cells than saplings in the remaining groups. In a study comparing wood formation in *Pinus halepensis* L. saplings subjected to different irrigation regimes, de Luis et al. (2011) reported that saplings in the control group presented narrower tree-ring width and fewer tracheids. Under water stress, cell division rates decrease (Balducci et al. 2016) and fewer tracheids are produced.

Water availability also plays a crucial role in the tracheids final size, especially during the cell enlargement phase. The pressure exerted by the water present in the vacuoles of the developing tracheids is partly responsible for forcing the primary wall to expand until the final size is reached (Kutschera and Niklas 2013). Saplings receiving the extra irrigation in September (irrigation and exclusion-irrigation groups) formed tracheids with larger lumen diameter in the last third of

the ring. These results indicate that the cambium is highly responsive to soil water availability, forming tracheids with higher water transport capacity. Previous studies on maritime pine have shown that this species presents a bimodal pattern of growth, resuming wood formation when rainfall returns after the summer (Vieira et al. 2015; Vieira et al. 2017). The increase in the intensity and duration of summer drought will limit both primary and secondary growth in maritime pine. However, the expected increase in autumn precipitation can have the opposite effect by providing the optimal conditions for a second period of growth (cf. Campelo et al. 2018). The ability of maritime pine to quickly adjust its cambial activity and wood properties to current environmental conditions could enhance the species survival under the predicted climate change scenarios. Regarding the first two-thirds of the tree ring, corresponding to earlywood, there were no differences in lumen diameter between groups. Earlywood presents a stronger genetic control than latewood, being less affected by changes in environmental conditions (Gaspar et al. 2008). Although there were significant differences in soil water content between treatment groups, from May to August soil water content was below 10% in all groups. It is most likely that to trigger a response in earlywood tracheid anatomy, the differences in soil water content between control and exclusion should have been higher and started earlier.

Regarding the overall duration of xylogenesis, enlarging cells were observed until October and the differentiation of xylem cells (cell wall deposition) lasted until the end of the growing season, in February 2018 (Fig. 9). The last enlarging cells were observed in October, when the photoperiod is less than 12 h and continues to decrease, suggesting that the end of cambial activity is triggered by decreasing photoperiod, as observed in conifers from cold environments (Rossi et al. 2006b). Besides duration of growth, xylogenesis is also characterized by the rate of cell division (Lupi et al. 2010; Rossi et al. 2012; Cuny et al. 2014; Cuny et al. 2015). Balducci et al. (2016) observed that *Picea mariana* (Mill.) B.S.P. saplings subjected to water deficit presented a reduced rate of cell production, but compensated for it by increasing the duration of xylem differentiation. Similarly, the long duration of xylogenesis in our study could also be a compensatory mechanism for the low soil water content observed in treatment groups during the summer.

Sapling mortality is often observed in water manipulation studies (Balducci et al. 2015), but the mortality observed in this study was residual (<1%). The low mortality and the fact that differences in the number

of tracheids were only observed in the exclusion treatment, confirm the drought-tolerant nature of maritime pine saplings. In a study comparing the response of seedlings of different *Pinus* spp. from the Mediterranean region to drought and temperature increase, it was concluded that maritime pine was the most drought-tolerant species (Matías et al. 2017). Our results support that maritime pine is a resilient, drought-resistant tree species and that its saplings can withstand intense spring droughts. To accurately predict the response of maritime pine saplings to drought, it will be interesting to study the effect of recurrent and more intense droughts.

5 Conclusions

In the context of the climate change scenarios predicted for the Mediterranean region (increased spring drought and autumn rainfall), we found that maritime pine saplings can experience spring drought, despite the reduction in primary and secondary growth and resume cambial activity to take advantage of the return of favorable conditions after the summer. This plasticity in cambial activity adapted to the Mediterranean climate, with two potential growing periods, separated by summer drought, gives this species an advantage to face the predicted climate change scenarios, with longer dry seasons and a more unpredictable precipitation pattern.

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Data availability The dataset generated during the current study are not publicly available but are available from the corresponding author upon reasonable request

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Annex

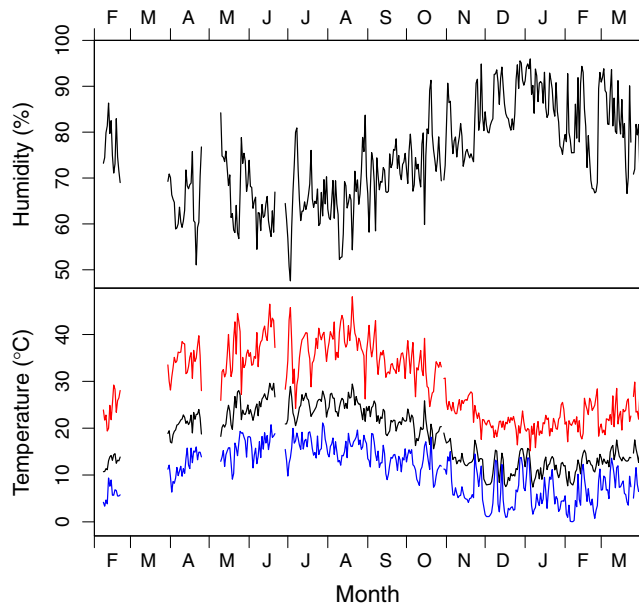


Fig. 7 Daily mean air humidity and maximum, mean, and minimum air temperature measured in the greenhouse during the study period, using iButtons

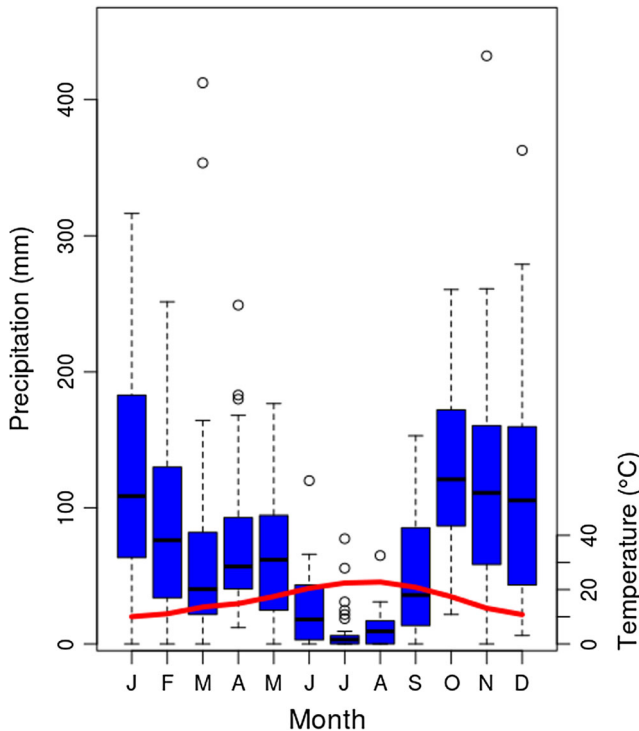


Fig. 8 Climatic diagram representing monthly average precipitation (mm) and temperature (°C) registered in the last 30 years in the grid point closest to Coimbra (40.38 N, 8.63 W; <http://www.climexp.knmi.nl>). Precipitation is represented in the box plots, horizontal lines represent the median, and the circles are outliers; average temperature is represented by the red line

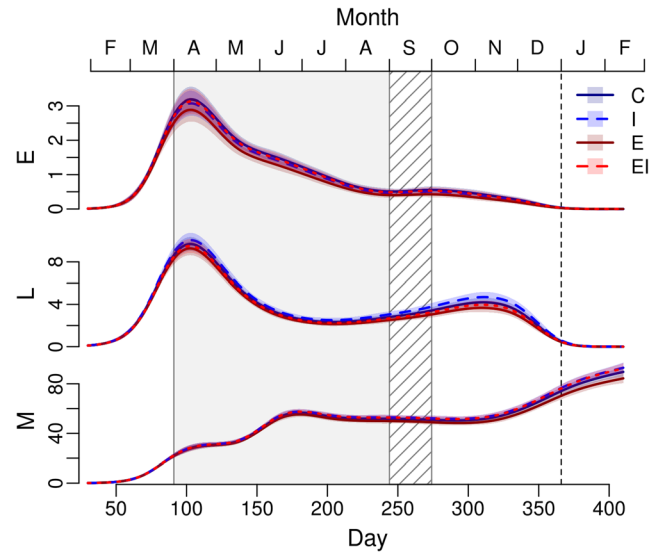


Fig. 9 Number of enlarging (E), cell wall deposition (L), and mature cells (M) in the four water manipulation treatment levels during the 2017 growing season fitted with generalized additive models. Treatment levels: C, control; E, exclusion; I, irrigation; EI, exclusion–irrigation. Solid and dashed lines represent the average number of enlarging, cell wall deposition, or mature cells and the shaded colored areas denote the 90% pointwise confidence interval. Significant differences between treatment levels were found whenever the confidence interval of the models did not overlap. Gray-shaded area corresponds to the water exclusion period and the oblique lines area to the extra irrigation treatment

Table 2 Generalized additive models resume table comparing the dependent variable, soil water content (SWC), to a set of explanatory variables (e.g., day and treatment levels). Treatment levels: *C*, control; *E*, exclusion; *I*, irrigation; *EI*, exclusion–irrigation. *df*, degrees of freedom; *edf*, effective degrees of freedom; *Ref. df*, reference degrees of

freedom; *GCV*, generalized cross-validation score, an estimate of the mean square prediction error based on a leave-one-out cross-validation estimation process; *family*, data distribution; *link function*, relates the conditional mean to the linear predictor; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

SWC	Parametric terms		<i>df</i>	<i>F</i>	<i>p</i>		
		TREAT		3	13.61	< 0.001	***
	DAY:TREAT		4	11.19	< 0.001	***	
	Smooth terms		<i>edf</i>	<i>Ref. df</i>	<i>F</i>	<i>p</i>	
	s(DAY):TREAT_C		8.668	8.964	38.38	< 0.001	***
	s(DAY):TREAT_I		8.890	8.996	36.56	< 0.001	***
	s(DAY):TREAT_E		8.671	8.962	67.53	< 0.001	***
	s(DAY):TREAT_EI		8.904	8.997	76.440	< 0.001	***
	Family			R^2_{adj}		0.70	
	Gamma			Deviance explained		67.9%	
	Link function			GCV		0.264	
	Log			<i>n</i>		719	

Table 3 Generalized additive models resume table comparing sapling height and diameter (dependent variables) in the four treatment levels. *df*, degrees of freedom; *edf*, effective degrees of freedom; *Ref. df*, reference degrees of freedom; *GCV*, generalized cross-validation score, an estimate of the mean square prediction error based on a leave-one-out cross-validation estimation process; *family*, data distribution; *link function*, relates the conditional mean to the linear predictor; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Height	Parametric terms		<i>df</i>	<i>F</i>	<i>p</i>		
		DAY		1	621.65	< 0.001	***
	DAY:TREAT		3	17.62	< 0.001	***	
	Smooth terms		<i>edf</i>	<i>Ref. df</i>	<i>F</i>	<i>p</i>	
	s(DAY)		3.519	4.511	67.080	< 0.001	***
	Family			R^2_{adj}		0.58	
	Gamma			Deviance explained		65.7%	
	Link function			GCV		0.035	
	Log			<i>n</i>		495	
Diameter	Parametric terms		<i>df</i>	<i>F</i>	<i>p</i>		
		DAY		1	1679.26	< 0.001	***
	DAY:TREAT		3	17.63	< 0.001	***	
	Smooth terms		<i>edf</i>	<i>Ref. df</i>	<i>F</i>	<i>p</i>	
	s(DAY)		7.324	7.861	31.860	< 0.001	***
	Family			R^2_{adj}		0.81	
	Gamma			Deviance explained		83.4%	
	Link function			GCV		0.017	
	Log			<i>n</i>		495	

Table 4 Generalized additive models resume table comparing the number of cambial cells (C) and total number of xylem cells (ELM) in the four treatment levels. *df*, degrees of freedom; *edf*, effective degrees of freedom; *Ref. df*, reference degrees of freedom; *GCV*, generalized cross-

validation score, an estimate of the mean square prediction error based on a leave-one-out cross-validation estimation process; *family*, data distribution; *link function*, relates the conditional mean to the linear predictor; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

C	Parametric terms		<i>df</i>	<i>F</i>	<i>p</i>		
		DAY		1	8587.149	< 0.001	***
	DAY:TREAT		3	3.377	0.0183	*	
	Smooth terms		<i>edf</i>	<i>Ref. df</i>	<i>F</i>	<i>p</i>	
	s(DAY)	8.884	8.996	1052.000	< 0.001	***	
	Family		R^2_{adj}		0.66		
	Gamma		Deviance explained		70.7%		
	Link function		GCV		0.028		
	Log		<i>n</i>		482		
ELM	Parametric terms		<i>df</i>	<i>F</i>	<i>p</i>		
		DAY	1	629.908	< 0.001	***	
		DAY:TREAT	3	9.332	< 0.001	***	
	Smooth terms		<i>edf</i>	<i>Ref. df</i>	<i>F</i>	<i>p</i>	
		s(DAY)	8.225	8.722	88.330	< 0.001	***
		Family		R^2_{adj}		0.88	
		Gaussian		Deviance explained		88.1%	
		Link function		GCV		60.529	
	Identity		<i>n</i>		482		

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