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Irrigation of Young Cork Oaks under Field Conditions—Testing the Best Water Volume

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Abstract: This study is the beginning of the first long-term study on cork oak irrigation under field conditions, with a structural-functional approach. Cork oaks are currently facing disturbances affecting cork quality and quantity, jeopardizing the future of the economic sector. There is a need for new production techniques that maximize cork oak growth and vitality. In this study, irrigation was implemented in a new intensive cork oak plantations to test the best irrigation volume. The long-term goal is to improve tree growth with minimum water requirements. A 6 ha intensive plantation was installed in Coruche, Portugal. The experimental plot consisted of a subsurface drip fertigation system, buried 40 cm deep; with five independent irrigation treatments. It was tested four irrigation volumes during the dry period—21 weeks in the summer of 2016—ranging from 1.88 mm to 5.62 mm a week. Information on meteorological conditions, soil moisture profile and leaf stomatal conductance were gathered periodically and dendrometric measurements were performed before and after the treatments. Cork oaks' structural and functional parameters were associated with irrigation volume Response to irrigation showed an inflection point in treatment 2, corresponding to a water supply of 3.12 mm per week: below the inflection point, stomatal conductance was reduced by 15% and relative diameter growth at the base was reduced by 10%. Stomatal conductance also showed a positive relationship with soil moisture below the irrigation tubes and with plants' stem diameter. In conclusion, irrigation supply during the period of water stress improved function and structure of cork oaks seedlings under field conditions. These results suggest that irrigation can be a viable alternative to improve cork oak growth in afforestation and reforestation.

Keywords: *Quercus suber*; tree growth; stomatal conductance; water relations; Mediterranean trees; precision irrigation

1. Introduction

Cork oak (*Quercus suber* L.) is a sclerophyllous evergreen Mediterranean tree of high conservation and socioeconomic value in its natural range area. This species covers about 2.2 M ha in the western part of the Mediterranean basin, growing well in acidic soils on granite, schist, or sandy substrates [1]. The main product obtained from this tree is its outer layer, cork. Portugal produces about half of the cork on the market (≈100,000 ton per year) and the remainder is mainly obtained in Spain, Morocco, Argelia, Tunisia, Italy and France [2]. Cork oak forests and the derived silvopastoral systems are ecologically and economically sustainable, serving as an important tool in preventing desertification [2].

However, severe cork oak mortality events have repeatedly occurred in the Mediterranean basin since the 1980s, disrupting the system in all its aspects [3–7]. Nowadays, cork oak dieback is considered a complex multifactorial phenomenon involving the combination of several factors acting together [8], where drought plays a role in causing trees to decline [3,4,9–11]. With a reduction in the raw material the future of the cork industry may be jeopardized, principally the production of natural cork stoppers. This is particularly relevant given that cork stoppers account for 70% of cork's market value [2], though they represent only 40% of cork production. In the search for new cork oak afforestation techniques, private companies and institutional research centers have established several cooperation projects between them. The long term goal is to develop sustainable production techniques capable of reducing seedling mortality, increasing tree growth and favoring tree health. Empirical observations and one cork oak field study [12] suggested that fertigation treatments may improve plant survival, health, and growth in the early stages of development. Enhancing plant growth is a key factor for research purposes due to the long period from planting until economically viable cork production. By Portuguese law the circumference at breast height (CBH) of cork trees must reach 70 cm before the first cork stripping, which usually occurs after 20 years' age or more. Afterward, the cork can be removed every 9 or 10 years. Under exceptional conditions, that period can be reduced to 7 or 8 years (Law 169/2001). Given that only the 3rd and subsequent harvests produce quality cork, a tree only becomes economically viable after 40 years' age or more. Therefore, the intent of this long-term project is to reduce the time until the 1st or 2nd cork extraction through fertigation. Research must also consider potential water supply constraints, particularly under current climate change scenarios. Consequently, the major scientific question of the long-term project is: what is the lowest requirement in water and fertilizers still improving cork oak growth? The objective is to maximize cork oaks' growth with efficient use of water, i.e., with as little water as possible. The hypothesis is that cork oaks have a threshold in water requirements –both in time and "space" (quantity)—above which there will be no significant growth. The specific aim of this study was to find the best irrigation volume for young cork oaks in intensive plantations under a particular soil type. As the crop coefficient (Kc, a widely used parameter for irrigation scheduling) of cork oaks is not known, the approach was the use of differential irrigation treatments and monitoring water relations between soil-plant-air. Stomatal conductance is considered to be related to plant water status in cork oak species [13–16] and can be used as a functional trait parameter. With dendrometric measurements accounting for structural parameters, the expected output will be a functional-structural response of the plants to different irrigation treatments.

2. Materials and Methods

2.1. Study Site

The study took place at "Herdade do Corunheiro", near Coruche, Portugal. The region is characterized by a typical Mediterranean subhumid climate with hot and dry summers. The normal annual average for rainfall in the region is 704 mm and for the annual temperature it is 15.1 °C (1971–2000, according to Portuguese Institute for Sea and Atmosphere data). Cork oaks cover 69% of the forest area in this region, representing to the largest area occupied by this species in Portugal [17]. The farm is mainly covered with forest, especially by cork oaks. The 6-hectare experimental plot was installed in 2014 in a former cropland for domestic use. A stream stands 130 m from the lower part of the experimental area, with a difference in level of 6 m. The plot has a slope of about 7% facing 12° north. Soil profile evaluation and sampling were carried out in eight locations by a soil science expert from the University of Evora: soil profiles were unstructured and presented sandy texture with loose tenacity and friability, non-stickiness, no plasticity and minimal compaction. Samples were sent to a specialized laboratory (the Agricultural Chemical Laboratory of the University of Evora) for physical and chemical evaluation. More than 75% of the particles were gross sand (Table 1a). Organic matter was very low (0.32%) and nitrogen was not detectable (Table 1b).

Table 1. Main results (Mean ± SD) of the physical (**a**) and chemical (**b**) properties of the soil profile at eight experimental field locations.

a: Physical Properties				
Density (g cm^{-3})	Gross Sand (%)	Fine Sand (%)	Silt (%)	Clay (%)
1.60 ± 0.11	77.55 ± 3.48	11.47 ± 1.78	4.48 ± 0.97	6.50 ± 0.97

b: Chemical Properties									
O.M. (%)	pH (H_2O)	P_2O_5 (ppm)	N (%)	K_2O (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
0.37 ± 0.30	5.39 ± 0.53	72.25 ± 33.03	traces	95.17 ± 76.57	27.33 ± 12.66	13.64 ± 7.75	17.20 ± 15.99	0.77 ± 0.65	0.07 ± 0.06

2.2. Plot Installation

The soil was tilled to clear the weeds and planting lines were deep-ripped with a 1 m ripper tooth. A total of 3606 1.5-year-old nursery seedlings were transplanted in April 2014 to the experimental plot in a 4 × 4 m spacing. Prior to planting, plants were selected according to the vitality indicated by leaf color and similarity in height. Average total height was 20 cm. Surface drip irrigation was installed in the first year of planting to promote seedling survival. Irrigation tubes were installed near the planting lines, following the slope of the terrain. Each planting line had one irrigation tube with 1.6 L/h drippers every 1 m. After one year, 20 plants were excavated and the root system analyzed [18]. It was observed that cork oak seedlings developed their roots down to the ripper line depth (±60 cm). Therefore, in April 2015 irrigation tubes were buried 40 cm down and 60 cm east from the planting line. Weeds and shrubs were annually cut down without soil tillage.

2.3. Experimental Plot Design

The study site corresponds to 4 ha in the center of the plantation and the remaining 2 ha belong to the edge. The experimental plot was prepared to undergo four treatments plus control, grouped into blocks with four to five irrigation lines. The treatment blocks were replicated four times and randomly distributed throughout the study site (Figure 1).

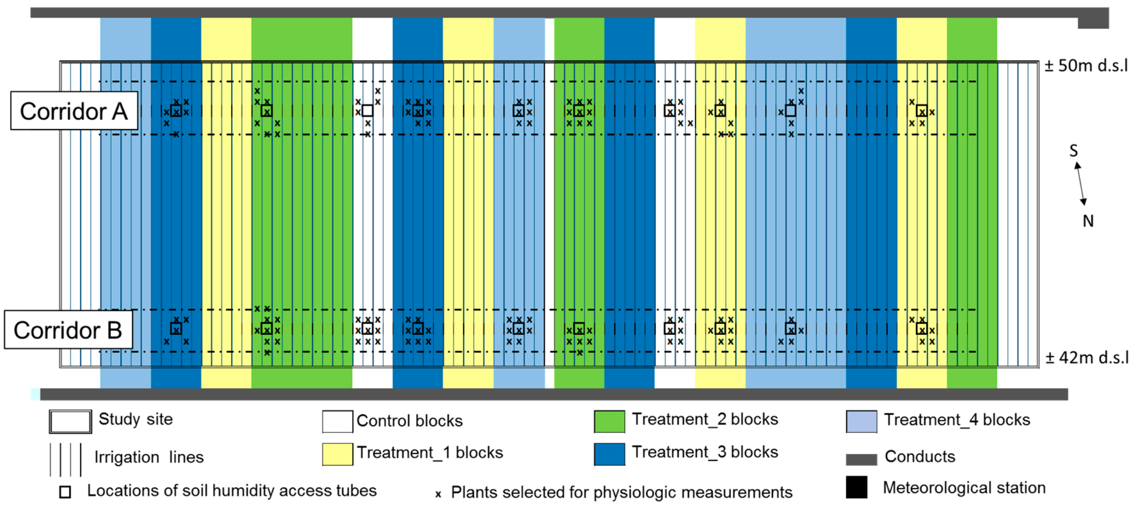

Figure 1. Schematic figure of the experimental plot.

2.4. Irrigation Treatments

During the first two summers (2014, 2015), the irrigation controller was scheduled to equally irrigate all the planting lines 12 h per week, totaling 5 mm of monthly irrigation. The experimental period began in 2016 and irrigation treatments started on June 2nd after the rainy months. Treatments consisted of four irrigation volumes ranging from 1.88 mm to 5.62 mm per week divided by three weekly irrigation periods; control blocks received survival irrigation once a month (Table 2). The latter was irrigated three times every four weeks to avoid plants' mortality. Twenty minutes before the end of each irrigation period, irrigation water was supplied for 10 min with Nutrifluid© NPK 12:6:6, corresponding to 11.5 kg ha^{-1} $week^{-1}$. Nutrients supply did not vary among treatments. Plants were irrigated overnight to allow rehydration when stomata are closed. Fertigation ended with the first autumn rains on October 26th, lasting 21 weeks.

Table 2. Irrigation treatments applied on the experimental plot in summer 2016, per week and total season.

Period	Irrigation Parameters	Treatments				
		Control	1	2	3	4
Weekly	Total volume (mm) *	**	1.88	3.12	4.38	5.62
	Frequency (x)	**	3	3	3	3
	Drip fertilization (min.)	**	30	30	30	30
Total season	Volume (mm)	21	39.48	65.52	91.98	118.02

* The increase in water volume was obtained by increasing each irrigation period. ** control was irrigated 3 days by month (every four weeks).

2.5. Data Collection

2.5.1. Meteorological Data

A portable meteorological station with data logger placed near the center of the experimental plot (Figure 1) permanently gathered and store information every ten minutes about air temperature (Ta in °C), rainfall (mm), relative air humidity (raH in %), air pressure (EA in mbar), wind direction and velocity (ms^{-1}). Air vapor pressure deficit (VPD in Pa) was calculated with the following equation [19]:

$$\text{VPD} = \text{Saturated Vapor Pressure (ES)} - \text{Actual Vapor Pressure (EA)} \tag{1}$$

where ES = 0.6108 EXP ((17.27 × Ta)/(Ta + 237.3)) and EA = (raH × ES)/100

2.5.2. Soil Moisture

Soil water profile was monitored at four locations per treatment along two elevations, totaling 20 locations (Figure 1): half of the monitoring points were at 47 m a.s.l and the remaining at 43 m a.s.l. Specific tubes for measuring soil water profile were installed between the plant and the irrigation tube 30 cm apart. Volumetric water content (%) was measured weekly in each location at six depths down to 100 cm with a Profile probe (PR2, Delta-T Devices). Between measurements, soil profile equipment was permanently installed in the field and locations were changed every 15 days.

As irrigation tubes were placed 40 cm deep, soil moisture measurements were grouped into two classes: sub-surface (0 down to 40 cm) and deep (40 cm to 100 cm deep) soil water storage (mm), according to the following equations:

$$\text{Sub-surface water storage (mm)} = (\theta_{0.1} \times 0.15\text{ m} + \theta_{0.2} \times 0.10\text{ m} + \theta_{0.3} \times 0.10\text{ m}) \times \text{CF} \tag{2}$$

$$\text{Deepwater storage (mm)} = (\theta_{0.4} \times 0.20\text{ m} + \theta_{0.6} \times 0.25\text{ m} + \theta_{1.0} \times 0.20) \times \text{CF} \tag{3}$$

where: θ_a is the volumetric water content by depth (a in meters) and CF is the conversion factor of water volume (m^3 m^{-3} to mm m^{-2}) = 10

2.5.3. Dendrometric Measurements

All plants were measured before and after treatments in February 2016 and 2017 (previously to the spring growing season). At the same time, plants' vitality was accessed visually (defoliation and drying). Stem diameter at the base (Db) was measured with millimeter-precision using caliper and total height (tH) was centimeter-accurate with a measuring tape. Plants' crown area was not measured as most plants had no apical dominance yet. Relative growth was calculated using the following equations:

$$\text{Relative diameter growth: Rg.Db2016 (\%)} = (\text{Db2017} - \text{Db2016})/\text{Db2016} \times 100 \quad (4)$$

$$\text{Relative height growth: Rg.tH2016 (\%)} = (\text{tH2017} - \text{tH2016})/\text{tH2016} \times 100 \quad (5)$$

2.5.4. Stomatal Conductance

Plants located at corridors A or B near soil moisture tubes (Figure 1) were selected for physiological measurements. From 7th July until 8th September 2016 stomatal conductance (mmol m^{-2} s^{-1}) was measured weekly with a portable diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, UK) in a total sample of 147 plants. Four fully expanded, south-oriented/sun-exposed leaves of the current-year spring flushing with appropriate size and smooth were selected from each plant. On each day of measurements, stomatal conductance was monitored in about 55 plants between 10:00 h and 16:00 h.

2.5.5. Statistical Analysis

Statistical analysis were made using the SPSS v.22 software package (IBM Corp., Armonk, NY, USA). Before statistical modeling, all variables were graphically explored regarding distribution patterns and outliers. No transformation was required and values that clearly corresponded to errors in measurements were removed. Analysis of Variance (ANOVA) was performed for each block to compare dendrometric parameters (diameter, height or relative growth) between plants from the center and the margin. As the results were not statistically different, all the plants were included in the following dendrometric models. A General Linear Mixed model was applied to analyze: (1) if deepwater storage with repeated measurements over the summer was related to treatments, to elevation (two classes), and to time (day of measurement); (2) If stomatal conductance, grouped by soil moisture locations and with repeated measurements over the summer was related to treatments, to elevation (two classes), to deepwater storage, to hour of the day, to time (day of measurement) and to several meteorological variables (air temperature, relative air humidity, calculated air vapor pressure deficit, wind velocity); Interactions were tested; (3) If each dendrometric parameter (diameter or height, or their relative growth), grouped by plants within blocks was related to the initial dendrometric parameter, to the distance to the stream, to elevation, or to treatments. In all the mixed models, non-significant independent variables were removed and, additionally, interaction between significant variables was tested. Several covariance structures were tested, selecting the one that best fit the data according to the information criteria. If the variance related to the random variables (block or locations) was not statistically significant, a model was tested without grouping plants within blocks or locations. Estimated marginal means of fitted model were requested, comparing the main effects with all the available methods (Least Significance Difference, Bonferroni, and Sidak). When applying General Lineal Models (when subjects were not grouped and there was no repeated measurements) contrast tests were performed. Treatment variable was reclassified according to the significance of the estimates of fixed effects, grouping those that were not statistically different and a new mixed model was performed.

3. Results

3.1. Meteorology

The ombrothermic diagram (Figure 2) indicates monthly precipitation/irrigation and mean temperature in the study field since the first summer after planting. During the four-month irrigation treatments, the daily temperature averaged 20.9 ± 3.5 °C and total precipitation was only 42 mm. Irrigation provided monthly water inputs ranging from 4 mm (control) to 24.4 mm (treatment 4). The month before the treatments was the wettest of 2016, where precipitation exceeded 120 mm.

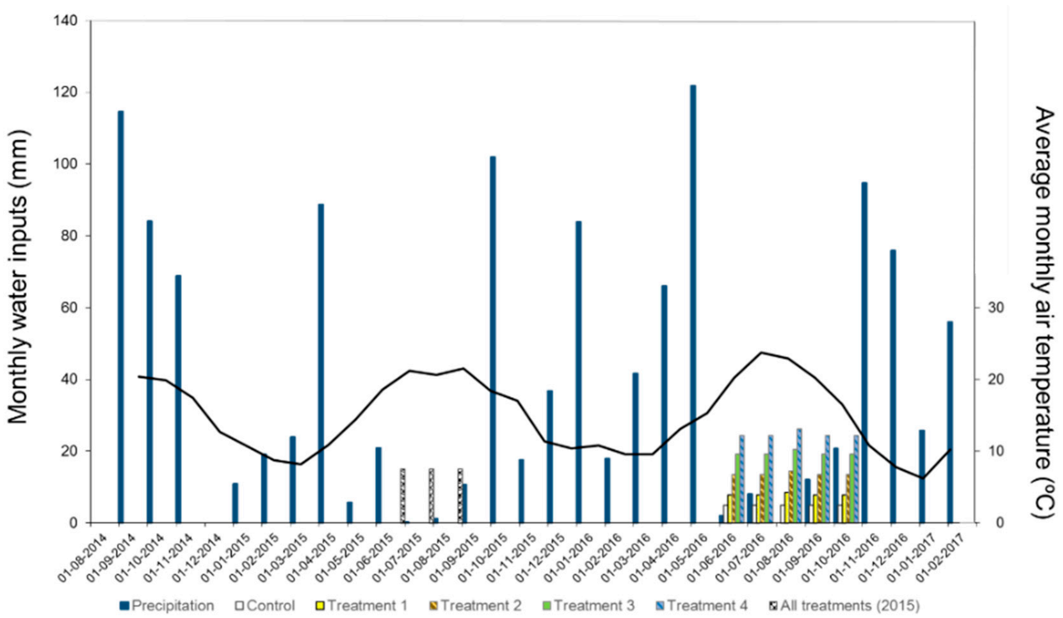

Figure 2. Ombrothermic chart of monthly rainfall and mean temperature that occurred in the experimental plot since planting, including monthly irrigation water inputs per treatment. Control received survival irrigation.

3.2. Soil Moisture

During 2016, surface soil moisture (above irrigation tubes) ranged from ≈0% to 13%; Changes in moisture were closely associated with daily precipitation (Figure 3).

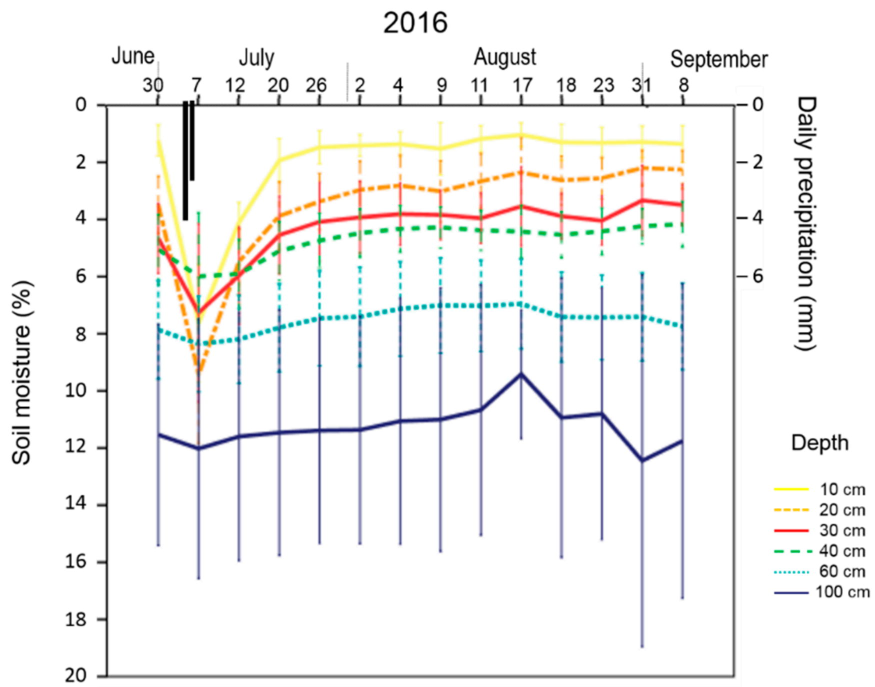

Figure 3. Average (lines) and standard deviation (vertical lines) of instantaneous soil moisture measured at six depths in all the locations from the experimental plot, and daily precipitation (black bars) during summer 2016.

In deep layers (60 and 100 cm) soil moisture fluctuated between 4% and 46%. Water soil storage reached 116.3 ± 18.8 mm in spring and slowly halved throughout the summer ($p < 0.001$, Table A1) despite irrigation treatments. The upper part of the experimental field presented lower values for deepwater storage (28–86 mm) than down the hill ((41–142 mm), $p < 0.001$). However, when soil was saturated (June) no differences between elevations were observed (t: −1.77, $p = 0.103$). There was no significant relationship between irrigation treatments and deep soil water storage ($p = 0.057$, Table A1). Additionally, continuous measurements at several locations showed no variation in soil water profile after or during the irrigation periods (data not shown).

3.3. Functional Parameters: Stomatal Conductance

Stomatal conductance varied between 34 mmol m^{-2} s^{-1} and 449 mmol m^{-2} s^{-1}, with mean + standard deviation = 191 ± 72 mmol m^{-2} s^{-1}. Using the mixed models' procedure (that considers non-independence of the data measured on the same plant on different days and plants grouped by location) there was no statistical significance between treatments and stomatal conductance ($p > 0.05$). However, since the variance between locations did not explain the observed variance ($p = 0.092$; Table A2), the random grouping within locations was removed. Standard errors and confidence intervals decreased and treatments were statistically significant ($p = 0.001$). Stomatal conductance in control and treatment 1 were significantly lower than in the remaining treatments which, in turn, were similar between them (Table A3). Therefore, treatments were reclassified and the first model was again performed. A statistically significant relationship between stomatal conductance and treatments was obtained with this model (Table 3). This physiological parameter was about 15% lower in control and treatment 1 than in the remaining treatments (176.91 ± 6.32 mmol m^{-2} s^{-1} and 203.51 ± 5.53 mmol m^{-2} s^{-1}, respectively). Additionally, stomatal conductance tended to decrease over the summer and was positively related to plants' dimension (initial diameter at the base). Deepwater storage was also associated with stomatal conductance: for each 1 mm increase in deepwater storage, leaf stomatal conductance increased 1.4 mmol m^{-2} s^{-1}. Meteorological data and hour of the day were not associated with this physiological parameter and removed from the model. Regarding hour of the day, graphical analysis showed that stomatal conductance displayed little variation between 10:00 h and 16:00 h.

Table 3. Independent fixed effects of the generalized linear mixed model (GLMM) with two treatment classes on the stomatal conductance (mmol m^{-2} s^{-1}) measured in 147 plants grouped by "location" as a random effect with a first-order autoregressive structure, in the experimental plot during the irrigation period (summer 2016).

Parameter		Estimate	S.E.	*t* Value	95% Confidence Interval		
					p Value	Lower	Upper
Intercept		116.46	25.04	4.65	<0.001	66.30	166.63
Time (day of measurement)		−1.29	0.18	−6.95	<0.001	−1.65	−0.92
Initial diameter_base (cm)		1.86	0.51	3.65	<0.001	0.85	2.87
Deep water storage (mm)		1.41	0.38	3.67	0.001	0.62	2.18
Treatments	Control + 1	−26.23	8.76	−2.99	0.009	−44.88	−7.57
	2 + 3 + 4	0 [a]					

[a]. This parameter is set to zero because it is redundant. S.E: Standard error of the mean.

3.4. Structural Parameters: Dendrometry

After two years of planting and before the beginning of the treatments (February 2016), there was already considerable variation in the plants' dendrometric values (see Figure 4 for diameter): mean and S.D. of diameter at the base (Db) was 1.53 ± 0.74 cm ranging from 0.51 to 1.91 cm, and of total height (tH) was 52.33 ± 25.04 cm ranging from 6 to 197 cm. All plants entered the following models since their position within blocks (center or margins) had no significant effect on growth. The mixed model indicated that irrigation treatments positively influenced the linear relationship between Db2016 and Db2017, though in the limit of significance ($F = 3.21$, $p = 0.049$).

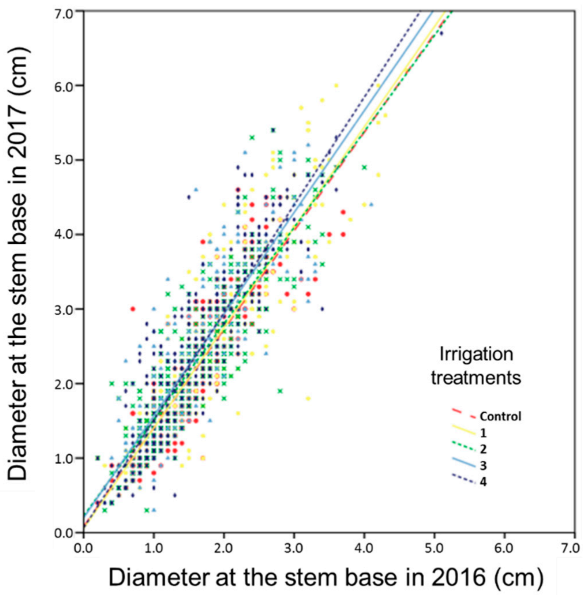

Figure 4. Linear relationship between initial (2016) and final (2017) diameter at the base, separated by irrigation treatments.

The results were quite similar for relative diameter growth (F = 3.23, p = 0.047). However, this parameter is dimensionless and, therefore, a better growth indicator of plants with disparate initial sizes. In addition, using relative increments rather than absolute values allowed measuring the effect of the initial values on growth. Since the random variable blocks was not significant (Wald Z: 1.26, p = 0.210), a general linear model was applied without grouping the plants into blocks. In this model (Table A4), irrigation treatments were more associated with increments (F = 7.15, p < 0.001) after a reduction in the standard errors and confidence intervals, but the tendency remained the same (Figure 5). Treatment contrasts of the last model reinforce the results (Table A4a).

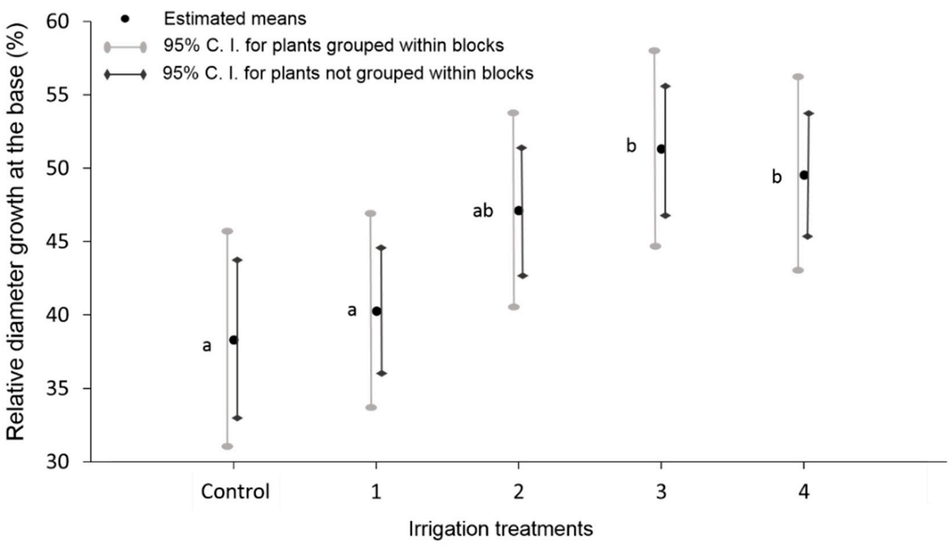

Figure 5. G.L.M.M. estimated marginal means of the relative diameter growth at the base (%) by irrigation treatments, with plants grouped or not within blocks. Different letters denote statistically significant differences at the 5% level without grouping plants within blocks as a random factor.

Considering the significant differences between treatments, the inflection point was on treatment 2 corresponding to an average increment of 47% (Figure 5). Again, control and treatment 1 were different from the remaining treatments (Table A4) and, similar to stomatal conductance models, treatments could be classified into two classes. The new mixed model with plants within blocks showed that the increase in diameter was ≈10% inferior in plants from control and treatment 1 (Table 4). Additionally, the relative increment was lower in larger plants ($t = -4.965$, $p < 0.001$). Distance to the stream was also negatively associated with diameter growth, though elevation was not ($p > 0.05$, removed from the model). Nevertheless, one should note that the variance in residuals was very large (Table A5). In regards to total height, average + S.D. was 66.6 ± 35.7 cm, relative increment was ≈22% and treatments were not significant ($p > 0.05$). Initial height values negatively influenced the relative growth ($t = -11.171$, $p < 0.001$) and distance to the stream showed a stronger negative relation with height than with relative diameter growth ($t = -6.857$, $p < 0.001$).

Table 4. Generalized linear mixed model (GLMM) Estimates of the Independent fixed effects on the relative diameter growth at the base (%)", grouped by plants within blocks as a random effect with a first-order autoregressive structure and combining treatments into two classes, in the experimental plot after the irrigation period.

Parameter		Estimate	S.E	*t* Value	95% Confidence Interval		
					***p* Value**	**Lower**	**Upper**
Intercept		78.70	7.85	10.02	<0.001	63.29	94.10
Initial diameter_base (cm)		−0.72	0.15	−4.94	<0.001	−1.10	−0.44
Treatments	Control + 1	−9.95	2.87	−3.47	0.003	−16.03	−3.87
	2 + 3 + 4	0 [a]	0.00				
Distance to the stream (m)		−0.08	0.03	−2.59	0.010	−0.14	−0.02

[a]. This parameter is set to zero because it is redundant. S.E: Standard error of the mean.

4. Discussion

4.1. Water Volume for the Best Structural Functional Response

In this study, cork oak afforestation was implemented in a 6 ha field under natural conditions, with fertigation during the dry season. Despite intrinsic plant variability and some soil heterogeneity, mostly related to groundwater access, cork oaks directly respond to irrigation treatments. The effect of irrigation on plants was both functional and structural, where the physiological response of plants to treatments closely matched with the structural response, particularly in diameter thickness. A 3-fold increase in water during the irrigation campaign (control + treatment 1 ≈ 31 mm; treatments 2 + 3 + 4 ≈ 96 mm) resulted in an average increase of 15% on stomatal conductance and a 10% increase in stem diameter. The irrigation inflection point was at 3.12 mm per week, above which the increase in diameter was not significant. This value applies to the particular conditions of the plot and to plant size. In [12], same size plants responded to a higher irrigation regime (12 mm vs. 4 mm by week). It is likely that in the sandy soil of the experimental plot, deep irrigation water is not fully available to the plants. An increase in irrigation regime can result in more gravitational water instead of capillary water—which is lost by percolation [20]. These results strongly indicate that a summer subsurface drip irrigation campaign with a total of 655 m^3 of water supplied 3 times a week is applicable to cork oak seedlings growing on deep sandy soils.

4.2. Soil Water Profile as an Indicator of Irrigation Requirements

Characterization of soil water profile is an important procedure for inferring water available to plants and is widely used in irrigation water management to meet crop needs. However, soil water measurements presented some constraints in this study: the coarse sandy soil of the plot limited the formation of a large wet-bulb due to the low water holding capacity [21]. Thus, the irrigation bulb did not extend horizontally to the locations where soil moisture was monitored (about 30 cm away). As soil moisture was monitored between the irrigation tubes and the plants, this means the wet-bulb did not reach the plants. Therefore, soil moisture measured in the locations was not directly related to irrigation inputs. In fact, continuous measurements also failed to distinguish water inputs from irrigation, confirming that the wet-bulb did not extend horizontally up to 30 cm. This parameter was not useful for a future irrigation water management in the sandy soils of the experimental site, however, other information was possible to obtain from soil water profile. Both soil analysis and soil water monitoring allowed inferring the soil water capacity of the experimental plot: measurements performed after rainy periods indicated that field capacity down to 1 m varied slightly across all profiles. On the other hand, measurements performed in summer highlighted two conditions: (1) surface moisture (0–30 cm, Figure 3) was below the permanent wilting point for this soil type (<5%) [22] constraining superficial roots functioning or even survival; (2) Elevation accounted for differences in water profile across the plot. This may be due to two reasons: the lower part of the plot is more prone to obtain gravitational water from the upper part of the slope; and/or proximity to the stream may be associated with groundwater availability. Finally, soil water measurements also indicated that deepwater storage was probably originated from groundwater capillary rise and not directly from irrigation—suggested by no statistical differences in soil moisture from control and irrigated lines.

4.3. Stomatal Conductance as an Indicator of Irrigation Requirements

Isohydric plants such as cork oaks strongly regulate stomatal opening when facing water stress, thus limiting carbon assimilation and growth [9,23]. Therefore, stomatal conductance in response to water deficit can eventually be used to assess irrigation needs [24]. In this study, several parameters related to soil moisture were associated with stomatal conductance: directly by the significant association with deepwater storage and treatments and indirectly by its reduction over time. Meteorological conditions may also influence stomatal conductance opening, such as temperature and air dryness [25–28]. Since none of them was statistically significant, the stomatal conductance decrease over time may be associated not to with weather but with some groundwater depletion as the dry season extends—though not detected through soil water measurements. In that case, the only variable independent of soil moisture that affected stomatal conductance was plants' diameter. Larger plants tended to show more stomatal conductance, at least at this stage of development. It may derive from a higher water absorption capacity due to larger root length. Finally, leaf stomatal conductance was also associated with treatments (Table 3). This significant relationship indicates that plants had access to the irrigation water at the magnitude of their supply. Roots are expected to growth unrestrictedly large distances from the stem, as observed in adult cork oaks [29], thus reaching the irrigation water. For example, plants from the study field excavated one year before [18] had already roots as far as 70 cm distant from the stem. These results suggest that stomatal conductance may be an indicator of irrigation efficiency in the subsequent studies on this subject.

4.4. Plants Structural-Functional Variability

Even with all the significant relations with the independent factors, most of the stomatal conductance variance was residual, indicating high variability between subjects. Variability in cork oak physiological parameters under natural conditions are usually expected [30–32]. Constrains in methodology may also account for some of the variability observed. Measurements could not be performed in all the sun exposed leaves, because they were too small and curled. Choosing the right size and shape of the leaves narrowed the options, and eventually, some selected leaves might not be fully exposed to sunlight. In fact, stomatal conductance varied within the same plant. Despite the methodological constraints and high residual variance, treatments had a small but significant effect on plants. The average values were in accordance [9,15,33], or even higher [30], with ones found in cork oaks with no water restrictions. Similarly, variation in diameter residuals was also very high. This may reflect intrinsic characteristics of each plant, such as genetics, initial acorn reserves, root development, etc., as well as nursery conditions or site-specificity: initial growth may be subject to several conditions other than water availability [34–37]. High variability in plant growth was also observed in several studies [38,39]. Regarding height, the non-significant response to irrigation treatments can be associated with the reduced apical dominance observed in the plants at the study site. This condition may change over time.

5. Conclusions

Field experiments usually pose more challenges, but the results have better external validity, and are applicable to natural conditions. Despite the constraints related to soil conditions, particularly the low soil water retention, irrigation was significantly associated with the plants' functional-structural response. A 3-fold increase in water corresponded to a 15% increase in stomatal conductance and a 10% increase in stem diameter. Hence, stomatal conductance may be the link between water availability and plant growth in the following studies. These results are promising in the analysis of the best irrigation regime for cork oaks under natural conditions. Plant needs and their resource use efficiency change over time, as well as abiotic factors such as meteorological conditions. Therefore, the study should be a long-term project in order to meet to the main objective of the best irrigation regime for all scenarios.

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Appendix A

Table A1. Independent fixed effects of the General Linear Mixed Model applied to the dependent variable "deepwater storage" in 20 locations of the experimental field during the irrigation period.

Parameters	*F* Value	df1	df2	*p* Value
Corrected model	5.14	6	214	<0.001
Treatments	2.33	4	214	0.057
Elevation (m a.s.l.)	9.52	1	214	0.002
Time (days)	12.30	1	214	<0.001

df: degrees of freedom.

Table A2. Generalized linear mixed model (GLMM) estimates of covariance parameters on the stomatal conductance measured in 147 plants subjected to four treatment classes, grouped by "location" as a random effect with a first-order autoregressive structure (AR), in the experimental plot during the irrigation period (summer 2016).

Parameter		Estimate	S.E.	Wald Z	95% Confidence Interval		
					p Value	Lower	Upper
Repeated Measures	AR diagonal	4043.20	243.78	16.58	<0.001	3592.55	4550.38
	AR rho	0.34	0.05	7.47	<0.001	0.25	0.43
Intercept (location)	Variance	346.26	205.55	1.68	0.092	108.17	1108.42

S.E: Standard error of the mean.

Table A3. Generalized linear mixed model (G.L.M.M.) estimates of covariance parameters on the stomatal conductance measured in 147 plants subjected to irrigation treatments, NOT grouped by "location", in the experimental plot during the irrigation period (Summer 2016).

Parameter		Estimate	S.E.	*t* Value	95% Confidence Interval		
					p Value	Lower	Upper
Intercept		183.76	17.25	10.65	<0.001	149.79	217.72
Time (day of measurement)		−1.96	0.18	−10.62	<0.001	−2.32	−1.60
Deep water storage (mm)		1.12	0.28	4.00	<0.001	0.57	1.67
Initial diameter_base (cm)		1.40	0.40	−10.62	0.001	0.61	2.20
Irrigation water (mm week^{-1})	≈1 *	−33.26	8.54	−3.90	<0.001	−50.08	−16.44
	1.88	−24.17	8.90	−2.72	0.007	−41.71	−6.64
	3.12	−8.33	8.60	−0.97	0.333	−25.28	8.61
	4.38	−4.44	9.33	−0.48	0.635	−22.83	13.95
	5.62	0 [a]					

* Control was irrigated every months, corresponding to an average 1 mm week^{-1} of water. S.E: Standard error of the mean.

Table A4. Generalized linear model (G.L.M.) on the relative diameter growth at the base (Rg.Db2016 %), in the experimental plot after the irrigation period (Summer 2016). (**a**) Repeated contrast Results (K Matrix), (**b**) Estimates of the Fixed Effects.

(a) Repeated contrast Results (K Matrix)

Water (Treatment) Repeated Contrast			Dependent Variable: Rg.Db2016 (%)
control vs. Level 1	Contrast Estimate		−2.36
	Std. Error		3.51
	***p* value.**		0.501
	95% Confidence Interval for Difference	Lower Bound	−9.25
		Upper Bound	4.52
Level 1 vs. Level 2	Contrast Estimate		−6.29
	Std. Error		3.11
	***p* value.**		0.044
	95% Confidence Interval for Difference	Lower Bound	−12.40
		Upper Bound	−0.18
Level 2 vs. Level 3	Contrast Estimate		−4.76
	Std. Error		3.20
	***p* value.**		0.138
	95% Confidence Interval for Difference	Lower Bound	−11.04
		Upper Bound	1.52
Level 3 vs. Level 4	Contrast Estimate		1.66
	Std. Error		3.14
	***p* value.**		0.598
	95% Confidence Interval for Difference	Lower Bound	−4.50
		Upper Bound	7.81

(b) Estimates of the Fixed Effects

Parameter		Estimate	S.E.	*t* Value	95% Confidence Interval		
					p Value.	Lower	Upper
Intercept		77.85	8.01	9.72	<0.001	62.14	93.56
Initial diameter_base (cm)		−0.69	0.15	−4.77	<0.001	−0.98	−0.41
Distance to the stream (m)		−0.08	0.03	−2.47	0.014	−0.14	−0.02
Irrigation water (mm week^{-1})	**≈1 ***	−11.75	3.50	−3.35	<0.001	−18.63	−4.88
	1.88	−9.39	3.06	−3.07	0.002	−15.38	−3.39
	3.12	−3.10	3.11	−1.00	0.319	−9.20	2.99
	4.38	1.66	3.14	0.53	0.598	−4.50	7.81
	5.62	0 [a]					

* Control was irrigated every months, corresponding to an average 1 mm week^{-1} of water. [a]: This parameter is set to zero because it is redundant. S.E: Standard error of the mean.

Table A5. Generalized linear mixed model (GLMM) estimates of the covariance parameters on the relative diameter growth at the base (%), grouped by plants within blocks as a random effect with a first−order autoregressive structure and combining treatments into two classes, in the experimental plot after the irrigation period.

Parameter	Estimate	S.E	Wald Z	*p* Value	95% Confidence Interval	
					Lower	Upper
Residual Variance	1683.86	59.69	28.21	<0.001	1570.84	1805.02
Intercept (subject = block)	17.24	15.00	1.15	0.250	3.13	94.84

S.E: Standard error of the mean.

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