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Root Growth Dynamics and Structure in Seedlings of Four Shade Tolerant Mediterranean Species Grown under Moderate and Low Light

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Abstract: Specific functional traits such as shade tolerance or leaf habits can enhance root growth dynamics and structure of planted seedlings in the understory of planted forests. We assessed how low and moderate light levels (17 and 33% of full sunlight, mimicking after-thinning stocking) affect the root growth dynamics and structure of four late successional trees, three deciduous (Acer monspessulanum L., Quercus pyrenaica Willd and Sorbus torminalis (L.) Crantz) and one evergreen (Quercus ilex L.) species. Rooting depth, dynamics and structure were mainly explained by species functional differences. Roots of deciduous trees elongated faster and deeper and were larger than the roots of the evergreen Q. ilex. Among deciduous trees, S. torminalis had the lowest root growth. Specific leaf area and nutrient concentration were positively related to root growth, highlighting the importance of traits related to the plant economic spectrum, as determinants of species root growth differences. Moderate light level slightly enhanced root growth and decreased the specific leaf area (SLA). Species differences in water potential under drought were positively related to rooting depth, evidencing the importance of its role in overcoming drought stress during seedling establishment. These findings can guide the selection of late successional, shade tolerant tree species for underplanting thinned Mediterranean plantations and provide insights into their ecology.

Keywords: late successional species; rhizotron; root architecture; rooting depth; shade tolerance; specific leaf area

1. Introduction

Tree seedlings are sensitive to abiotic stress factors such as extreme temperatures, high radiation, or drought [1]. These stress factors are common in Mediterranean forest ecosystems, but water stress during summer drought is the main cause of mortality of plant juveniles, either naturally regenerated [2] or planted [3]. Root growth is a key ecophysiological process for overcoming drought stress and the establishment of seedlings in xeric environments [4,5]. Development of an extensive and deep root system favors extensive soil exploration and access to deep soil layers, which retain moisture during dry periods [6,7]. Root soil colonization and resource uptake ability depend on growth dynamics, elongation rate of the root system and root architecture, i.e., how plants spatially organize their root systems both at a fine and at a coarse scale [8–10]. Understanding how these traits vary both at the intra- and at inter-specific levels and interact with the environment is important for planning forest restoration [11].

Root growth dynamics and architecture vary widely among species, provenances, season and plant age [12–14]. For instance, woody species differ in the relative development
of taproots and the distribution of coarse and fine roots within the soil profile. These differences in root architecture affect rooting depth, nutrient and carbohydrate storage and soil resource foraging [15,16]. In a global root analysis, Jackson et al. [17] reported that root systems distribution in depth varies according to functional group and biome. In the case of dry and temperate species, 50% of roots grow beyond 30 cm of depth in the soil profile. Functional differences among plant species are frequently linked to differences in root properties [18,19]. Some evidence indicates that the leaf and plant economic spectrum is linked to specific root properties [20]. Thus, fast growing plants, whose leaves usually have short life span and high specific area [10,21], tend to produce a high number of fine roots with ephemeral tissues of low density, which results in a high specific root length (SRL). These types of roots are very efficient in soil resource acquisition [22].

Within the forest restoration framework, underplanting late successional, shade tolerant tree species to diversify monospecific plantations is currently undertaken in Mediterranean [23,24], tropical [25] and temperate [26] sites. In these plantations, seedlings are mostly exposed to moderate and intense shade conditions. Moderate shade can facilitate the establishment of shade tolerant trees in xeric climates, through mitigation of photoinhibition and other physiological stresses provoked by high temperatures and radiation [24,27]. However, intense shade can inhibit seedling root growth [15,28], which prioritizes photosynthate allocation to shoot growth [29], hindering seedling establishment in dry climates [30–32].

The effect of low light availability on root development depends on species shade tolerance and functional plasticity to variations in light intensity [33]. For instance, moderate to high light triggers a rapid and vigorous root growth after planting in the Mediterranean trees *Quercus ilex* L. and *Pinus halepensis* Mill. However, under intense shade, the light-demanding species, *P. halepensis*, shows stronger root growth inhibition in response to intense shade than the shade tolerant *Q. ilex* [28,34]. There is prolific scientific literature on the effects of light on the morphological and physiological performance of shoot and total growth of ecologically contrasting woody plants from Mediterranean ecosystems [35–37], tropical ecosystems [38,39] and wet temperate ecosystems [40]. However, little is known about the effects of light on the root growth dynamics of trees. Specifically, few comparative studies have analyzed the effect of light environment at levels like those of the understory on the root performance of late successional species with different functional traits. Knowledge of the light levels that maximize rooting depth and the expansion of the root system in forest species is important for species selection and designing forest thinning treatments to maximize performance of understory plantations.

This study addresses (1) the root growth dynamics and architecture and (2) the physiological performance in seedlings of four late successional Mediterranean tree species in response to light reduction under moderate levels of radiation. Studied species are: *Quercus ilex* L. ballota (Desf.) Samp (here after *Q. ilex*), *Quercus pyrenaica* Willd, *Sorbus torminalis* (L.) Crantz and *Acer monspessulanum* L. These species differ in their functional attributes; *Quercus ilex* is evergreen while the rest are deciduous and thrive in wetter sites than *Q. ilex* [41]. According to available literature, the species have different shade tolerance, therefore they could have a different root growth response [33,37,42]. Moreover, the different leaf habits of oaks allow us to assess the importance of this trait on root growth while minimizing phylogenetic bias. The objective of this study is to provide sound basis for species selection when diversifying planted forests. We tested the following hypotheses: (1) Species having high specific leaf area and biomass allocation to foliage and low tissue density have higher root growth than species with the opposite traits; (2) shade tolerance positively affects root growth and rooting depth capacity under moderate and low light conditions. To test these hypotheses, we performed an experiment with seedlings transplanted into rhizotrons and grown under two shade levels mimicking the light conditions in the understory of a Mediterranean pine plantation after two thinning treatments of distinct intensity.
2. Materials and Methods

2.1. Seedling Cultivation and Plant Material

Seedlings of the four broadleaf, late successional Mediterranean trees, *S. torminalis*, *Q. ilex* ballota, *Q. pyrenaica*, and *A. monspessulanum* were cultivated in the nursery of the School of Forestry, Technological University of Madrid (40°27′N; 3°43′W, 664 m a.s.l.). Seeds were collected in two seed zones in the Guadalajara province (Central Spain), namely, *S. torminalis* and *Q. ilex* seeds in the Alcarrias Region, whereas *Q. pyrenaica* and *A. monspessulanum* in the Sierra de Guadarrama-Ayllon (RIUS 21 and 20, respectively) [43]. Pregerminated acorns of *Q. pyrenaica* were sown in January 2017 while acorns of *Q. ilex* were sown in March. Prior to sowing, seeds of *S. torminalis* and *A. monspessulanum* were stratified following methodology described by [41] and then sown in March and April, respectively. All species were raised in Forest Pot® 300 containers (50 cavities of 300 mL per tray and 15 cm of height, Nuevos Sistemas de Cultivo S. L., Girona, Spain). Growing medium consisted of a mixture of 80% white peat (Kekkila® White 420 F6, Finland) and 30% vermiculite (Projar, S.A., Valencia, Spain) plus a slow-release fertilizer (12–14 months duration; N:P:K 18-6-8, plus micronutrients) (Nutricote Total®, Arista LifeScience Tokyo, Japan) at a 7 g L\(^{-1}\) rate. After emergence, seedlings were moved outdoors in late April 2017. From then, seedlings were exposed to full sun (except in August, when an 80% shade cloth was extended to reduce light to 20%) and well-watered every 1–3 days until April 2018.

In May 2018, we randomly harvested five seedlings per species prior to transplanting non-harvested seedlings into the rhizotrons (see below) and froze them at −20 °C until processing. Shoot height and diameter at the cotyledon insertion point were measured. Then, biomass was separated into leaves, stems, and roots, except in *Q. pyrenaica*, which had no leaves at harvest and was weighed after drying at 62 °C for 48 h. We calculated the shoot/root ratio and assessed nitrogen (N) and phosphorus (P) concentrations through the standard Kjeldahl method using an auto-analyzer (CFA SAN++, Skalar, Breda, The Netherlands). Nitrogen and P content were calculated by multiplying the concentration of each nutrient in each seedling compartment by their mass. The morphological and nutritional information of the plants at planting is shown in Table S1 (Supplementary Material). Before transplanting in the rhizotrons, seedling height followed the order *Q. ilex* > *A. monspessulanum* > *S. torminalis* > *Q. pyrenaica*, whereas the total mass was *Q. ilex* > *Q. pyrenaica* > *S. torminalis* > *A. monspessulanum*. Finally, N and P concentrations were higher in *A. monspessulanum* and *Q. pyrenaica* than in *Q. ilex* and *S. torminalis* while the nutrient content was higher in *Q. pyrenaica* and *Q. ilex* compared to *A. monspessulanum* and *S. torminalis*.

2.2. Experiment Design

On 9 May 2018, ten seedlings per species were transplanted into rhizotrons, which consisted in 1 m length transparent methacrylate tubes, exterior diameter of 12 cm, and a wall thickness of 0.6 cm. To allow water drainage and prevent substrate loss, all tubes were closed with a perforated lid. The bottom of the rhizotrons was filled with 10 cm of gravel as a drainage layer and the rest of the rhizotron was filled with a 2:1 mixture of sieved washed sand and perlite. Once filled, rhizotrons were inserted into a second opaque plastic tube, 1.1 m in length and 16 cm in diameter that was buried in the ground. This allowed the rhizotrons to keep a similar temperature to the surrounding soil and facilitated their easy extraction from the ground. The opaque tube was buried with a 30° inclination to force the roots to grow along the transparent wall of the rhizotron [44,45]. Then, one seedling was planted into each rhizotron placing the plug against the wall to facilitate measuring the elongation of new roots. Once transplanted into the rhizotrons, we randomly assigned the seedlings to two light level treatments, each treatment encompassed five seedlings per species. Shoots were shaded using two types of cylindrical meshes 70 cm in height and 12 cm in diameter with different light transmissivity levels. Light levels were chosen to mimic the understory light of an even-aged *Pinus pinaster* 55-year-old plantation in Guadalajara (Central Spain) where different thinning and underplanting experiments.
are being conducted. The two light levels tested (low and moderate) correspond to an unthinned (basal area 35.7 m$^2$ ha$^{-1}$) and heavy thinned (35% basal area removed to a residual value of 23.2 m$^2$ ha$^{-1}$) stands, respectively. We selected meshes with light transmissivities similar to the Global Site Factor previously estimated by hemispherical photos using software Gap Light Analizer (GLA) Version 2.0 [46], under the plantation canopy in both levels of cover canopy, i.e., 22.8 ± 2.4 and 41.3 ± 5.5 for unthinned and the heavy thinned treatments, respectively. Daily Light Integral (DLI) over the experimental site was 60.5 mol m$^{-2}$ d$^{-1}$. Light levels of the two mesh types were measured with a quantum sensor (model SQ-212, Apogee Instruments, Logan, UT, USA) connected to a data logger (HOBOn U12, Onset Computer Corporation, MA, USA) for 3 days in late June 2018. Mean and standard error values were 17.34 ± 0.28% and 33.14 ± 0.43% for the low and moderate light levels, respectively (Figure 1).

![Figure 1. Illustration of a rhizotron used for measuring root growth dynamics under controlled two light conditions.](image)

At the beginning of the experiment, rhizotrons were irrigated to saturate the growing medium to mimic soil moisture in spring. Afterwards, each plant was irrigated weekly with 1 L. Irrigation was conducted with a water-soluble fertilizer (N:P:K 20-20-20; Scotts Co., Marysville, OH, USA) at a 1 mM N concentration to simulate the natural nutrient availability in soils where these species usually inhabit [47]. After 8 weeks, irrigation frequency was reduced to once every two weeks to induce a drought period according with the rainfall regime and soil moisture at Mediterranean sites.

The experiment was performed in a field plot at the School of Forestry, Technological University of Madrid. During the experiment, the mean temperature was 21.7 °C, and the mean maximum and minimum temperatures were 33.1 and 13.9 °C, respectively.

2.3. Root Measurements

Two weeks after the beginning of the experiment, most seedlings had visible new roots protruding and growing against the rhizotron wall. Every week, new roots were drawn on 29.7 × 21 cm$^2$ acetate sheet placed on the rhizotron wall in the same position. Measurements were made for 11 weeks until 1 August 2018, when the roots of most seedlings reached the bottom of the rhizotron, and the experiment finished. New roots
were identified, and the new segments of existing roots were drawn with different colors each week. The minimum root length registered on sheets was 0.5 cm and the roots that did not grow for two weeks were considered dead. Root growth dynamics was assessed through image analysis of drawn roots. Acetate sheets were scanned (Scanjet G3110, Hewlett Packard, CA, USA) at a 300 dpi resolution and analyzed with the plugin Smart Root version 4.1 [48] by semi-automated image analysis through the vectorial quantification of root growth for a complex root system. Thus, the number of roots and the length of each root segment or unit (hereafter root unit) per week was measured. The elongation rate of the whole root system and per root unit was calculated as the sum of the root length increments of all individual roots after one week of growth and divided by the number of elongating (live) roots, respectively. The number of elongating roots was quantified as the number of roots that showed growth in the previous two weeks. Rooting depth at a specific date was measured as the vertical distance from the bottom of the root plug to the tip of the deepest root according to [44,45].

2.4. Root and Leaf Morphological Traits

On 1 August 2018, we harvested seedlings by carefully extracting the whole root system from the rhizotron using low-pressure water. Plants were separated into leaves, stems, and roots. Leaves were scanned, and the area was measured with an image analyzer Image J version 1.52a [49]. Then, samples were oven-dried at 60 °C for 48 h and weighed to assess their mass. For root morphology analysis, we only used the new emerging roots, which were separated by cutting the protruding roots at the plug surface; for total root mass we also considered the plug, previously washed and dried (Supplementary Material Figure S1). Seedling parts were stored in a freezer at −20 °C prior to measuring the morphological root traits. The defrosted and clean new roots growing out of the plug were digitalized with a scanner (Epson Perfection V800 Pro Scanner, Seiko Epson Corporation, Nagano, Japan) at a 1200 dpi resolution and converted to TIFF format using the software Arc Photo Studio version 6.0 (Arc Soft, Inc., Freemont, CA, USA). The length, surface area, average root diameter and volume of new roots were determined using with WinRhizo System Version 3.1b (Instruments Regent Inc., Chemin Sainte-Foy, Quebec, QC, Canada). Specific root length (SRL) was calculated as the ratio between the length and mass of protruding roots per plant. Root tissue density was determined as the new root dry mass to new root volume ratio [50]. Specific leaf area (SLA) was calculated as the ratio between the leaf area and its dry mass. Finally, the surface root area to surface leaf area ratio was calculated for each plant.

2.5. Physiological Measurements

Species physiological response to light levels was assessed in five seedlings per species by measuring water potential and chlorophyll fluorescence. Mid-day water potential (ψ) was measured at the end of the experiment (week 11) between 14:00 and 15:00 h solar time on the upper third of the shoot. Measurements were conducted using a pressure chamber (1000 PMS Instruments, Albany, OR, USA). Additionally, the quantum yield efficiency of the photosystem II (φPSII) was measured at week 11 between 10:00 and 11:00 h solar time with a portable pulse modulated fluorimeter using standard protocols (FMS2, Hansatech Instruments, Kings, Lynee, UK).

2.6. Data Analysis

All analyses were performed using the software R, version 3.6.2 [51]. We used linear mixed models for repeated measures to analyze root dynamic traits (rooting depth, number of elongating roots, whole root system elongation rate and the elongation rate per root unit). The species and light levels were fixed factors and individual seedlings was the random effect. We used the lme4 package [52] to perform these analyses. Morphological traits at the end of the study (total root, stem and leaf mass, the leaf, stem and root mass fraction, the root average diameter and surface area, SRL, SLA, and ratios of surface root area/surface
leaf area) and physiological traits (ψ and φPSII) were analyzed by a two-way ANOVA using the stats package [51]. In this case, species and light levels were fixed factors. Values of significance per factor were tested using the car package [53]. Additionally, analysis of covariance (ANCOVA) was developed for φPSII response to species and light levels and its interaction considering ψ as covariate. Data normality and homoscedasticity were checked through Shapiro–Wilk and Levene’s tests, respectively. Significant differences (α = 0.05) among species and treatments were tested using the Tukey post hoc test with the lsmeans package [54]. Finally, ψ was related to rooting depth and φPSII. Additionally, we related φPSII to mass of new roots and rooting depth at the end of the experiment through linear regression. Non-normality and heterogeneity of variance were corrected using logarithmic or Box Cox transformations.

3. Results
3.1. Root Growth Dynamics

Species strongly differed in rooting depth, but differences were observed only after week 3 and increased continuously until the end of the study (Species × Time interaction, Table 1, Figure 2). We found two couples of species were differentiated: roots of *A. monspessulanum* and *Q. pyrenaica* deepened more than the roots of *Q. ilex* and *S. torminalis*. Rooting depth after 11 weeks of *A. monspessulanum*, the species with the deepest roots, was 66% higher than the rooting depth of *Q. ilex*, the species with the shallowest root system (Figure 2). Light level did not significantly affect rooting depth.

**Table 1.** Statistical results of the effect of species and light level on root dynamic traits in rhizotrons. Data are F-values and p-values in brackets.

| Traits                     | Species (S) | Light (L) | Time (T)     | S × L | S × T | L × T | S × L × T |
|----------------------------|-------------|-----------|--------------|-------|-------|-------|-----------|
| Rooting depth              | 8.8 (<0.001)| 0.17 (0.49)| 307 (<0.001) | 0.41 (0.74) | 7.20 (<0.001) | 0.48 (0.65) | 0.97 (0.50) |
| Number of elongating roots | 24.4 (<0.001)| 1.46 (0.24)| 74.8 (<0.001) | 0.09 (0.96) | 6.93 (<0.001) | 3.37 (0.003) | 1.67 (0.02) |
| Root system elongation rate| 27.9 (<0.001)| 1.10 (0.30)| 52.7 (<0.001) | 0.16 (0.92) | 6.01 (<0.001) | 2.24 (0.016) | 1.95 (0.003) |
| Elongation rate per root unit| 7.10 (<0.001)| 0.088 (0.93)| 13.5 (<0.001) | 0.26 (0.85) | 2.71 (<0.001) | 0.50 (0.89) | 0.86 (0.67) |

**Figure 2.** Rooting depth (left) and elongation rate per root unit (right) in seedlings of four broadleaf Mediterranean tree species grown in rhizotrons. Data are mean and ± one SE.

Elongation rate per root unit showed a time-dependent response that differed among species (interaction Species × Time), regardless of light level (Table 1). Overall, *A. monspessulanum* showed the highest values of elongation rate per root unit while *Q. ilex* had the lowest. Species differences were more notorious between weeks 1 and 8. After week 8, elongation rate per root unit was progressively reduced until the end of the experiment (Figure 2).
Species differed in the number of elongating roots and the elongation rate of the whole root system, although significant interactions with time and light level occurred (Table 1). On average, species ranking for both root variables was \textit{A. monspessulanum} > \textit{Q. pyrenaica} > \textit{S. torminalis} > \textit{Q. ilex} (Figure 3). For instance, the average number of elongating roots for \textit{Q. ilex} was 6.8 while for \textit{A. monspessulanum} it was 33.9. Similarly, whole root system elongation rate ranged from 7.49 in \textit{Q. ilex} to 61.61 cm week$^{-1}$ in \textit{A. monspessulanum}. The trend of the average number of elongating roots and root system elongation rate across time varied among species (Species \times Time interaction). For instance, differences between \textit{A. monspessulanum} and the rest of species occurred from the second week, while \textit{Q. pyrenaica} had more new roots than \textit{Q. ilex} after week 6 and \textit{S. torminalis} after week 8. Overall, light increased the number of elongating roots and the root system elongation rate, but differences were only significant after the tenth week (Light \times Time interaction) when moderate light increased these variables by 39 and 36%, respectively, and by 58 and 47% at the final week of the experiment. Incident light affected the number of elongating roots and root elongation rate only in \textit{Q. ilex}, which produced 1.5 times more elongating roots and had 8.5 times higher root elongation rate under moderate light than under low light level after the ninth week (interaction Species \times Light \times Time, Table 1).

### 3.2. Plant Morphological Traits at the End of the Experiment

Species differed in most root variables, except for the SRL (Figure 4). Interactions between species and light was not significant for any root morphological trait. Overall, \textit{A. monspessulanum} had higher total root length, surface area, dry mass and diameter and ratio of surface root area: surface leaf area than the rest of the species, which either did not differ among them or \textit{Q. ilex} had significant lower values than \textit{Q. pyrenaica} and \textit{S. torminalis}. Root tissue density was the highest in both oaks, which had similar values, whereas values of root tissue density in \textit{A. monspessulanum} and \textit{S. torminalis} did not differ. Specific leaf area was greater in \textit{A. monspessulanum} and \textit{Q. pyrenaica} than in \textit{S. torminalis} and \textit{Q. ilex}. The rest of the variables did not differ among species.

Light level exposure effects on root and SLA were smaller than the species effects, with significant or marginally significant negative effects on the mass, surface area and length of new roots and positive effects on specific leaf area (Figure 4).

### 3.3. Physiological Traits

At the end of the experiment, $\psi$ differed among species ($p = 0.017$) with \textit{A. monspessulanum} and \textit{Q. pyrenaica} having higher values ($-3.13 \pm 0.09$ and $-3.48 \pm 0.30$ MPa, respectively) than \textit{Q. ilex} and \textit{S. torminalis} ($-4.54 \pm 0.56$ and $-5.07 \pm 0.16$ MPa, respectively). We found a strong positive relationship between seedling $\psi$ at the end of the experiment and rooting depth. Seedlings with higher $\psi$ (fewer negative values) had deeper roots, with \textit{A. monspessulanum} and \textit{Q. pyrenaica} seedlings having overall higher values of both variables than \textit{S. torminalis} and \textit{Q. ilex} seedlings (Figure 5).
Figure 3. Number of elongating roots (left) and root system elongation rate (right) in seedlings of four broadleaf Mediterranean species grown into rhizotrons under low (17%) and moderate (33%) light levels. Data are means ± one SE.
values of root tissue density in *A. monspessulanum* and *S. torminalis* did not differ. Specific leaf area was greater in *A. monspessulanum* and *Q. pyrenaica* than in *S. torminalis* and *Q. ilex*. The rest of the variables did not differ among species.

Light level exposure effects on root and SLA were smaller than the species effects, with significant or marginally significant negative effects on the mass, surface area and length of new roots and positive effects on specific leaf area (Figure 4).

**Figure 4.** Morphology or structure of protruding roots and leaf variables of four broadleaf Mediterranean tree species grown under two light levels: low (irradiance of 17%) and moderate (irradiance 33%). Data are mean ± one SE. *p*-values of the main effects are given in each subplot. Means not sharing common letters are significantly different after Tukey HSD test, $\alpha = 0.05$. *Am*: *Acer monspessulanum*, *Qp*: *Quercus pyrenaica*, *St*: *Sorbus torminalis*, *Qi*: *Quercus ilex*.
A strong positive relationship between seedling \( \psi \) at the end of the experiment and root depth. Seedlings with higher \( \psi \) (fewer negative values) had deeper roots, with \( A. \) monspessulanum and \( Q. \) pyrenaica seedlings having overall higher values of both variables than \( S. \) torminalis and \( Q. \) ilex seedlings (Figure 5).

Plants grown in moderate light tended to have lower \( \psi \) than the plants grown in low light in all the species except for \( Q. \) pyrenaica, which showed the reverse response (marginal Species \( \times \) Light interaction; \( p = 0.055 \)). The mean \( \psi \) for \( A. \) monspessulanum, \( S. \) torminalis and \( Q. \) ilex plants grown under moderate light was \(-3.90 \pm 0.32 \) MPa while the \( \psi \) of the plants grown under low light was \(-4.64 \pm 0.36 \) MPa.

The \( \phi \)PSII only showed significant differences among species at the end of the experiment (week 11) (interaction Species \( \times \) Time; \( p = 0.021 \)), when \( A. \) monspessulanum and \( Q. \) pyrenaica had significantly higher \( \phi \)PSII values than \( Q. \) ilex and \( S. \) torminalis (Figure 6). A strong relationship (\( r^2 = 0.42, p < 0.001 \)) was found between \( \psi \) and \( \phi \)PSII. However, species differences in \( \phi \)PSII disappeared (\( p = 0.13 \)) when \( \psi \) was included in the model as a covariate (significance of \( \psi \) \( p = 0.003 \)), with no significant effect of shade level (\( p = 0.35 \)) or the Species \( \times \) Light interaction (\( p = 0.84 \)). We also found a stronger relationship between \( \phi \)PSII and rooting depth (\( r^2 = 0.53, p < 0.001 \)) than \( \phi \)PSII and mass of new roots (\( r^2 = 0.19, p < 0.003 \)) at the end of the experiment.

Figure 5. Linear regression between the predawn water potential (\( \psi \)) and rooting depth at the end of the study (week 11) in four broadleaf Mediterranean tree species growing in rhizotrons. Each point represents the data from an individual.

\[
\begin{align*}
\text{Acer monspessulanum} & \quad P < 0.001 \quad R^2 = 0.54 \\
\text{Quercus pyrenaica} \\
\text{Sorbus torminalis} \\
\text{Quercus ilex}
\end{align*}
\]
Figure 6. Quantum yield efficiency of the photosystem II (ϕPSII) in four broadleaf Mediterranean tree species grown under two shade levels at the week 11 after transplanting into rhizotrons. Data are mean ± one SE. Means not sharing common letters are significantly different after Tukey HSD test, α = 0.05.

4. Discussion

Root growth dynamics and structure were more influenced by species functional differences than by light reductions. Contrary to our hypothesis, all species showed a similar root growth response pattern to light reduction despite their reported ecological differences [42].

4.1. Interspecific Differences in Root Development and Its Relationship with Species Functional Attributes

Tree species showed notable differences in rooting depth, growth dynamics and the structure of roots, which likely reflect the distinct functional characteristics of species [19]. Thus, roots of *Acer monspessulanum* and *Quercus pyrenaica* grew faster and deeper than the roots of *Sorbus torminalis* and *Quercus ilex*. Our results suggest that species differences in traits related to the leaf and plant economics spectrum [55], especially in SLA, can modulate the observed root growth differences. In general, plants with low SLA and high tissue density incur high construction costs [9,56] and have low photosynthetic rates, which result in long leaf life spans and slow growth capacity [21,55,57]. In accordance with this idea and our first hypothesis, the evergreen species *Q. ilex* showed the lowest SLA, the highest root tissue density, and the slowest root growth. Contrary to *Q. ilex*, the deciduous species, especially *Acer monspessulanum*, had high SLA values (Figure 4), a trait that can also optimize light interception in shaded environments [37]. Consequently, the deciduous species can potentially invest more resources in root growth [29]. This pattern is also shown within the *Quercus* group: although both species produce strong tap roots and similar root tissue density (Figure 4), *Q. pyrenaica*, which has high SLA, showed higher root growth and grew in depth faster than *Q. ilex*. Finally, the potential role of SLA as a modulator of root growth is also supported among the deciduous species: *Acer monspessulanum* and *Q. pyrenaica* had higher SLA and root growth than *Sorbus torminalis* (Figure 4). Moreover, tissue nitrogen and phosphorus concentration in *Acer monspessulanum* and *Q. pyrenaica* were higher than
the concentration in *S. torminalis*, which can confer a higher photosynthesis rate [21] and therefore a higher root growth capacity [58,59].

We did not find any relationship between species root growth capacity and SLR, which is contrary to results shown in other studies [22]. Interspecific differences in root growth dynamics and structure can also be due to species differences in resource allocation among plant parts and key functional processes such as storage, stress tolerance and growth [44,45,60]. The importance of resource allocation differences is out of the scope of this paper but deserves future study.

*Acer monspessulanum* and *Q. pyrenaica* had the highest root biomass and length, rooting depth and root mass fraction at the end of the study. These root properties are explained by their faster root growth, and it seems unrelated to species habitat characteristics. Compared to plant species from mesic habitats, plants inhabiting dry habitats usually grow faster in depth and allocate proportionally more biomass to roots, which is considered an adaptation to avoiding drought stress [5,19,61]. *Acer monspessulanum* and *Q. pyrenaica* thrive in sub-Mediterranean habitats, which are wetter than the habitats of *Q. ilex* [62]. High rooting capacity of *A. monspessulanum* and *Q. pyrenaica* can provide highly competitive and colonization capacity in forest understories [63]. The comparatively and unexpectedly low rooting capacity and surface root area/leaf area ratio of *Q. ilex* seedlings during the establishment might hinder drought avoidance capacity [3]. This limitation, however, might be offset by the development of low SLA leaves and high tissue density, which are traits related to drought and high-temperature stress resistance [64,65].

We showed the importance of rooting depth as a mechanism to avoid drought stress [66]. *Acer monspessulanum* and *Q. pyrenaica* developed the deepest roots and showed the lowest water stress after stopping irrigation (Figure 5). Higher hydration allowed these species to keep higher quantum yield efficiency of the photosystem II (Figure 6), a surrogate of photosynthesis activity [16]. In seasonal dry climates, juvenile roots of woody plants need to grow deep during the wet season to get established and avoid water stress mortality during the dry season [5,19].

Irrespective of the differences in growth rate, elongation of the root system in all species depended more on the production of new root units (number of elongating roots) rather than on the elongation rate of each root unit. A similar growth pattern was found in different pine species [44,45] and suggests a common root soil colonization pattern in trees based on the production of new root units that likely maximizes root foraging effectiveness [67].

Although our study was developed under controlled conditions and in rhizotrons to allow seedlings to express their root growth potential by reducing physical obstructions, it is possible that species have lower rooting potential under field conditions. Future studies should assess if the root growth pattern under field conditions is similar to under controlled conditions.

### 4.2. Responses to Light Gradient

All species showed similar root growth responses to the tested shade gradient: changes from low to moderate light levels provoked a small increase in the elongation rate of new roots, leading to significantly higher total root biomass and a trend to higher total root surface area and length at the end of the study (Figure 4). In contrast, rooting depth and physiological responses were unaffected by the light environment. The weak root elongation response might be explained by the low light gradient in this experiment (from 17 to 33% light transmissivity) [68], but also due to the similar shade tolerance of the studied species. More intense responses of root growth in species such as *Acer opalus* Mill. have been observed under stronger light gradients (from full sun to 13% light transmissivity [36]. In general, shade tolerant late successional species tend to show a low plasticity to changes in light environment compared to early successional species [13], particularly in Mediterranean environments [28]. Additionally, moderate and low light levels in our study might have inhibited the allocation of resources from shoot to roots,
contributing to reduce the growth plasticity in response to light [61]. As per root biomass, SLA also decreased from moderate to low light levels (Figure 4). This response is part of the shade avoidance syndrome aimed to maximize the efficiency of carbon investment into foliage production [69,70].

Reduction of post-planting root elongation can limit water and nutrients uptake along the soil profile. This can hinder the establishment of planted seedlings [4], particularly in dry summer climates such as the Mediterranean [58]. Therefore, moderate increments in light within the understory could benefit seedling drought stress avoidance in species underplanted to enrich forest plantations.

5. Conclusions and Practical Implications

Moderate light increases, such as that which occurs after forest plantation thinning, may enhanced root growth in the studied species. However, the main differences of root growth dynamics and structure were mainly explained by intrinsic species differences. Irrespective of light level, A. monspessulanum, followed by Q. pyrenaica, developed larger and deeper root systems than S. torminalis and Q. ilex. Higher rooting capacity let A. monspessulanum and Q. pyrenaica remain more hydrated under drought conditions, which could potentially confer a higher performance in understory plantations, particularly after thinning. Underplanting is an adaptative management tool to restore ecological functions and the diversity of monospecific pine plantations [71]. Therefore, our results provide guidelines for the selection of late successional, shade tolerant tree species for underplanting. From an ecological perspective, we demonstrate that despite regenerating in late stages of forest succession, the studied species might differ in their regeneration niche and colonization capacity [36]. Additional studies are necessary to understand the interaction of moderate light conditions with other abiotic and operational factors, such as soil fertility and preparation on the rooting capacity and establishment ability of late successional trees in Mediterranean plantations.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12111540/s1, Figure S1: Leaf, stem, and total root mass allocation in four broadleaf Mediterranean species after growing in rhizotron, Table S1: Morphological and nutrient status of one-year-old seedlings of the four broadleaf species prior to transplanting into rhizotrons.

Author Contributions: Conceptualization, software, and formal analysis, writing—original draft preparation, J.L.G.-P. and J.E.G.; writing—review and editing, supervision, project administration and resources, funding acquisition, J.A.O. and P.V.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research is part of the project FORADMIT “Gestión Forestal para la Adaptación y Mitigación: diversificación estructural y específica de pinares mediterráneos de repoblación” (AGL2016-77863) 2016–2020 funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe” and co-financed by the REMEDINAL network of the Madrid Autonomous Government (S2018/EMT-4338). José Luis García Pérez was supported by the National Council of Science and Technology (CONACyT) and National Institute for Forestry, Agriculture, and Livestock Research (INIFAP) from the Mexican Government through a doctorate grant.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We give thanks to Enrique Andivia Muñoz and Andrei Toca for providing assistance in root image analysis and acknowledge the inputs of José A. Sigala and Raquel Benavides that helped to improve the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.
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