



Drought and fire stress influence seedling competition in oak forests: fine-root dynamics as indicator of adaptation strategies to climate change

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Abstract

Increased summer drought and wildfires as a consequence of continuing climate change are expected to lead to disturbance of Mediterranean ecosystems. Seedlings recruitment is sensitive to both stresses and, therefore, any adaptation and restoration strategy devised to protect these forests should take into account a careful study on their effects on seedling development. As a substantial fraction of net primary productivity of forested ecosystems is channelled in the belowground compartments, the knowledge of how roots behave under stressful conditions becomes of primary importance to select the right management strategy to be implemented. This work tries to enlighten the events occurring in the fine root portion of the root system in young seedlings of three co-existing oak species (*Quercus ilex*, *Quercus trojana* and *Quercus virgiliana*) under controlled conditions. We have made a comparative analysis of the effect of these two stresses, alone or in combination, with the aim to evaluate the tolerance level of these seedlings and, therefore, to obtain an indication of their recruitment potential in the field. The parameters investigated were biomass and a number of morphological traits. Data obtained suggest that a decrease in diameter could be part of a tolerance strategy in all three oaks tested together with a reduction of root length. In addition, tolerance to water shortage could require a reduction of carbon allocated belowground, in particular in the very fine roots, which leads to an overall reduction of the root system dimension. *Q. trojana* seedlings seem to be the fastest in resuming growth after stress interruption but a good recovery was also found for the remaining two oak species. Although our study provides interesting information regarding a possible tolerance strategy taking place in the fine root compartment when seedlings of these three oak species undergo water stress and fire treatment, more information is needed before any suggestion can be made as to which species would be best suited to make these forests more resistant to global changes.

Keywords

Quercus spp, Abiotic Stress, Fine-Root Biomass, Fine-Root Length, Diameter Class, Climate Change, Forest Adaptation

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1 Introduction

1.1 Effect of climate change on Mediterranean forests

In the Mediterranean basin water availability is subject to seasonal variability and for this reason forest ecosystems during summer often undergo mild and/or extreme drought leading to the induction in plants of various adaptive mechanisms (Chaves et al. 2003; Montagnoli et al. 2012, 2014; Tognetti et al. 1999, 2000). Over the last century, this situation has worsened with an increase in temperature of about 0.8°C and a decrease in rainfall of about 5 % (Begni et al. 2001; Xoplaki et al. 2004), leading to an overall increase of evapotranspiration and aridity (De Luis et al. 2003; Piñol et al. 1998). Under these conditions it is not surprising that Mediterranean forests undergo to a) a decrease in productivity (Chaves et al. 2002; Dreesen et al. 2012; Faria et al. 1998; Pereira et al. 2004, 2007), b) variations in morphological and physiological traits (Dreesen et al. 2012; Qaderi et al. 2012; Peñuelas et al. 2002), and c) an increase of tree mortality rates (Lloret et al. 2004; Martínez-Vilalta and Piñol 2002). In addition, several other physiological events are affected by drought, such as 1) plant reproduction through the arrest of reproductive development (Moya et al. 2008), 2) seedling establishment (Lloret et al. 2004), and 3) seedling mortality (Neilson and Wullstein 1985). Furthermore, hot and dry summers are often associated with forest fires, which represents an even more severe threat (Sanchez-Humanes and Espelta 2011) for their frequency and severity have increased due to climate change (Giorgi and Lionello 2008; Vilagrosa et al. 2003). It has been shown that drought affects seedlings more than saplings or mature trees, as they are more susceptible to environmental stresses (Chiatante et al. 1999; Chiatante et al. 2005; Di Iorio et al. 2011; Margolis and Brand 1990). In this scenario, in order to evaluate the natural recruitment rate in Mediterranean forests under climate change (Limousin et al. 2009; Ozturk et al. 2010; Sabaté et al. 2002; Scarascia-Mugnozza et al. 2000) any adoption of a forest adaptation

strategy (Chapin et al. 1990) should begin from the knowledge of how seedlings respond to concurrent drought and fire.

1.2 Tree response to water availability: the role of fine roots

Independently of the climate region considered, the scientific community agrees that, in forest ecosystems, the belowground biomass accounts for a mean of 13–25% of the stand biomass, and fine roots (Helmisaari et al. 2002) represent a percentage of 2–15%. This fact indicates that a substantial fraction of net primary productivity in forested ecosystems is exported belowground in order to produce short-living fine roots (McClougherty et al. 1982; Joslin and Henderson 1987). Therefore, a better knowledge of forest root dynamics is important for measuring, modelling, and predicting the value of ecosystem services (carbon storage for example) as well as providing useful indications when programming any measure of forest adaptation and/or restoration at landscape level (Stanturf et al. 2014).

Very fine ($d < 0.5$ mm) and fine ($0.5 < d < 2$ mm) roots (Zobel and Waisel 2010) represent the most dynamic component of a root system (Hendrick and Pregitzer 1992; Barlow 2010), and they comprise the majority of the length and water/nutrient-absorbing surface area of a root system (Bauhus and Messier 1999; Guo et al. 2008; Xia et al. 2010; Rewald et al. 2011; in Comas et al. 2013). Furthermore, fine roots are characterized by a rapid turnover which is influenced by a variety of internal (e.g. genotype of plant species) and external (e.g. temperature, precipitation, soil properties, nutrient availability and competition between plants) factors (Teskey and Hinckley 1981; Kuhns et al. 1985; Burke and Raynal 1994; Steele et al. 1997; Tierney et al. 2003; Chiatante et al. 2005; Majdi et al. 2005; Scippa et al. 2006; Montagnoli et al. 2012). Thus, fine-root dynamics set limits on shoot functioning by assuring the maintenance of forest plant productivity even under water deficit (Comas et al. 2013). Given their simple anatomical organization, fine roots are also the most sensitive component within the overall root system and, thus, respond rapidly to variations occurring in the rooting environment (Helmisaari et al. 2002). Thin roots are believed to be the belowground equivalent of thin leaves, which have lower metabolic cost (Withington et al. 2006; Ostonen et al. 2007).

Several studies on plant nutrition demonstrated that plants continuously adapt the growth of different organs to nutrient availability in the soil (Metcalf et al. 2008). Moreover, different tree species appear to adopt different strategies in the attempt to maximize their nutrition capacity (Comas et al. 2002; Curt and Prevosto 2003; Comas and Eissenstat 2004). This species-specific adaptation to nutrient availability in case of shortage can be resumed in two strategies: a) tolerance or b) avoidance of the stressful conditions (Manes et al. 2006 and references therein). In the case of stress tolerance the plant adopts an 'extensive' strategy (Ostonen et al. 2007), with a shift of carbon allocation towards the roots, where photosynthates are used to increase water uptake capability. This hypothesis fits with the demonstration (Ostonen et al. 2011) that across a European climate gradient, Norway spruce forests adapt to low N availability by increasing biomass, length and number of root tip ectomycorrhizas. When the limiting factor is water availability in the soil, the tolerance strategy leads to an increase in root mass and length in the fine-root system as soil moisture declines (Manes et al. 2006; Ostonen et al. 2007; Di Iorio et al. 2011; Montagnoli et al. 2012). In the case of stress avoidance, plants respond to a reduction in water availability by closing their stomata

thereby reducing CO₂ assimilation and diffusion into the plant (Manes et al. 2006), which leads to a decline in root mass production (Metcalf et al. 2008). Therefore, it is reasonable to assume that root length is proportional to resource acquisition (benefit) whereas root mass is proportional to construction and maintenance (cost) (Eissenstat and Yanai 1997). In particular, when considering water availability in the soil it is known that the necessity to optimize water/nutrient uptake stimulates finer roots production, explaining why there is a relatively greater root length per unit mass under drier conditions (Metcalf et al. 2008). In previous studies conducted in Turkey oak and European beech forests, we found that fine roots respond to water content and soil temperature variations independently of their diameter (Montagnoli et al. 2012, 2014). In beech forest these studies suggested that the increase in very fine root length results from elongation of their primary anatomical-zone unlike thicker fine roots which grow radially in consequence of vascular cambium activity (Barlow 1997; Montagnoli et al. 2014). In another study, (Di Iorio et al. 2011) we showed that *Quercus pubescens* seedling survival under severe drought stress might be entrusted on shedding the thinner roots. Variations in diameter of fine roots in response to changes in moisture and/or temperature in the soil have also been demonstrated in boreal trees (Ostonen et al. 2007).

1.3 Adaptation and restoration in Mediterranean forests

Several oak species characterizing Mediterranean forests are present in the Apulia region (Southern Italy). In particular, the association Teucro siculi-Quercetum trojane (Bianco et al. 1998) presents the coexistence of three oak species: *Quercus virgiliana*, *Quercus ilex* and *Quercus trojana*, with the latter being the dominant species. It constitutes one of the habitat types (*Q. trojana* woods, code 9250) of Murgia di Sud-Est, which has been declared Site of Community Importance (SCI, code IT9130005) by the European Commission and has been included in the Natura 2000 network of protected areas. These three oak species have very different morphological and physiological traits with *Q. ilex* being an evergreen species whereas *Q. virgiliana* being a deciduous and *Q. trojana* have a semi-deciduous habit. The wood quality is completely different too with *Q. trojana* timber mostly used for ship construction, and timber of the remaining two species prevalently used for energy purpose. The millennium-long exploitation history and the technological advancements have probably imposed repeated changes in management strategy of these forests over time. Therefore, it cannot be excluded that changes in vegetation compositions have been introduced anthropologically (Thompson et al. 2009). More recently, drought and fire events are increasing in this region, both numerically and qualitatively, affecting species distribution and production at both local and regional scales (Matías et al. 2012) thereby putting at risk the very survival of this plant community. The three coexisting *Quercus* species are likely to exhibit different adaptive responses to climatic constraints. Since *Q. trojana* represents the dominant species, its seedlings are expected to have fine-root morphological traits that allow them to cope better than the other two species with stressful environmental conditions of drought and fire. In the present paper we test this hypothesis by measuring the variation in fine-root length and mass in relation to two fine root diameter classes (very fine root $d < 0.5$; fine root $0.5 < d < 2$ mm). Our goal is to predict, through fine-root dynamics, the possible responses and adaptation of these species to future aridity increases of Mediterranean ecosystems. Finally, in order to

avoid the potential associated impact on forest ecosystems such as a decline in productivity, we evaluated the seedlings' recruitment potential which will allow for a better planning of management strategies for the adaptation to climate change and the maintenance of ecosystem integrity and environmental benefits.

2 Material and methods

2.1 Plant material and growth chamber characteristics

Approximately 200 mature seeds were randomly collected from 15-20 individuals of each species (*Q. ilex*, *Q. virgiliana* and *Q. trojana*) in a forest situated in the district of Martina Franca named Contrada Lanzo and located within the protected area Murgia Sud-Est (40°39'25"N, 17°18'25"E). In the area where seeds were collected, *Q. trojana* displays a deciduous habit, which might be related to harsh winter condition. Mean annual precipitation of the site measured over an 80-year period is 470 mm. According to Bagnouls and Gausson (1953), this site experiences a five-month dry season from May to September, with monthly mean precipitation of 26, 18, 13, 20 and 32 mm, respectively. In order to avoid pregermination, immediately after their collection seeds were stored in a cold room at 4°C for 2 weeks before being used in experiments. One hundred seeds for each species were weighed and sown in 3.5 L pots filled with a 3:1:1 mixture of peat, clayey loam, and pumice (440 g dry mass each pot), with the addition of 20 g of a slow-release fertilizer (NPK 14-7-14), and placed in a growth chamber. Seed germination was 58 % for *Q. ilex*, 60% for *Q. virgiliana* and 85% for *Q. trojana*. A single growth chamber was used to allow for a strict control of environmental factors (uniform conditions) and seedling development (coetaneous cohort). Temperature, humidity, light intensity and period, and water availability were regulated to mimic summer weather conditions occurring at the site, with temperature and relative humidity following a diurnal cycle. A maximum temperature of 27°C was applied, which corresponds to the average maximum temperature recorded during the summer season. In fact, these seedlings grow in the shade of a thick canopy and, therefore, the air temperature is relatively low. Temperatures and photoperiods are shown in Chiatante et al. 2015, Table 1. Daily relative humidity cycled from 35 to 76%. Photosynthetically active radiation (PAR, 400-700 nm) at plant height was 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Light intensity was established as the medium irradiance value corresponding to 17% of full sunlight, which is the maximum light intensity recorded under the canopy cover in natural condition during repeated 4 h measurements (from 11.00 to 15.00 h) in July. Mean seedling age at the initiation of each experiment was 2 months (± 18 days) after bud protrusion above the soil, with a seedlings height of 8.50 ± 0.31 cm for *Q. virgiliana*, 13.82 ± 0.72 cm for *Q. ilex* and 11.79 ± 1.03 cm for *Q. trojana* [mean \pm standard error (SE)].

2.2 Drought experiment

We applied sequentially to the same seedlings (55 for each species) two levels of water shortage indicated, respectively, as mild drought stress (M-stress) and severe drought stress (S-stress). The rationale of using different levels of water shortage, one after the other, was that comparing morphological and physiological traits (Thomas and Gausling 2000) of three different species at two different dose–response treatments

during a given period of time (Poorter et al. 2012) allows for a more correct evaluation of stress tolerance and avoidance mechanisms. Soil water potential (Ψ_{soil}) was daily measured by gypsum blocks (Delmhorst KS-D1 Digital Soil Moisture Tester) inserted in the middle of the pot (8 cm from the top of the pot). Six gypsum blocks were used for each treatment. Gypsum blocks measure Ψ_{soil} from -0.03 MPa to about -1.5 MPa. More accurate measurements of field capacity (-0.033 MPa) and wilting point (-1.5 MPa) were obtained with a pressure membrane extractor and then related to soil moisture. The experimental design is represented in Figure 1.

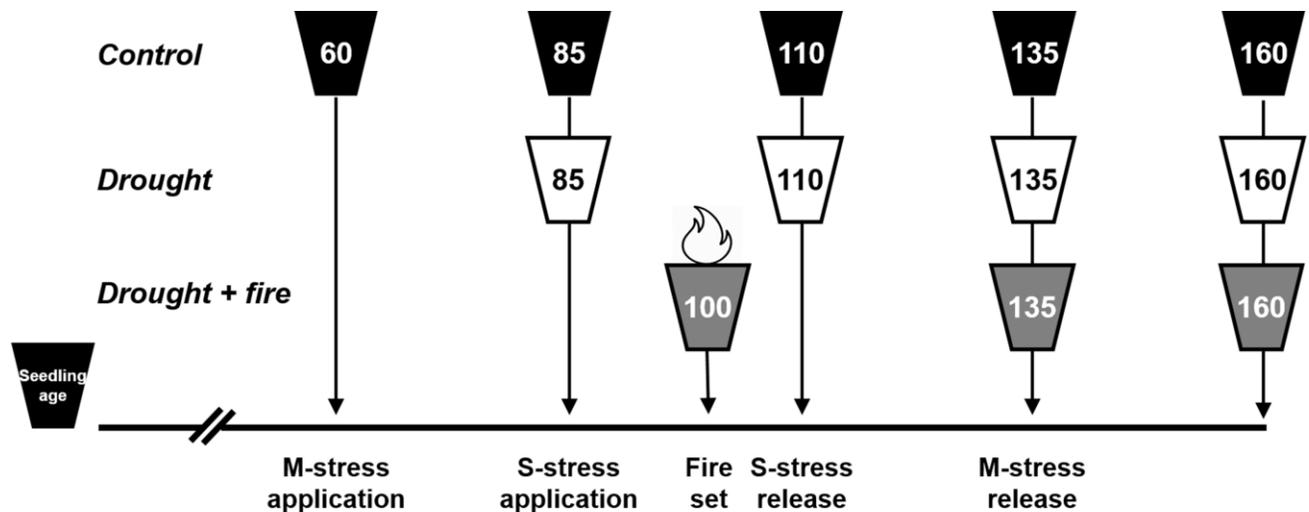


Figure 1. Schedule and sequential connection among the three drought/fire treatment combinations. Numbers indicate the experimental day, corresponding to seedling age, of control (black pots), drought stress (white pot) and drought+fire (gray pots); stresses application and release is indicated by the downward arrow. M-stress, mild-drought stress application, S-stress, severe drought stress

Five plants per treatment-species combination were collected at each of five harvest points. After 2 months of growth (day 60), a drought stress was applied. To this purpose, watering was completely withheld for a period of 16 days until gravimetric soil water content reached 86.6%. This stage was considered to be a moderate drought treatment (M-stress application; Fig. 1) corresponding to a Ψ_{soil} of -0.19 MPa. From this point on, each pot was weighed daily and a small amount of tap water was added to maintain a constant weight. From day 85 on, watering was withheld again for a period of 10 days until gravimetric soil water content reached a value of 48.2%. This was considered to be a severe drought treatment (S-stress application; Fig. 1), with Ψ_{soil} below wilting point (-1.5 MPa corresponds to a soil water content of 76.1%). Subsequently, soil moisture was kept constant for 15 days by adding tap water when necessary to keep pot weight constant. At day 110, soil moisture was increased to 86.6% (S-stress interruption; Fig. 1) and kept constant for a further 25 days. At day 135, soil moisture was increased to field capacity and kept constant for the final 25 days (M-stress interruption; Fig. 1). Control pots were maintained at constant field capacity ($\Psi_{\text{soil}} = -0.033$ MPa).

2.3 Fire treatments

To investigate the combined effect of drought and fire, a sub-sample of 10 seedlings for each species was burned 40 days after the beginning of drought treatment and 15 days after the beginning of severe drought stress (day 100; Fig. 1 and Fig. 2). To simulate a fire effect, the pot surface was covered with straw, which was set on fire for 15 seconds. After fire treatment, seedlings remained under S-stress and recovery of this cohort started at the same time as the remaining seedlings, which had undergone only drought treatment.

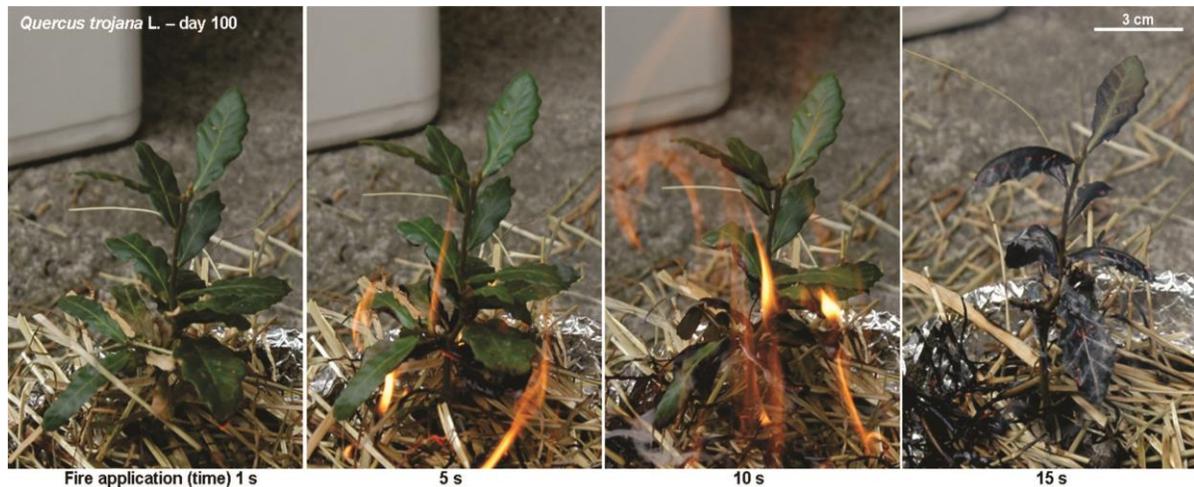


Figure 2. Time-course pictures in seconds (s) of the fire application to *Q. trojana* seedling from fire set (1 s) to completely seedling burnt (15s)

2.4 Fine root measurements

After removing each seedling from the soil, roots were rinsed repeatedly with running tap water, and scanned at a resolution of 400 dpi with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Epson Expression 10000 XL; Fig. 3). Images were analyzed by WinRhizo Pro V. 2007d (Regent Instruments Inc. Quebec) in order to measure 1) measure fine root length and 2) group the different fine root fragments in two diameter classes (very fine $d < 0.5$ mm, fine $0.5 < d < 2.0$ mm). Fine-root biomass was determined by collecting five seedlings for each species at each sampling point every 25 days starting on day 60. Fine roots were detached from each seedling and biomass was calculated as dry mass after oven drying (52 h at 75°C).

2.5 Statistical analysis

Morphological measurements were square root or log transformed to ensure normal distributions and equal variances for the use of parametric statistics. A three-way MANOVA was used to compare different species, experimental steps and drought treatments. Post-hoc Bonferroni tests were conducted to detect overall differences between species at each experimental step and between control and drought-treated seedlings for each species at each experimental step. Analyses were applied on a 95% significance level. Statistical analysis was carried out using statistical software package SPSS 17.0 (SPSS Inc, Chicago IL, USA).

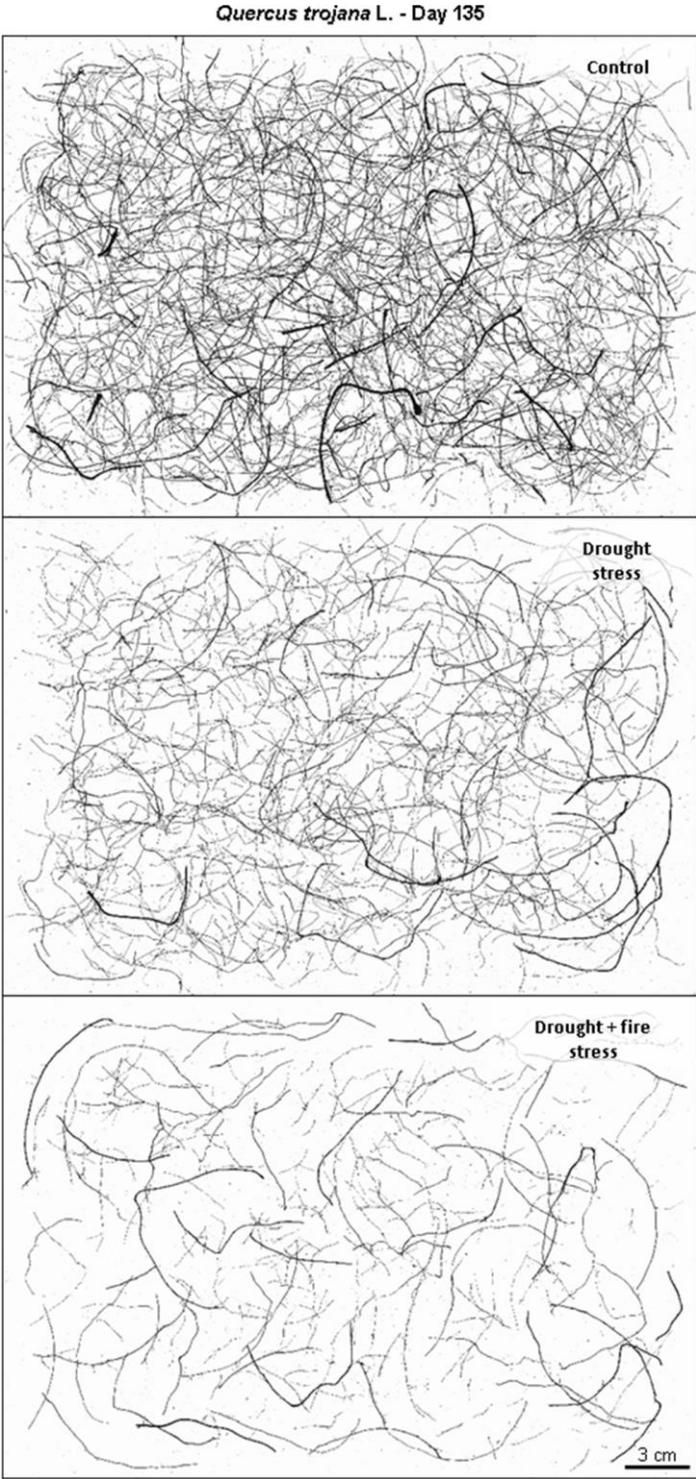


Figure 3. High resolution image of *Q. trojana* seedling root system acquired by Epson Expression 10000 XL at day 135 in control, drought and drought + fire stressed seedlings.

3 Results

The effect of different stresses (drought; drought + fire) on plant fine root system development, was investigated by measuring fine-root traits such as biomass (dry weight; g) and length (cm) for two diameter classes (vFR - very fine roots, $d < 0.5$ mm; FR - fine roots, $0.5 < d < 2.0$ mm) for each oak species.

3.1 Root biomass

In control seedlings of all three considered species, vFR biomass increased significantly ($p < 0.001$) throughout the experiment (Fig. 4a, b, c). *Q. virgiliana* showed a slower increase than the other two species reaching at the end of the experiment (day 160) an almost two-fold higher biomass compared to the beginning of the experiment (day 60; Fig. 4a). *Q. ilex* and *Q. trojana* both showed a linear increase reaching, respectively, a three-fold and two-fold increased root biomass at day 160 (Fig. 4b, c). An opposite trend was observed when considering FR biomass ($2.0 < d < 0.5$ mm) which increased throughout the experiment in control seedlings of *Q. virgiliana* (Fig. 4d) while both *Q. ilex* and *Q. trojana* stopped their growth at day 85 and 110 respectively (Fig. 4e, f). *Q. virgiliana* seedlings showed significant higher ($p < 0.001$) values of vFR biomass than control seedlings at day 85, after 25 days of M-stress application (Fig. 4a). During S-stress and recovery from severe to mild stress, vFR biomass decreased to values lower than those of control seedlings. At day 135, when mild stress was released, vFR biomass increased rapidly and reached the values of control seedlings (Fig. 4a). *Q. ilex* seedlings under drought stress showed a continuous increase of vFR with values similar to those of control seedlings. Moreover, during the severe stress application *Q. ilex* drought stressed seedlings keep increasing their vFR biomass reaching values significantly higher ($p < 0.05$) than control seedlings at day 135. When mild stress was released, vFR biomass decreased reaching values significantly lower ($p < 0.05$) than those of control seedlings. *Q. trojana* drought stressed seedlings showed a continuous increase of vFR biomass throughout the experiment with values always significantly lower ($p < 0.001$) than those of control seedlings (Fig. 4c).

In all three oak species FR biomass did not increase during M-stress and S-stress (Fig. 4d, e, f) resulting in significant lower ($p < 0.05$) values compared to controls. *Q. virgiliana* started to recover after S-stress release (day 110) and FR biomass values dramatically increased when M-stress was also released resulting in no significant difference to control values at day 160 (Fig. 4d). FR biomass in *Q. ilex* showed a slight decrease at the beginning of the drought period when M-stress was applied (day 60) and then remained stable until the end of the experiment (day 160) resulting in a biomass significant lower ($p < 0.05$) than that of control seedlings (Fig. 3e). *Q. trojana* seedlings under M-stress showed a FR biomass increment similar to control seedlings (Fig. 4f) however, when S-stress was applied (day 85) FR biomass decreased significantly (Fig. 4f; $p < 0.001$). At day 110, when S-stress was released, FR biomass increased rapidly reaching almost the same values as those of control seedlings but remaining significantly lower (Fig. 4f; $p < 0.05$). At the end of the growing period, the highest biomass values, i.e. at least two-fold higher compared to the beginning of the experiment, were found in *Q. trojana* for both root diameter classes and irrespective of stress treatment. *Q. ilex* showed vFR values similar to those of *Q. virgiliana* but significant lower values of FR ($p < 0.001$).

Forty days after the beginning of drought treatment (day 100), 10 seedlings per species were subjected to fire treatment for 15 seconds. Although fire treatment was brief, leaves and stems were burned completely and all seedlings showed crown dieback a few days after resumption of the normal watering regime. Seedlings did not show fire-induced mortality. Indeed, on day 135 (35 days after fire treatment), new stems resprouted from dormant buds present at the root collar and new leaves developed in all burned seedlings of all species except for one single *Q. virgiliana* seedling. After fire treatment, biomass of both diameter classes decreased significantly ($p < 0.05$) for *Q. ilex* and *Q. trojana* seedlings. *Q. virgiliana*, on the other hand, showed a decrease of vFR at day 160 while FR maintained similar values (Fig. 4a, d). At the end of the growing period, 60 days after fire disturbance, all fire-drought stressed seedlings showed significantly lower ($p < 0.001$) values of root biomass than control and drought seedlings for both diameter classes in all three oak species.

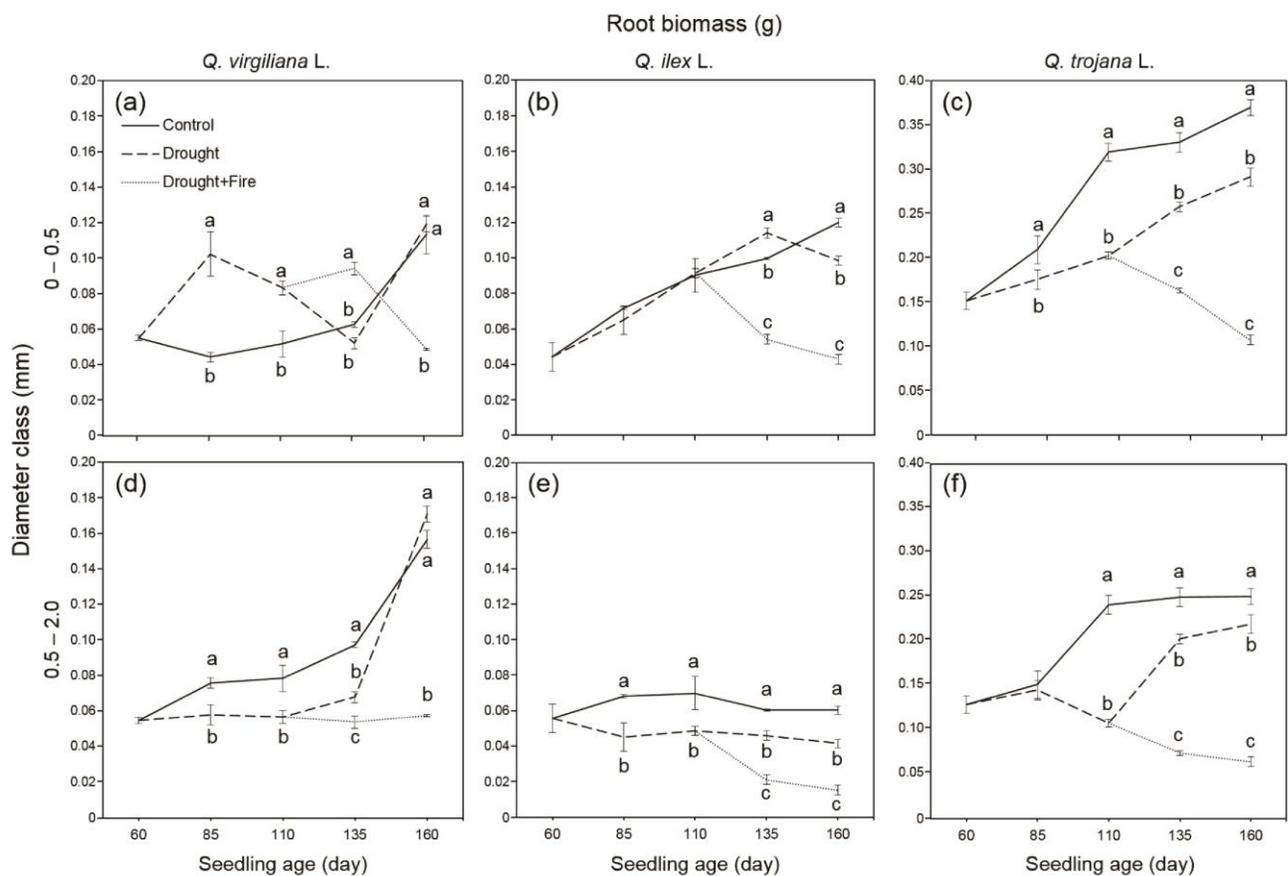


Figure 4. Very fine and fine -root biomass (rows), of *Q. virgiliana*, *Q. ilex* and *Q. trojana* seedlings (columns) at days 60 (harvest 1), 85 (harvest 2), 110 (harvest 3), 135 (harvest 4) and 160 (harvest 5) under three treatment combinations (see Section 2). Continuous line (—) and dashed lines (- - -) refer to watering and no-watering conditions, respectively; dotted line (...) refer to burnt conditions. Values are the mean of 5 replicates; standard error of the mean is indicated (\pm) if it is larger than the symbol. Within each harvest, if written, the means of the different treatment combinations without a common letter are significantly different ($P < 0.05$).

3.2 Biomass allocation by diameter classes

The allocation of biomass between the two diameter classes of fine roots considered showed differences between species and between control and stressed seedlings (Fig. 5). Control seedlings showed three different patterns of allocation depending on the oak species. *Q. virgiliana* seedlings showed an increase in the percentage of FR biomass at the beginning of the experiment that remained stable until the end of the growing period (day 160; Fig. 5). *Q. ilex* seedlings, on the other hand showed a continuous increase of the vFR biomass percentage throughout the whole experiment (Fig. 3b). The same pattern, but less intense, was found in *Q. trojana* (Fig. 5). Drought-stressed seedlings showed differences in allocation pattern in the two diameter classes in comparison with control seedlings. In particular, *Q. virgiliana* drought-stressed seedlings showed an opposite pattern compared to control seedlings with an increment of vFR biomass when M-stress and S-stress were applied, recovering the same values of control seedlings when stresses were released (Fig. 5). In the case of *Q. ilex*, the drought-stressed seedlings pattern did not differ from control seedlings, showing a continuous decrease through the whole experiment but with higher percentage of vFR (Fig. 5). Drought-stressed *Q. trojana* seedlings only showed a slight increase of vFR biomass percentage when S-stress was applied, recovering the percentage of control seedlings right after S-stress was released (Fig. 5). When fire was applied all the three species showed the further increase of vFR biomass compared to drought stressed seedlings. In particular, *Q. virgiliana* and *Q. trojana* seedlings showed the highest percentage of vFR biomass 35 days after fire treatment (day 135; Fig. 5) and *Q. ilex* seedlings 60 days after fire treatment at the end of the experiment (day 160; Fig. 5).

3.3 Root length

In control seedlings of all three considered species, vFR length showed a significant increase ($p < 0.001$) throughout the experiment (Fig. 6). *Q. virgiliana* showed a lower increase than the other two species reaching at the end of the experiment (day 160) a two-fold higher root length compared to the beginning of treatment (day 60; Fig. 6a). Both *Q. ilex* and *Q. trojana* reached, respectively, a three-fold and four-fold increase in root length at day 160 (Fig. 6b, c). In the case of FR, control seedlings of *Q. virgiliana* showed an opposite trend as FR increased throughout the experiment (Fig. 6d) while both *Q. ilex* and *Q. trojana* stopped FR growth at day 85 and 110, respectively (Fig. 6e,f).

When the same morphological traits were measured in drought-stressed seedlings, *Q. virgiliana* showed significant higher vFR values ($p < 0.05$) than those of control seedlings at day 85, 25 days after of M-stress application (Fig. 6a). During S-stress and the recovery from severe to mild stress vFR length decreased to values lower than those of control seedlings. At day 160, despite a recovery during normal watering, drought-treated seedlings showed significantly lower ($p < 0.05$) values of vFR length compared to control seedlings (Fig. 6a). Drought-stressed seedlings of *Q. trojana* and *Q. ilex* showed lower values of vFR length compared to controls throughout the experiment (Fig. 6b, c). In particular, *Q. ilex* stopped vFR growth during drought treatment at day 85, which resulted in significant lower ($p < 0.05$) vFR values than those of control seedlings at day 160 (Fig. 6b). *Q. trojana*, despite an increase of vFR length during M-stress and recovery after S-stress interruption, showed significant lower ($p < 0.05$) values compared to control seedlings at day 110 and day 160 (Fig. 6c).

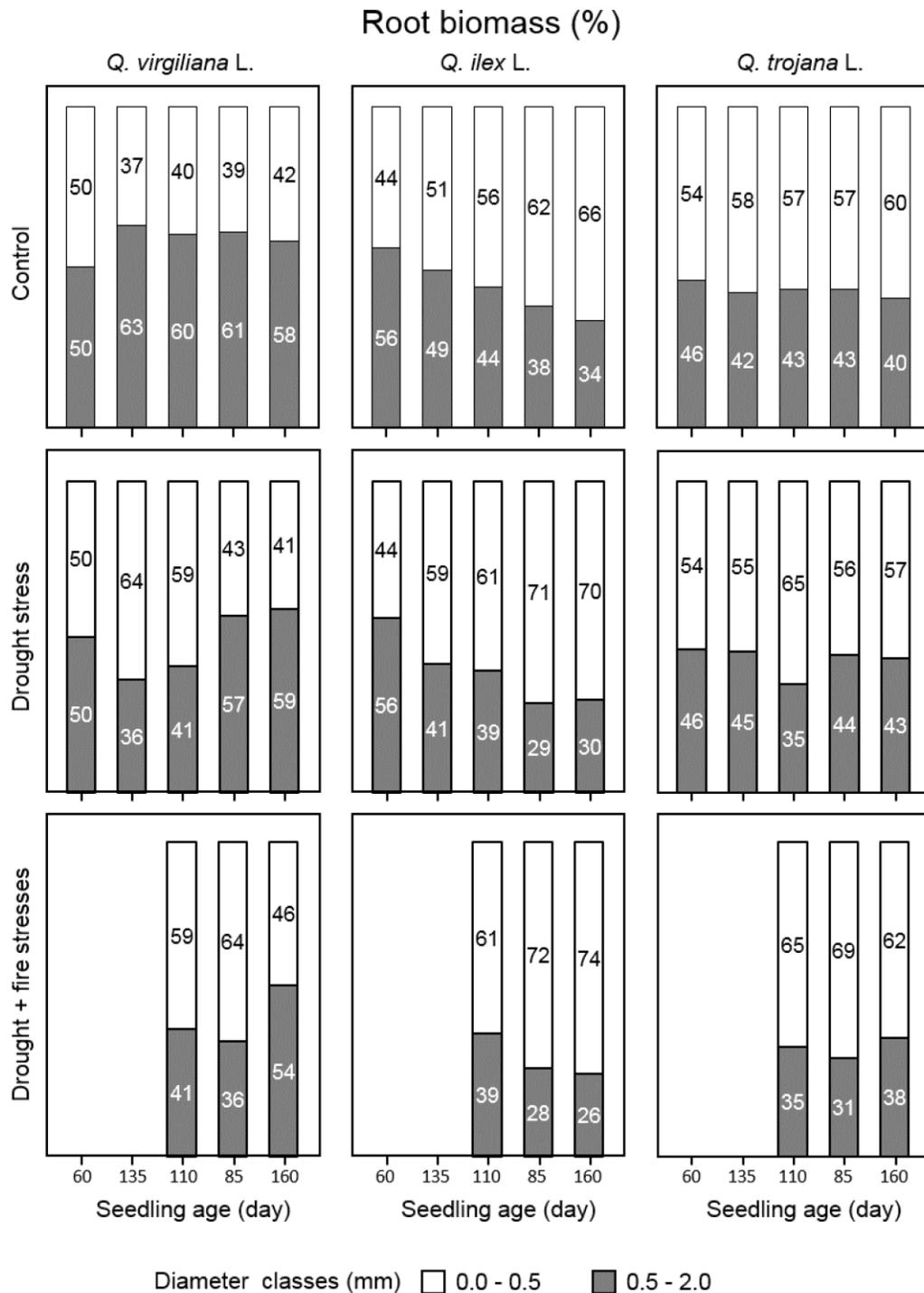


Figure 5. Fine (white bar) and very fine (gray bar) – root biomass percentage variation in *Q. virgiliana*, *Q. ilex* and *Q. trojana* seedlings (columns) at days 60 (harvest 1), 85 (harvest 2), 110 (harvest 3), 135 (harvest 4) and 160 (harvest 5) under three treatment combinations (rows).

In all three oak species FR length did not increase during M-stress and S-stress (Fig. 6d, e, f) resulting in significant lower values ($p < 0.05$) compared to controls. *Q. trojana* started to recover after the end of S-stress treatment showing FR values that were not significant lower than those of control seedlings at day 135 and 160 (Fig. 6f; $p = 0.535$ and $p = 0.728$, respectively). *Q. virgiliana* started to recover after day 135 during normal watering reaching FR values not significantly lower than those of control seedlings at day 160 (Fig. 6d; $p = 0.324$). In *Q. ilex* FR length continued to decrease after S-stress interruption and started to recover only during normal watering but values remained significantly lower (two-fold) than those of control seedlings (Fig. 6e; $p < 0.05$). At the end of the growing period both vFR and FR length values were highest in *Q. trojana* seedlings irrespective of stress treatment ($p < 0.001$; Fig. 6c, f). In *Q. ilex* seedlings vFR length values were intermediate between those of *Q. trojana* and *Q. virgiliana* whereas FR length of *Q. virgiliana* showed intermediate values between those of *Q. trojana* and *Q. ilex*.

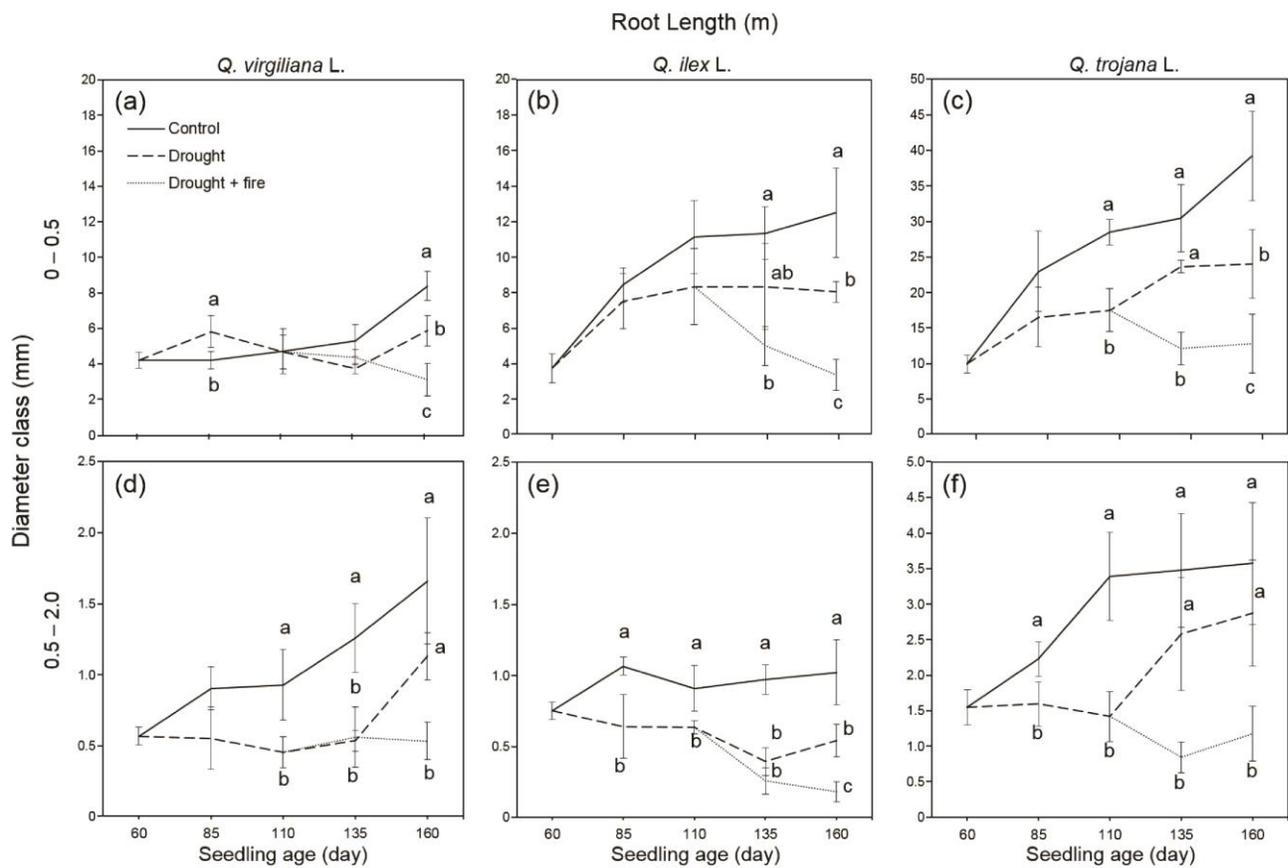


Figure 6. Very fine and fine -root length (rows), of *Q. virgiliana*, *Q. ilex* and *Q. trojana* seedlings (columns) at days 60 (harvest 1), 85 (harvest 2), 110 (harvest 3), 135 (harvest 4) and 160 (harvest 5) under three treatment combinations (see Section 2). Continuous line (—) and dashed lines (- - -) refer to watering and no-watering conditions, respectively; dotted line (....) refer to burnt conditions. Values are the mean of 5 replicates; standard error of the mean is indicated (\pm) if it is larger than the symbol. Within each harvest, if written, the means of the different treatment combinations without a common letter are significantly different ($P < 0.05$).

4 Discussion

This paper analyses fine root dynamics in seedlings of three co-existing oak species (*Quercus ilex*, *Quercus trojana* and *Quercus virgiliana*) when subjected to drought or/and fire treatment in a growth chamber. This study has been conducted under controlled conditions in order to exclude the influence of other potential environmental variables. The long-term aim of this study is a) to throw light upon the role of fine roots in supporting seedling survival potential of these tree species in a context of global change and b) to collect data useful to predict the fast-cycling fraction of carbon stored in below-ground organs when seedlings are under normal or stress conditions. The latter point could be used to better estimate the carbon stored below ground in the assumption that the fast-cycling fraction of seedling fine roots could remain proportionally the same for belowground biomass of adult trees. In addition, this type of study regards the possibility to use the turnover rate as an indicator of root efficiency in resource acquisition and sustain plant metabolism (Mainero et al. 2009; Montagnoli et al. 2014). Finally, a better knowledge of seedling survival potential under stressful conditions could help decision makers in choosing the most appropriate management actions (Frelich and Reich 1998; Gauthier et al. 2008; in Thompson et al. 2009).

The data presented here indicate that the fine root fraction in seedlings of the three oak species considered respond differently to drought and/or fire stress. In particular, data referring to *Q. virgiliana* control seedlings indicate that thicker fine roots increase their biomass and length contemporaneously with temperature increase as has been found in other plant species (Pregitzer et al. 2000; Kaspar and Bland 1992; Larson 1970; Wilcox and Ganmore-Neumann 1975; Teskey and Hinckley 1981; Bevington and Castle 1985; Kuhns et al. 1985; Lieffers and Rothwell 1986; McMichael and Burke 1998; King et al. 1999; Weltzin et al. 2000; Montagnoli et al. 2014; Barney 1951; Merritt 1968; McMichael and Burke 1998; Pregitzer et al. 2000). Different is the pattern in very fine roots where biomass and length show a variation which seems to be largely independent of temperature except the final stage of the experiment. *Q. virgiliana* seedlings respond to water withdrawal with a rapid surge of very fine root biomass which is higher than that observed in unstressed control seedlings. This response could be an attempt to increase the volume of soil exploited per unit mass invested in fine roots (Ostonen et al. 2007; Montagnoli et al. 2012; Comas et al. 2002; Curt and Prevosto 2003; Comas and Eissenstat 2004). However, the peak reverses with the application of severe stress suggesting that there is probably a threshold-value whose exceedance leads to a steep decrease in fine root growth. According to Eissenstat and Yanai (1997), when exceeding such a threshold value the metabolism of fine roots might be arrested and a root shedding induced to achieve a more functional overall root system dimension. Furthermore, our data indicate that as soon as water becomes available again, metabolic and physiological functions of fine roots are fully resumed.

In *Q. ilex* control seedlings, the temperature-dependent increment of biomass and length seems to be limited to the very fine root fraction indicating that in oak species the response of this root fraction could be really species dependent. In *Q. ilex* seedlings water withdrawal affects mainly the length parameter independently of the root diameter class whereas biomass is affected only in thicker fine roots. These results could be explained assuming that very fine roots continue to accumulate organic matter even if elongation or new emission of roots is arrested. The thicker fine roots of this oak

species are affected by water withdrawal in both their capacity to accumulate organic matter and to undergo further growth. The discrepancy observed compared to increase of biomass and length in the finer root fraction could represent a specific tolerance strategy to counteract lack of water availability (Nardini and Tyree 1999) through starch accumulation as was shown by Deans and Ford (1986) in the root apparatus of Sitka spruce and by Di Iorio et al. (2015) in beech saplings. It would be interesting to investigate whether this tolerance strategy has any advantage when harsh environmental conditions end.

Fine roots of *Q. trojana* control seedlings respond to temperature increase with very slight differences depending upon their diameter. The absolute values for biomass and length parameters achieved at the end of experiment are twice as high as those found in the other two oak species examined here. Unfortunately, the meaning of this difference in terms of recruitment potential under normal or stress conditions remains unclear at present. When water is withdrawn the effect on biomass and length became clearly visible at a later stage probably when root growth stops and several roots die, as found by Cudlin et al. (2007) in other plant species. Also in the case of *Q. trojana* seedlings, there could be a threshold value for water withdrawal tolerance before starting an overall reduction of the root system dimension by arresting root growth and/or by starting a root shedding event (Joslin et al. 2000; Chiatante et al. 2005, 2006; Di Iorio et al. 2011). This finding is similar to data reported by us regarding a *Q. cerris* forest (Montagnoli et al. 2012). However, the data collected here indicate that even when these effects on the fine root fraction are induced, the survival of seedlings at the end of the stress treatment remains preserved as suggested by the resumption of growth in all fine root categories.

The data referring to the effect of fire superimposed on water withdrawal clearly indicate that the addition of this further stress worsens the situation by arresting both biomass and length in a similar way independently of the oak species considered. At the same time it is important to underline that even by coupling two stresses the seedlings of all three oak species resume growth as shown by the emission of new branches and leaves (Chiatante et al. 2015). The resumption of growth in the aboveground organs contrasts considerably with the resumption of growth in the fine root fraction. In fact, even at the end of the experiment a clear-cut resumption of biomass and length growth has not been observed in fine roots. However, it is reasonable to speculate that the carbon stored in the roots and in the stem is used immediately to build new leaves and branches to the detriment of roots. Probably a longer recovery time would have shown the resumption of root growth as was found by us in different experiments conducted in the nursery or in the growth chamber with another oak species (Chiatante et al. 2005; Di Iorio et al. 2011).

5 Conclusions

The study presented here demonstrates that all three oak species are sensitive to temperature which stimulates an increase in fine root biomass and length independently of their diameter. Both fine root length and biomass are affected when water is withdrawn but a good level of tolerance was observed as long as a threshold limit is not exceeded. In terms of tolerance strategy these data suggest that: 1) all three oak species are able to adjust fine root morphology in response to water shortage by inducing a general decrease of mean diameter; and 2) the very fine roots are probably

the most dynamic and responsive root fraction able to respond to climate change. However, the observation that all three oak species tolerate water stress and fire (both alone or in combination) does not provide clues as to which species has a better recruitment potential and, therefore, should be preferred in adapting strategies for this forest in response to the foreseen worsening of climate change conditions. From a biomass point of view, *Q. trojana* seedlings show the highest biomass storage potential in the fine roots compartment but this trait should be compared with for example the coarse roots. Moreover, only a complete calculation of carbon storage accumulated in the roots of adult trees could provide a more important indication regarding their contribution to a specific ecosystem service. Certainly, the highest values of both biomass and length in fine roots of *Q. trojana* suggest that resumption of root growth following soil rewetting could be more rapid in this oak species. Furthermore, *Q. trojana* productivity under recurrent drought could be higher in comparison with the other two oak species. However, all these considerations should be evaluated against other data obtained in a previous study (Chiatante et al. 2015) where we showed that the deciduous or semi-deciduous oak *Q. trojana* is less resistant to cavitation due to the presence of xylem vessels with a very large diameter. The balance between the contrasting properties of this oak species should take into consideration that it is able to sustain hydration at a less negative value of water potential (Vilagrosa et al. 2012) by developing a larger root system able to explore deeper soil layers. Finally, even though our data suggest that *Q. trojana* is an adequate candidate for a good natural seedling recruitment under a climate change worsening, the final decision on the correct adaptive strategy for managing this type of forests needs further investigations.

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