

Seasonality of fine root dynamics and activity of root and shoot vascular cambium in a *Quercus ilex* L. forest (Italy)[☆]



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ABSTRACT

We investigated the effects of seasonal changes in soil moisture and temperature on the morphological growth traits of fine roots (< 2 mm in diameter) and vascular cambium activity of stems and coarse roots in a mature *Quercus ilex* L. stand in the South of Italy. Fine roots were sampled by a soil core method, and cambium tissues were carefully collected by hammer and chisel. Mean annual fine root mass and length were 115 g m⁻² (live 45 g m⁻²; dead 70 g m⁻²) and 471 m m⁻² (live 244 m m⁻², dead 227 m m⁻²), respectively. Mean diameter size of fine root necromass was higher than for fine root biomass. Mean specific root length (SRL) was 6.8 m g⁻¹ and turnover rate was 3 year⁻¹. Fine root traits displayed a complex pattern related to season. In particular, biomass and length peaked in summer and late autumn. The summer maximum was characterized by an increase of the thinner part of the root population (smallest diameter size and highest SRL) and was mainly driven by soil temperature. Our results suggest that *Q. ilex* adopted an intensive strategy modifying the root length per unit mass, channelling carbon preferentially into the production of very fine roots. This allowed trees to exploit transient periods of low soil water content by accessing a greater soil volume and thereby facilitating nutrient and water uptake. The autumn maximum was characterized by an increase in mean diameter size of the fine root population (largest mean diameter size and lowest SRL). Thus, once precipitation sufficiently recharged soil moisture, it is reasonable to state that in addition to trees producing new roots, their percentage of very fine roots that did not die after the summer flush continued their growth in a radial pattern to function for starch storage. Shoot and root cambial activity strongly varied during the season from the winter minimum (4.8 shoot and 4.7 root cambial cell number) to three- and two-fold higher values measured during the summer maximum, and higher values but of lesser magnitude measured in autumn. Periods of cambial activity significantly matched fine root phenology. Matching these growth traits with soil temperature and water content within a natural stand of *Q. ilex* enables this species to survive the typically dry summer in the Mediterranean area, which is likely to become drier and longer given the increase in temperature expected this century.

1. Introduction

In perennial woody plants, the vascular cambium is a coherent lateral sheet of meristematic tissue just a few cells thick occurring between the secondary phloem and the secondary xylem. This tissue occurs from the roots, through the stem, and up to the tips of branches. For trees outside the belt of tropical rain forests, the cambium generally undergoes a seasonal activity cycle with a dormant and an active period

each year. Following winter dormancy, the cambium of temperate zone trees is reactivated, forming new phloem cells to the outside and xylem cells to the inside through cell divisions. New annual increments of xylem and phloem are thus inserted between old layers of these tissues, causing the stem, branches, and major roots to increase in thickness (Pallardy, 2008). Tree cambium produces an enormous amount of biomass. FAO (2016) estimates the global aboveground woody biomass is 531 billion m³. In the complex process of cambial activity, exogenous

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and endogenous factors are interacting and responsible not only for the quantitative wood formation but for also anatomical features such as vessels and fibers. Indeed, declines in water availability during the growing season have been shown to affect xylem quality and quantity (Balducci et al., 2013, 2014), as well as many phenological events, such as leafing, flowering (Bernal et al., 2011; Peñuelas et al., 2002), and fine root elongation (Montagnoli et al., 2012a, 2014). Effects of drought stress on wood formation have shown a seasonal co-dependency as well as an inter- and intra-specific component. For example, in *Populus* only one or two cambial cell derivatives occurred under drought stress in early summer and none subsequently occurred under drought stress in late summer (Arend and Fromm, 2007). In *Pinus halepensis* L., the number of differentiated tracheids as well as the cambial cell production was characterized by two major growth phases, one in spring and another in autumn, interrupted during the summer drought period when the cambium might remain active but cell divisions occur at a very low rate (de Luis et al., 2011). In *Pinus sylvestris* L. and *Betula* spp. L., cambium dynamics measured along a south-north transect in Finnish Lapland were characterized by the highest growth rate in the second half of June and the first half of July, respectively (Schmitt et al., 2004). Recently Liang et al. (2016) reviewed that precipitation occurring at the beginning or during the earliest part of the growing season is crucial for tree-ring growth in semiarid areas. Under Mediterranean climate, xylem growth tends to show a typical bimodal pattern caused by subsequent cambial reactivations closely following spring and autumn precipitation (Camarero et al., 2010). Investigations that link cell structure and variations in precipitation or drought stress are numerous (Balducci et al., 2013; Eckstein, 2004; Eilmann et al., 2011; Fonti et al., 2010; Gea-Izquierdo et al., 2012; Giovannelli et al., 2007; Lautner, 2013; Liang et al., 2016; Liang and Eckstein, 2006). On the contrary, studies examining cambial activity in roots and its relation to cambial activity in shoots, as well as investigations concerning the effect of moisture and temperature on the temporal dynamics of cambial activity are scarce or absent. Thus, xylogenesis requires more examination, especially in regions or ecosystems where drought events occur regularly.

Fine roots (< 2 mm in diameter) of forest trees are short-lived, non-woody, associated with mycorrhizae (Finér et al., 2011) and a good indicator of forest adaptation to climate change (Eissenstat et al., 2000, 2013; Brunner et al., 2015; Montagnoli et al., 2016). Their function of absorbing water and nutrients plays a crucial role in plant survival potential and seedlings establishment after out planting (Montagnoli et al., 2018). In addition, fine root exudation of carbohydrates stimulates microbial decomposition of soil organic matter, induces heterotrophic respiration (Sun et al., 2017), and improves nutrient availability in the rhizosphere (Kaiser et al., 2015). Similar to cambial cell activity, fine root development is subject to seasonal fluctuations because of endogenous (e.g., genotype of plant species) and exogenous (e.g., temperature, precipitation, soil properties, nutrient availability, and competition among plants) factors (Burke and Raynal, 1994; Chiatante et al., 2005; Kuhns et al., 1985; Majdi et al., 2005; Steele et al., 1997; Teskey and Hinckley, 1981; Tierney et al., 2003). For *Quercus cerris* L. and *Fagus sylvatica* L., we previously demonstrated that fine root occurrence has a multimodal pattern related to soil temperature and water content (Montagnoli et al. 2012a, 2014). Changes in soil moisture may also induce changes in the diameter of the root population (Amendola et al., 2017; Ostonen et al., 2007). Under drier soil conditions, plants produce longer and finer roots, the belowground equivalent of thin leaves (Ostonen et al., 2007; Withington et al., 2006), which results in a relatively greater length per unit mass thereby leading to an increase in specific root length (Metcalfe et al., 2008). Indeed, specific root length (SRL mg^{-1}), intended as the length-to-mass ratio of a root fragment, is a good indicator of the benefit/cost analysis (Ostonen et al., 2007) where root length is assumed to be proportional to resource acquisition (benefit) and root mass to construction and maintenance (cost) (Eissenstat and Yanai, 1997). Thus, a stress-tolerant plant

adopts an ‘extensive’ strategy, shifting carbon allocation toward roots or an ‘intensive’ strategy with morphological adaptation of the fine roots to increase soil exploitation area and thus water uptake under harsh soil conditions (Montagnoli et al., 2012a; Ostonen et al., 2007).

Very little is known about the belowground compartment of Mediterranean ecosystems (Canadell and Rodà, 1991) and even less about the root systems of sclerophyllous species, such as *Quercus ilex* L., which is able to tolerate the summer and winter drought periods that characterize the Mediterranean climate (López et al., 1998). In the light of on-going and projected climate change, having an improved understanding of the turnover rate at which fine roots die and contribute to soil carbon pools is important. In addition, woody plants undergo cycles of cambium dormancy and reactivation that are cued by environmental signals (Chaffey, 1999; Savidge, 1996, 2001), but studies that link the growth resumption of the cambial zone to moisture availability are scarce (Ren et al., 2015). This is unfortunate because such information may provide important insight regarding possible species-specific drought tolerance strategies, which would allow for a better planning of management approaches for forest adaptation to climate change. To date and the best of our knowledge, no literature concerning the comparison of fine root dynamics and cambium activity of stems and roots exists. Thus, in the present work, we hypothesized that seasonal dynamics of the fine root system, devoted to provide water and nutrient uptake during the drought period and to accumulate starch just prior to the onset of the dormancy period, is supported by cambial activity in both stems and roots. To test our hypothesis, we evaluate the seasonal variation of (1) cambial activity through the measurement of cell numbers in stems and roots, (2) fine-root mass and length, (3) specific root length and fine root diameter. Furthermore, additional information on the annual fine-root production and turnover rates was evaluated.

2. Materials and methods

2.1. Site description, study plots, and sampling schedule

In the Mediterranean Basin and Middle East, *Q. ilex* extends longitudinally from Portugal to Syria and latitudinally from Morocco to France. The species occurs throughout Italy, preferring acid soils and forming pure forests or mixed forests with other broadleaved species: *Quercus pubescens* Willd., *Fraxinus ornus* L., and *Ostrya carpinifolia* Scop. (Pignatti, 1982; Pirone, 1995). Our study was conducted at the Oriented Natural Reserve – Bosco delle Pianelle (Puglia region, Murge, southeastern Italy, 40° 38' 36"N, 17° 14' 2"E) at an altitude of 440 m with little slope. The soil is classified as Luvisols, (Haplic Luvisols (Chromic), IUSS/ISRIC/FAO 2006) characterized by shallow bedrock and abundant stoniness; these soils were the traditional source of rocks used to build walls around fields (Costantini et al., 2013). The overstory is dominated by *Q. ilex* (canopy cover > 75%) forming a high forest stand that was unmanaged for 5 years prior to our experiment and having a mean tree density of 1066 trees ha^{-1} , a mean DBH of 13.6 cm, and a mean height of 10.6 m. The main understory species are *Viburnum tinus* L., *Phillyrea latifolia* L., *Arbutus unedo* L., *Ruscus aculeatus* L., *Pistacia lentiscus* L., *Asparagus acutifolius* L., and *Cyclamen neapolitanum* Ten. During our sampling period (2013–2014), Mediterranean climatic conditions prevailed, with temperatures and precipitation (concentrated in autumn and spring with a summer drought spanning from May to September; Bagnouls and Gaussen, 1953) in accordance with the general trend and magnitude of the past 75 years (Fig. 1a, weather data from Rete Agrometeorologica Regionale – MTA32 – Martina Franca, 40°38'04" N, 17°16'40" E).

We established eight, permanent, 10 m × 10 m plots that were separated by a mean distance of 40–50 m, equal to 4.25 times the mean height of the *Q. ilex* on the site. Each plot was considered an independent replicate based on Sudmeyer et al. (2004). Plots were sampled approximately every 30 days from September 2013 through September 2014; 13 sampling periods total. On each sample date, soil

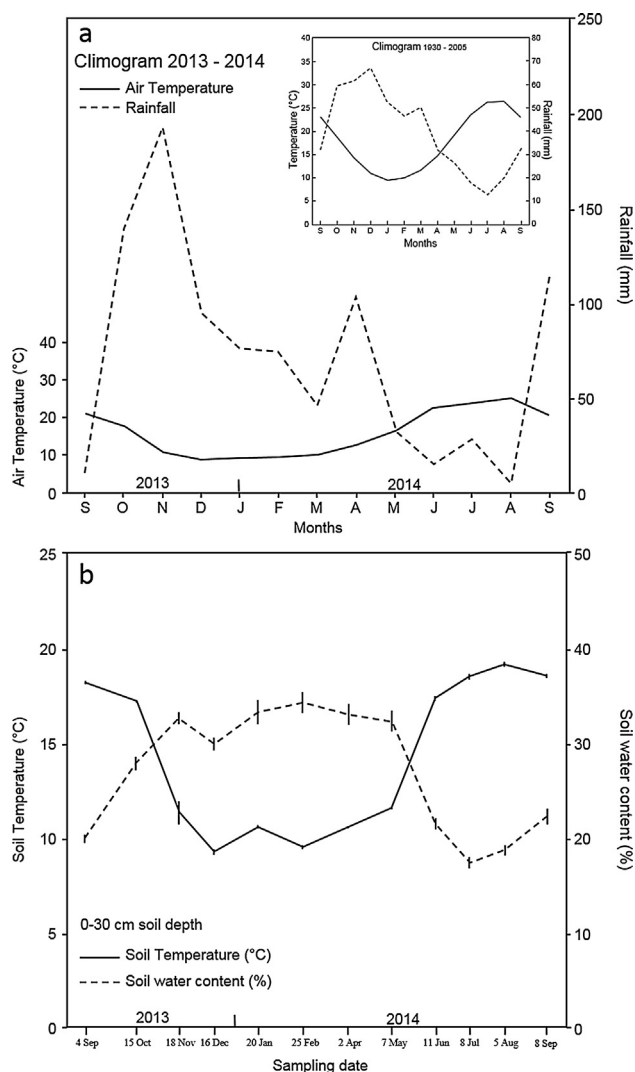


Fig. 1. Inner panel (a) Monthly average air temperature (solid line) and rainfall (broken line) for the period 1930–2005. Data from www.agrometeopuglia.it Assocodipuglia; (a) Monthly average air temperature (solid line) and rainfall (broken line) during the 2013–2014 experimental period. (b) Seasonal variation of soil water content (broken line) and temperature (solid line) at each sampling date plotted for 0–30 cm soil depth. Data are means \pm 1SD.

moisture and temperature were measured in each plot at three depths (0–10, 10–20 and 20–30 cm) hereafter reported as mean 30 cm depth, proximal to a soil core sampling point described below. Volumetric soil water content (%) was recorded with a ThetaProbe type ML2 (Delta-T Devices, Cambridge, UK). Soil temperature ($^{\circ}\text{C}$) was recorded with Checktemp 1 thermometer with an NTC thermistor sensor ($\pm 0.3^{\circ}\text{C}$; Hanna Instruments, Villafranca Padovana (PD), Italy).

Table 1

Mean (\pm SE) seasonal fine root characteristics of *Quercus ilex*. Total dry mass values include live and dead tissue. Net production is according to the minimum–maximum method. Turnover rate is the quotient of net production and standing biomass.

Season	Total dry mass		Net production ($\text{g m}^{-2} \text{ year}^{-1}$)	Standing biomass (g m^{-2})	Standing length (m m^{-2})	Specific root length (m g^{-1})	Turnover rate (year^{-1})
	Peak minimum	Peak maximum					
	(g m ⁻²)						
Autumn	66.9	149.2	82.3				
Summer	77.6	130.9	53.3				
Annual			135.6	Live: 45.0 \pm 2.6 Dead: 69.8 \pm 4.4	Live: 244 \pm 16 Dead: 227 \pm 13	Live 6.8 \pm 0.6 Dead 3.7 \pm 0.2	3.0

Table 2

General linear model values (two-way ANOVA) obtained testing effects of time and sampling plot on different fine-root traits and cambial cell number.

Parameter	Source of variations			
	Time		Sampling plot	
	F	p value	F	p value
<i>Biomass</i>				
Fine root biomass (FRB)	3.698	< 0.001	1.398	0.206
Fine root length (FRL)	6.210	< 0.001	1.310	0.247
Specific root length (SRL)	3.202	0.001	1.248	0.281
Mean root diameter (d)	4.100	< 0.001	0.812	0.607
<i>Necromass</i>				
FRB	1.278	0.255	5.647	0.152
FRL	2.227	0.022	4.325	0.223
SRL	1.864	0.059	1.101	0.373
d	2.099	0.031	1.683	0.109
<i>Cambial cell number</i>				
Shoot	8.219	< 0.001	1.519	0.229
Root	14.345	< 0.001	1.134	0.373

2.2. Fine-root measurements

On each sample date, we randomly collected two soil samples within each plot (8 plots \times 2 cores \times 12 dates = 192 cores). Due to the presence of stones, a motor-driven percussion hammer (BOSCH 5-40 DCE Professional, Stuttgart, Germany) with a 10-cm square-shaped stainless steel blade was used for cutting the soil and obtains cube-shaped soil cores (10 cm \times 10 cm \times 30 cm deep). After cutting edges of each core, the soil was carefully collected by hand. Samples were stored in plastic bags at 4 $^{\circ}\text{C}$ until processed (within 20 days of collection). For processing, each sample was placed in a nylon bag (300 μm mesh) and washed automatically using a washing machine (adapted from Benjamin and Nielsen, 2004). Fine roots ($d < 2 \text{ mm}$) were separated by hand from rocks and sand and were examined at the stereomicroscope and divided into two main groups: *Quercus* and other species. Fine roots from *Quercus* were classified “live” (hereafter biomass) or “dead” (hereafter necromass) depending on their color, texture, and shape (Vogt and Persson, 1991). All fine root samples were scanned submerged in water at a resolution of 800 dpi with a calibrated flatbed scanner coupled to a transparency unit for image acquisition (Epson Expression 10,000 XL) and analyzed by using WinRhizo Pro V. 2007d (Regent Instruments Inc., Quebec, Canada). Live and dead fine root lengths were calculated together with a mean diameter of the fine root population. Samples were then oven-dried and weighed to obtain biomass and necromass. Finally, specific root length (SRL), defined as the fine root length to dry mass ratio was calculated.

Annual fine root production was estimated using the minimum–maximum method. This method calculates, and sums in case of multimodal seasonal pattern, only significant differences between seasonal minimum and maximum fine-root dry mass (live biomass plus necromass) (Edwards and Harris, 1977; Hertel and Leuschner, 2002;

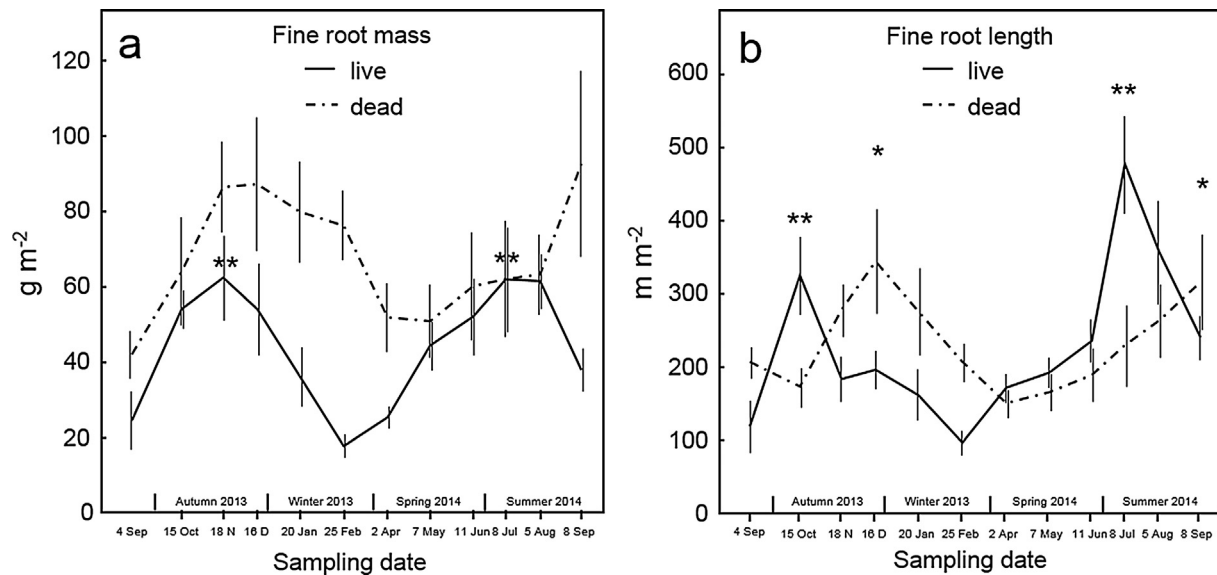


Fig. 2. (A) Seasonal patterns of live (solid line) and dead (broken line) fine root biomass ($g\ m^{-2}$). (B) Seasonal patterns of live (solid line) and dead (broken line) fine root length ($m\ m^{-2}$). Data refer to 0–30 cm soil depth. Each sampling date is represented as means ($n = 8$) \pm 1 SE. Asterisks indicate statistically significant peaks (Dunnnett's test, * $p < 0.1$, ** $p < 0.05$).

McLaugherty et al., 1982). Mean standing biomass was calculated as the average of annual live fine root standing crop. Fine root turnover rates of live biomass were calculated as annual root production divided by mean standing biomass (Brunner et al., 2013).

2.3. Cambium tissue sampling and measurements

On each sample date, one tree from each plot (8 trees total) was randomly selected for cambium tissue sampling. Using a hammer and chisel, we removed a 30 mm \times 10 mm \times 10 mm sample from the stem at breast height and from a main, coarse root at 20–30 cm from the root collar. Each sample contained the inner part of the living bark, the cambium, the current xylem increment, and at least one previous fully formed xylem growth ring. Sampling was done with care to avoid compression of cambial tissue or separation of the bark from the wood. Immediately after removal, samples were fixed in formalin–acetic acid–alcohol (FAA, 5:5:90). Wood samples were further reduced in size (about 5 mm \times 5 mm \times 5 mm) and tender and heterogeneous tissues (e.g. bark–cambium wood) were dehydrated and embedded in Technovit 7100 (Bio-Optica, Milan, Italy) before cutting. Fixed wood was immersed in a series of technovit–ethanol solutions (progressively 30, 60, 100%) and left in each solution for 24 h. After polymerization, a sliding microtome (Leica SM 2400, Leica Biosystems Nussloch GmbH, Germany) was used to produce cross-sectioned cuts with a 15 μ m-thickness. Sections were photographed using an Olympus BX63 light microscope equipped with an Olympus DP72 camera. Images were analyzed by ImageJ 1.41o software (Wayne Rasband, National Institute of Health, USA). For each root section, the cambial cell number was calculated considering all cells having a thin wall and a small radial diameter (Morel et al., 2015).

2.4. Statistical analysis

Each of the eight permanent plots was considered a replicate. At each sampling date and within each plot, the two soil cores were pooled. Fine root and cambial cell data were not normally distributed nor did they meet the assumption of homoscedasticity. Thus, fine root data were square-root or log-transformed to ensure normal distributions and equal variances to allow the use of parametric statistics. Analysis of variance (one-way ANOVA) for effect of time on fine-root traits (biomass, necromass, live root length, dead root length) and cambial cell

number was carried out with time as a fixed effect and sampling plot as a random effect. To test the significance of each peak in the fine root seasonal pattern, the Dunnnett's test (unilateral alternative, $p < 0.05$ and $p < 0.1$) was applied to differences among the maximum value of the peak (reference mean) and both prior and subsequent first minimum values (Montagnoli et al., 2012a, 2012b). A post-hoc Bonferroni test was applied to test differences in cambial cell number among each sampling date. Differences were considered significant at $p < 0.05$ unless otherwise stated. Statistical analyses were carried out with SPSS 17.0 (SPSS Inc, Chicago IL, USA).

3. Results

3.1. Soil moisture and temperature

As a result of fall rainfall events in 2013 (Fig. 1a), volumetric soil water content (SWC) increased from 4 September (20%) to a maximum on 18 November (35%), and then remained constant to 7 May 2014 (35%; Fig. 1b) even as rainfall decreased. Then, SWC dropped to its lowest recorded value (19%) on 8 July 2014. Thereafter SWC increased again to 25% on 8 September 2014. Soil and air temperatures showed the same seasonal variation: decreasing from 4 September to 16 December 2013 and then being constant until the 2 April 2014. Air and soil temperatures warmed significantly from 7 May 2014 to the maximum values recorded on 5 August 2014. Summer drought (i.e. precipitation is insufficient to compensate for losses through evapotranspiration) spanned from early September to half October in 2013 and from end of May to early September in 2014 (Fig. 1a, b).

3.2. Fine root production and morphological characteristics

Annual fine root production was 135 $g\ m^{-2}$; the average lifespan of fine roots was 4 months (i.e., a turnover rate of 3 year $^{-1}$). The annual mean fine root biomass was lower (65%) than the annual mean fine root necromass (Table 1). Mean annual length of live roots was slightly higher than the mean length of necromass (Table 1). Mean annual specific root length was 6.8 and 3.7 $m\ g^{-1}$, for live and dead fine roots, respectively (Table 1). Time significantly affected fine root biomass and length ($p < 0.001$; Table 2), while the random effect of sampling plot was not significant ($p = 0.20$ and 0.25 respectively; Table 2). From the beginning of the season to the seasonal maximum, fine root biomass

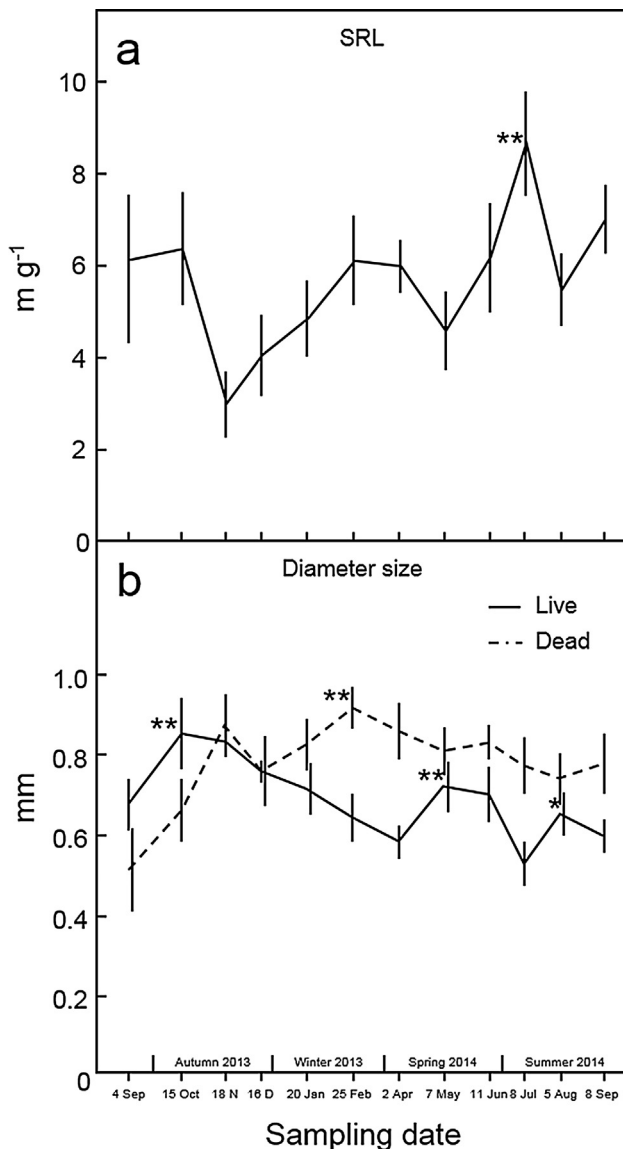


Fig. 3. (A) Seasonal pattern of specific root length (SRL; m g^{-1}) of live fine root. (B) Seasonal pattern of live (solid line) and dead (broken line) mean diameter size of fine root population (mm). Each sampling date is represented as means ($n = 8$) ± 1 SE. Asterisks indicate statistically significant peaks (Dunnnett's test, * $p < 0.1$, ** $p < 0.05$).

and length increased their values almost three and five-fold respectively with two statistically significant ($p < 0.05$) distinct peaks (Fig. 2a, b). In particular, fine root biomass and length increased from the beginning of September to mid-October and then decreased until the end of February. This fall peak occurred during the transition from the dry to the wet season on 18 November for biomass and 15 October for length (Fig. 2a, b). Subsequently, fine root biomass and length peaked a second time ($p < 0.05$) on 8 July (Fig. 2a, b) during the summer transition from the wet to dry season. Then, fine root biomass and length decreased up to the end of the sampling period (8 September 2014). Similarly, to the live fine roots, length of dead fine roots was significantly affected by time ($p = 0.022$; Table 2), showing two peaks ($p < 0.1$) corresponding in timing with fine root necromass. Specific root length showed a complex seasonal variation (Fig. 3a) with three peaks occurring 15 October, 25 February–2 April, and 8 July, although only the latter was statistically significant ($p < 0.05$). Specific root length increased slightly, however, throughout the growing season. The diameter size of the fine root population also showed seasonal variation

with three distinct significant peaks ($p < 0.05$) occurring in fall, spring, and summer, with a general tendency of decreasing throughout the growing season (Fig. 3b, solid line). Throughout the growing season, mean diameter size of fine root necromass showed higher values than those measured for fine root biomass (Fig. 3b, dotted line).

3.3. Cambial cell number

Shoot and root cambial cell number were significantly affected by time ($p < 0.001$; Table 2) but not by the random effect of the plot ($p = 0.229$ and 0.373 , respectively; Table 2). From 4 September, we observed a significant increase in the number of cells in the cambial zone of the roots and shoots, which peaked markedly to 15 October 2013 (Figs. 4 and 5a, b). Shoot and root cambial cell number, after their peak in October declined until minimum values were reached on 16 December (4.8 ± 0.2) and 20 January (4.7 ± 0.1), respectively (Figs. 4 and 5c, d). Thereafter, shoot and root cambial cell numbers increased significantly throughout 2014, 11 June and 8 July ($p < 0.05$) respectively (Fig. 4). On these dates, cambial cell number reached maximum, i.e., about three and two fold the minimal number (Fig. 5e, f). After this, for shoot and root, the numbers of new cells formed declined significantly to 5 August ($p < 0.05$; Fig. 5g, h) and reached the same value measured during the previous year's fall (Fig. 4).

4. Discussion

The activity of vascular cambium of roots has been scarcely investigated (Evert, 2006) in comparison with the stem (De Micco et al., 2016; De Swaef et al., 2013; Sanchez et al., 2012), and, to the best of our knowledge, its seasonal pattern has yet to be described. Thus in our study, each month through one seasonal cycle, we explored the dynamics of shoot and root cambial activity of *Quercus ilex* growing in a natural forest in relation to fine roots, soil water content, and soil temperature. Cambial cell numbers measured in shoots and roots significantly changed during the season. From 16 December onward, the increase in soil temperature together with high soil water content corresponded to a continuous increase in the number of cells in the cambial zone of shoots and roots. The activity of root cambium seemed to be shifted in time in respect to the shoots. Indeed, shoot cambial cell number reached its minimum on 16 December with a first significant increase occurring at the following sampling point (20 January). In the case of roots cambial cell number, seasonal minimum was reached on 20 January while the first significant increment occurred right after on 25 February. Such a time-shift might be due to the buffer effect of the soil that, in comparison with air, has a larger storage term especially with a higher content of water (Al-Kayssi et al., 1990; Campbell and Norman, 1998), having a strong influence on all biological process (Koorevaar et al., 1983; Pregitzer et al., 2000). A significant growth increment of root and shoot cambium occurred with a dramatic increase in temperatures after 7 May and cambial cell number reached its maximum, i.e. three- and two-fold the minimal number in winter. Thereafter, cambium cell number declined through August until a second increment of lower magnitude was detected after rainfall resumed in August and September. This bimodal seasonal growth pattern concurs with the classification of tree-ring formation in Mediterranean environments proposed by Cherubini et al. (2003): cambial activity, triggered by the increase in temperature and soil water availability from spring rainfall, stop when extreme and prolonged drought conditions occur. The cambium is reactivated when soil water becomes available again but deactivates once temperature becomes prohibitive during the winter. Moreover, while cambial activity of the stem has been linked to radial increments (Oberhuber et al., 2014; Steppe et al., 2006), environmental factors (Battipaglia et al., 2010, 2014; De Micco and Aronne, 2012), and leaf phenology (Morel et al., 2015), our study can now also offer some insight into the linkage of cambial growth and

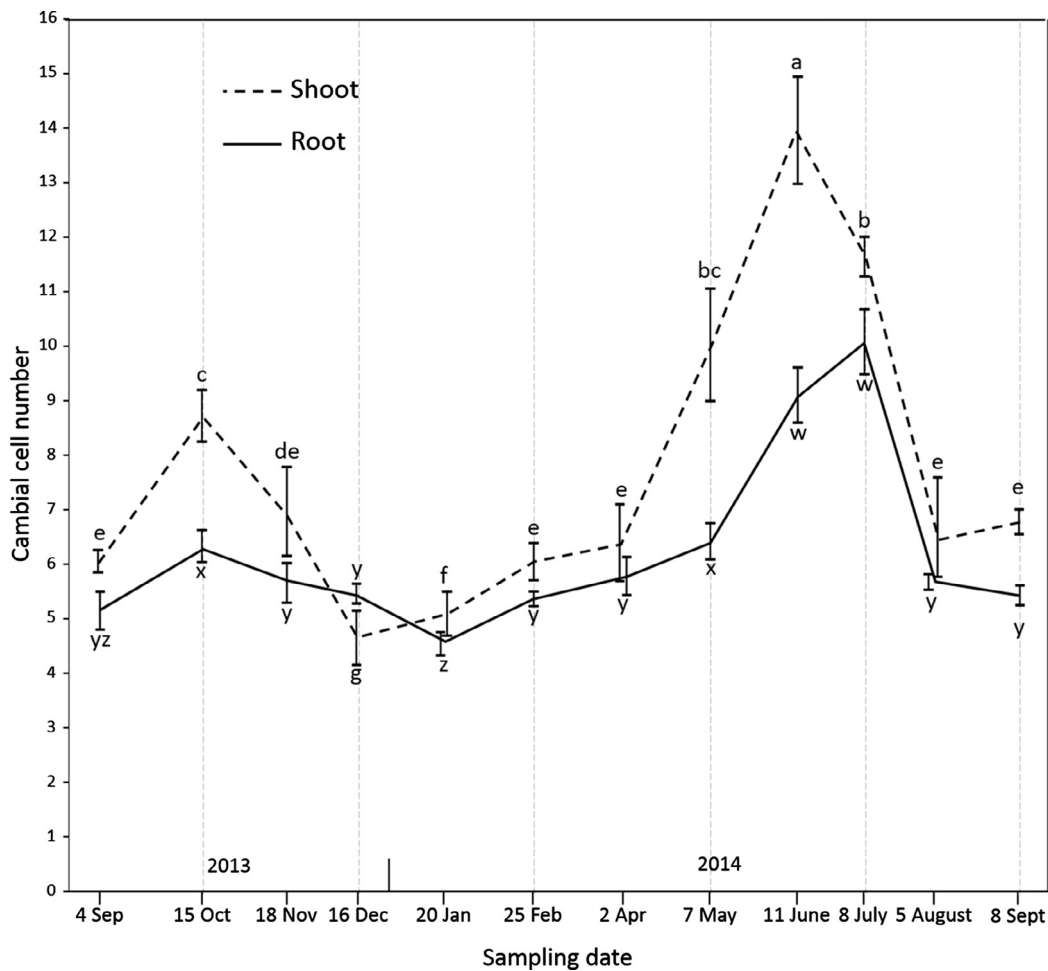


Fig. 4. Seasonal pattern of cambial cell number for shoots (broken line) and roots (solid line). Each point is the mean of eight samples ($n = 8$) \pm 1 SE. Different letters indicate significant difference for shoots (a–g) and roots (w–z) between each sampling date (Bonferroni test, $p < 0.05$).

fine root phenology.

We found that fine root activity (mass and length) also followed a well-defined bimodal seasonal growth pattern that overlapped cambial cell growth, with significant peaks in summer and autumn. Our findings concur with the general pattern of fine root growth observed in northern temperate forests (Brassard et al., 2010) and in Italy for *Q. cerris* (Montagnoli et al., 2012a) and *F. sylvatica* (Montagnoli et al., 2014). Specific root length for *Q. ilex* also showed a complex seasonal variation as in the case of fine root biomass and fine root length. Comparing summer and autumn, specific root length was greater in summer than in autumn, whereas fine root diameters were smaller in summer than in autumn. This suggests that the higher summer soil temperature seemed to trigger fine root growth. During summer in the Mediterranean environment, when soil water content decreases and temperature increases and subsequent carbon gain is lowered by the reduction in stomatal conductance, carbon is preferentially channeled into fine root elongation (longitudinal growth) (Dickson and Tomlinson, 1996; Di Iorio et al., 2011; Montagnoli et al., 2012a, 2014; Thomas and Gausling, 2000). Thus, at the root level, *Q. ilex* adopted an intensive strategy by increasing specific root length (i.e. increase of the volume of soil exploited per unit biomass) when soil moisture content decreased to the lowest value of the growing season (Comas et al., 2002; Comas and Eissenstat, 2004; Curt and Prevosto, 2003; Montagnoli et al., 2012a; Ostonen et al., 2007). This concurs with Bjork et al. (2007) and Makita et al. (2011) who reported a morphological plasticity of roots, especially in the finest fine root fraction. On the contrary, in autumn, when water is available and temperatures are

cooler, the growth rate of fine root biomass was greater than that of length, with the lowest values of specific root length and the highest values of mean diameter measured during the season. Thus, fine root growth is mainly of the radial type (Amendola et al., 2017; Montagnoli et al., 2012a, 2014) as plant production is primarily invested in starch accumulation (Terzaghi et al., 2016). In our study, the seasonal peaks of fine roots lasted only few weeks, suggesting that most of the newly produced fine roots are of a ‘short-lived’ type. If so, the amount of very fine and fine roots surviving the winter and those at the beginning of the growing season (spring) might represent the ‘long-lived’ fine root portion of the root system. Similarly to previous works (Eissenstat and Yanai, 1997; Guo et al., 2008; Montagnoli et al., 2012a; Montagnoli et al., 2014), our results show that *Q. ilex* fine roots borne in winter generally lived longer (ca. 130 days) than roots borne in early fall (ca. 75 days) resulting in differently aged fine root pools (Joslin et al., 2006).

Thus, the almost complete overlap of fine root phenology with seasonal activity of both vascular cambia observed in our study demonstrates that they are interrelated, but the variation in fine root morphology suggests that the nature of this interrelationship differs between spring and autumn. Both cambial activity and fine root growth are synchronously triggered by the increase in temperature and soil water availability from spring rainfall, entering into a stasis when extreme and prolonged drought conditions occur during summer, and resuming growth again in late summer with lower temperature and soil water recharges. In particular, we infer that during the late spring–summer period, with a further rise in temperature and rainfall events,

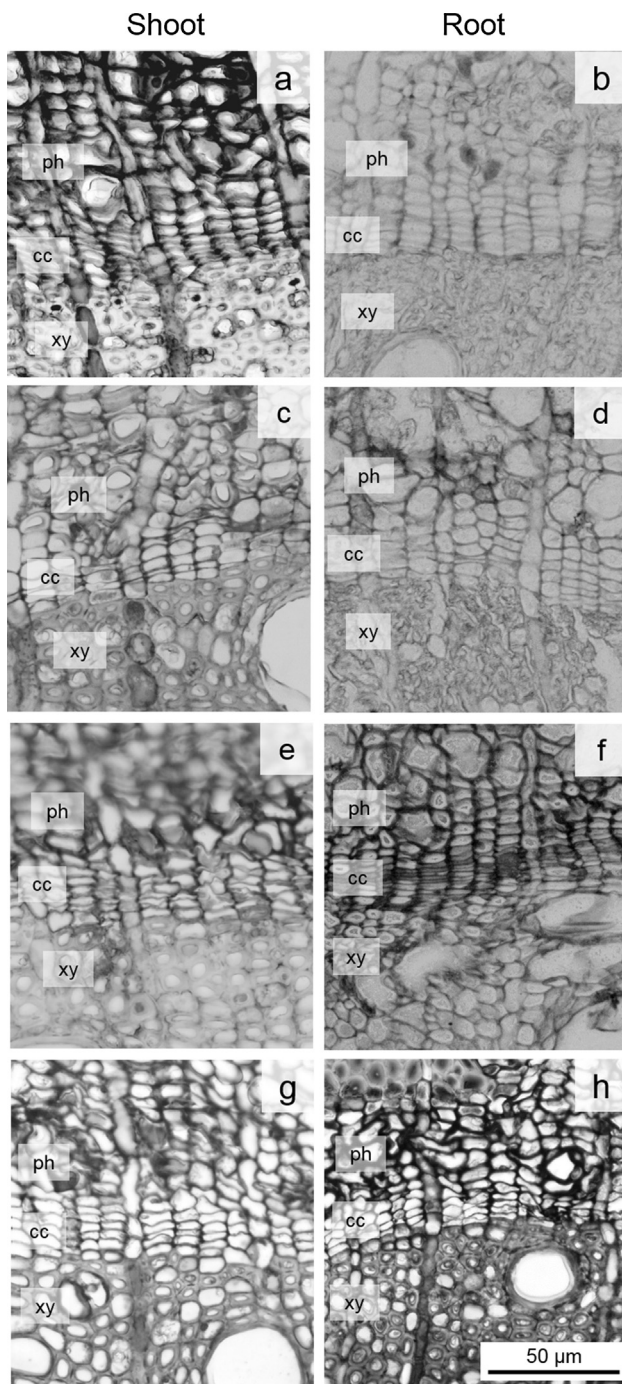


Fig. 5. Anatomical sections of shoot and root sampled between 2013 and 2014. Cambial cells (cc), phloem cells (ph) and xylem cells (xy) measured for shoot and root (columns) at different sampling date (rows). (a) and (b) 15 October 2013; (c) 16 December 2013; (d) 20 January 2014; (e) 11 June 2014; (f) 8 July 2014; (g) and (h) 5 August 2014.

fine root development, along with an enlarging xylem component (Larson, 2012; Pallardy, 2008), provides the means for water and nutrient transport to the plant canopy. In autumn, when air temperature began to decline, photosynthates produced in the canopy are directed basipetally through the newly enlarged phloem (Larson, 2012; Pallardy, 2008) toward growth of fine roots that serve to store starch.

In our stand, annual production of fine roots (135 g m^{-2}) was only about half that measured by López et al., (2001a) Mean standing fine root mass (115 g m^{-2}), including dead and live roots, was only about 20% to 72% of that reported for *Q. cerris* (Montagnoli et al., 2012a;

Claus and George, 2005), *Q. robur* (Bakker, 1998), *Fagus* (Montagnoli et al., 2014), and other forest species (Jackson et al., 1997; Finér et al., 2011). The mean total live fine root biomass we observed (45 g m^{-2}) was of the same magnitude but slightly lower than that measured by López et al. (2001a). These low values could be related, however, to the high content of rock fragments that reduce the soil available to root colonization (López et al., 2001a; Burke and Raynal, 1994). In our study, biomass of dead fine roots was 50% greater than living ones, and this might be the result of the high turnover rate (3 year^{-1}), similar to that reported by López et al. (2001b), which implies high dead mass accumulation (Godbold et al., 2003).

To more accurately predict ecological factors on a biome scale, such as carbon storage or adaptation to climate change, it is necessary to link root biomass with fine root length (Jackson et al., 1997), but such an estimation is still frequently missing in the literature. Our field fine root length (244 m m^{-2}), was almost four-fold higher than that reported for *Q. ilex* in the north of Spain at the same soil depth (López et al., 2001c). For specific root length in trees, the meta-analysis by Ostonen et al. (2007) found fine root ($d < 2 \text{ mm}$) values ranged widely from about 4 to 32 m g^{-1} and our observation (6.8 m g^{-1}), while falling within the range for broad-leaved species, was lower than values found for other oak species (Bakker, 1998; Montagnoli et al., 2012a; Claus and George, 2005). Certainly more work is required to improve the quality of root-related research.

5. Conclusions

To the best of our knowledge, we describe for the first time an almost complete overlap of fine root phenology with seasonal activity of shoot and root vascular cambia. Our study demonstrates that cambial growth and fine roots phenology are interrelated, but the variation in fine root morphology suggests that the nature of this interrelationship differs between spring and autumn. During the late spring–summer period, with a further rise in temperature and rainfall events, fine root development, along with an enlarging xylem component (Larson, 2012; Pallardy, 2008), provides the means for water and nutrient transport to the plant canopy, whereas in autumn, when air temperature began to decline, photosynthates produced in the canopy are directed basipetally through the newly enlarged phloem (Larson, 2012; Pallardy, 2008) toward growth of fine roots that serve to store starch.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.foreco.2018.06.044>.

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