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Root growth dynamics linked to above-ground growth in walnut (*Juglans regia*)

Maria Loreto Contador[1,*], Louise H. Comas[2], Samuel G. Metcalf[1], William L. Stewart[1], Ignacio Porris Gomez[1], Claudia Negron[1] and Bruce D. Lampinen[1]

[1]University of California, Department of Plant Sciences, One Shields Avenue, Davis, CA 95616, USA and [2]USDA-ARS Water Management Research Unit, 2150 Centre Avenue, Bldg D, Suite 320, Fort Collins, CO 80526, USA
** For correspondence. E-mail mlcontador@ucdavis.edu*

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• **Background and Aims** Examination of plant growth below ground is relatively scant compared with that above ground, and is needed to understand whole-plant responses to the environment. This study examines whether the seasonal timing of fine root growth and the spatial distribution of this growth through the soil profile varies in response to canopy manipulation and soil temperature.
• **Methods** Plasticity in the seasonal timing and vertical distribution of root production in response to canopy and soil water manipulation was analysed in field-grown walnut (*Juglans regia* 'Chandler') using minirhizotron techniques.
• **Key Results** Root production in walnuts followed a unimodal curve, with one marked flush of root growth starting in mid-May, with a peak in mid-June. Root production declined later in the season, corresponding to increased soil temperature, as well as to the period of major carbohydrate allocation to reproduction. Canopy and soil moisture manipulation did not influence the timing of root production, but did influence the vertical distribution of roots through the soil profile. Water deficit appeared to promote root production in deeper soil layers for mining soil water. Canopy removal appeared to promote shallow root production.
• **Conclusions** The findings of this study add to growing evidence that root growth in many ecosystems follows a unimodal curve with one marked flush of root growth in coordination with the initial leaf flush of the season. Root vertical distribution appeared to have greater plasticity than timing of root production in this system, with temperature and/or carbohydrate competition constraining the timing of root growth. Effects on root distribution can have serious impacts on trees, with shallow rooting having negative impacts in years with limited soil water or positive impacts in years with wet springs, and deep rooting having positive impacts on soil water mining from deeper soil layers but negative impacts in years with wet springs.

Key words: Canopy removal, *Juglans regia*, pruning, rhizotron, root dynamics, root production, root vertical distribution, walnut, water deficit.

INTRODUCTION

Understanding mechanisms governing plant growth and functioning is critical for maintaining productivity in managed ecosystems under fluctuating climatic patterns as well as economically driven shifts in crop or forest management. The interdependence of above- and below-ground organs highlights the importance and need to understand further the role that endogenous and exogenous factors play in root growth dynamics and their response to development of reproductive and vegetative organs above ground. The allocation of photosynthates in woody perennials is thought to be controlled in part by resource availability, carbon requirements for growth and maintenance of each organ and the ability of organs to compete for carbohydrates (DeJong, 1999). The plasticity of root growth dynamics in response to endogenous and exogenous factors, however, remains an open question (Comas *et al*., 2005).

Patterns of root growth have important implications for tree crop management, yet information commonly found in text books is based on limited data sets (Eissenstat *et al*., 2006). Unlike the above-ground system, the study of root growth and development either through non-destructive direct observation or by digging holes to observe roots is difficult, and therefore the available data on root growth dynamics are limited to a few species. Head (1967) found that, in apple and plum trees, the vigour of shoot and root growth was influenced by pruning, and that pruning also affected the periodicity of new root growth. For instance, in plum, a bimodal pattern of root growth was observed. In late spring, pruning stimulated strong shoot growth during June/July, while concurrently reducing root growth during the same period, and inducing a second peak of root growth later in August/September. Similarly, in apple, pruning stimulated shoot growth, reduced root growth during the most active phase of tree growth in June/July and prolonged inactivity in summer. Atkinson (1980) reported an apparent bimodal growth pattern for apple roots, where root growth was initiated earlier than shoot growth and had alternating periods of active and less active root growth. A bimodal root production curve is also described for walnut, but the origin of the data set used for the curve is unknown and is unlikely to have come from walnut trees (Catlin, 1998). Recently, advances in technology and techniques have made it easier to study roots with minimal

disturbance to the soil profile and control over spatial variability. For instance, the minirhizotron technique was found to be a good method to quantify root biomass in surface and deep soil layers for tree species (Jose *et al*., 2001). The time needed to process the huge amount of data provided by minirhizotrons has shortened due to the development of new software for collecting data on fine roots in minirhizotron images (Zeng *et al*., 2008). In addition, discrimination between live and dead roots in minirhizotron images using root pigmentation has made it possible to estimate the time of root death with greater accuracy (Comas *et al*., 2000).

These advances have facilitated recent studies on fundamental aspects of environmental and biological controls on root dynamics and functioning in many tree species. For example, Tierney *et al*. (2003) studied the relationship between environmental variables, such as temperature, water and nutrient availability, and fine root dynamics in a northern hardwood forest. A strong relationship was found between fine root production and mean monthly air temperature, while natural variation in soil moisture content and nitrogen availability did not appear to predict fine root production. Psarras *et al*. (2000) observed root growth on apple trees for 2 years and found one main peak of root production during late June and early July followed by a smaller peak of root production during August to September. Basile *et al*. (2007) found that in peaches, fine root production was lowest during winter and highest during spring and summer, declining during the final stages of fruit growth. Comas *et al*. (2005) found one main mid-season peak in root production for grapevines, with lower root production rates later in the season when carbohydrate demands for reproduction increased.

Canopy pruning and deficit irrigation can affect root growth in several ways. Field studies have shown that canopy pruning can cause root growth to decrease or stop until shoots regrow (Richards, 1984; Eissenstat and Duncan, 1992; Comas *et al*., 2005). However, the duration of decreased root growth and time needed for the plant to reach a new root to shoot ratio equilibrium depends on the amount of defoliation, the species, plant size and timing of pruning (Eissenstat and Duncan, 1992). In a study of canopy and environmental control on root production of Concord grape (*Vitis labruscana*) with two levels of winter canopy pruning and irrigation, root production was greater under minimal canopy pruning and was initiated earlier in the season compared with heavy pruning (Comas *et al*., 2005). Root production in this study was also reduced in dry years without irrigation (Comas *et al*., 2005). In general, under limited water availability, root growth in wetter soil is favoured over that in the dry areas. For example, in olive trees, standing root length was found to be concentrated around the wetted zone under drip irrigation and uniformly distributed under flood irrigation (Fernandez *et al*., 1992). In almond trees, root growth was favoured in a heavily watered treatment compared with a less irrigated one (Abrisqueta *et al*., 1994). In grapevines, Bauerle *et al*. (2008) found that high and low shoot vigour rootstocks have similar tolerance to soil moisture deficits according to root survivorship in dry soil, but high vigour rootstocks presented enhanced morphological plasticity to lateral soil moisture heterogeneity.

In walnut, *Juglans regia*, the physiological responses of above-ground organs to deficit irrigation have been studied previously (Cochard *et al*., 2002; Rosati *et al*., 2006; Sun *et al*., 2011), yet basic information of root growth dynamics of trees in walnut orchards is limited to a few studies (Kuhns *et al*., 1985; Sun *et al*., 2011). In 2010, unpruned treatment trees were found to have a more favourable hydraulic status (based on mid-day stem water potential) compared with minimally or heavily pruned treatment trees from August until the end of the season. One hypothesis was that pruning interfered with root growth and resulted in trees with reduced capacity for water uptake. Thus, we studied the fine root system dynamics of walnuts, specifically of the cultivar Chandler grafted on Paradox seedling rootstock. We determined seasonal patterns of fine root growth and distribution through the soil profile. Our main objective was to test the hypotheses that the seasonal timing and spatial distribution of root growth varied in response to canopy manipulation. Specifically, we tested if (1) pruning reduced root growth and/or delayed root production; and (2) if deficit irrigation promoted root growth in deeper soil layers, which remain wet for longer periods.

MATERIALS AND METHODS

Experimental site and plant material

A field trial was carried out at an experimental walnut orchard located at the Nickels Soil Laboratory (Arbuckle, CA, USA) (38 °58'04·2''N, 122 °04'21·0''W) during 2011 and 2012. Soil at the site was a combination of Arbuckle Hillgate Complex, Arbuckle Sandy Loam and Hillgate Loam (Beaudette and O'Geen, 2010).

The plant material used was the walnut variety 'Chandler', a moderately vigorous cultivar with a lateral bearing habit and high yield, grafted onto Paradox seedling rootstock, a hybrid obtained from the crossing of Northern California black walnut (*Juglans hindsii*) and English walnut (*Juglans regia*). Trees were planted in 2008 on 0·35 m high by 1·50 m wide berms at a tree spacing of $4{\cdot}57 \times 6{\cdot}70$ m (326 trees ha^{-1}). Nutrition, pest and weed management, and other orchard operations, were consistent with common commercial walnut orchard practices.

Pruning and irrigation treatments

Four treatments that included three pruning and two irrigation levels were imposed in a randomized block experimental design containing five replicated plots of each treatment. Each plot consisted of three rows of trees and five trees within each row. The imposed pruning and irrigation treatments were: heavily pruned with control irrigation (T1); minimally pruned with deficit irrigation (T2) in which irrigation was restricted to 75 % of control levels; minimally pruned with control irrigation (T3); and unpruned with control irrigation (T4). Pruning treatments were patterned after commercial procedures. A full factorial of pruning and irrigation treatments was not possible due to space limitations. Pruning began at the end of the first growing season and was completed during dormancy in March each year. The orchard was irrigated with a double line drip system with inline emitters spaced 56 cm apart. The amount of water applied under control irrigation matched calculated evapotranspiration over the season. In the spring of 2012, one emitter was re-positioned

to the top of the berm in order to produce a uniform wetting pattern on the observational area for root growth.

For the heavy pruning treatment, in the first year one central leader was chosen and headed at a height of 1·8 m; other shoots were removed. In the second year, a central leader and 4–6 primary scaffolds were selected and headed by removing one-third of the previous season's growth. The remaining unselected scaffolds were removed if they were large, in order to avoid competition with the selected scaffolds. Forked branches were reduced to a single branch, and branches below 90–120 cm were removed. Any remaining branches were removed if they were too close to another branch or otherwise tipped (about 10 cm removed). In the third year, the chosen central leader had 40 % of the growth from the previous year removed. Branches that were competing with the leader were removed. In season branching points were removed on secondary scaffolds. Secondary scaffolds were chosen to be well spaced in all directions and headed by 40 %, making sure no secondary scaffold was taller than the central leader. All twisted, crossed or rubbing branches were removed. In the fourth year, little pruning was done and only the leader and major scaffolds were headed.

For the minimal pruning treatment, in the first year the main scaffold was headed at 1·8 m. In the second year, a central leader was selected and one-third of the previous season's growth was removed. Four to six primary scaffolds were selected and headed below the height of the central leader, removing a quarter to a third of the length of the previous season's growth. Forked branches on chosen scaffolds were reduced to a single branch. The remaining unselected branches and small caliper fruit wood were left unpruned and unheaded to create early fruiting wood. In the third year, the strongest, tallest scaffold was chosen as the leader and one-third of the previous season's growth was removed. Other scaffolds were left alone if they were growing in a vertical position. One or two strong scaffolds were chosen on the sides of the canopy, one in each cardinal direction, and one-third of the new growth was removed. In the fourth year, if needed, minimal pruning was done and the leader and scaffolds were headed by removing one-third of the growth from the previous season.

For the unpruned treatment in the first year, lower branches were removed and one main leader was selected, but it was not headed. In the following years, no pruning or heading was done, unless lower branches needed to be removed for reasons of safety or ease of orchard access.

Installation of minirhizotron tubes and soil monitoring equipment

In March 2011, minirhizotron acrylic tubes of 140 cm length were installed on the south side of the two central trees in each replication, 60 cm south from the centre of the trunk and 20 cm to the east of the centre of the berm at a 60 ° angle from the orchard floor using a steel guide frame (140 cm tubes; 4 treatments × 5 blocks × 2 tubes/block = 40 tubes). The bottoms of the tubes were sealed with a plug. Tops of tubes protruding from the ground were wrapped with one layer of black pipe wrap tape followed by a double layer of white electrical tape to block light. The tops of tubes were insulated with a 14 cm long foam plug and capped with a white-painted cap in order to reflect sunlight as much as possible and prevent water and animals from entering the minirhizotron tubes. Rubber gaskets painted white were also placed around the tops of the tubes at the soil surface to minimize soil erosion, light penetration into the tubes and water running down the external tube surface. Watermark soil temperature and moisture sensors were installed at three depths (30, 60 and 90 cm) on two trees in each treatment (total of eight trees) and attached to data loggers (Irrometer Watermark Monitor 900 m data loggers, Irrometer Company Inc., Riverside, CA, USA).

Data collection

Digital images were captured from 79 fixed windows in each tube with a minirhizotron digital camera system (BTC-2 Bartz Technology Co., Santa Barbara, CA, USA). Images were collected through the soil profile from 0 to 82 cm every 14 d during the growing season and every 30 d during the dormant season for two consecutive years. The number of roots, date of appearance, date of death (according to root pigmentation), and depth were tracked using Rootfly 2.0.2 software (Clemson University, Clemson, SC, USA).

Above-ground measurements included mid-day stem water potential, yield and nut quality. Mid-day stem water potential was measured approximately every 14 d during the growing season on the three centremost trees of each plot by placing the terminal leaflet in a Mylar (Dupont Teijin Films, Chester, VA, USA) polyester film bag for 15 min, cutting the leaf and placing it in the pressure chamber (Fulton *et al*., 2001). Dates of key phenological stages of the trees were recorded during the season following standard protocols (C. Leslie, University of California at Davis, USA, pers. comm.). Crop yield was recorded for each treatment for both growing seasons using a load cell-equipped harvest trailer.

Mean, maximum and minimum air temperatures were obtained from the meteorogical station (Campbell Scientific CR10X) located at the Nickels Soil Laboratory (Arbuckle, CA, USA) (38 °58'04·2''N, 122 °04'21·0''W) of the University of California Statewide Integrated Pest Management Program (UC IPM).

Root vitality measurement

In order to classify roots as dead or alive, root pigmentation was related to metabolic activity. Root samples were excavated and classified into four pigmentation categories: white, light brown, dark brown to black, and boiled control, which included all the pigmentation categories, but roots were boiled to ensure no metabolic activity would be present. Each root sample was reduced with triphenyl tetrazolium chloride (TTC) to red formazan, which was later extracted and photometrically measured at 490 and 520 nm using a spectrophotometer (UV/VIS) following the protocol described by Comas *et al*. (2000). Spectral analyses of root extractions in 95 % ethanol were previously done in order to verify that root pigmentation would not interfere with the absorption of formazan at the wavelengths measured.

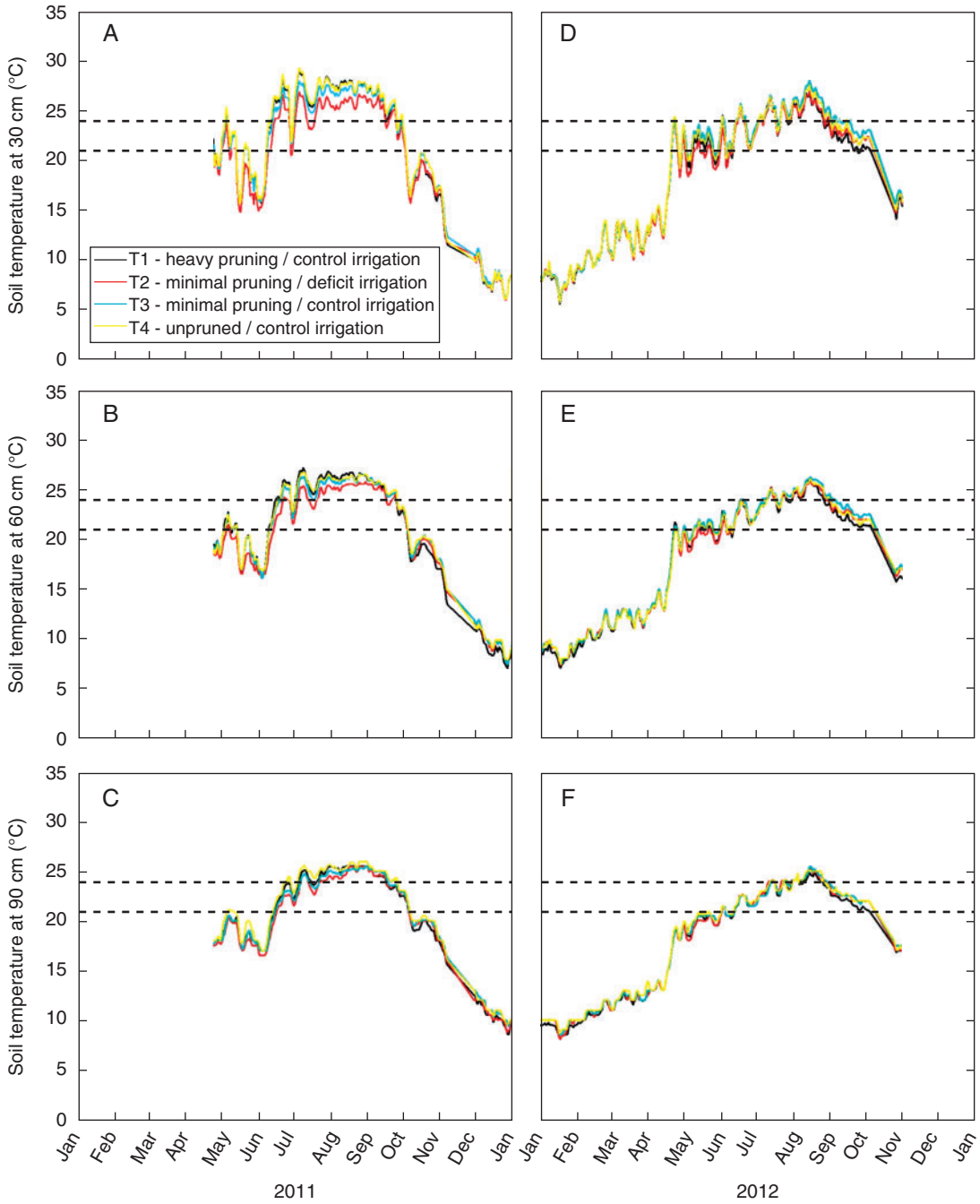

FIG. 1. Seasonal patterns of soil temperature at depths of 30 cm (A, D), 60 cm (B, E) and 90 cm (C, F) adjacent to minirhizotron tubes in 2011 and 2012. Horizontal dashed lines indicate the previously reported ideal range (21 and 24 °C) of soil temperature for maximum root growth (Haas, 1939; Kuhns *et al.*, 1985). Values are means.

Experimental design and statistical analysis

Statistical analysis was done using the statistics package SAS/STAT 9.3 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA; PROC GLM) using a generalized linear model split plot analysis with the main plot as treatment and time as the sub-plot was used to examine the effect of treatment and date on the appearance of roots, death of roots and number of living roots during the growing season. The effect of treatment and depth on total root production was also tested with an ANOVA using a generalized linear model (PROC GLM) with a split plot analysis, in which the main plot was the combined pruning/irrigation treatment and the sub-plot was depth. The effect of treatments, season and depth on root production during each season and distribution through the soil profile was tested with an ANOVA using a generalized linear model (PROC GLM) with a split–split plot analysis, with the

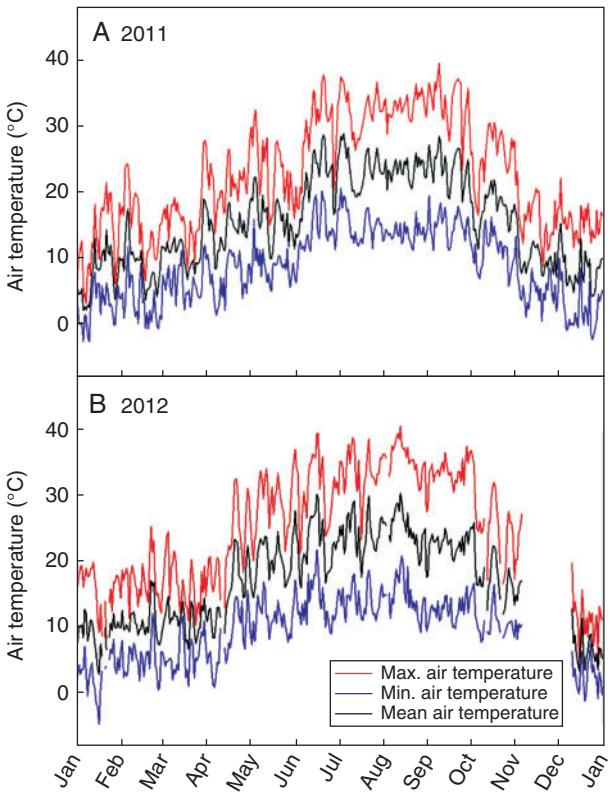

FIG. 2. Seasonal patterns of maximum, mean and minimum air temperature for (A) 2011 and (B) 2012.

main plot being the combined pruning/irrigation treatment, the sub-plot depth, and the sub-sub-plot season. Whenever the ANOVA had significant interactions between the factors studied, simple effects were tested by comparing means among sub-plots within main plots and main plots within each sub-plot. If no significant interactions were found, then the main effects were tested by comparing the main plot and sub-plots separately at all levels of each factor. When significant differences were found, Tukey's honestly significant difference test was used for mean separation.

RESULTS

Soil and air temperature, tree status and yield

Soil temperatures in spring 2011 increased slowly due to cloudy skies, reaching 21 °C in early June. Soil temperature stayed in the range of 21–24 °C until July, and then increased above 24 °C until the end of September (Fig. 1). In 2012, soil temperatures reached 21 °C in early May and stayed in the 21–24 °C range until early October. Soil temperature peaked above 24 °C from mid-July through August. Seasonal patterns were similar among treatments in both years (Fig. 1). Maximum, minimum and mean air temperatures are shown in Fig. 2.

In 2011, mid-day stem water potential measurements indicated that plant water status was above the fully watered baseline, as calculated from the value of mid-day air vapour pressure deficit (McCutchan and Shackel, 1992), from 5 May through 13 June, and all treatments experienced three cycles of stress during July and August (Fig. 3A). Pruning tended to increase water stress, and, overall, the unpruned treatment with control irrigation (T4) was the least water stressed throughout the year, and minimal pruning with deficit irrigation treatment the most stressed (T2). In 2012, plant water status stayed at or below the fully watered baseline to a greater extent than in 2011, but plant water status was closer among treatments, especially between 27 July and 20 September (Fig. 3B). Similar to 2011, however, the unpruned trees with control irrigation (T4) were generally the least water stressed, and those minimally pruned with deficit irrigation treatment (T2) the most stressed (Fig. 3B).

There were no significant differences in crop yield between treatments in either year ($P = 0{\cdot}1218$ and $P = 0{\cdot}3081$ for 2011 and 2012, respectively; data not shown).

Vital staining

Root metabolic activity varied with root pigmentation, with the highest activity in white roots, intermediate in light brown-pigmented roots and the lowest in dark brown/black and control roots (Fig. 4). Measurements at 520 nm gave similar results to those reported at 490 nm (data not shown).

Annual root growth

In 2011, new root growth started in late April and peaked in mid-June (Fig. 5D). In 2012, new root growth started in early May and peaked in early to mid-June for the control irrigation treatments and late June for the deficit irrigation treatment (Figs 5H and 6B), but no significant differences were found among treatments in roots produced ($P = 0{\cdot}981$), present ($P = 0{\cdot}999$) or dying ($P = 0{\cdot}947$) by date. In both years, the initiation of root growth coincided with the pre-formed leaf growth, flowering and initiation of nut growth periods (Fig. 5A, D, E, H). In both years, a decline in new root growth and living root population coincided with the neoformed leaf growth and kernel fill period (Fig. 5).

In 2011, all treatments had significantly higher numbers of living roots from 7 June to 19 July compared with the rest of the growing season ($P < 0{\cdot}05$). A small second peak observed in autumn 2011 can be explained by the fact that the minirhizotron equipment had a technical problem and measurements could not be taken for a period of 6 weeks, leading to an accumulation of roots, which is reflected on the next set of measurements taken in the early November period (Fig. 5B–D). There was a unimodal peak of living roots in 2012, which was similar among treatments but occurred between 1 June and 11 July, slightly earlier than in 2011 ($P < 0{\cdot}05$).

Root populations remained low through autumn and winter (Fig. 5). New roots continually grew through the growing season as older roots died (Fig. 5B, D, F, H). Total mean root production differed among treatments in 2011 (Figs 5D and 6A). The minimally pruned trees with control irrigation (T3) produced significantly more roots than the minimally pruned trees with deficit irrigation (T2) and the unpruned trees with control irrigation (T4) treatments in spring and summer ($P < 0{\cdot}05$),

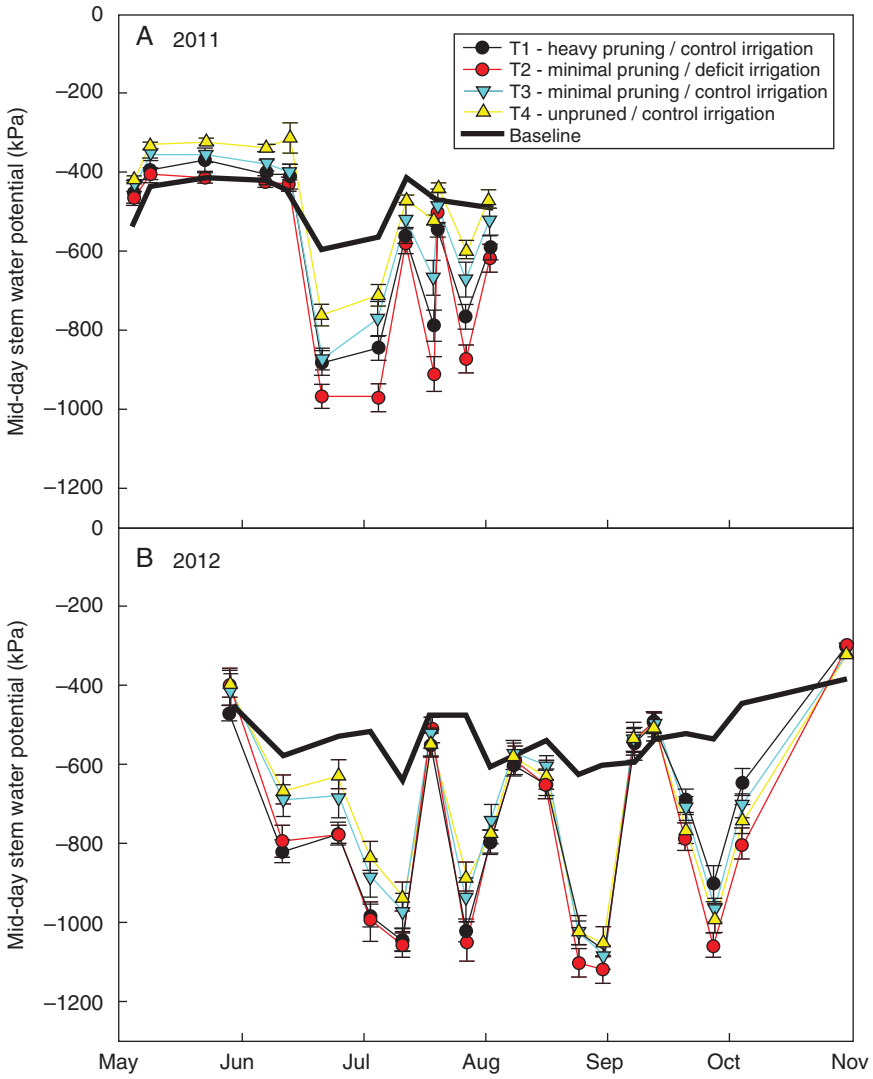

FIG. 3. Mid-day stem water potentials for (A) the 2011 season and (B) the 2012 season by treatment. Values are means ± s.e. Solid black line represents the fully watered baseline calculated from mid-day air vapour pressure deficit (McCutchan and Shackel, 1992).

with no significant differences among the other treatments (Fig. 6B). In 2011, living root populations were significantly larger in the minimally pruned trees with control irrigation (T3) compared with the heavily pruned trees with control irrigation (T1) ($P < 0{\cdot}05$; Fig. 5C). Total mean root production did not differ significantly among treatments in 2012 ($P = 0{\cdot}914$; Fig. 5H). However, the minimally pruned trees under control irrigation (T3) produced marginally more roots in spring and significantly fewer in summer compared with the minimally pruned trees with deficit irrigation (T2) in spring (spring, $P = 0{\cdot}071$; summer, $P < 0{\cdot}05$; Fig. 6).

Root production with soil depth

In both years, substantial root production was observed down to 82 cm, the deepest depth observed (Fig. 7A, E). Root production differed through the soil profile, with the greatest root production at 23–42 cm soil depth ($P < 0{\cdot}05$; Fig. 6A, E).

Minimally and heavily pruned trees with control irrigation (T1 and T3) produced more roots at 23–42 cm compared with minimally pruned trees with deficit irrigation (T2) and unpruned trees with control irrigation (T4) in 2011 ($P < 0{\cdot}05$; Fig. 7A). The heavily pruned trees with control irrigation (T1) also produced significantly more roots at 23–42 cm than at 63–82 cm ($P < 0{\cdot}05$; Fig. 7A). In 2012, patterns were similar but not as strong ($P = 0{\cdot}081$; Fig. 7E). In both years, root production in spring and summer was primarily responsible for these patterns (Fig. 7).

Deficit irrigation stimulated root production at deeper soil depths, although patterns were slightly different among years (Fig. 7). Trees under deficit irrigation produced marginally more roots at 43–62 cm soil depth compared with the 3–22 cm depth in 2011 ($P = 0{\cdot}0725$; Fig. 7A), mostly due to spring root production (Fig. 7B). Trees under deficit irrigation produced significantly more roots at 63–82 cm in 2012 compared with the 3–22 cm depth in 2012 ($P < 0{\cdot}05$; Fig. 7E), mainly due to summer growth (Fig. 7G).

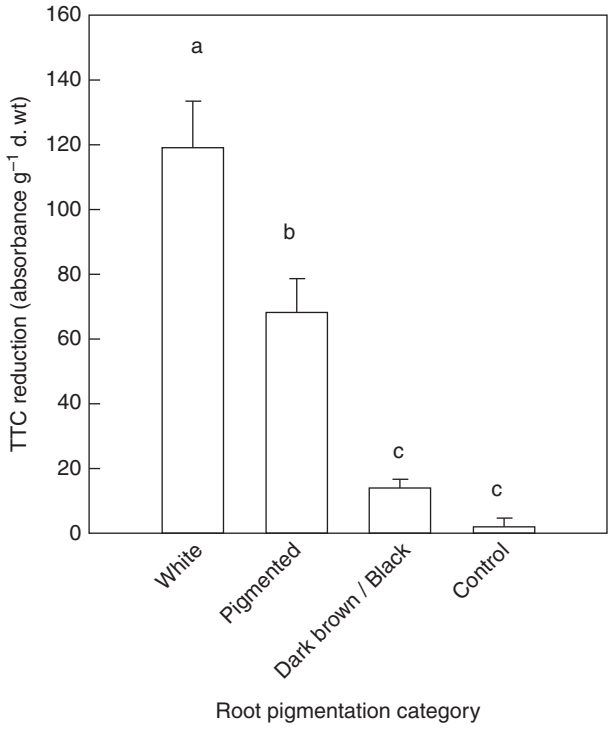

FIG. 4. Mean absorbance of triphenyl tetrazolium chloride (TTC) reduction to red formazan at 490 nm among root pigmentation categories. Values are means ± s.e. Different letters denote significant differences ($P < 0{\cdot}05$).

DISCUSSION

Root production in walnuts followed a unimodal curve with one marked flush of roots, rather than two flushes as previously reported. Root production began in spring and peaked in early summer when soil temperature reached 21 °C, concurrent with the growth of pre-formed leaf and nut expansion. This concurrence in timing suggests that initiation of root production in walnuts may be influenced by soil temperature and/or plant demand for soil resources. Root production and the size of metabolically active root populations declined in mid to late summer as neoformed shoot growth and kernel fill was developing above ground. This decline in root populations also coincided with rising soil temperatures. Counter to expectations, pruning and irrigation did not strongly affect the timing of root production. The distribution of fine roots through the soil profile, however, showed plasticity to canopy and irrigation treatments. Water deficit was associated with less root production in shallow soil layers and more growth in deeper soil layers, while canopy pruning was associated with increased shallow root production.

The unimodal curve of root production that was found was contrary to what has been observed in apple and plum and previously reported for walnut (Head, 1967; Atkinson, 1980; Caitlin, 1998). In temperate environments where plants become dormant shortly after crop harvest, a unimodal pattern was found for grape (Comas *et al*., 2005) and for apple (Psarras *et al*., 2000). Likewise, dormancy also followed soon after crop harvest for walnut in our system. Unimodal patterns in these temperate systems may be explained by competition for carbohydrates, with the crop preventing root production during the late season, and dormancy quickly following crop harvest, effectively limiting further root production. Declining soil temperatures may also play a role.

Neoformed shoot growth and kernel fill phases may have high demands for carbohydrates, and limit root production due to limited carbohydrate supplies. Although a decline in root production in response to high soil temperature (>24 °C) cannot be separated from declines due to competition for plant carbohydrates in the current study, findings are consistent with previous findings, which demonstrated that a decrease in root production was related to the presence of growing fruit in peach (Williamson and Coston, 1989). Similarly, Grossman and DeJong (1995) found that during the phase of maximum fruit sink strength, photosynthate availability for new root production can be limited. We expected to find differences in the timing of root production and number of roots produced among the different treatments, but no significant differences were found. We anticipated that spring heavy pruning would stimulate shoot growth and/or reduce root growth, as has been observed in other species (Head, 1967; Richards, 1984; Eissenstat and Duncan, 1992), or shift root production to later in the growing season (Comas *et al*., 2005, 2010). An explanation for the lack of differences in the current study may be that the capacity of a tree for growth after defoliation depends on specific tree physiology. Species with indeterminate capacity for neoformed growth such as walnut have a larger potential for compensatory growth if they have the ability for nitrogen remobilization (Millard and Grelet, 2010). Walnut accumulates large pools of non-structural carbohydrates which are never fully depleted (Gauthier and Jacobs, 2011), potentially providing the capacity to allocate resources for the growth of organs above and below ground.

In a previous study on citrus, after pruning at the beginning of the season, the carbohydrates used to re-establish the canopy were apparently used at the expense of root growth, and later on in the season at the expense of fruit production (Eissenstat and Duncan, 1992). However, in the current study there were no significant differences in yield among treatments in either year, potentially explaining why there were no effects of yield on root growth. Again, it is possible that the impact of pruning on yield was ameliorated by the large pools of non-structural carbohydrates that walnuts are known to accumulate, which could be redistributed to the different growing organs (Gauthier and Jacobs, 2011).

The period of rapid root production in the current study coincided with the period in which soil temperature reached 21 °C (Fig. 1A–H). According to previously published data, maximum root growth in walnut was observed between 21 °C (Kuhns *et al*., 1985) and 24 °C (Haas, 1939). Lyr and Hoffman (1967) found that root growth temperatures in the literature ranged between 2 and 35 °C, with broad variation in both minimum and optimum temperatures among species. Greenhouse and field studies have shown that the root growth rate initially increased with temperature in annual and perennial crops when other factors, such as water availability, remain equal (Lyr and Hoffman, 1967; Ericsson *et al*., 1996; Pregitzer *et al*., 2000). For maize, root growth started at around 9 °C and reached a maximum growth rate at 28 °C (Barber *et al*., 1988). In conifer seedlings, root growth was observed to begin when soil

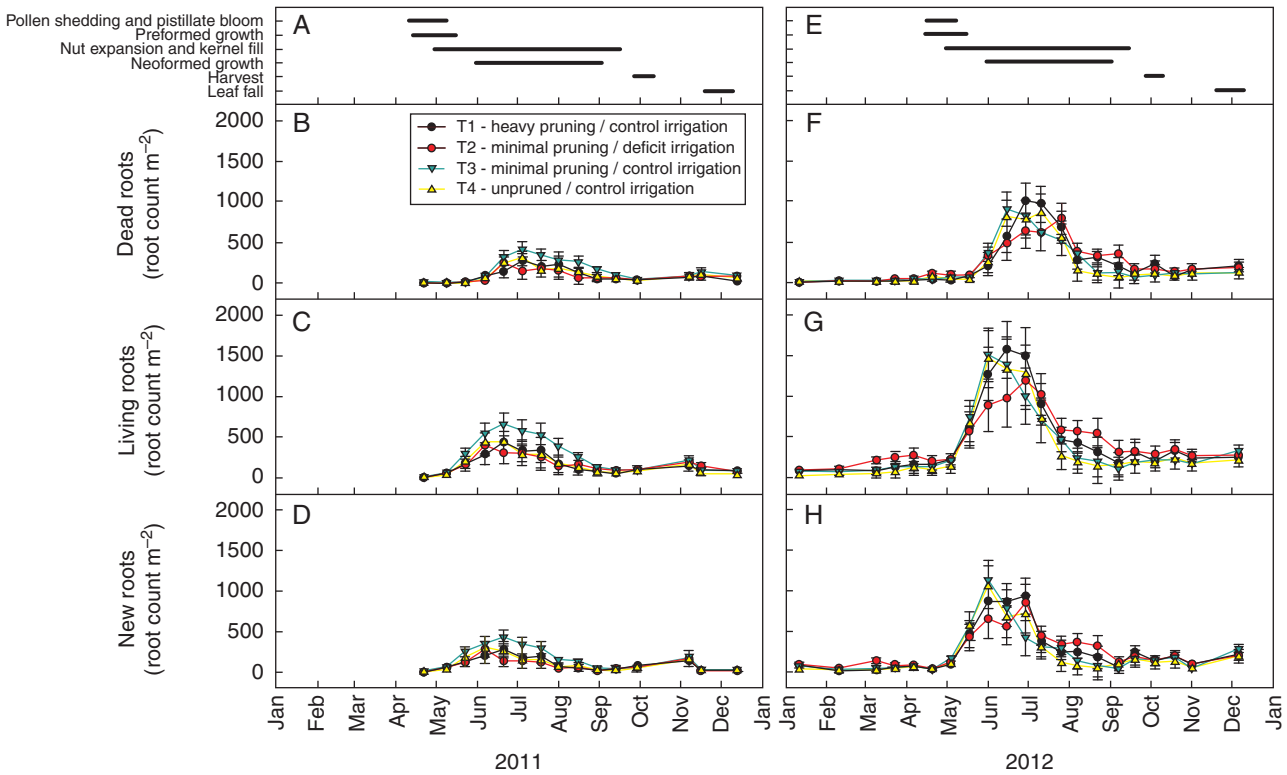

FIG. 5. Seasonal patterns of walnut above- and below-ground growth. (A, E) Phenological stages of walnut tree for 2011 and 2012, respectively. (B, F) Seasonal patterns of new dead root counts by treatment between 0 and 82 cm soil depth for 2011 and 2012, respectively. (C, G) Seasonal patterns of standing live root counts by treatment between 0 and 82 cm soil depth for 2011 and 2012, respectively. (D, H) Seasonal patterns of new root counts by treatment between 0 and 82 cm soil depth for 2011 and 2012, respectively. Values are expressed as count per m^2 minirhizotron tube surface and are means ± s.e.

temperature was >5 °C, intensified once soil temperature was >10 °C and reached maximum growth rate at 20 °C (Lopushinsky and Max, 1990). Increasing soil temperatures can promote root growth; nonetheless, high temperatures (>24 °C) have been observed to inhibit root growth in walnut (Haas, 1939).

Total annual root production differed significantly among treatments only in 2011, when the minimally pruned trees with control irrigation treatment had more root production compared with the other three treatments. A possible explanation for the observation of lower root production in the deficit irrigation treatment could be that water deficit restricted root growth (Abrisqueta *et al*., 1994; Abrisqueta *et al*., 2008) since mid-day stem water potential was significantly lower in the deficit irrigation treatment compared with control irrigation treatments in 2011. Lower root production with deficit irrigation was not observed in 2012, but water status was more similar among treatments in 2012. Interestingly, unpruned trees with control irrigation were the least water stressed throughout the season, and also produced relatively small root populations.

The majority of roots in the current study were found between 23 and 62 cm deep in the soil profile. This is in contrast to what was observed in a restored English walnut forest under monsoonal influence, in which 60 % of total root mass was observed in the upper 30 cm of the soil profile (Sun *et al*., 2011). The differences could be due to several factors, such as different precipitation patterns and irrigation systems among the studies (monsoonal rainfed vs. drip irrigation), the fact that the orchard in the current study is situated in a warmer climate and was planted on berms which expose the shallow soil layers to higher temperature fluctuations and drier soil, or it could be due to different growth patterns through the soil profile of the rootstocks (English walnut vs. Paradox seedling rootstock).

Root distributions found here in response to treatments were only partially expected. The observed difference in root distribution between the two irrigation levels was expected, since it has been reported that water stress promotes root growth in deeper soil layers (Abrisqueta *et al*., 1994, 2008; Burkart *et al*., 2004). We anticipated that root distribution in walnut would be particularly responsive since walnut root growth was found to be sensitive to soil water availability (Kuhns *et al*., 1985). Following an irrigation event, soil moisture varies through the soil profile and over time. Usually the upper layers dry first and the deeper soil layers remain wet for longer periods (Bauerle *et al*., 2008). Root system plasticity may be especially important for water uptake from soils with heterogeneous moisture availability, such as when water is available at deeper soil layers. This is particularly true when water is limited, as it has been observed that root growth decreases in drier areas and is favoured in wetter areas of soil (Coutts, 1982; Richards and Crockroft, 1975; Fort, 1998; Comas, 2005; Abrisqueta *et al*., 1994; Bauerle *et al*., 2008; Comas *et al*., 2010). In contrast, shallower root growth in the minimally and heavily pruned trees, compared with those minimally pruned with deficit and

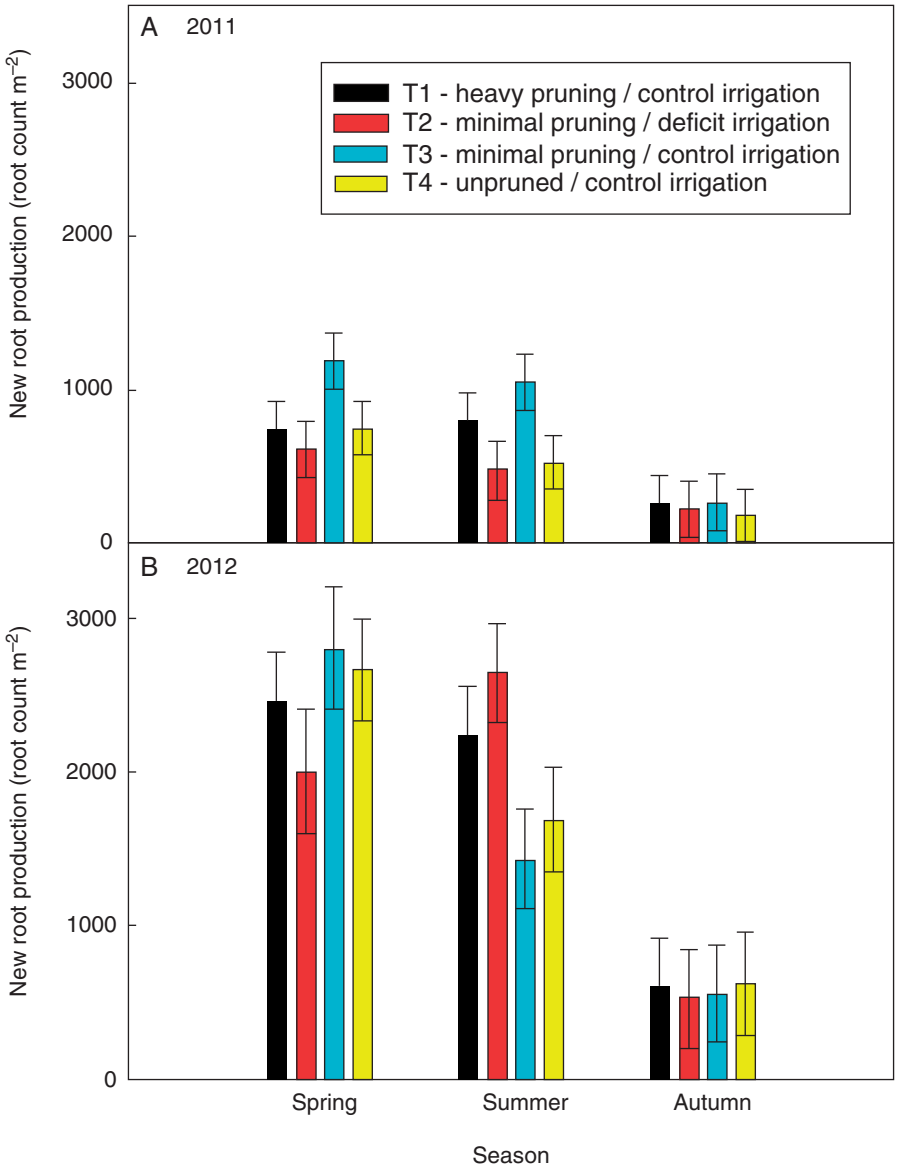

FIG. 6. Mean number of roots produced in minirhizotron viewing areas by season for 2011 (A) and 2012 (B). Values are means ± s.e.

unpruned, was consistent in both years of the current study and unexpected. Shallow root growth in response to pruning may be due to fewer resources allocated to woody root structures, which could have many negative implications for walnut trees, including greater reliance on irrigation, need for more irrigation and greater potential damage to trees from mechanical harvesting. However, it is also possible that the shallow root growth could be a benefit in heavy rainfall years since the shallow roots would have better aeration.

In all treatments, root populations in both growing seasons were composed of white, light brown, and dark brown to black roots as previously reported for grape roots (Comas *et al.*, 2005). Root metabolic activity decreased with increased root pigmentation, such that pigmentation could be used as an indication of root vitality to identify living and dead roots as in previous studies (Comas *et al.*, 2000). Interestingly, the presence of living and dead root populations closely followed that of root growth, with a similar offset throughout the season, suggesting that root life span was consistent throughout the season and independent of soil temperature and tree carbohydrate status.

Through the use of a satellite-sensed normalized difference vegetation index, it has been found that the growing season has been extended, with an earlier spring and longer autumn, in Eurasia and North America by as much as 18 and 12 d, respectively (Zhou *et al.*, 2001). Similarly, a meta-analysis of 19 European countries indicated that on average spring/summer advanced 2·5 d per decade for the period of 1971–2000 (Menzel *et al.*, 2006). The extension of the growing season has brought shifts in plant phenology; for example, a study of two late walnut cultivars 'Franquette' and 'G-139' presented a phenological model showing an earlier onset of bud-breaking dates, 7 and 3 d earlier for 'Franquette' and'G-139' respectively, over

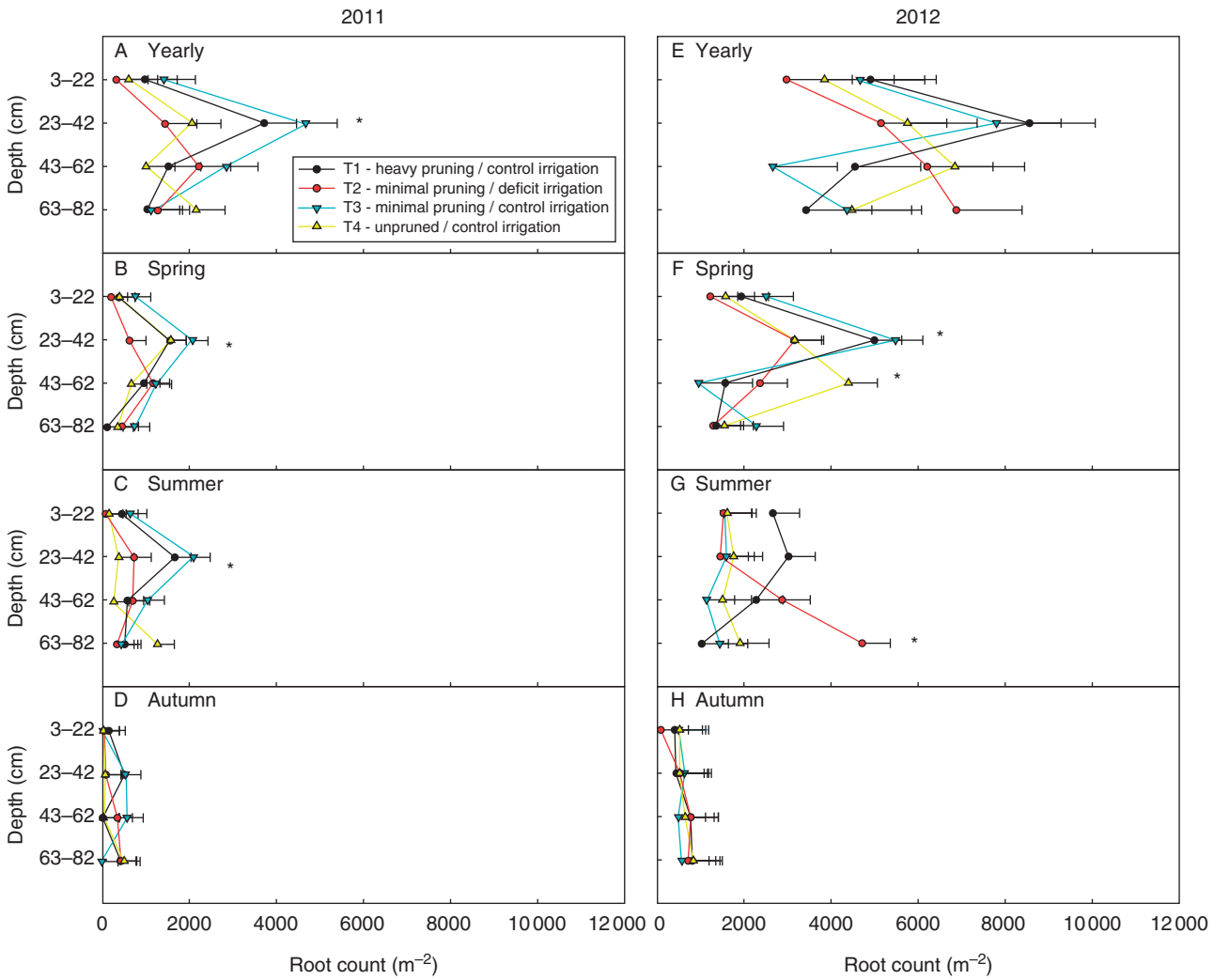

FIG. 7. Mean root counts produced in minirhizotron viewing areas by depth for 2011 and 2012. (A, E) Mean annual root production for 2011 and 2012, respectively. (B, F) Mean spring root production for 2011 and 2012, respectively. (C, G) Mean summer root production for 2011 and 2012, respectively. (D, H) Mean autumn root production per m^2 for 2011 and 2012, respectively. Values are means ± s.e. Asterisks denote significant differences at $P < 0{\cdot}05$ between treatments at each depth.

the past decades (1984–2006) closely correlated with an increase of 0·9 °C in spring air temperature (Črepinšek *et al.*, 2009); a similar study in hazelnut found an advance of 2·5–3·9 d in leafing, 7·0–8·8 d in male flowering and 6·3–8·9 d in female flowering after an increase of 1 °C in air temperature over the past decades (1969–1979 and 1994–2007) (Črepinšek *et al.*, 2012); first flowering advanced 4·5 d on 385 British plant species in the 1991–2000 decade compared with the period 1954–1990 (Fitter and Fitter, 2002); a 26 d advance of blooming over the last century for *Populus tremuloides* was found in western Canada (Beaubien and Freeland, 2000); and a 7–8 d and 10–11 d flowering advance for apple and pear, respectively, since 1989 in France (Atauri *et al.*, 2010). The alteration of the timing of plant growth and development by global climate change may have important implications in resource acquisition for plant development and productivity. Shifts in the length and timing of above- and below-ground growth may affect uptake of soil mobile and immobile resources (Nord and Lynch, 2009). Shifts in soil temperature may impact root growth, and do so differently among species. This highlights the importance of exploring factors governing root growth to understand possible implications of climate change in the productivity and management of cropping and wild ecosystems.

In conclusion, the results presented add to growing evidence that root growth in many ecosystems follows a unimodal curve with one marked flush of root growth in co-ordination with the initial leaf flush of the season. Root vertical distribution appeared to have greater plasticity than timing of root growth in this system. While we found an anticipated response of water deficit promoting root growth in deeper soil layers for mining soil water, we also found that canopy removal promoted shallow root growth, which could have negative implications for managed ecosystems, especially those experiencing late season shortfalls. This could also be potentially beneficial in years with wet springs when the shallow roots would be better aerated, although it may not replace proper water management in orchards as a vital aspect of maintaining healthy root systems. We observed that root growth declined with increased soil

temperature and carbohydrate allocation to plant reproduction, suggesting that temperature and/or carbohydrate competition constrained root growth. However, further experiments controlling soil temperature and reproductive growth will be needed to separate these effects. More studies on root growth responses and regulation of these responses in more locations are required to understand the potential effects of a shifting climate on cropping and wild ecosystems.

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