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Publication Date

2022

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The Impact of Soil Hypoxia on Almond Fine Root Production During the Growing Season in California Orchard System

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Horticulture & Agronomy

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2023

ABSTRACT

Soil hypoxia occurs when soil oxygen is used by roots and soil organisms at a faster rate than it is replenished by diffusion through the soil. Once soil oxygen levels fall below the amount necessary to support aerobic cellular respiration, new root production is reduced, water and nutrients uptake decline and energy demand switches from active ion uptake to cellular maintenance. In the Mediterranean climate of California, soil hypoxic conditions happen typically in periods of high rainfall, but they can also occur during the growing season if a high amount of irrigation water is applied. As the diffusion of oxygen through water filled pores is approximately 10,000 times slower than through air filled pores, the concentration of soil oxygen can drop rapidly, especially if root respiration is high. To test the impact of hypoxia on root growth and activity at different phenological stages, we flooded the root systems of young (5th leaf) almond (*Prunus dulcis*) trees three times for 3 days during the growing season and measured root population dynamics and root/soil activity.

We found that there were no impacts of saturating the rootzone of almond trees for three consecutive days in either late spring, summer, and fall, even though the treatments succeeded in creating hypoxic conditions. We had hypothesized that hypoxia would have the greatest negative impact in the spring when most new fine root production was expected to occur. However, due to very dry conditions and resulting little root production prior flooding, we observed that most root production occurred in the two weeks after each spring and summer saturation event. We concluded that this response is likely due to drought alleviation reducing soil strength and enhancing root production, rather than a response to the short-term hypoxic conditions. Future field studies will need to incorporate higher irrigation and perhaps longer saturation treatments.

In memory of my parents Maria and Aaron.

In memory of my grandparents Agustin and Piedad.

ACKNOWLEDGMENTS

I would like to express my deepest appreciation to Dr. Astrid Volder for her support, patience, motivation, and knowledge. The discussions and feedback further my research and abilities to perform it. I would also like to thank Dr. Thomas Gradziel and Dr. Helen Dahlke for helping me develop my research project and facilitating field equipment. My master's degree journey would have not been possible without their guidance and support.

I am also deeply embedded to Hana You for all her immense support in the field and lab and all her guidance during my masters. I would also like to express my gratitude to my brother Agustin for being my right hand during my field work. This research project would have not been possible without them.

I would also like to thank Tad for providing technical expertise, and the Linquist lab for providing equipment. I am also thankful to UC Davis Plant Sciences department for providing the facilities and funds for my project. Also, I would like to thank GGHA coordinator Lisa Brown for all her encouragement, patience, and support.

Above all, I could have not undertaken this journey without my family's love, support, and encouragement, throughout my studies, specially from my sister Jenny.

TABLE OF CONNTENTS

Chapter 1 – Overview: Soil Hypoxia and Fine Roots

There are various studies that have demonstrated that in general, the more water is applied to tree crops over the growing season, the greater the achieved yield for that year (Hutmacher et al. 1994, Girona et al. 1993, Stewart et al. 2011). However, when large amounts of water are applied, soil conditions can become hypoxic. Soil hypoxia occurs when air found in soil pores is displaced by water and remaining oxygen is consumed by roots or soil organisms (Bailey & Voesenek, 2009). This can strongly negatively affect the root system specially when energy demand in the root system is high, for example during periods of high fine root production and /or nutrient uptake (Pimentel et al. 2014, Slowick et al., 1979). Fine roots (usually defined as those <2 mm in diameter) are crucial to trees as they are responsible for most of the water and nutrient uptake during the growing season (Smucker, 1993). However, they are susceptible to hypoxic conditions as young fine roots are the roots with the greatest rates of respiration (Koumanov et. al, 2006, Volder et al. 2006). Root respiration is the process in which the plant converts carbohydrates into energy in the form of adenosine triphosphate (ATP) required for active nutrient uptake, root growth, and root maintenance, 65%, 25%, and 10% respectively (Munns, 1999). In aerobic respiration there is a production of 2mol ATP per mol of glucose consumed compared to the yielding of 32 mol ATP per mol of glucose in aerobic respiration (Colmer & Voesenek, 2009), which leads to a loss in energy production of 94%. Therefore, when root respiration is inhibited, there is an immediate reduction in nutrient uptake and new fine root production, while prolonged lack of oxygen could result in root death. In this overview, I will be focusing on the response of fine roots to soil hypoxic conditions throughout the growing season.

Soil hypoxic conditions lead to declining soil oxygen levels as oxygen use will be greater than the re-supply of oxygen since oxygen moves 10,000x slower through water filled pores than through air filled pores (Armstrong, 1980). As soil oxygen falls below necessary levels to support aerobic cellular respiration, new root production and root nutrient uptake decrease (Kozlowski, 1986) and energy allocation to root maintenance. At this point aquaporins in the root cell membranes close and water uptake into the root is much reduced (Tournaire-Roux et al., 2003, Toro et al. 2018, Lambers et al., 2008). This leads to stomatal closure thus, paradoxically, fully saturated soils hinder water uptake by reducing root permeability and transpiration often leading to drought-like symptoms the leaves and more negative water potentials in the plant (Kozlowski, 1984, Verlag et al., 2002; Pérez-Jiménez et al., 2018; Kreuzwieser et al., 2004).

For example, in sweet cherries (*Prunus avium*) hypoxic conditions led to the wilting and yellowing of leaves (Pérez-Jiménez et al., 2018, Ziegeler et al. 2017). Misinterpretation of this leaf wilting as drought stress could encourage unnecessary irrigation which may further exacerbate the problem. The reduction in transpiration could make the whole tree water potential more negative, reducing the amount of turgor pressure available for cell elongation and thus reducing tissue growth rates (Lambers et al., 2008, Olien, 1989). As roots cell elongation is decreased (or stopped), and less lengths is produced per root mass (i.e., a reduced specific root length), a lower volume of soil is explored by the root system. Longer term effects depend on the time of year when hypoxia occurs. A reduction of root length produced in the spring would mean a much-reduced capacity for water uptake during the subsequent summer. In Mediterranean climate with little natural rainfall this can lead to enhanced water stress for the whole tree. However, if soil hypoxic conditions were to occur in fall or winter, perhaps consequences would be minimal as these are generally not periods of very high root production and trees water use activity.

Similarly, nutrient uptake is impaired during soil hypoxic conditions. Nitrate (NO_3^-) uptake requires energy since its transport into plant cells occurs against a chemical and electrical gradient (Epstein et al., 2004). Uptake of ammonium is less energy intensive, however, ammonium is toxic to plants and needs to be immediately converted to amino acids in the glutamine synthetase-glutamine-2-oxoglutarate aminotransferase (GS-GOGAT) cycle which is a highly energy intensive cycle (Kishorekumar et al., 2020). During the glutamine synthetase (GS) cycle, glutamate, ammonium, and ATP are used to form glutamine which is then used in the glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle along with 2-oxoglutarate to synthesize glutamine (Hawkesford et al., 2012). Also, the fate of nitrate depends on plant species, some plants covert a large proportion of nitrate to amino acids in the leaves, however in majority of plants have a substantial portion of the nitrate required converted into amino acids using the GS-GOGAT cycle in the root cells. The uptake of nutrients uses about 65% of ATP produced in root aerobic respiration therefore, under anaerobic conditions when respiration is 94% less efficient producing at ATP, nutrient uptake decreases strongly.

Generally, tree crops have two fine root growth events or flushes, 1) from the end of winter dormancy until full canopy 2) after crop harvest (Wells, 2002; Baddeley, 2004). Usually, the spring root flush is larger than the summer flush. The first root flush coincides with the time of the year when nutrients demand to support growth is the highest. Nutrients and water are needed throughout the growing season but there is higher demand for nutrient uptake during leaf out and fruit set, while water demand is generally greatest at full canopy and high vapor pressure deficits (mid-late summer conditions). Therefore, having soil conditions favorable for fine root growth is particularly important during spring as that is when nutrient demand is highest (and most fine roots are produced). In terms of water uptake, favorable conditions for root growth in the spring are essential as that is when roots can explore deeper soil layers to ensure access to water from deeper soil layers later in the season when demand increases substantially.

In this thesis I will assess the impact of low soil oxygen conditions in the root zone during the trees most active growing period versus periods when we expect less active root growth. Specifically, based upon the above review of the literature, I hypothesize that if hypoxic conditions in the rootzone reduces the efficiency of root respiration, then the trees in hypoxic conditions will show decreased new root growth and increased root mortality after the hypoxic event occurred. Also, we expect that if hypoxia has the greatest impact on active roots, then the impact of hypoxia will be greatest for the event in the late spring when roots have the greatest amount of new roots production and demand for active nutrient uptake is the highest.

<u>Hypothesis and objective:</u>

1. If hypoxic conditions in the rootzone reduces the efficiency of root respiration, then we expect that:
    a) Trees in hypoxic conditions will show decreased new root growth and increase root mortality in the week after the hypoxic event occurred.
    b) Roots of trees exposed to hypoxic conditions will show reduced activity as demonstrated by lower rates of soil CO_2 efflux immediately after the event.
2. If hypoxia has the greatest impacts on active roots, then we expect that:
    a) The impact of hypoxia will be greatest for the event in the spring when almond root systems have the greatest amount of new root production and respiratory oxygen demand to support active nutrient uptake is the highest.

b) Reduced spring root production due to hypoxia will lead to water stress for those trees in the subsequent summer months as overall reduced new root production will lead to reduced water uptake capacity during summer drought conditions.

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Chapter 2 – The effects of soil hypoxia on almond fine root length production during the growing season in California.

INTRODUCTION

Soil hypoxia happens when oxygen found in soil air space is displaced by water through rainfall or irrigation, and remaining oxygen is used by roots and soil microbes thus leaving low level of oxygen in the root zone (Bailey & Voesenek, 2009). Roots will begin decreasing respiration rates when soil oxygen concentrations reach 4-6% of partial pressure (Morard & Silvestre, 1996). Soil hypoxia largely depends on soil properties such as soil texture, structure, compaction, and obstructive soil layers (Huang, et al., 1999). Once soil has been saturated, soil oxygen replenishment is minimal due to the 10,000 times slower diffusion of oxygen through water compared to air (Armstrong, 1980). Excess precipitation and irrigation in agricultural land can lead soil hypoxic conditions. Reduced growth and damage to root systems occurs once soil oxygen levels fall below the amount necessary to support aerobic cellular respiration (Drew & Lynch, 1980).

Aerobic respiration is the process in which glucose is broken down to create energy in the form of ATP for growth, maintenance, and active nutrient uptake by roots. It is composed of three biochemical processes: glycolysis, tricarboxylic acid cycle, and electron transport chain in which the latter two produce the most ATP and need oxygen to work (Plaxton et al., 2007; Lambers et al., 2008). When oxygen is not present, the amount of ATP produced per molecule of glucose decreases from approximately 32 ATP to 4 ATP (Colmer & Voesenek, 2009). Such decrease in energy production will lead to a decrease in fine root production and active nutrient uptake, and remaining energy is used for maintaining the existing root tissues (Toro et al., 2018; Lambers et al., 2008).

Fine roots (the most external root order with a diameter less than 2 mm) are the roots responsible for most of the water and nutrient uptake during the growing season (Jackson et. al., 1997). Due to the comparatively high rates of respiration needed for nutrient uptake and root growth, the activity of the most external fine roots is more susceptible to low oxygen levels than higher order, coarser, roots (Koumanov et al., 2006; Volder et al., 2006; Pimentel et al. 2014; Rewald et al., 2014). Besides a lack of energy production to maintain nutrient uptake, hypoxia also leads to the closure of aquaporins in fine roots, therefore limiting water uptake (Tournaire-Roux et al., 2003). Overall, prolonged hypoxic conditions are detrimental to plants since it reduces the number of fine roots being produced and limits those already established fines roots in their ability to acquire water and nutrients.

The soil CO_2 efflux is composed by two main processes: root respiration and soil microbial respiration (Vodnik et al., 2009). On average, roots can contribute to at least 50% of soil CO_2 efflux, which can range between 10 to 90% based on active plant growth (Cardon & Whitbeck, 2007). Since fine roots are the most active, they have an important role in soil respiration rates. For example, it has been demonstrated in maple trees that their fine roots (< 5mm in diameter) can have 2.5-3.5 higher respiration rates compared to the thicker roots (Pregitzer et al., 1998). As a result, soil CO_2 efflux can serve as an indicator of fine root metabolic activity.

Generally, tree crop fine root production is greater, 1) at the start of leaf flush and 2) after harvesting (Wells et al., 2002a; Baddeley et al., 2004). Usually, the spring root flush is much larger than the post-harvest flush. In California, growers of almond (*Prunus dulcis*) orchards emphasize maintaining a high amount of available water in the soil during nut development. Applying excess water, particularly during periods of high root production, may cause a decrease of root activity and growth, making almond orchards vulnerable to abiotic stress factors, such as drought, in subsequent months (Insausti & Gorjón, 2013; Parolin et al.,2010; Irfan et al., 2010; Colmer & Voesenek 2009). Leaf wilting, yellowing, and browning symptoms can all be a response to reduced nitrogen uptake and/or water uptake because of excess water in the rootzone (Yordanova et al., 2005; Pérez-Jiménez et al., 2018; Ziegeler et al., 2017; Domingo et al., 2002). The impact of over-irrigation likely depends on the phenological stage of the tree when hypoxia occurs. For example, saturated conditions in the dormant season, when there is minimal root production and activity and thus low oxygen demand, has been shown to have minimal impact on root production and long term three physiology and yield (Ma et al., 2022). However, over irrigation during crucial periods of root production (e.g. spring) could strongly reduce root production and rooting depth – potentially resulting into problems later in the season if shoot water demand exceeds root system capacity for water uptake. In California, most natural precipitation occurs during the winter when deciduous trees are dormant however, during the growing season over-irrigation of trees has been shown to compromise yields (Morales-Olmedo et al., 2021).

In this project we expose field grown mature almond tree root systems to hypoxic conditions for a brief period (3 days) at three different periods during the growing season - late summer (August, at nut maturity), in the fall (October – after nut drop) and in the late spring (June, at full canopy). We expect that creating hypoxic conditions will be detrimental to new root production and that this effect will be strongest during the period of expected highest root growth and demand for nutrient uptake (i.e., late spring). We also expect that by negatively impacting spring root growth, trees exposed to spring soil hypoxia will experience greater water stress during the summer as they will have produced less roots. Overall, we expect that periods of saturated soils during periods of lower expected root production and activity (i.e., summer and fall) will have minimal impacts on new root production or root death.

MATERIALS AND METHOD

Study Site and Experimental Design

The experiment was conducted at the UC Davis Plant Sciences field facility in Davis CA during 2019 and 2020. This location has a predominantly Mediterranean climate with wet winters (December to March) and dry summers (June to September). In the past 30 years, the region has received during the winter (January, February, and March) an annual average of 250 mm of rain and has had an average annual temperature of 10.5 ^{o}C with average high temperature of 16 ^{o}C (National Weather Service). Additionally, during the summer the region has had an average of 6.8 mm of rain with an average temperature of 24 ^{o}C with average highs of 34 ^{o}C (National Weather Service). The soil is a Yolo silt loam (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents) that has an average saturated hydraulic conductivity of 15 mm hr^{-1} (UC Davis Soil Web) and the trees used for this study were twelve five-year-old almond trees (Non pareil on Krymsk 86 rootstock).

Minirhizotron tubes and root processing

Trees were arranged in a single row and tree spacing was 3 meters. In 2018, each tree had a 90 cm long minirhizotron tube (clear acrylic tube) installed 50cm away from the center of the tree at a 45^{o} angle for a vertical depth of 60 cm. Images were collected at 0-15cm, 15-30cm, 30-45cm, and 45-60cm with the CI-600 In-Situ Root Imager (CID Bio-Science, Camas, Washington, USA) at two-week intervals during the dormant season and frequency increased during the growing season to one-week intervals. During saturation events root images were taken three days before saturation and four times at three-day intervals after saturation. Root tracing was performed using Rootfly software (Clemson University, Clemson, SC, US) to determine root length, root color, root diameter, and root longevity. The criteria used to determine root death was complete root disappearance and only fine roots (<2mm in diameter) were used for data analysis.

Experimental design

An experiment was designed to test four saturation treatments and the non-saturated control. Each saturation treatment applied the saturation event in one of the seasons of the year (except for winter). Trees were divided into three groups according to stem diameter and then one tree from each stem diameter group was randomly assigned to a saturation treatment to be applied in fall, spring, or summer, or no flooding (Figure 1). Flooding treatments were applied in 2019, Summer I (Sept. 3-Sept. 7) and Fall (Oct. 15-Oct. 19) and in 2020, Spring (June 23-June 27) and Summer II (Aug. 11-Aug. 15). The rate of water application necessary to maintain soil saturation was determined by measuring in-situ soil hydraulic conductivity. Two irrigation lines were then placed parallel to each other on each side of the tree and drippers with spaghetti tubing were placed in a zig zag on the two main irrigation lines to evenly irrigate an area of 18 m^2 around the tree. Extra tubing was included to ensure ponding water during treatment. To maintain standing water, barricades were made around flooded trees. During the Summer I and Fall treatments, the treatment trees received 8hrs of continuous irrigation per day for three days. In this period, water accumulated on the soil surface but drained during the nighttime when irrigation was turned off and resumed the following morning. Trees selected for the Spring and Summer II saturation treatment received continuous irrigation for three days without nighttime drain. The water accumulated around the trees and there was 3-5 cm of ponded water for the length of the treatments. Water began to accumulate 4-5 hours after irrigation started. Control trees were irrigated once for 8 hours using a micro sprinkler. The orchard irrigation regime changed between 2019 and 2020. In 2019, all trees were irrigated with a single micro sprinkler at a rate of 21.4 gph for two days every two weeks before the first treatment. The Summer I control trees did not receive irrigation during the flooding treatment but Fall control trees did. In 2020, none of the trees were irrigated until a week before Spring flooding treatments began.

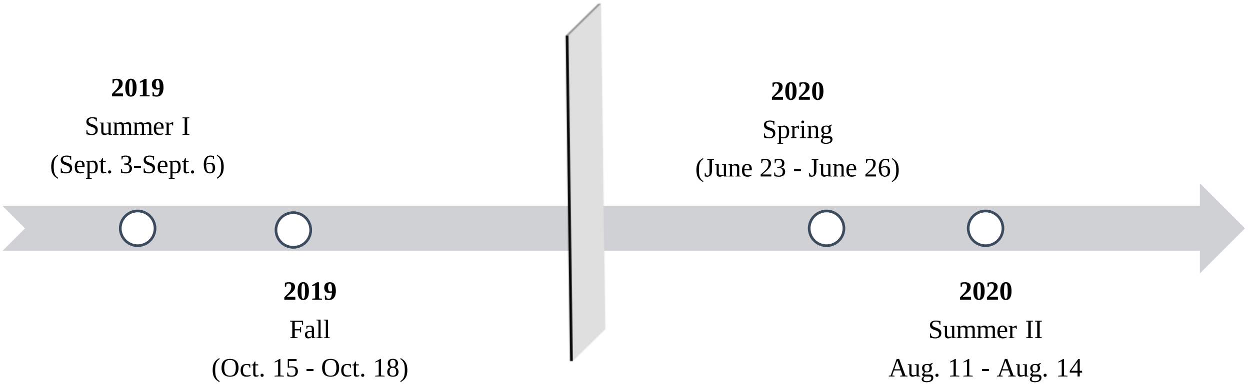


Figure 1. Timeline for experiment with dates when flooding irrigation treatments were applied.

Soil water content, soil temperature, and predawn stem water potential

Volumetric water content (VWC) and soil temperature were measured hourly with sensors (GS3, METER, Pullman, Washington, US) installed at each tree at 20cm and 50cm depths. VWC and soil temperature were measured hourly for the length of the experiment. In addition, soil redox was measured during Fall 2019 treatment and compared to VWC to be used as a reference to determine soil hypoxia at the other experimental dates. Predawn stem water potential of the trees was measured using a pressure chamber (Soil Moisture Equipment Corp., Goleta, California, US) before and during flood irrigation.

Soil Redox Potential

Redox potential sensors were made with platinum (Pt) electrodes (UC Davis- Dahlke Lab) and Campbell Scientific CR800 dataloggers (Campbell Scientific, Logan, Utah, US) were used to take measurements at five-minute intervals. Two Pt electrodes were placed at the depth of 20 cm and another two at 50 cm in one of the saturation plots that was saturated for three days. This data was used along with soil VWC during saturation to estimate soil hypoxia in all saturation treatments, whereby the soil redox potential threshold for soil hypoxic conditions was determined to be at 300 mV (Ganot & Dahlke, 2021). Soil saturation lasted 10 hours each day.

Root and soil activity

Root and soil activity was determined by measuring diurnal CO_2 flux from the soil using LI-COR 8100A Soil CO_2 Flux System and LI-COR 8150 Multiplexer (LI-COR Biosciences, Lincoln, Nebraska, US). Soil efflux rates were measured one day before and after, and during saturation treatments. In each saturation event, two saturated trees and two control trees had automated chambers installed 1m away from the center of the tree and adjacent to the minirhizotron tube. CO_2 efflux rates were measured hourly for three minutes with one-hour intervals.

Statistical Analysis

For each saturation event, average root length production per tube and depth was calculated starting 2-3 weeks prior to the event, during the event, and 2-3 weeks after the event. Root length production of the treatment tubes was then compared separately with those of the long-term control only (i.e., data collected for the other two seasonal treatments were not included). A three-way ANOVA for each saturation event composed of the treatment, timing, and depths was performed first. We then performed a two-way ANOVA for treatment and timing within each soil depth. Also, a two-way ANOVA was performed for predawn stem waster potential (PSWP) in each saturation event and was composed with treatment (control vs. treatment) and days during the saturation event. A Tukey’s post-hoc test at 95% confidence was performed on the control and treatment PSWP means for each day in all saturation events. This analysis was performed with R Studio.

RESULTS

Root growth seasonal dynamics

For control trees, there was a peak of fine root length production at 0.10 m_{root} m^{-2} day^{-1} in early April, followed by two more fine root flushes of 0.13 and 0.08 m_{root} m^{-2} day^{-1} in July and August (Fig.2a). In 2020, there was no obvious flush of fine root length production at the beginning of the growing season but there was an increase in fine root production in early July and August, after irrigation was restarted. There were not clear peaks in root deaths rates in 2019 and root death was mostly stable from April through August at 0.04 m_{root} m^{-2} day^{-1}. In 2020, similar fine root death rates to those of the previous year occurred (0.04 m_{root} m^{-2} day^{-1}) but there was a spike of root death at the end of spring of approximately 0.11 m_{root} m^{-2} day^{-1} and towards the end of the summer 0.06 m_{root} m^{-2} day^{-1}. The first spike of root death occurred after irrigation was restarted (Fig. 2b).

Soil moisture and temperature

Max VWC was measured as 0.40 and 0.37 m^3 m^{-3} at depths 20 and 50 cm, respectively. In December 2018 and January 2019 there were rain events that replenished soil water and maintained soil moisture above 80% max VWC but rain events were not prolific that winter. Control trees were regularly irrigated in 2019, however, they were not regularly irrigated in 2020 (Fig. 2b). In February 2020 soil water content began to decline and reached a low value of 50% max VWC in June at both depths, just prior to the late Spring saturation treatment.

The mean annual soil temperature was 19.7 and 18.8 ^{o}C at 0-30 cm and 30-60 cm depth for 2019 and 21.4 and 21.6 ^{o}C for 2020 respectively (Fig. 2c).

Summer I – Root growth rates, soil water status, and root activity

There was no significant difference in root length production before, during, and after the first summer saturation event (Summer I) between control and treatment trees (Table 1, Fig. 3a). Soil hypoxia was determined based on the timing soil redox potential dropped below 300 mV and was compared to the volumetric water content at that time (Supplementary Data, S1) which resulted in a 0.37 m^3 m^{-3} VWC threshold above which soil hypoxia occurred. In Summer I, soil hypoxia was achieved for only 5 hours on the third day of treatment at the depth of 20 cm and not at all at 50 cm depth (Fig. 3b, 3c).

Throughout the event, soil CO_2 efflux rates were greater from the saturated soils than the control soils (Fig. 3c). Average soil CO_2 efflux rates were 1.81 and 3.43 µmol CO_2 m^{-2} s^{-1}, for the control and saturated soils during the saturation event.

Fall– Root growth rates, soil water status, and root activity

For the Fall saturation event, there were no significant treatment or depths effects on root length production rates for control and treatment trees (Table 1, Fig. 4a).

The average soil VWC during the saturation event at depths 20 and 50 cm was 0.35 and 0.34 m^3 m^{-3}, respectively with the maximum reaching 0.37 m^3 m^{-3} in both depths for 4 hours, thus the threshold for soil hypoxia (0.37 m^3 m^{-3} was barely reached) (Fig. 4b).

The average soil CO_2 efflux rates were 1.98 and 2.48 µmol CO_2 m^{-2} s^{-1}, respectively for control and saturated plots during the saturation event. Throughout the saturation event, treatment soil CO_2 efflux rates were higher in the saturation treatment than the control treatment (Fig. 4c). Within 24 hours after water application was stopped, soil CO_2 efflux from the treatment plots was the same as that from the control plots (Fig. 4c).

Spring– Root growth rates, soil water status, and root activity

There was no significant difference in root length production between treatment and control trees before, during, and after saturation event (Table 1, Fig. 5a). There was no root length production for control trees at either depth until after the saturation event, when there was an increase in the 0-30 cm depth only (0.045 m_{root} m^{-2} day^{-1}). In the treatment plots there was a small production of root length production before the event at 30 cm only and after the saturation event at both depths (0.009 m_{root} m^{-2} day^{-1}) (Fig. 5a).

The average VWC for Spring saturation plots at depths 20 and 50 cm was 0.38 and 0.33 m^3 m^{-3} with maximums reaching 0.39 and 0.367 m^3 m^{-3}, respectively. At depth 20cm there was continuous soil saturation for the duration of the treatment (three days) without overnight drainage. At depth 50 cm, maximum soil water content was reached at the start of the saturation treatment and did not reach the hypoxia threshold of 0.37 m^3 m^{-3} (Fig 5b.).

Soil CO_2 efflux rates reached higher values in the control plots compared to treated plots withing hours after the saturation event started (Fig. 5c). The average soil CO_2 efflux rates for control and treatment plots during the treatment period were 2.51 and 1.58 µmol CO_2 m^{-2} s^{-1}, respectively.

Summer II– Root growth rates, soil water status, and root activity

Root length production in the second summer period was not affected by treatment or soil depth (Table 1, Fig. 6a).

The average soil at depths 20 and 50 cm were 0.395 and 0.378 m^3 m^{-3} with maximums reaching 0.40 and 0.38 m^3 m^{-3}, respectively. Irrigation for saturation treatment was continuous for the length of treatment (three days) without overnight saturation and both depths demonstrated soil saturation and remained above 0.37 m^3 m^{-3} for more than 60 hours (Fig. 6b).

The average soil CO_2 efflux rates for control and treatment plots were 2.37 and 1.99 µmol CO_2 m $^{-2}$ s^{-1}, respectively. Once water application was stopped and soil VWC started declining (Fig. 6b), soil CO_2 efflux rates peaked at 5.5 µmol CO_2 m $^{-2}$ s^{-1} from the treatment plots withing 10 hours (Fig. 6c).

Predawn stem water potential during saturation

Predawn stem water potential (PSWP) was measured before each saturation event to obtain baseline data. There was no significant effect of the saturation treatments on PSWP (Table 2, Fig. 7a, & 7b). In the late spring of 2020, the saturation treatment trees had a significantly less negative PSWP (-0.85 MPa) that the control trees (-1.6 MPa) (Fig. 7c). During the second summer event, the saturation events increased PSWP for treatment trees at the end of the saturation period (Fig. 7d). On average, control and treated trees had a PSWP of -0.88 and -0.78 MPa during saturation.

Redox potential

Soil redox potential was measured to estimate the soil VWC needed to have soil hypoxic conditions. The soil redox potential threshold to determine soil hypoxia was 300 mV. Soil hypoxic conditions were reached withing 8 hours at the soil depth of 20 cm and remained during saturation event with the maximum VWC of 0.37 m^3 m^{-3} or 37% (Supplemental Data, S1).

DISCUSSION

In this study the root length production trends for the control trees we different from what has been reported in other tree crops such as peaches, apples, cherries where most root growth occurs in the spring and another small root flush during fall (Wells et al., 2002a; Wells et al., 2002b; Baddely et al., 2005). In our study we found greater root length production in late summer (August) in both years. In 2019, we found the top lowest % max VWC at the depths 20 and 50 cm during the month of July (75%) when the summer fine root flushed occurred compared to that of April (65%) when the spring fine root flush happened (Fig 2b). A lack of consistent irrigation during spring and early summer, which allowed the soil to dry below 70% maximum VWC for prolonged periods of time likely hindered normal root production pattern.

Similarly in 2020, no irrigation was applied until mid-June. However, in 2020 winter rain was 8.06 inches compared to the 22.66 inches in 2019. This led to very low soil VWC between March and early June (55% Max VWC). The lack of water in the soil profile stopped root length production until irrigation started for the Spring and Summer II treatments. The root length flush happened primarily after applying saturation treatments since during these treatments, control trees were also being irrigated for brief periods starting two weeks before (Fig. 5b and Fig. 6b). This resulted in an increase of root production during this time of the growing season since better soil moisture conditions became available compared to the early spring when it was drier. Although, there was root growth occurring during the summer, the highest peak of fine root growth rate in the 2020s was 23% lower compared to highest rate in the summer of 2019.

Saturation treatments creating soil hypoxic conditions

During the first two saturation events, soil saturation was only achieved at 20 cm depth on the last day of Summer I. Technical issues with the irrigation system meant that irrigation was turned off overnight, allowing enough drainage to occur that soils remained very wet, but did not reach prolonged saturation levels. During the 2020 Spring treatment saturation was achieved at the 20 cm depth for three days (Fig. 6b). Although the soil did not remain saturated at 50cm depth, there was an initial spike of VWC at that depth showing the irrigation water had reached that depth, however drainage was faster than infiltration rate. There could have been a sandier layer at depth 50 cm and therefore was draining faster than the rate the water was infiltrating on the topsoil since the neighboring soil was a sandy loam with a sand content of 60% and a saturated hydraulic conductivity of 100 mm hr^{-1} (UC Davis Soil Web). During summer treatment, soil saturation was achieved at both depths and was maintained for three days (Fig. 6b).

The effect of saturation treatments on root length production

There was no difference in root length production based on soil saturation treatment, timing (before, during, and after saturation), and soil depth (Table 1). We expected root production to be the same before saturation and diminished during and after treatment at both depths within all seasons (fall, spring, and summer). Most of the root production in each season occurred during and after the saturation treatment. During the first two events (Summer I and Fall) this could have occurred because there were no soil hypoxic conditions created during the treatment and instead irrigation events just replenished soil moisture, enhancing soil conditions for root growth rather than damaging root production. Soil CO_2 fluxes during the first two events had a similar response, fluxes were higher in the saturation treatment, likely because prolonged saturation conditions and hypoxia were never reached, and microbial activity was stimulated rather than diminished.

Just before and during the late Spring saturation event, there was very little to no root production occurring at 0-30 cm depth (Fig. 5a). Leading up to the later Spring saturation event were very dry soil conditions that led to severely reduced root length production for the preceding months (Figs, 2a, 2b, 5a). Drought conditions reduce overall tree growth and limit metabolic activity (Tixier et al., 2020) and will cease fine root production and reduce metabolic activity in roots that are present. For example, in plums (*Prunus cerasifera*), low-order (most external, finest roots) root production was particularly inhibited by sever water stress (Cochavi et al., 2019). Before irrigation began, treatment and control trees reached an average PSWP of -1.1 MPa (Fig. 7c), which is very negative for orchard trees. For comparison, young almond trees that were not irrigated for 28 days reached a PSWP of -0.8 MPa during the summertime while well-watered trees had a PSWP of -0.4 MPa (Torrecillas et al., 1996, Nortes, et al., 2005).

Our trees exhibited much more negative PSWP. However, once the saturation treatment irrigation began trees increased their PSWP to -0.78 MPa (Fig. 7c), while normally irrigated trees, which received much less water, remained at a PSWP of -1.1 MPa. Although, we expected the greatest negative effect of soil hypoxia to occur in the late Spring (when we expected the highest root length production), we could not assess this hypothesis because we did not find any significant pre-treatment root length production.

The impact of drought on root and soil processes was substantial as evidenced by increased root length production after the saturation and irrigation (control) events (Fig. 5a), as well as the increased breakdown of root material after the event (Fig. 2, root death). Saturated conditions at this time did not significantly reduce soil CO_2 efflux (Fig. 5c) which was the opposite than what we observed int the summer and fall events in 2019. This was caused by our ability to maintain saturated conditions (Fig. 5b) by irrigating 24 hours in the late Spring 2020, while irrigation was turned off overnight in the summer and fall of 2019 (Fig. 3b, 4b). As there was very little root production in the spring of 2020, these responses are mostly due to soil microbial responses to water and oxygen availability (Fig 5) (Bogati & Walczak, 2022).

Most of the root production occurred during Summer 2020 at both depths compared to the other seasons (Fig. 6a). After the later Spring saturation event, 7 more irrigation events were applied prior to the start of the Summer II saturation event to stimulate root length production. We succeeded in maintaining soil saturation and hypoxic conditions at 20 cm and 50 cm for three days (Fig. 6a, b), which caused a significant drop in soil CO_2 efflux (Fig. 6c). However, contrary to what we hypothesized, new root length production during the saturation event was not negatively affected (Fig. 6a). If any, those trees exposed to soil saturated conditions at 0-30 cm depth produced more root length during and after the event than the control trees.

In our study, the highest average soil CO_2 efflux was measured in the summer of 2019, however, the highest maximum flux was observed in the summer of 2020 after the saturation event ended (Fig. 3c and Fig. 6c). In summer 2020, we were able to apply enough water to create sustained saturated conditions, which reduces the rate at which gas exchange happens between the soil and the atmosphere resulting in soil CO_2 accumulation in the soil during the saturation event. Once continuous irrigation was stopped and the soil pores allowed to drain, this soil CO_2 accumulation in the soil led to a spike in CO_2 escaping from the soil (Fig. 6c). High root production occurring during and immediately after the saturation event (Fig. 6a), could have contributed to the high soil CO_2 efflux in the treatment plots after irrigation was stopped.

CONCLUSIONS

Unfortunately, root length production metrics in our study were so low that we were unable to properly assess the impact of the saturation events on root growth. During the first two events (Summer 2019 and Fall) we did not reach complete soil saturation for three days of soil hypoxic conditions. Short burst of soil saturation (<12 hours) increased soil CO_2 efflux in response to the first two events, particularly in the summer of 2019 when new root production was also slightly increased by a short period of soil saturation. In spring and summer of 2020, we were able to maintain three days of complete soil saturation, with surface water ponding, in the treatment plots. Although, saturation did significantly decrease soil CO_2 efflux rates in the spring and summer of 2020 during the saturation events, this did not have a measurable effect on root production during or after either event as pre-event root production rate was likely limited by soil water availability.

FIGURES AND TABLES

Table 1. Three-way ANOVA for each saturation event. Soil saturation treatment (saturated vs. control), timing within each treatment (before, during, and after saturation), measurement depth (0-30 and 30-60 cm) and their interactions did not have a significant ($P<0.05$) impact on root length production.

	Summer I		Fall		Spring		Summer II	
Factors	F	P	F	P	F	P	F	P
Trt	1.537	0.227	2.539	0.124	0.160	0.693	1.086	0.308
Timing	1.007	0.380	1.081	0.355	1.495	0.244	1.673	0.209
Depth	0.005	0.944	0.055	0.817	1.353	0.256	0.127	0.725
Trt x Timing	0.436	0.652	0.248	0.782	0.531	0.595	1.083	0.355
Trt x Depth	0.018	0.895	0.469	0.500	0.568	0.458	0.002	0.962
Time x Depth	1.783	0.190	0.337	0.717	0.764	0.477	0.898	0.421
Trt x Timing x Depth	0.469	0.631	0.655	0.529	1.157	0.331	1.377	0.272

Table 2. A Tukey Test was performed for at each saturation event to compared PSWP means between control and treatment trees. There was no significant difference between control and treatment trees each day during the saturation event ($P<0.05$). Day 1, PSWP was measured before the saturation treatment and on Day 5, a day after stopping the treatment.

	Summer I	Fall	Spring	Summer II
Day	P	P	P	P
1	0.99	0.99	1.00	0.99
2	0.99	0.25	0.96	0.99
3	1.00	0.99	0.98	0.99
4	0.99	0.95	0.35	0.48
5	0.99	0.99	0.80	0.06

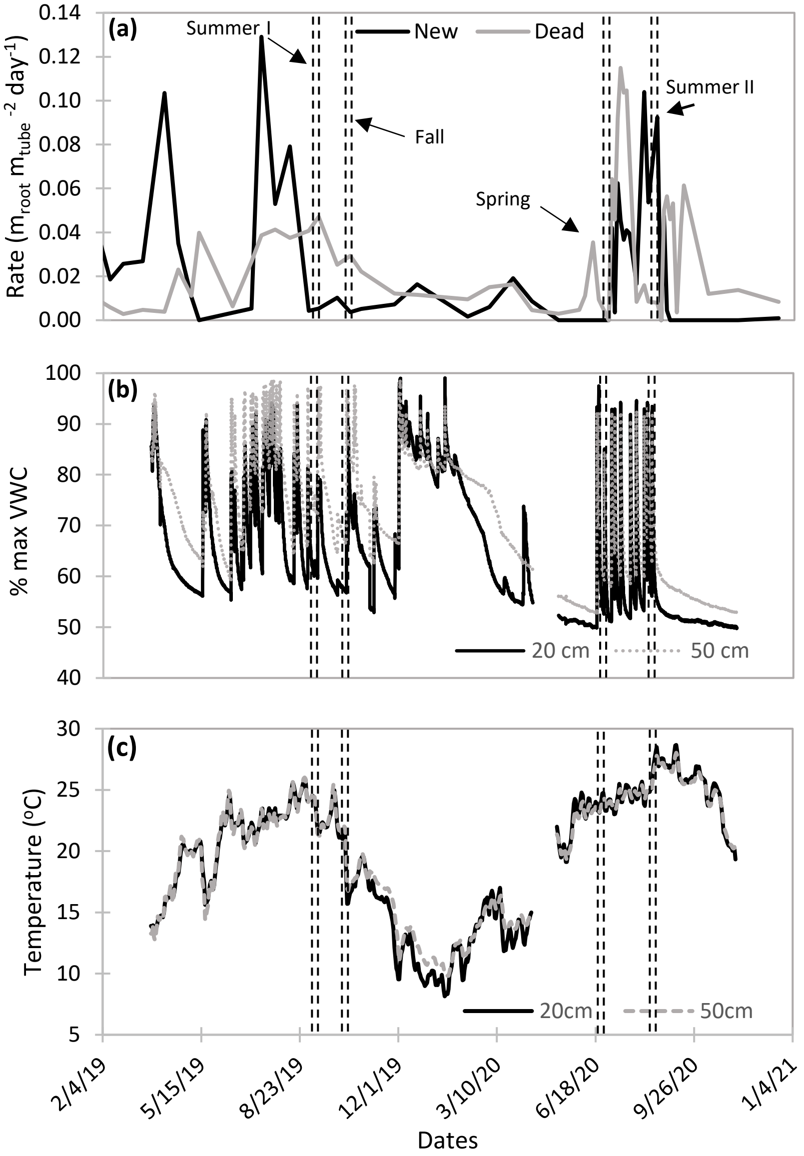


Figure 2. (a) Average new root length production in m per m^2 tube surface (black line) and root death pattern (grey line) throughout the experiment for the control trees that were not exposed to flooding events. Data were averaged across the whole tube (0-60 cm depth) n=3. (b) Daily averages of % max VWC for the control trees throughout the experiment. 100% max VWC for 20 cm = 40 m^3 m $^{-3}$, for 50 cm = 37 m^3 m^{-3}. (c) Daily average soil temperatures at 20 cm and 50 cm for the control trees throughout the experiment.

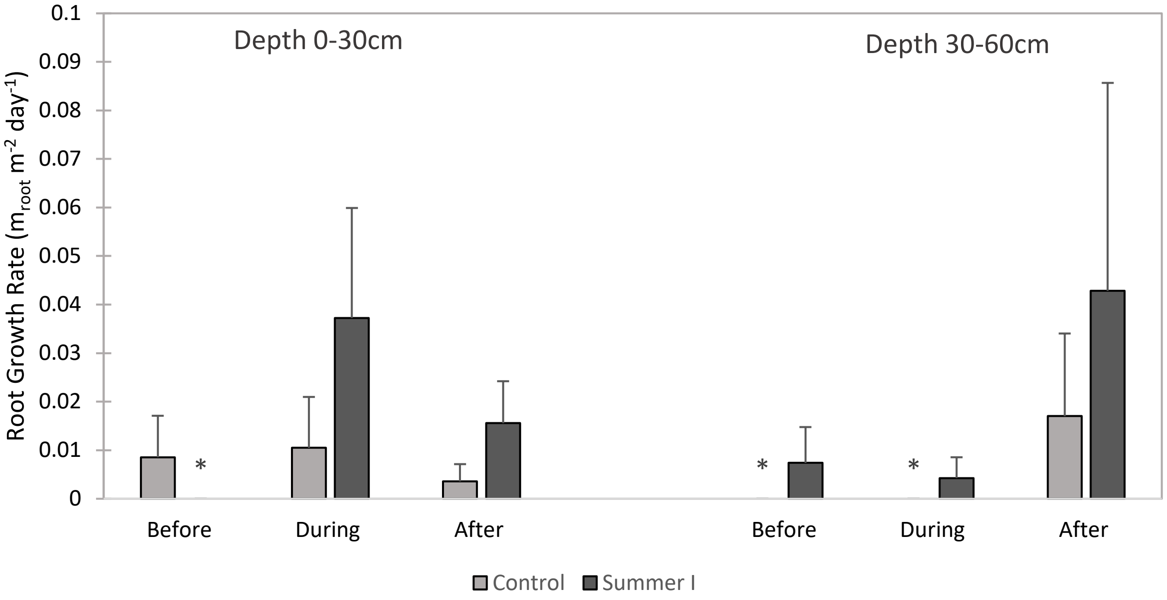
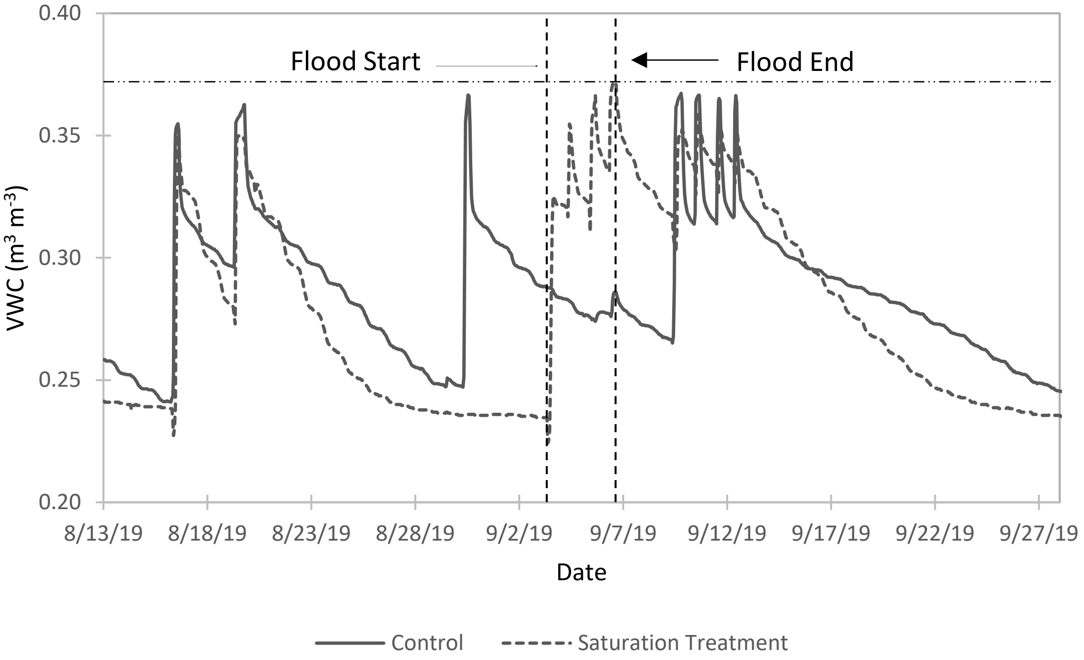


Figure 3a. Root length production (m_{root} m^{-2} day^{-1}) in summer 2019 (Sept. 3rd to Sept. 9th) for tubes that were saturated (Summer I, dark grey bars) compared to the control (normal irrigation, light grey bars) at 0-30 cm and 30-60 cm soil depth. “Before” is average root production at 2 weeks preceding the saturation event, “during” is average root production during the saturation event, and “after” is average root production for 2 weeks after the saturation event. The * indicates no roots observed. Error bars represent SE.



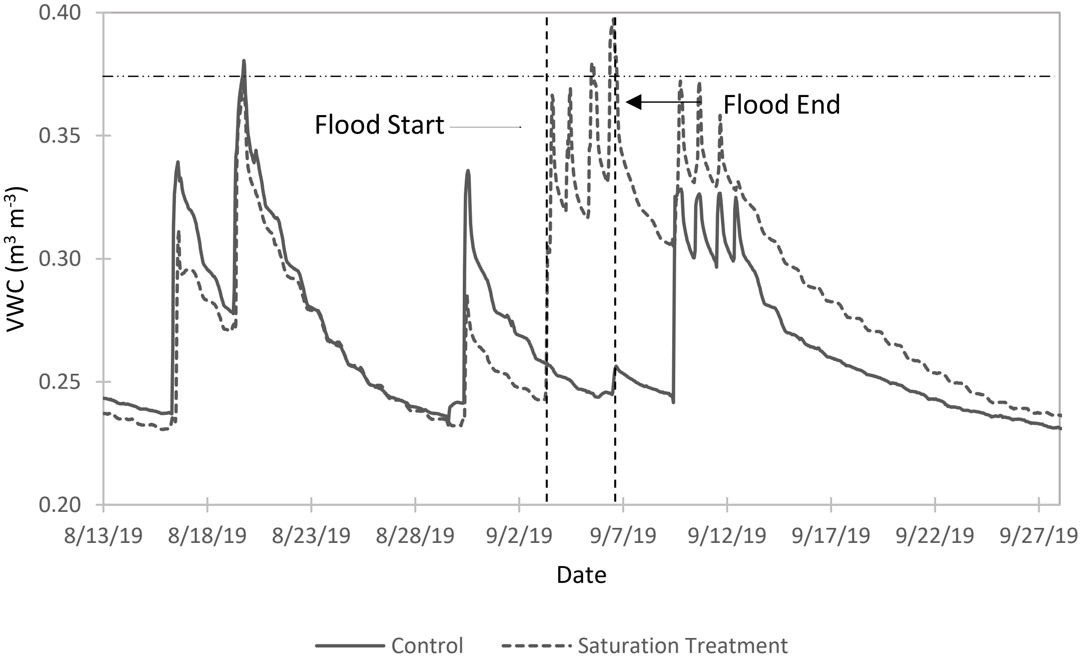


Figure 3b. Summer 1 (Sept. 3rd to Sept. 7th) average hourly % max. VWC at depths 20cm (top) and 50cm (bottom). It includes three weeks before and after treatment. Horizontal dashed line represents saturated VWC (0.37 m^3 m^{-3}).

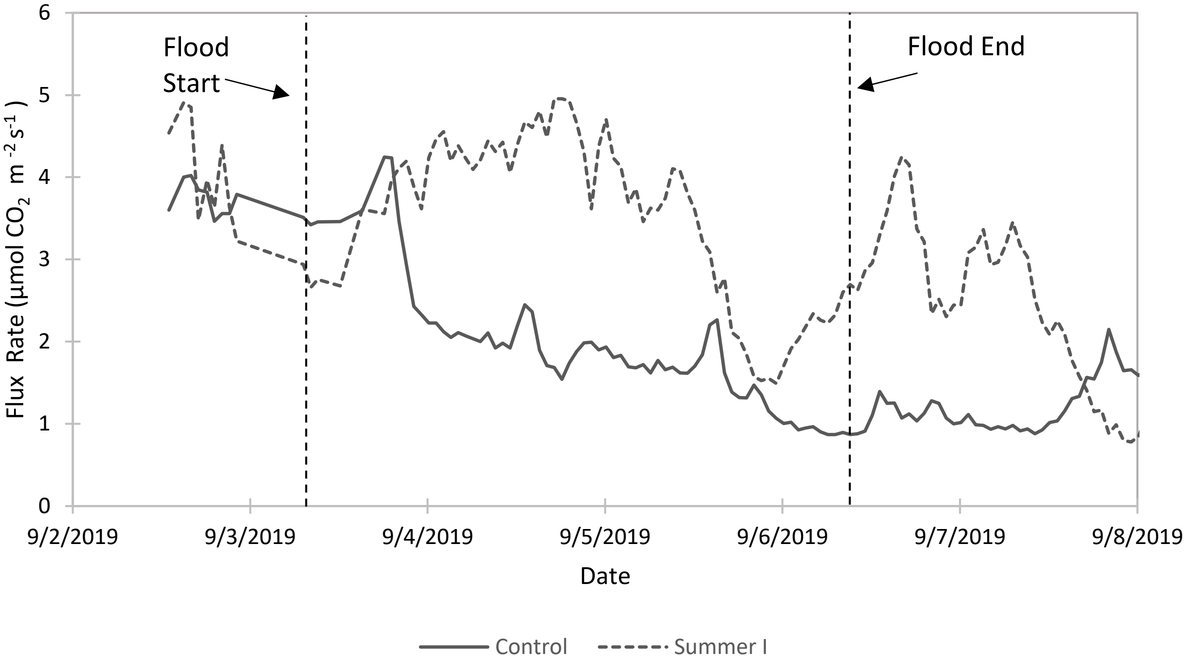


Figure 3c. Average hourly soil CO_2 flux rates during Summer I saturation event. Vertical dashed lines mark the beginning and end of treatment.

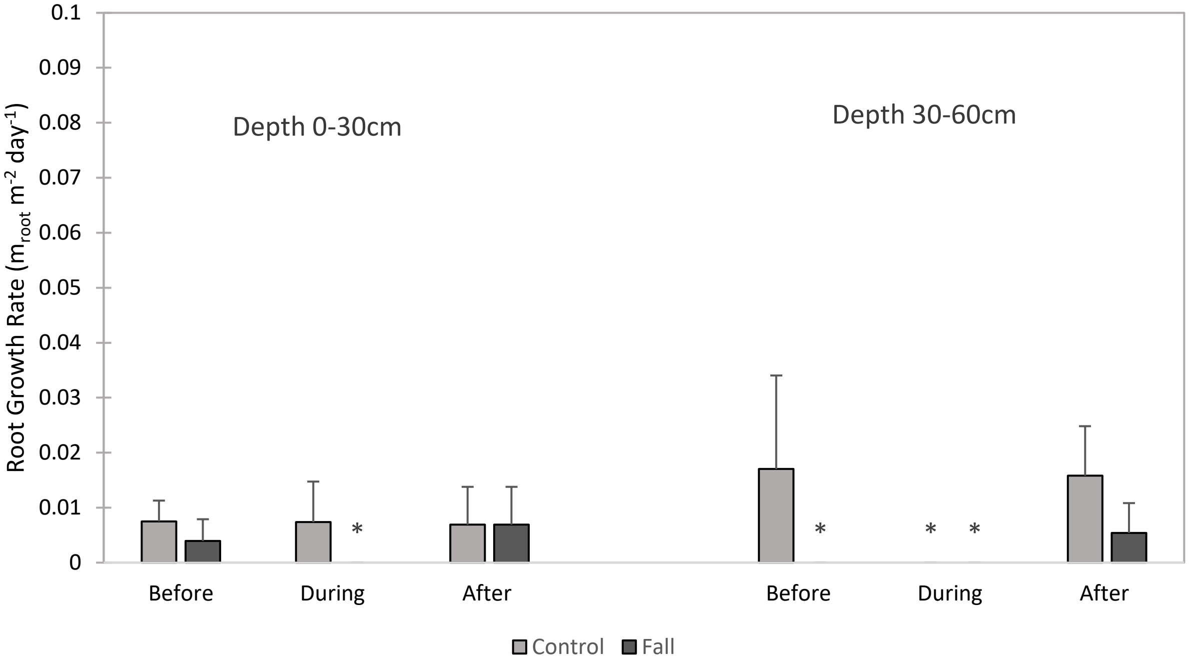


Figure 4a. Root length production (m_{root} m^{-2} tube day^{-1}) in Fall 2019 (Oct. 15th to Oct. 19th) for tubes that were saturated (dark grey bars) compared to the control (normal irrigation, light grey bars) at 0-30 cm depth, 30-60 cm depth. "Before" is average root production for the 2 weeks preceding the saturation event, "during" is average root production during the saturation event, and "after" is average root production for 2 weeks after the saturation event. The * indicates no root growth measured. Error bars represent SE.

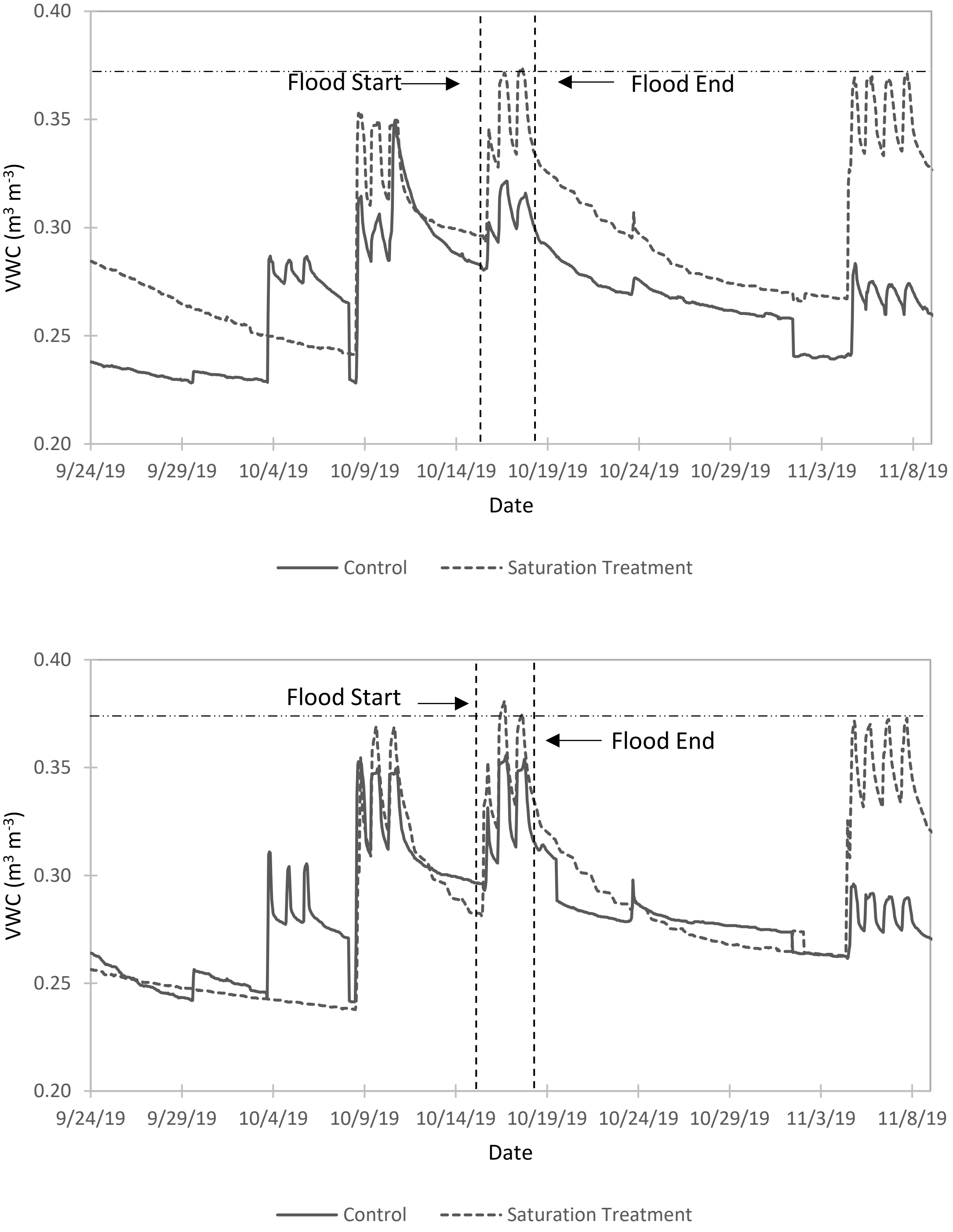


Figure 4b. Fall (Oct. 15th to Oct. 19th) average hourly %Max. VWC at a depths 20cm (top) and 50cm (bottom). Horizontal dashed line represents saturated VWC (0.37 m^3 m^{-3}).

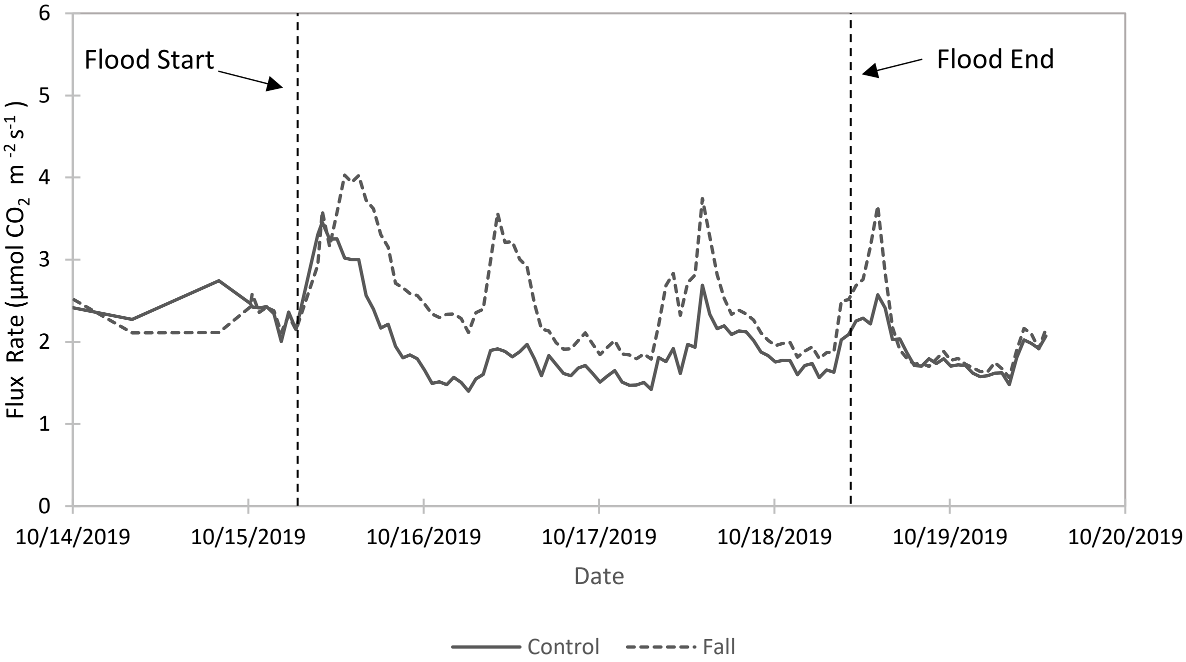


Figure 4c. Average hourly soil CO_2 flux rates during Fall saturation event. Vertical dashed lines mark the beginning and end of treatment.

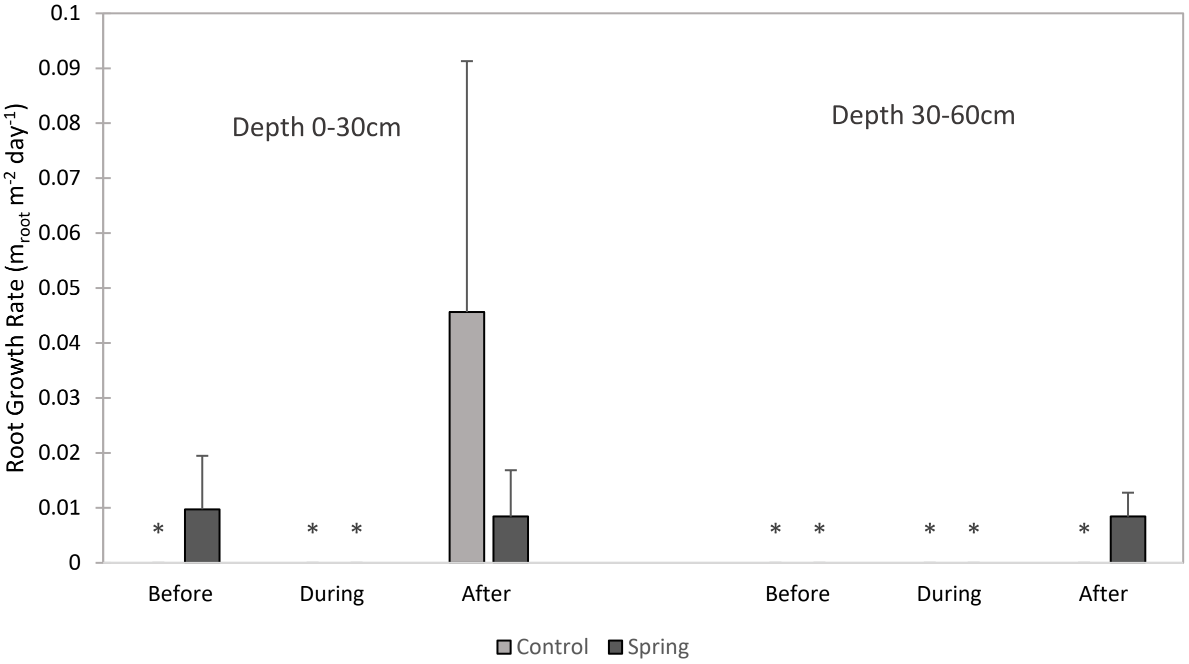


Figure 5a. Root length production (m_{root} m^{-2} tube day^{-1}) in Spring 2020 (Jun. 23rd to Jun. 27th) for tubes that were saturated (dark grey bars) compared to the control (normal irrigation, light grey bars) at 0-30 cm and 30-60 cm soil depths. "Before" is average root production for the 2 weeks preceding the saturation event, "during" is average root production during the saturation event, and "after" is average root production for 2 weeks after the saturation event. The * indicates no root growth measured. Error bars represent SE.

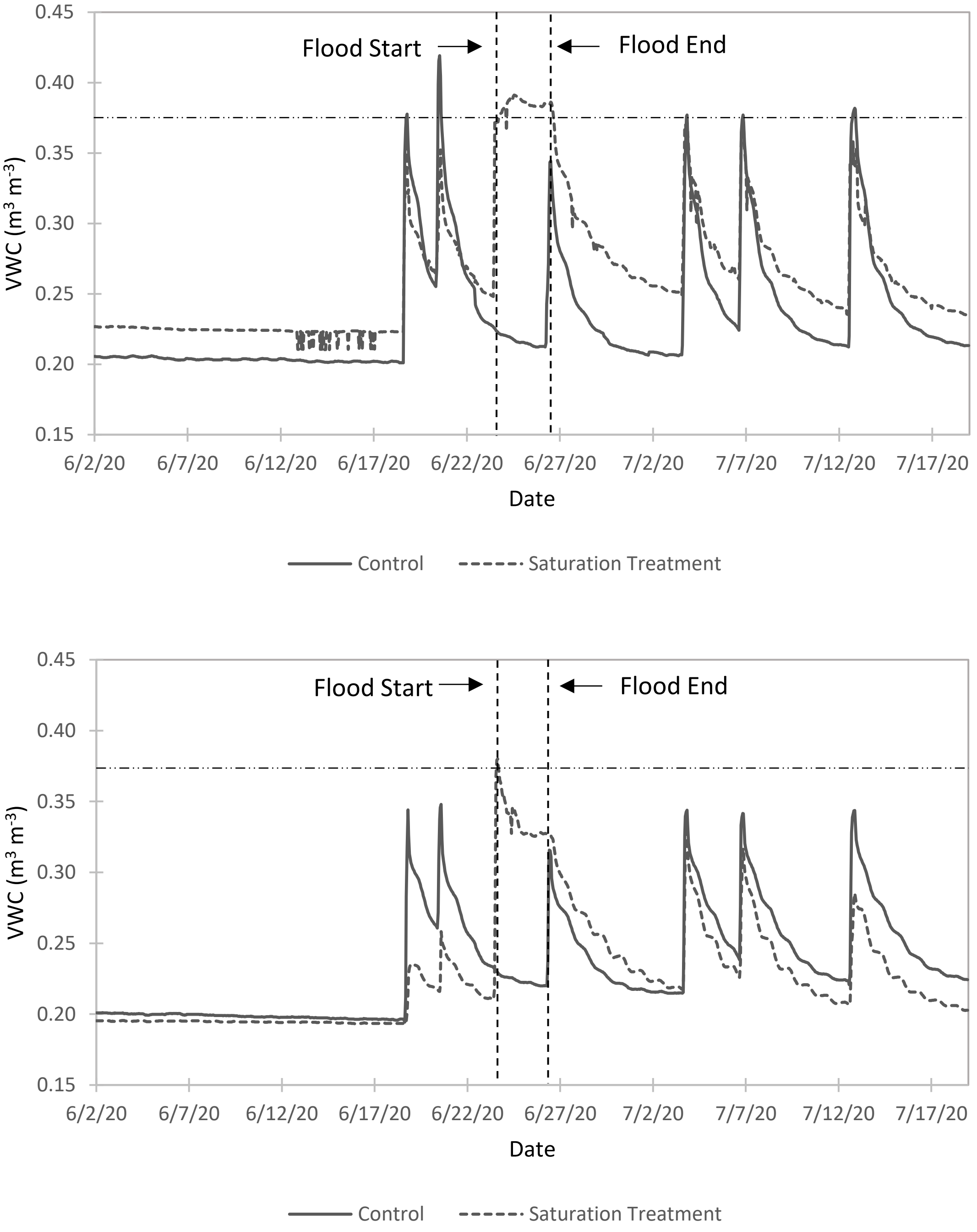


Figure 5b. Spring (Jun. 22nd to Jun. 26th) average hourly %Max. VWC at a depths 20cm (top) and 50cm (bottom). It includes three weeks before and after treatment. Horizontal dashed line represents saturated VWC (0.37 m^3 m^{-3}). NOTE: VWC axis is in different proportion compared to Fig. 2b and Fig. 3b.

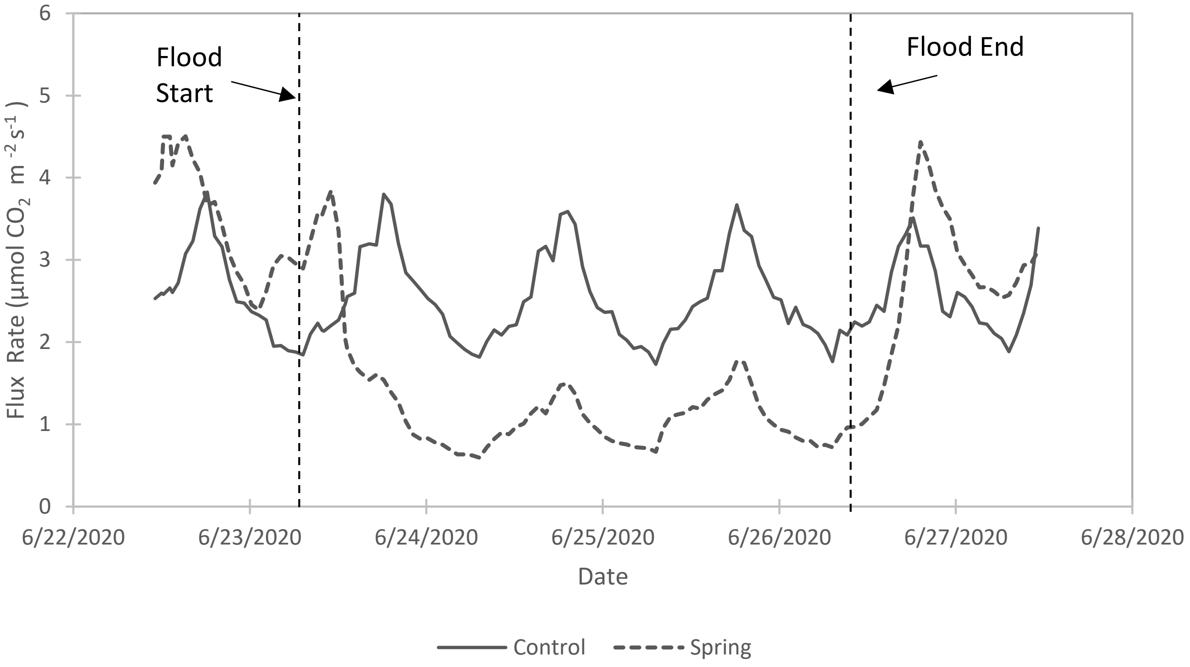


Figure 5c. Average hourly soil CO_2 flux rates during Spring saturation event. Vertical dashed lines mark the beginning and end of treatment.

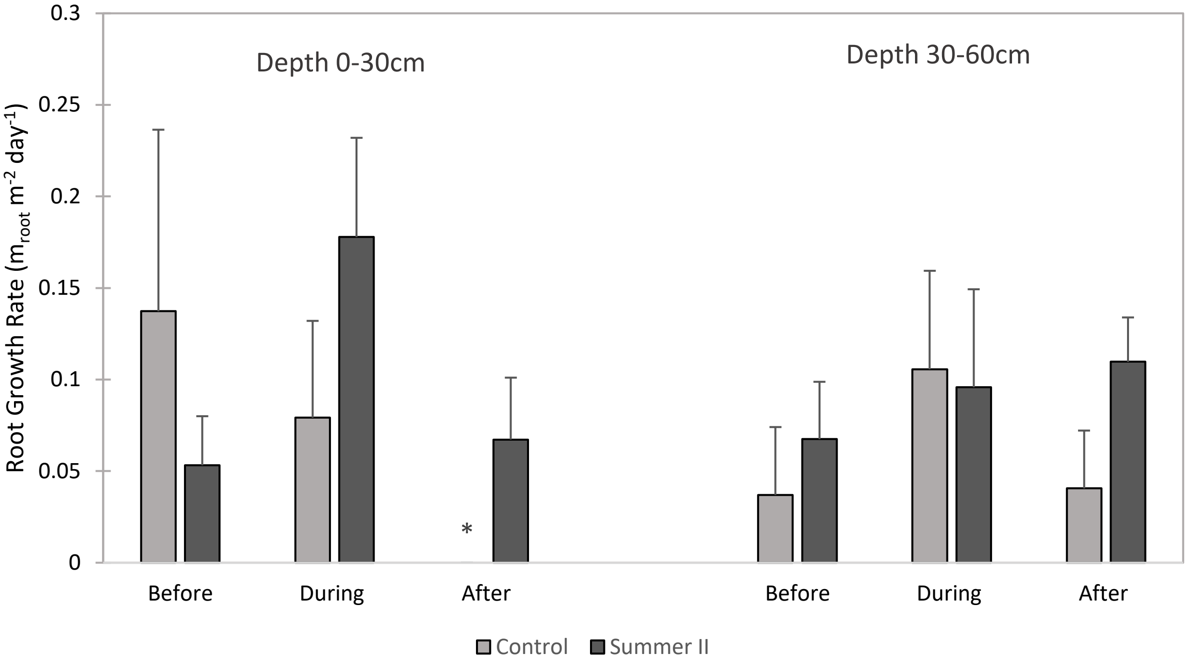


Figure 6a. Root length production (m_{root} m^{-2} tube day^{-1}) in Summer II 2020 (Aug. 10th to Aug. 14th) for tubes that were saturated (dark grey bars) compared to the control (normal irrigation, light grey bars) at 0-30 cm and 30-60 cm soil depths. “Before” is average root production for the 2 weeks preceding the saturation event, “during” is average root production during the saturation event, and “after” is average root production for 2 weeks after the saturation event. The * indicates no root growth measured. Error bars represent SE. NOTE: Root growth rate axis has different scale compared to Fig 3a, Fig. 4a, and Fig 5a.

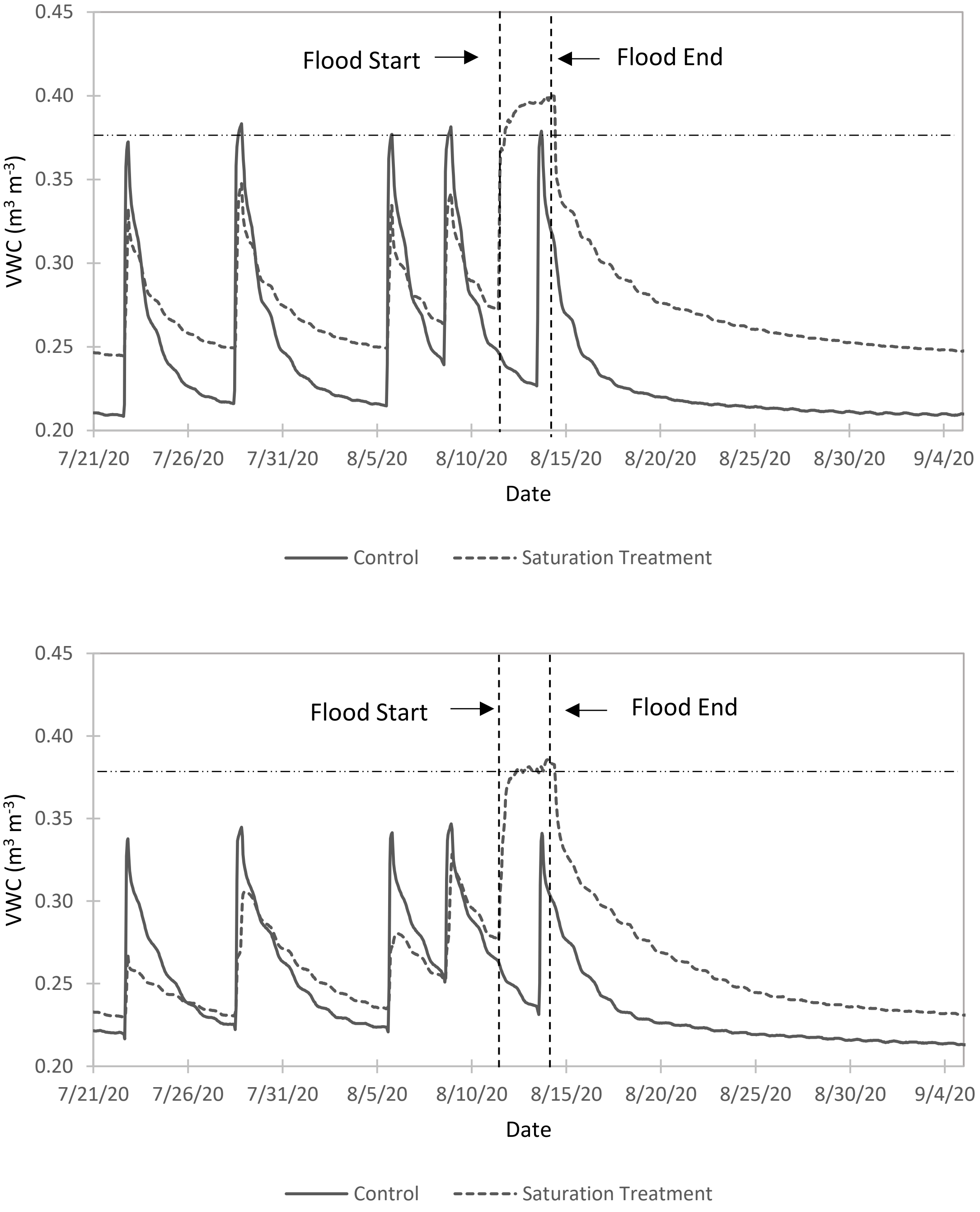


Figure 6b. Summer II (Aug. 10th to Aug. 14th) average hourly %Max. VWC at a depths 20cm (top) and 50cm (bottom). It includes three weeks before and after treatment. Horizontal dashed line represents saturated VWC (0.37 m^3 m^{-3}). NOTE: VWC axis is in different scale compared to Fig. 3b and Fig. 4b.

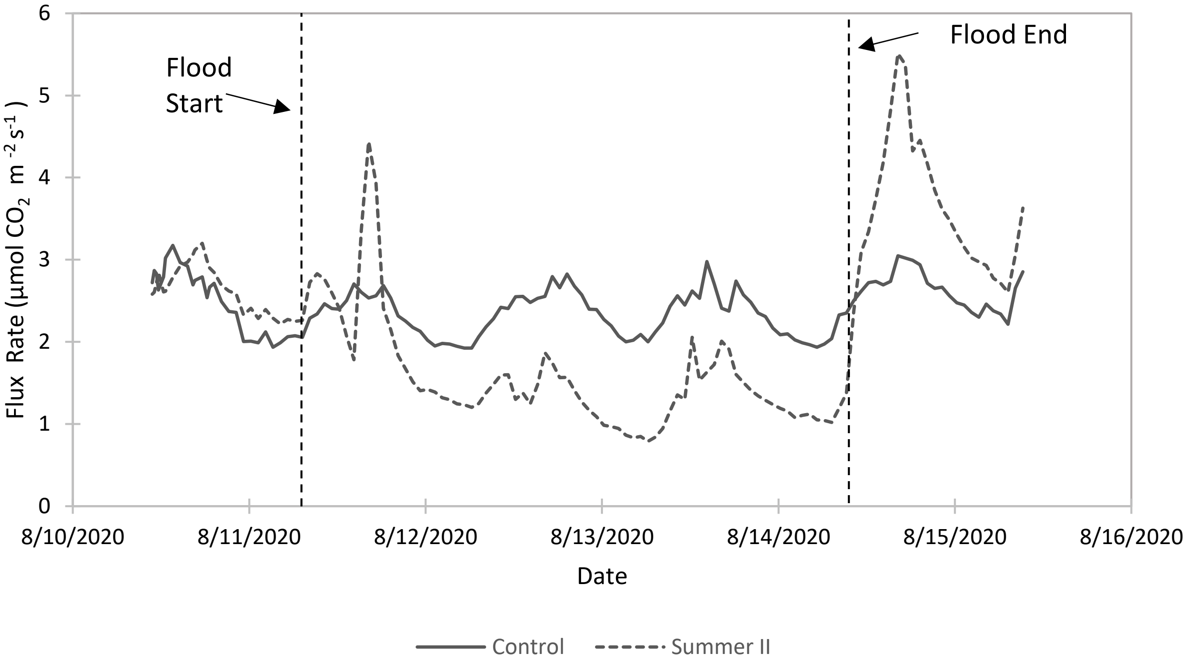


Figure 6c. Average hourly soil CO_2 flux rates during Summer II saturation event. Vertical dashed lines mark the beginning and end of treatment.

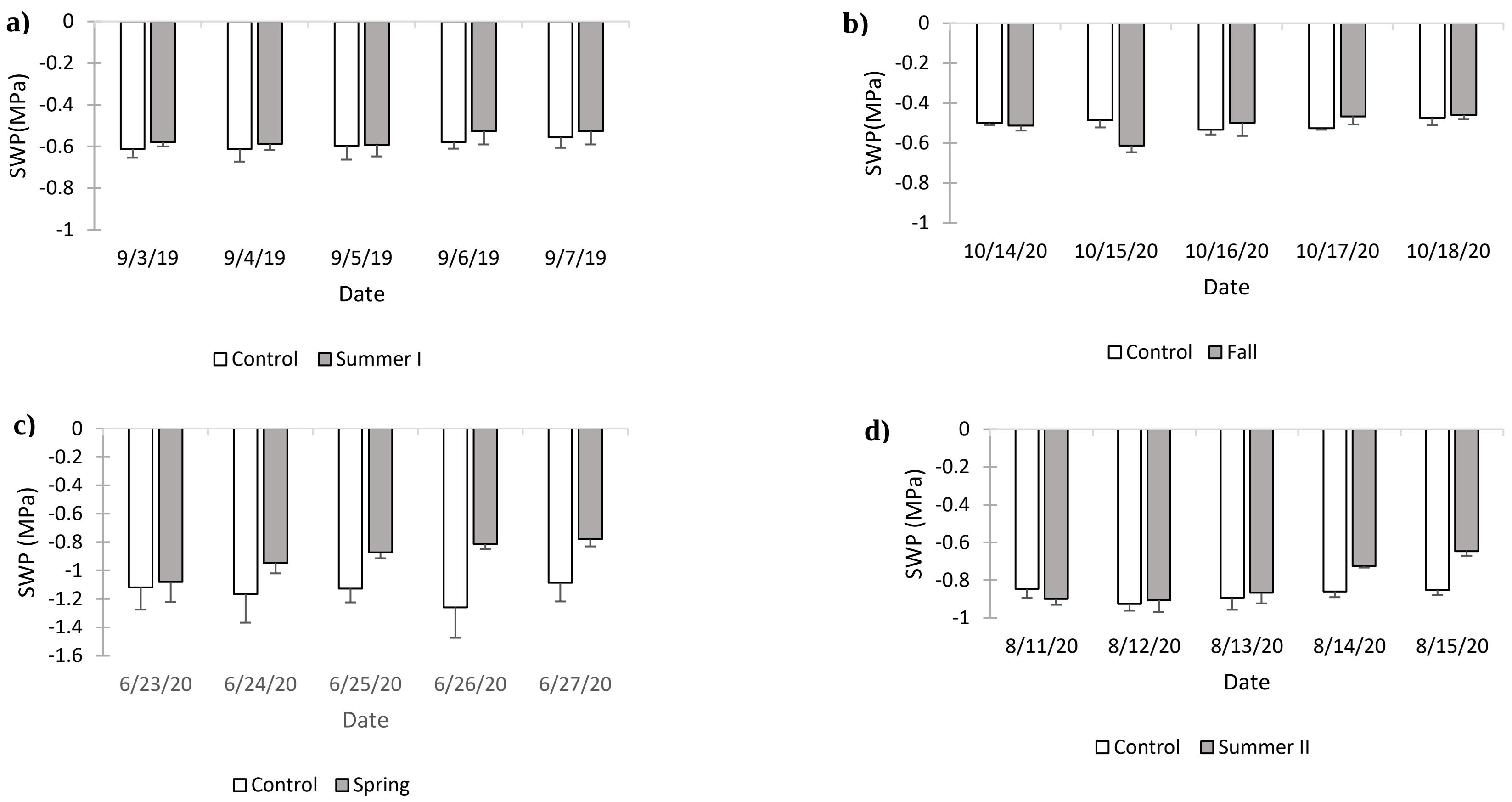


Figure 7. Average predawn SWP measured before and during flood irrigation treatments. a) Summer I, b) Fall, c) Spring, d) Summer II. First date was when irrigation started but PSWP were taken before treatment started. Also, Spring have different SWP scale. Error bars represent SE (n=3).

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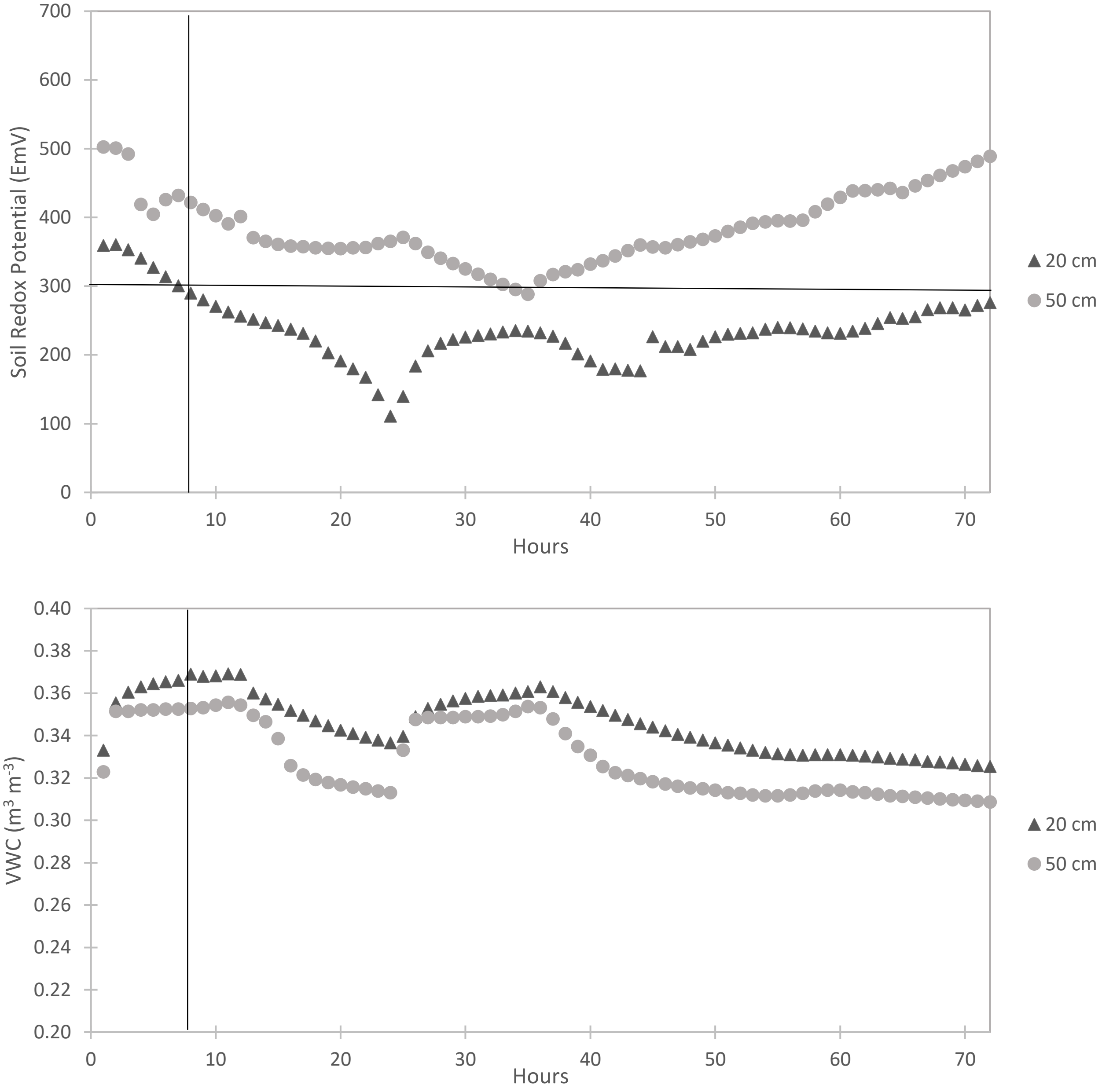
SUPPLEMENTARY DATA



S1. Soil redox potential (above) and soil VWC (below) at the depths of 20 cm and 50 cm during a 72-hour saturation event. This data was used to determine the amount of time soil become anaerobic (below 300 mV) and estimate the soil VWC for it.

Chapter 3 – Further Discussion.

I hypothesized that if hypoxic conditions in the rootzone reduced the efficiency of root respiration, trees in hypoxic conditions would show decreased root growth and increase root mortality after the hypoxic event had occurred. I also expected that if hypoxia had the greatest impact on active roots, then the impact of hypoxia will be greatest for the event in the late spring when almond trees normally have the greatest amount of new root production and oxygen demand to support new respiration would be the highest.

In this study the three-day saturation event did not have an impact on the production of fine roots in almonds during the growing season. Contrary to expectations, the trees did not exhibit higher root production in the spring and overall root production rates after the events, except summer 2020, were low. This was likely to be due to the lack of irrigation that our almond trees received prior to treatments due to technical limitations at the research site. This created extremely dry soil conditions that contributed to the low root length production. Therefore, when our treatments were applied in late spring and to a lesser extent during the summer, the excess water applied promoted root growth after the events as drought conditions were alleviated. This was mostly observed after treatment have been applied in the year 2020. Additionally, in summer and fall of 2019 we were not able to reach continuous soil saturation irrigation was turned off at night by research station staff. As the soils have very good drainage, this allowed oxygen replenishment and these hypoxic conditions were only rarely (and briefly) reached.

However, there were observations made on fine root production that were of interest. For example, we observed no significant difference in root length production by depth in control and treatment trees. This was a response we were not expecting because in most cases root production decreases with depth and in spring 2020 hypoxic conditions were of considerably shorter duration and depth than in the top soil. This was observed in our root data for each saturation event in both years and more interestingly, even after saturation events where we would have expected greater recovery production in the top soil. Additionally, we observed root production patterns that did not coincide with many of those reported for tree crops such as peaches and apples and even almonds themselves. The impact that drought (delayed irrigation in this case) in 2020 was such that no discernable spring root flush was measured, and root production only started after the first saturation event (Chapter 2, Fig. 2a).

For future experiments, longer continuous saturation events (at least one week) may be needed to potentially observe stunted root growth caused by soil hypoxia for almond trees growing in this well drain soil. Perhaps a better option would be to repeat the experiment with trees growing in poorly drained soil however, finding a site with the right characteristics where growers would allow such test is very difficult as there could be harmful repercussions of this treatments to the trees. Further field research is necessary to understand root response to soil conditions that almond trees will most likely experience in almond orchards, but the well drain soil conditions at the Plant Sciences research farm make creating prolonged hypoxic conditions technically very difficult. Another limitation in our study was the low irrigation applied to the control trees in 2020 which meant that the trees were exposed to deep drought conditions prior to being exposed to hypoxia. Unfortunately, management of the irrigation outside of the saturation events was not under our control. However, the study still contributes to the understanding of almond roots system and their response to drought alleviation when water is applied in large (saturation) events versus more controlled smaller events. Our data suggest the almond root system rebound better from imposed drought when a large saturating event, even when brief (<3 days) hypoxia, is applied compared to several shorter events as applied in the control plots. This observation warrants further research. Understanding the root systems for tree crops in response to irrigation events is crucial for plant breeders to develop rootstocks, plant pathologist to understand timing of root susceptibility to diseases, and extension agents to provide assistance to farmers regarding irrigation scheduling and nutrient management.