ISRAEL JOURNAL OF PLANT SCIENCES, 64 (2017) 3-4
https://doi.org/10.1080/07929978.2017.1288478

BRILL

Insights from “The Hidden Half”: The impact of root-zone oxygen and redox dynamics on the response of avocado to long-term irrigation with treated wastewater in clayey soil

David Yalin[a], Amnon Schwartz[b], Shmuel Assouline[c], Kfir Narkis[c], Amram Eshel[d], Adolfo Gabriel Levin[e], Anat Lowengart-Aycicegi[f], Jorge Tarchitzky[a] and Moshe Shenker[a]

[a]Department of Soil and Water Sciences, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel; [b]Department of Plant Sciences, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel; [c]Department of Environmental Physics and Irrigation, Institute of Soil, Water and Environmental Sciences, ARO Volcani Center, Israel; [d]Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv, Israel; [e]Northern R&D, Israel Ministry of Agriculture, Kiryat Shmona, Israel; [f]Extension Service Israel Ministry of Agriculture, Bet-Dagan, Israel

ABSTRACT

Treated wastewater (TWW) is a major source of water for agriculture in Israel; however, recent reports indicate a marked yield loss in TWW-irrigated avocado and citrus orchards planted in clayey soils. The association of the yield loss with clayey soils rather than sandy soils suggests that it is associated with conditions in the root zone, and specifically poor aeration. A three-year study (2012–2015) was conducted in an avocado orchard planted in clayey soil, comparing the oxygen and redox conditions in the root zone of TWW-irrigated plots with fresh water (FW)-irrigated plots, together with the physiological status of the trees. Soil parameters included: continuous *in-situ* measurement of soil-water tension (SWT), soil oxygen, and soil redox potential, and periodic measurements of soil solution composition. Physiological parameters included: mineral composition of plant tissue from the leaves, trunk xylem and roots, root growth, yield, fruit setting, plant volume, and yield. TWW-irrigated plots were found to endure longer periods of low SWT indicating higher water content, accompanied by lower oxygen levels and more reduced conditions in comparison to FW-irrigated plots. The differences in these soil parameters between treatments were greater during the irrigation season than during the rainy period. The more reduced conditions in the TWW plots did not lead to significant differences in Fe or Mn concentrations in the soil solution or in plant leaves. TWW soil solution had significantly higher Na levels compared with FW. This did not affect the leaf Na content, but was expressed in substantially higher Na content in the root and trunk xylem, with up to seven times more trunk xylem Na in TWW-irrigated plants compared with FW-irrigated plants. Root growth was significantly hindered in TWW-irrigated plots compared with FW-irrigated plots. A negative correlation was found between root growth and the duration of hypoxic conditions, and similarly between root growth and the Na levels in the roots. TWW-irrigated plants had greater fruitlet numbers at the initial fruit-setting stage, but had a smaller number of fruit and a lower yield at harvest. The yield (kg/tree) negatively correlated with the duration of hypoxic conditions in the root zone but not with the Na levels in the roots or xylem. Our findings point towards a substantial role of oxygen deprivation as a major factor leading to the damage to TWW-irrigated orchards in clayey soils. Based on the assimilation of data, we suggest that a downward cascade is instigated in the TWW-irrigated orchards by increased input of Na into the soil, leading to degradation of soil hydraulic properties and reduced aeration. Impaired physiological functioning of the roots due to limited oxygen supply results in less roots growth, lower water uptake and impaired selectivity against Na uptake, thus imposing a negative feedback to increase soil water content, reduce aeration and root-zone oxygen availability for the roots, and further impair plant resistance to the high Na levels.

ARTICLE HISTORY
Received 15 December 2016
Accepted 12 January 2017

KEYWORDS
Soil redox; soil oxygen; treated wastewater; effluent; *Persea americana*, salinity damage; *in-situ soil measurements*

Introduction

Wastewater reclamation is commonly practiced in much of the world today (Sato et al. 2013). A prevalent reclamation option is the irrigation of agricultural crops because treated wastewater (TWW) is a reliable, cheap source of water with high nutritional value for crops. In Israel this has led to extensive use of TWW for agriculture in the past 30 years, with over 80% of

CONTACT Moshe Shenker ✉ moshe.shenker@mail.huji.ac.il

This paper has been contributed in honor of Professor Uzi Kafkafi.

TWW apportioned to agricultural use, generating nearly 50% of the water used for agriculture (Futran 2013).

The use of TWW for agriculture is based on the widely accepted perception that it does not negatively affect agricultural production (Haruvy 1997). However, recent findings challenge this perception: a marked decrease in yield appeared in citrus and avocado trees following 5–10 consecutive years of irrigation with TWW in comparison to fresh water (FW)-irrigated trees, with up to 40% less yield in Hass avocado (Lahav et al. 2013; Assouline et al. 2015). To date, the reports of TWW-related yield loss are limited to orchards cultivated on clayey soils (>50% clay) rather than to sandy soils (Zilberstaine et al. 2011; J. Tarchitzky and A. Lowengart-Aycicegi, unpublished data). An initial study investigating the damage in avocado orchards planted in clayey soils indicated that the damage was not linked to toxic levels of boron (B), chloride (Cl) or sodium (Na) in the leaves (Lahav et al. 2013; Lowengart-Aycicegi et al. 2013), which are widely considered to be the main causes of damage in TWW-irrigated crops (Kafkafi 2010; Yermiyahu et al. 2010).

Long-term irrigation with TWW of orchards and field crops planted on clayey soils has led to increased Na levels in the soil in relation to FW irrigation (Tarchitzky et al. 2006), and this was shown to affect the soil hydraulic properties, reducing significantly the hydraulic conductivity and the sorptivity of the soil in the root zone (Levy et al. 1999; Levy & Assouline, 2010; Assouline & Narkis, 2011; Assouline et al. 2016; Bardhan et al. 2016). This negative impact on the soil hydraulic properties affected both the water and aeration regimes in the root zone and caused lower oxygen concentrations in TWW-irrigated orchards (Assouline & Narkis 2013). The association of the damage with clay soils rather than sandy soils and the fact that no noticeable damage appeared in field crops led us to hypothesize that the yield reduction could be linked to these soil aeration problems rather than to mineral toxicity (Na, Cl, or B toxicity), as was previously suspected. The basic assumption underlying our study is that in clayey soils the high sodium adsorption ratio (SAR) typical of TWW degrades the soil properties and leads to a pronounced decrease in soil aeration; this would be accentuated in orchards due to the fact that the soil is not tilled. Additionally, TWW adds biologically available organic material which affects microbial activity (Elifantz et al. 2011), effectively increasing the oxygen demand. The joint effect of limited oxygen supply with increased oxygen uptake is likely to lead to events of acute lack of oxygen in the root zone (hypoxia). Because of the high temporal variability of changes in soil oxygen that could happen at the hourly level, they may have not been noticed in previous studies that focused on the seasonal scale.

We outline here three plausible mechanisms of damage in hypoxic conditions which were the focus of this study:

A main cause of damage to plants in hypoxic conditions is a shift in soil redox potential. The soil redox potential is an intensity measure which describes the soil oxidizability, and is often perceived as an expression of “electron activity” (although free electrons do not exist in significant concentrations in solution). The measured potential is expressed relative to the standard hydrogen electrode as an “Eh” value; large Eh values favor the existence of electron-poor (i.e. oxidized) species, and small Eh values favor the existence of electron-rich (i.e. reduced) species (Sposito 2008). In a review of Eh measurements in a wide array of soils, Baas-Becking et al. (1960) depict the range of soil redox as varying between an Eh value of –350 mV in waterlogged soils and up to an Eh value of +640 mV in “normal” soils at $pH = 7$.

The redox-related damage to plants occurs as a result of chemical shifts in the solution composition typical of different redox states. For chemical interpretation of the redox potential a “pe” value is calculated from the measured Eh according to the Nernst equation. Because H^+ also takes part in many redox reactions the sum of $pe + pH$ is used to define the soil redox state (Fiedler et al. 2007). The damage to plants under conditions defined as suboxic ($9 < pe + pH < 14$) may occur due to nitrite accumulation, causing direct damage to roots (Van-Cleemput & Samater 1996), or due to reductive dissolution of Fe and Mn hydroxides which may lead to excessive levels of these elements (Fageria et al. 2002) and to changes in the availability of other nutrients such as P (Shenker et al. 2005).

Continuous measurement of the redox potential may be performed potentiometrically with an inert electrode positioned directly in the soil. Studies as early as the 1960s have shown the value of potentiometric soil redox measurements in evaluating Fe, Mn, and NO_3 availability under field conditions

(Baas-Becking et al. 1960; Meek et al. 1968; Meek & Grass 1975). However, little agronomic use of redox measurements has been made (Husson 2013). A major reason that explains this trend is a disagreement regarding the quantitative interpretation of redox measurements and the lack of clarity regarding their implications. Critics claim that quantitative interpretation of potentiometric measurements is not possible because electrodes are differentially affected by different redox couples and because natural solutions are always far from reaching redox equilibrium (Sposito 2008). Despite this criticism, it is widely accepted that low redox values represent a thermodynamically favorable environment for microbial reduction processes, and therefore redox measurements offer insight into the occurrence of such conditions, as demonstrated in numerous studies (Reddy & Patrick 1975; Schwab & Lindsay 1983a, 1983b; Patrick & Jugsujinda 1992; Peters & Conrad 1996; Yao et al. 1999; Mansfeldt 2004; Shenker et al. 2005; Yu et al. 2007).

To assess the effect of oxidation reduction processes in the soil, soil solution sampling is commonly used. To that end, soil solution samples must be acquired *in situ* to represent the prevailing redox conditions and precaution must be taken regarding sample handling. A commonly used sampling technique employs the use of porous soil solution samplers set in the soil connected to a suction and sample retrieval system (Litaor 1988). Rhizon samplers composed of a blend of polymers were shown to have negligible ion exchange capacity and thus faithfully represent soil solution composition (Meers et al. 2006; Shotbolt 2010). These samplers have an equivalent pore size of 0.15–0.2 microns, which offers on-site sample filtration. Another important approach to assess whether reductive dissolution of Fe and Mn interferes with plant nutrition is mineral analysis of the plant material (Grundon et al. 1997).

A second mechanism of damage to plants in soils with low oxygen is related to adverse effects on root physiology and activity. Poor Na exclusion may result from deteriorated physiological conditions. This may lead to toxic effects of even low salt concentrations in the soil solution which are regarded as nontoxic under aerated conditions (Barrett-Lennard 2003). Higher salinity levels are inherent to TWW-irrigated soils (Marcar et al. 2010). The adverse effects of salinity on plant growth in general (Chinnusamy et al. 2005 and references cited therein) and specifically on root growth (Bernstein & Kafkafi 2002 and references cited therein) have been studied extensively. Avocado is well known for its low salinity tolerance and specifically for its sensitivity to high Cl levels (Bar et al. 1997). Previous long-term studies on the effect of TWW on plants used leaf sample analysis as a diagnostic tool to assess Na damage; however, many woody plant species accumulate Na in the roots and stems rather than translocate it to the leaves (Bernstein 2013). Higher Na concentrations were found in the trunk of table grapes irrigated with TWW in comparison to FW-irrigated (Netzer et al. 2014) and a strong link between high Na levels in the trunk and plant mortality was identified (Y. Netzer, personal communication 2015), which supports the possibility of a “hidden” Na toxicity not detected in previous studies which focused on the leaf mineral content.

The third mechanism we refer to is related to the reduction of root growth caused by hypoxia (Armstrong & Drew 2002; Fagerstedt et al. 2013). Previous investigations into the damage to TWW-irrigated orchards in clay soils found lower stem water potential in TWW-irrigated trees as compared to FW-irrigated trees despite lower soil water potential in the root zone of the latter (Zilberstaine et al. 2011). These contradicting findings suggest that water flow to the stem is constrained, supporting the idea that the root system is underdeveloped in relation to the plant water demand. In order to measure root growth we used the ingrowth-core sampling, which is a direct method for estimating root growth which proved efficient in previous studies (Böhm 1979; Oliveira et al. 2000; Polomski and Kuhn 2002; Andreasson et al. 2016).

To date, no mechanistic explanation has been given to the negative impact of TWW irrigation on crop yields in clay soils. Although the negative effect of hypoxic conditions on plants and specifically on subtropical trees (such as citrus and avocado) has been long recognized (Oppenheimer 1978), no extensive study was done to investigate whether TWW indeed affects the root zone soil oxygen levels (Friedman & Naftaliev 2012). We thus aimed at detecting in-soil parameters and processes that act as the direct factors that hinder tree development in clay soils irrigated with TWW. For this purpose we set to characterize and quantify the effect of prolonged irrigation with TWW on the following parameters: (i) oxygen and redox regimes in the root zone and their relation to water content; (ii) the soil solution concentration of plant nutrients and other elements (e.g. Na) in the soil; (iii) Na and Cl content in various plant organs; (iv) plant root growth; (vi) overall plant size; (vii) early fruitlet abscission; and (viii) final yield.

Methods

The experiment was conducted during the years 2012–2014 in the Western Galilee Experiment Station, Acre, Israel (Akko, 32°55′52′′N 35°06′23′′E). The soil in the site is a grumusol containing approximately 60% clay, mostly smectite. The orchard selected included different avocado varieties irrigated with either TWW or FW consecutively since 1995. TWW is supplied by the Acre treatment plant which receives municipal wastewater from the city of Acre. The TWW is of secondary quality; major indices of the water quality are given in Table 1. Irrigation was performed using aboveground drippers and fertilizer application was performed through fertigation; the amounts and timing of irrigation and fertilizer application were conducted according to local agronomic recommendations. TWW-irrigated plots received the same water amounts as FW plots, and the N, P, and K concentrations in the treated wastewater were supplemented with fertilizer up to an equivalent amount to the fertilizer applied in the FW-irrigated plots (annual amounts average: 253 kg N/ha, 35.6 kg P/ha, and 267 kg K/ha). Measurements were performed in three plots of each treatment chosen to represent the entire orchard. Each plot consisted of three adjacent trees of Hass avocado on VC-66 West-Indian rootstock; these were chosen as the focus of research because they were most affected by TWW in previous experiments. The measuring station deployment in each plot is described in Figure 1.

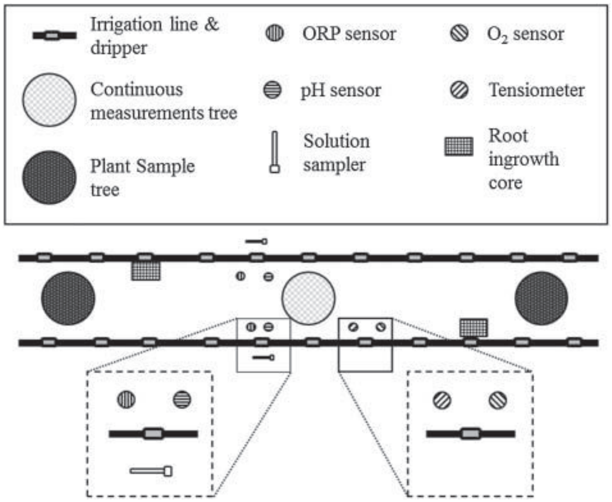


Figure 1. Typical deployment of measurement equipment in each plot. In the dashed rectangles are enlarged schemes of the points where oxygen, water tension, and redox were measured continuously and soil solution was sampled.

Redox potential and pH were measured in duplicate at a depth of 25 cm in proximity to the drippers approximately 50 cm north of the measurement tree. Redox was measured using combination Pt electrodes (ELH-031, Van London pHoenix Co., USA) with Ag/AgCl reference cells, and pH using glass combination electrodes (ELH-067, Van London pHoenix Co., USA) with Ag/AgCl reference cells. The electrodes were set into auger holes and pressed directly into moistened soil after field calibration with standard solutions. The soil extracted from the holes was then packed around the electrodes approximately to the original volumetric density. Data from the electrodes were logged using data-loggers (Multilog pro, Fourier-systems, Israel) once every half hour.

Soil oxygen was measured at a depth of 20 and 35 cm in proximity to the drippers approximately 50 cm south of the measurement tree. Oxygen was measured using a galvanic type cell with Pb-based electrodes (KE-50, Figaro Engineering Inc., Japan). The electrodes were placed in perforated plastic chambers and set into the soil. Data from the electrodes were logged using Campbell data-loggers once per three hours and data were later corrected according to air temperature (for more details see Assouline & Narkis 2013).

Soil water tension (SWT) was measured at a depth of 20 and 35 cm in proximity to the drippers approximately 50 cm south of the measurement tree, parallel to the soil oxygen sensors. Transducer-equipped digital tensiometers manufactured by Mottes

Table 1. Irrigation water quality in the experimental site (2013–2014 value ranges).

Parameter Treatment	pH	EC ds/m	K	Na meq/l	Ca+Mg	SAR $(meq/l)^{1/2}$	Cl	B mg/l	TSS	BOD
TWW	7.8–8.9	1.4–2.1	0.7–1.3	5.5–8.9	7.1–9.7	2.7–4.6	160–273	0.0–0.2	3.5–63.0	3.4–32.0
FW	6.3–7.6	0.7–1.0	0	0.7–1.3	6.3–9.8	0.4–0.6	39–75	0.0–0.1	–	–

Tensiometers Israel were used. Data were logged once per half hour directly onto the Mottes internet site through remote transmission.

Rhizosphere Rhizon samplers were set in proximity to the pH and redox electrodes. The solution was collected using a syringe, which was also used for the creation of the vacuum. The syringe was kept in an ice-cooled dark chamber during the sampling period of 2–12 hours. The sample was separated into different aliquots in the field: one aliquot was acidified on site using concentrated HNO_3 and was analyzed for total element composition using ICP-AES (ARCOS-SOP, Spectro, Germany); a second was dedicated to on-site measurement of pH and electric conductivity (EC) using field measurement devices (pH with pH-207 and EC with CD4303, Lutron Electronic Enterprise Co., Ltd, Taiwan).

Plant material was sampled from the trees adjacent to the measurement tree biannually (October and April). Between 5 and 10 of the oldest leaves of the last flush were sampled from each tree and then transported in an ice box to the lab where they were rinsed with double-distilled water (DDW). Xylem tissue was sampled in the main shoot of each tree using an electric 5 mm drill at a height of 0.5 m after removing the bark and the woodchips created by the drill were collected. Roots for mineral content analysis were manually separated from soil samples taken in proximity to the sampled tree. In the lab, the root samples were thoroughly washed in $CaSO_4$ solution. All plant material was dried in a 60 °C oven, ground and digested using concentrated HNO_3 followed by hydroperoxide at a temperature of about 120 °C. Elemental analysis was performed using ICP-AES (ARCOS-SOP, Spectro, Germany). Cl was measured in a water extract of the grinded samples using a coulometric Ag titration chloridometer (Chloride analyzer 926, Sherwood Scientific Ltd, UK).

Root growth was followed using the ingrowth core method. Metal wire (0.5 × 0.5 cm) cylinders (10 cm diameter × 30 cm length) were placed in drilled holes 1 m away from the tree trunk under the drip line and filled with local soil packed to approximately the same volumetric density as the local soil. After several months, depending on the tree phenology, the cores were extracted and replaced by new ones filled with fresh soil. The extracted cores were washed in tap water and the roots were stored in 20% ethanol in a cold room (4°C). The roots were scanned by an Epson Expression 1600 flatbed scanner with overhead lighting system (Epson America Inc., USA) in a tray full of water and the WinRhizo 2005b (Regent Instruments Inc., Canada) image-analysis program was used for determining root length, surface area, and volume of the roots extracted from each core. The roots were then dried in an oven (80°C, 48 h) and weighed. The measured values were divided by the time period (days) from insertion to extraction of the core to yield daily growth data.

Visual estimates of the trees' physiological condition were made on June 2014 by three agronomists specializing in avocado cultivation: they were asked to estimate the plant overall volume, foliage cover and leaf color on an ordinal scale of 1 (small) to 5 (large).

Fruit onset and fruitlet abscission was measured by marking 250 inflorescences on each of five trees from each treatment in May 2013, and then counting the number of fruitlets remaining on the marked inflorescences on a weekly basis up to October 2013.

Total fruit weight and fruit number were determined for each tree at harvest time (November 2013 and 2014) and average fruit size was calculated based on these data.

Statistical analysis

Statistical analysis was performed with JMP® pro statistical software version 2.0.1 (SAS Institute Inc., USA). Much of the collected data did not comply with many of the assumptions underlying the commonly used statistical tests. Firstly, because dry periods with high oxygen and Eh values were more prevalent, the oxygen and Eh data sets are negatively skewed rather than normally distributed (Figure 3). Secondly, TWW seemed to affect the values distribution, and thus the assumption of equal variance between groups is not valid. A third obstacle was the limited number of repetitions within each treatment due to the technical limitations of the experimental setup. We therefore performed non-parametric statistical tests in the analysis of these data (Wilcoxon's signed-rank test).

Results and discussion

Soil water tension (SWT), oxygen and Eh dynamics

A view of the daily dynamics in SWT, oxygen percentage in the root-zone atmosphere and in-situ redox potential (Eh) is given in Figure 2, which shows

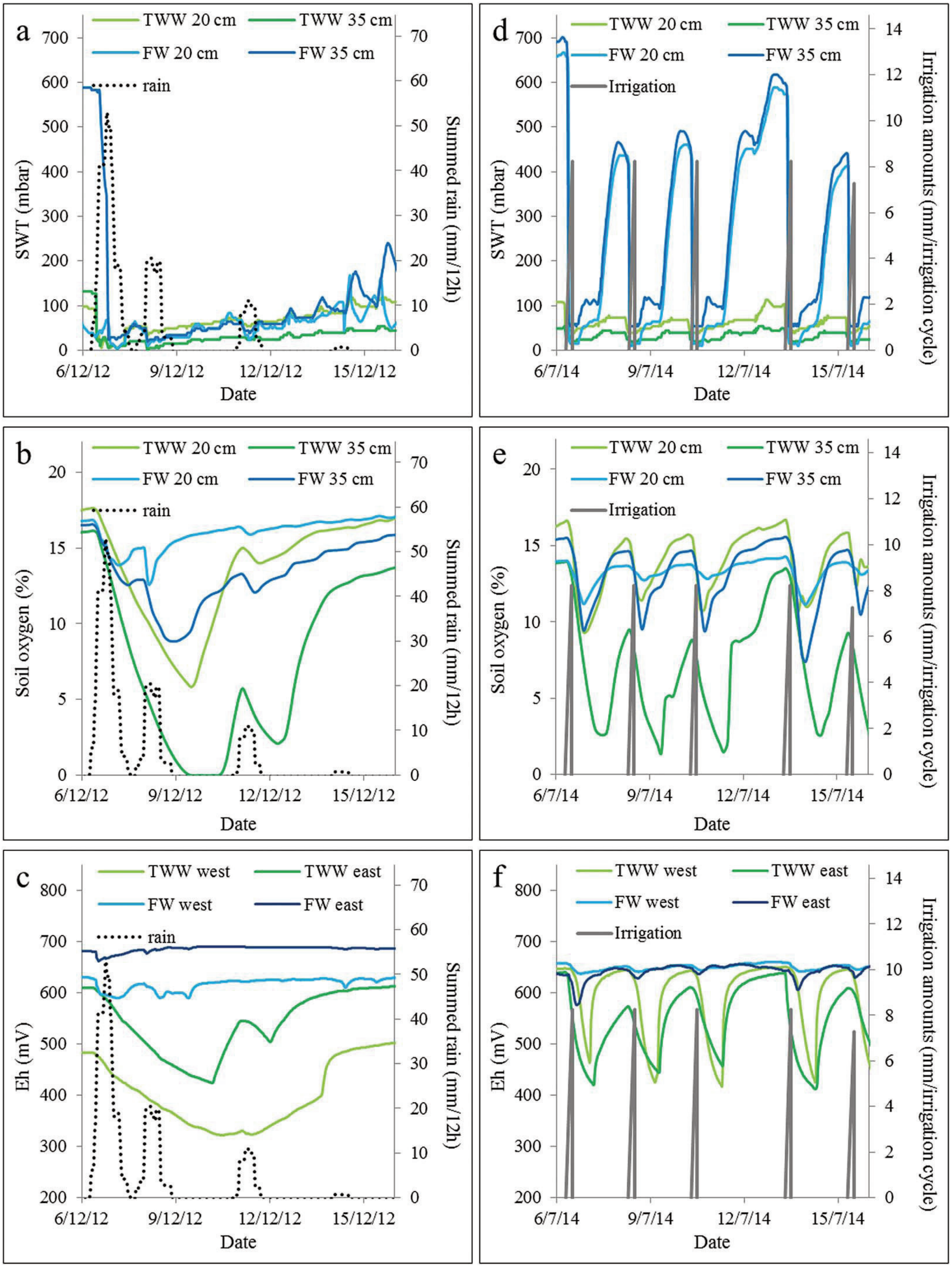


Figure 2. Soil conditions during 10 days of a rainy period with summary of rainfall during 12 h prior to each time point (a–c) and 10 days of an irrigation period with irrigation amounts (d–f), showing: soil water tension (SWT) at 20 and 35 cm depths (a, d), soil oxygen levels at 20 and 35cm depths (b, e), and redox potential at 25 cm depth, east and west of the measurement tree (c, f), in TWW-irrigated plot #3 and FW-irrigated plot #5. Rain data acquired from the Ministry of Agriculture online database (www.meteo.co.il/). Irrigation is depicted as starting at 07:00 on each irrigation day, and ending at 12:00, although the exact time of ending differed as a function of the irrigation volume.

10 days from the rainy period 2012 and 10 days from the irrigation season 2014. The periods displayed represent the observed trends during the study, in two adjacent plots distanced only 12 m apart but differing in water quality (i.e. TWW or FW) alone. A distinct pattern appears in both seasons and in both treatments: during dry periods, SWT, oxygen and Eh values reached maximum high values and remained nearly constant, then when the soil was wetted by rain or irrigation, the SWT values were lowered and this was followed by decreasing soil oxygen and Eh values. Subsequently, during the drying process oxygen and redox continued their decline while SWT values began to rise, until a critical water tension was achieved when presumably oxygen penetration into the soil depth was substantially increased, then oxygen and Eh values began to increase up until the next wetting event. This reoccurring pattern according to the expected mechanistic order supports the reliability of the oxygen and Eh measurement in the root zone. Diurnal changes are also noticed; these may be caused by daily changes in temperature and their effect on soil microbial activity (e.g. Clay et al. 1990).

The differences in SWT values between the two measured depths were not consistent. Differences in soil oxygen levels in the two depths were consistent during the summer months only with lower oxygen levels at 35 cm compared with 20 cm depth. The differences in Eh between the east and west measuring points in each plot were not consistent and represent the large variability in Eh within each treatment. This large variability is inherent to soil Eh because it is affected by localized factors such as plant activity (i.e. water consumption), microbial activity, chemical and physical properties and the complex interactions between them.

The evident differences between the TWW irrigated plots and the FW irrigated plots varied along the year and the trends will be discussed in the following segment where annual trends are shown.

Annual trends in soil water tension, oxygen and Eh

Figure 3 presents an overview of the soil water tension at 35 cm depth, soil oxygen at 35 cm depth and Eh at 25 cm depth of TWW- vs. FW-irrigated plots during the full period of the experiment. The figure shows monthly percentile values for each treatment. Percentile values were chosen to represent the data because of the non-normal distribution apparent in this figure. Furthermore, because data were collected on a fixed frequency the percentile data have a meaning of the percent time that the values of each parameter were below or above the presented value. For clarity the percentile values were determined for the monthly data set collected from all sensors of each treatment.

An annual oscillation appears in each of the SWT monthly percentile values in both treatments with the lowest values (i.e. the wettest conditions) occurring during the summer months when irrigation is most intense, and the highest values occurring during the beginning of spring (February–March) when rain was scarce and irrigation was not yet applied. The soil oxygen percentile values and Eh percentile values followed a similar annual oscillation, with the lowest values (i.e. the most oxygen-limited conditions) occurring during the summer months and the highest values occurring during the early spring period. The lower SWT, oxygen and Eh levels during the summer periods (compared with the winter periods) are in accordance with the high irrigation frequency during this period in comparison to the sparse occurrence of rain events during the late winter.

Regarding SWT, the 50th, 75th and 90th percentile values were substantially higher in TWW-irrigated plots in comparison with FW-irrigated plots, especially during the summer months, while the 10th and 25th percentile values did not differ consistently between treatments. The 50th to 90th percentile values represent periods in which aeration of the soil normally occurs and oxygen stress is relieved. The much lower 90th percentile values in TWW-irrigated plots compared to the FW-irrigated plots (e.g. a value of 118 mbar for the TWW-irrigated plots compared with 335 mbar for the FW-irrigated plots in August 2013) are an indication that for a great part of the time aeration was substantially impaired in these plots. The low SWT values in TWW-irrigated plots could be attributed to the increased water retention and the decreased water conductivity of the soil inflicted by the high sodium levels in the TWW (Assouline & Narkis 2011; Bardhan et al. 2016), but may also reflect decreased plant water uptake due to impaired physiological conditions. Work by Lahav and Kalmar (1977) showed that wetter soil condition are not advantageous to the production of avocado trees and may even hinder the yield of some varieties. Likewise, recent work examining the effect of different water-to-air ratios on avocado growth showed

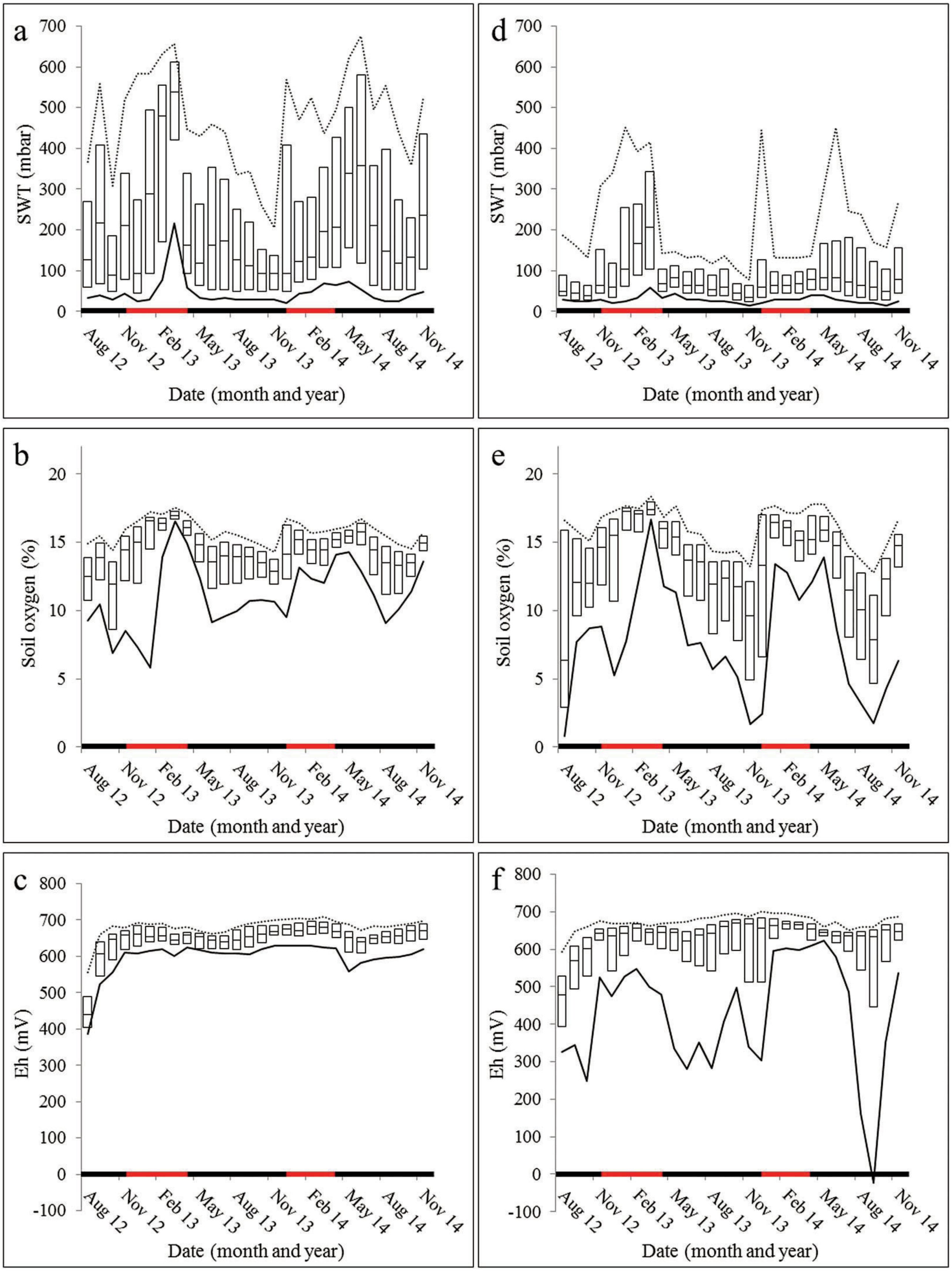


Figure 3. Soil water tension (SWT), oxygen levels and Eh between August 2012 and November 2014 in (a–c) TWW- vs. (d–f) FW-irrigated avocado plots, showing monthly values of: 0.1 percentile (solid black line), 0.9 percentile (dotted black line), 0.25 and 0.75 percentiles (box range) and median (inner box line); SWT at 35 cm depth (a, d; $n \sim 2800$), soil oxygen at 35cm depth (b, e; $n \sim 700$) and Eh at 25 cm depth (c, f; $n \sim 7700$). Red areas of the horizontal axis mark the winter periods when irrigation was not given.

poorer root and shoot growth of plants grown under high water contents (Gil et al. 2012).

Regarding soil oxygen levels, the 10th and 25th percentile values were substantially lower in TWW-irrigated plots in relation to FW-irrigated plots during the summer months, but the 50th, 75th and 90th percentile values did not differ in a consistent manner between treatments. The 10th and 25th percentile values reflect the conditions during the periods in which oxygen levels were most limiting, the lower values in TWW-irrigated plots indicate that oxygen stress in these plots was more severe. The 10th percentile values in TWW plots reached levels as low as 1.7% soil oxygen while FW-irrigated plots remained above 5.8%. A universal critical soil oxygen level for plant growth may not be defined (Armstrong & Drew 2002). Recent studies that focused on the oxygen requirements of avocado plants had defined critical values of air-to-water ratio or critical oxygen diffusion rate values (Ferreyra et al. 2007, 2008; Gil et al. 2012); however, these values do not indicate a critical soil oxygen level, and thus are not comparable with our measurements. In early studies, in which avocado seedlings were grown for two months in containers with controlled oxygen levels above the root surface, it was demonstrated that oxygen levels below 2% were lethal to the plants while 5% of oxygen lead to severe leaf burn and hindered root and shoot growth in relations to 10% or 21% of oxygen (Valoras et al. 1964; Stolzy et al. 1967). These data clearly point towards the hazardous potential of the low oxygen levels measured in the TWW-irrigated plots, but again a direct comparison might be erroneous because our data reflect the oxygen level within the root zone while the data of the above studies reflect the oxygen levels above-soil.

Regarding Eh, the TWW-irrigated plots also had substantially lower 10th percentile values in comparison to FW-irrigated plots during the summer months but did not differ consistently in the higher Eh percentiles. The 10th percentile values represent the periods with most reduced conditions and in the TWW-irrigated plots these values were below 420 mV for much of the summer months of 2013 and 2014. Values below 420 mV at the pH of the studied soil (ca. pH 7.5) are defined suboxic and are conducive of nitrite formation (Sposito 2008) and thus might be expected to be detrimental to plant roots. The 25th, 50th, 75th and 90th percentiles in TWW plots and all values in FW-irrigated plots remained at Eh values oscillating between 500 and 600 mV, representing oxic conditions; this implies that despite the clayey nature of the soil, for most of the time soil detrimental reduction processes are expected to have a negligible effect on the composition of the soil solution.

During the winter months the treatments did not differ significantly in oxygen or Eh percentile values. The lack of difference between treatments may result from the high SWT during this period. Another explanation for the high oxygen and Eh values during the winter months is that the low temperatures are less conducive of microbial oxygen consumption (Meek et al. 1968).

To understand the implications of the wet, hypoxic and reduced conditions described in Figure 3 a more straightforward description of the measure of time spent in these conditions is needed. For this SWT (at 35 cm depth), oxygen (at 35 cm depth) and Eh values were divided into four ordinal levels.The monthly percent of time (τ) spent at each level is presented in Figure 4. For each month the τ value is the number of readings within the range of each level out of the total number of readings for that month from all sensors of each treatment. The division into levels of SWT was performed arbitrarily because no critical SWT values exist for the experimental site. We divided the SWT values into the following levels: $<$50 mbar; 50–100 mbar; 100–150 mbar; and $>$150 mbar. For soil oxygen levels a similar arbitrary division was performed into the following levels: $<$5%; 5–10%; 10–15%; and $>$15%. Redox values were divided according to the accepted geochemical scale into three levels: anoxic ($pe + pH < 9$), suboxic ($9 < pe + pH < 14$), and oxic ($pe + pH > 14$); the oxic range was subdivided arbitrarily to specify very oxic conditions ($pe + pH > 16$). The τ values are the percent time that the soil parameter was at a certain range of values and is abbreviated as: $\tau^{\text{soil parameter}}_{\text{range}}$.

The annual oscillations are clear in all three parameters in both treatments: the months with the greatest $\tau^{\text{SWT}}_{<50\text{mbar}}, \tau^{\text{Ox}}_{<5\,\%}$, and $\tau^{\text{pe+pH}}_{<14}$ values were the summer months (June–October) of each year, when intense irrigation was applied. The months with the greatest $\tau^{SWT}_{>150\text{mbar}}, \tau^{Ox}_{>15\%}$, and $\tau^{pe+pH}_{>16}$ values were the spring months (February–May), a period with very little rain and no intense irrigation.

The difference in τ values between treatments was most prevalent in the summer months of each year, in

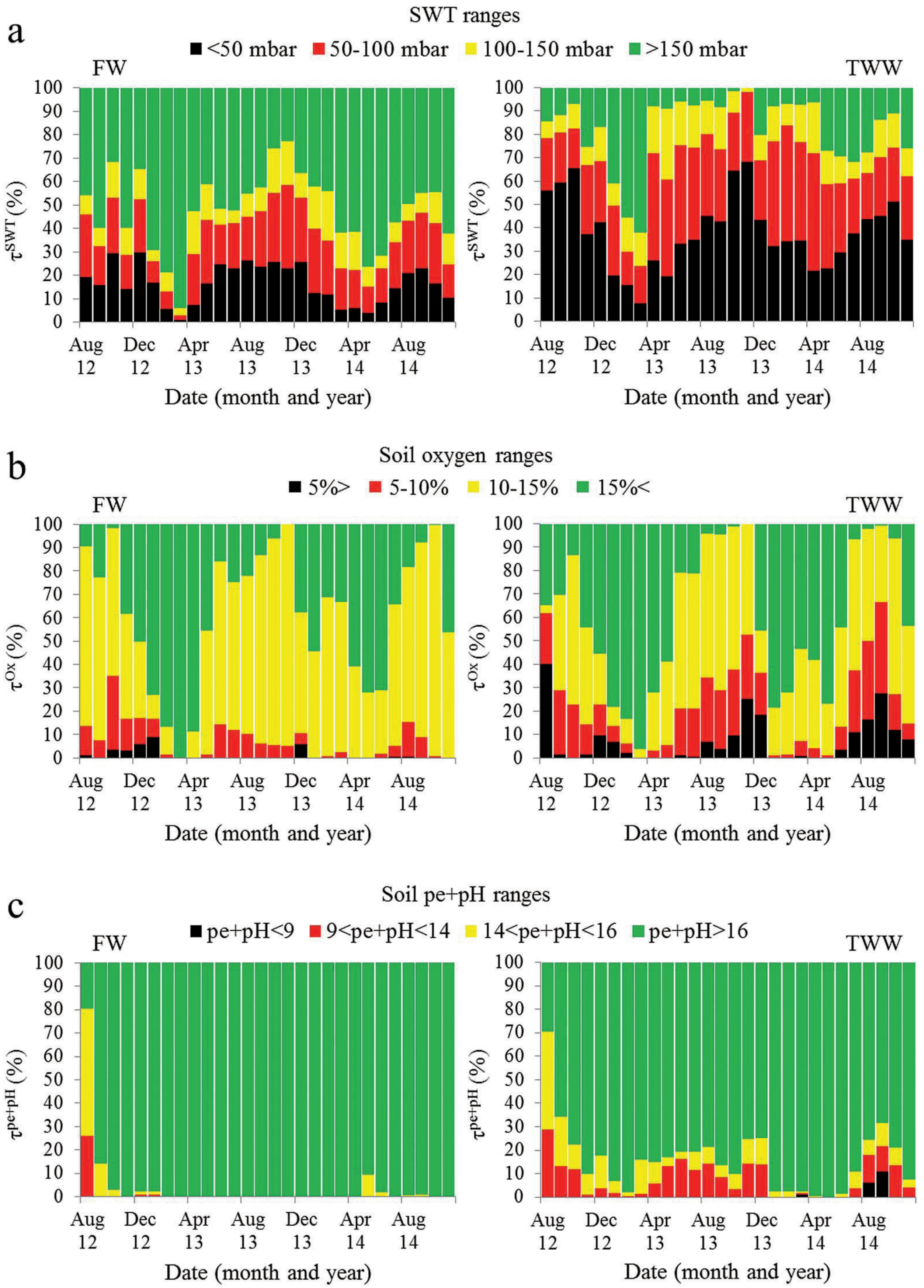


Figure 4. Monthly percent of time spent at different value ranges, of (a) SWT (τ^{SWT}) at 35 cm depth, (b) soil oxygen levels (τ^{Ox}) at 35 cm depth and (c) pe + pH (τ^{SWT}) at 25 cm depth, in FW- (left) vs. TWW- (right) irrigated plots, between August 2012 and November 2014.

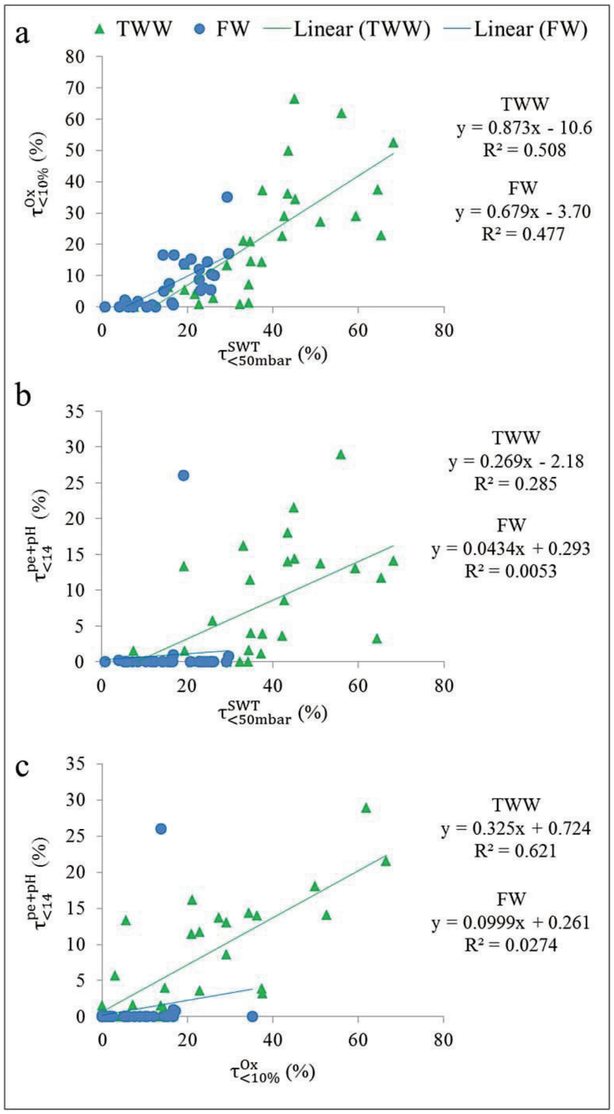


Figure 5. (a) Relations between percent of time below 50 mbar soil water tension ($\tau^{SWT}_{<50mbar}$) and percent of time below 10% soil oxygen ($\tau^{Ox}_{<10\%}$), (b) relation between $\tau^{SWT}_{<50mbar}$ and percent of time below pe + pH = 14 ($\tau^{pe+pH}_{<514}$), and (c) relations between $\tau^{Ox}_{<10\%}$ and $\tau^{pe+pH}_{<14}$ of TWW- vs. FW-irrigated plots, with linear regression equations and R^2 values.

which the $\tau^{SWT}_{<50mbar}$ values in TWW-irrigated plots were as high as 70%, compared to below 30% in FW-irrigated plots. Likewise, TWW-irrigated plots had $\tau^{Ox}_{<10\,\%}$ as high as 60% while in FW-irrigated plots it did not raise beyond 20% apart of one month. Regarding the redox conditions, it is evident that TWW-irrigated plots had much greater $\tau^{pe+pH}_{<14}$ values compared with the FW plots; however, it should be noted that this low pe + pH range seldom occurred for more than 20% of the month even in the TWW plots.

Figure 5 presents the relations between $\tau^{SWT}_{<50mbar}$ and $\tau^{Ox}_{<10\%}$ or $\tau^{pe+pH}_{<14}$ and the relations between $\tau^{Ox}_{<10\%}$ and $\tau^{pe+pH}_{<14}$. It appears that $\tau^{SWT}_{<50mbar}$ lower than 15% did not lead to an increase in $\tau^{Ox}_{<10\%}$ or $\tau^{pe+pH}_{<14}$ values; this gives a general hint to the duration of low SWT required to affect oxygen availability. Higher $\tau^{SWT}_{<50mbar}$ values lead to a linear increase in $\tau^{Ox}_{<10\%}$ in both treatments (p-value < 0.0001 for each of the regression lines). Regarding $\tau^{pe+pH}_{<14}$, the linear relations to $\tau^{SWT}_{<50mbar}$ were significant in TWW-irrigated plots (p-value < 0.0001 for the regression line) but not in FW-irrigated plots. Similarly, only in TWW-irrigated plots did $\tau^{Ox}_{<10\%}$ affect $\tau^{pe+pH}_{<14}$. The lack of correlation between $\tau^{pe+pH}_{<14}$ and the other two parameters in the FW-irrigated plots may point towards the tendency of the Eh to drop only at lower oxygen levels than 10%, which were more prevalent in the TWW-irrigated plots.

Plant mineral composition and nutrient availability

Figure 6 describes the soil solution, root, trunk and leaf concentrations of the elements which are widely considered as the main cause of damage in TWW-irrigated crops (namely Na, Cl, and B), together with the concentration of Fe and Mn, which are both redox-sensitive elements that may hinder plant nutrition in suboxic soil conditions.

TWW-irrigated plots had substantially and significantly higher levels of Na in the soil solution and in the lower tree parts, but not in the leaves, which seems to indicate a Na retention mechanism as known to exist in many woody plants (Bernstein 2013). Such a retention effect has previously been documented by Kadman (1964) in seedlings of avocado grown hydroponically. The vast differences between Na levels in the TWW-irrigated plant root and trunk tissues compared with the FW-irrigated plants are a novel finding. While it is well established that high Na levels cause toxic effects on plant tissue (Maathuis & Amtmann 1999), the consequence of the Na concentrations found in the TWW-irrigated plants are not yet clear and require further investigation. In a comprehensive survey of grape plants in Israel a direct link between vine wilt and Na concentrations in the branch tissue was found (Y. Netzer, personal communication); however, work is still required to establish such a link in avocado plants, and toxic threshold concentrations are yet to be determined.

Cl levels were substantially and significantly higher in the leaf tissue of TWW-irrigated plants but not in the root or shoots of these plants. The accumulation of Cl in avocado leaves is well known (Lahav & Kadman 1980), and unlike the Na it is shown here that Cl is not retained in the lower tree parts. Lahav and Kadman (1980) suggest leaf Cl concentration of 2500 mg/kg as excessive but note that Cl concentration as high as 7000 mg/kg may exist in healthy trees. We therefore do not suspect the Cl levels found in the TWW leaves (3060 ±450 mg/kg) to be toxic. Similarly to Cl, differences in B concentrations were found in the leaves but not in the lower plant parts, with significantly higher concentrations in TWW-irrigated plants; nonetheless, in both treatments the B concentrations in the leaves were well within the range considered adequate (Lahav & Kadman, 1980).

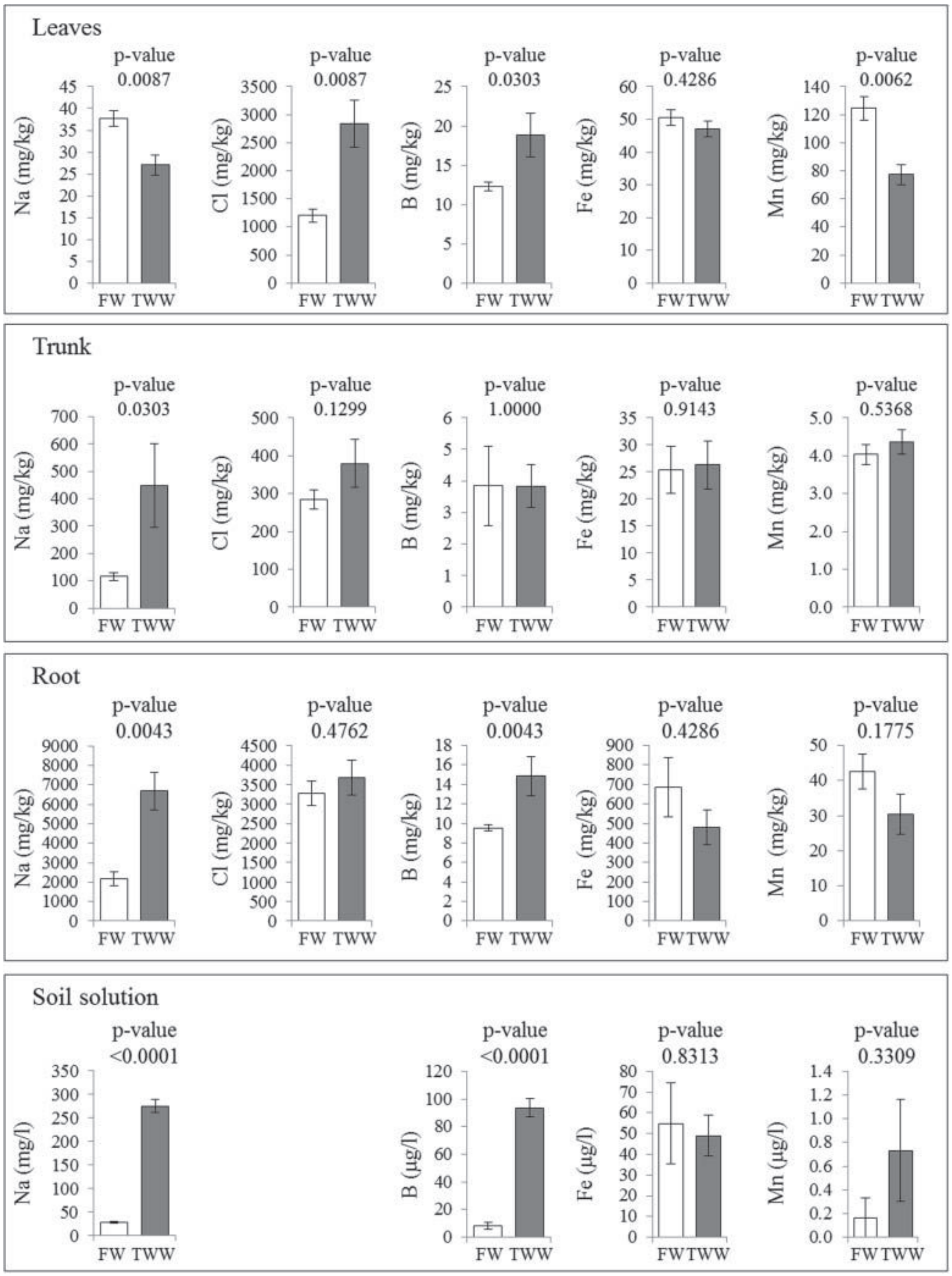


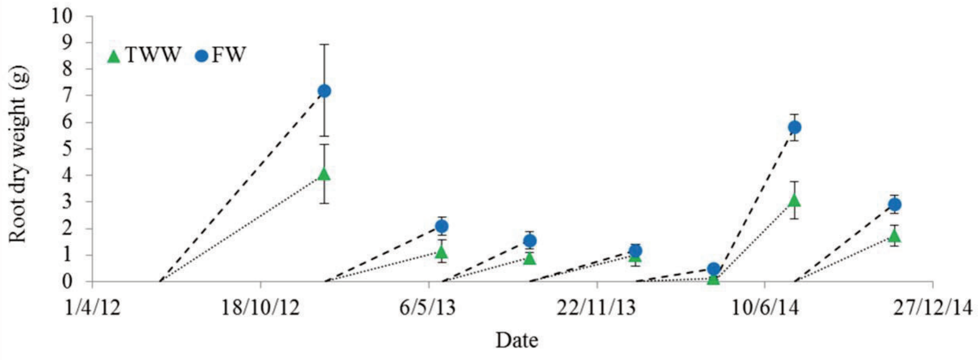
Figure 6. Mineral composition of plant leaves, trunk xylem, and root sampled during September 2014 together with soil solution composition sampled four times during July–September 2014 in TWW- vs. FW-irrigated plots. Means, standard error values and *p*-values for Wilcoxon's exact signed-rank test expressing prob≥|S-Mean|.

Regarding Fe and Mn, no significant differences appeared in their concentrations in the soil solution. Furthermore, the lack of difference in Fe leaf concentration and the higher Mn concentrations in in the leaves of FW-irrigated plants do not support any negative effect related to reductive dissolution of these elements in the root zone. Indeed, as shown in the previous segment (Figure 4), the measured Eh values in the soil were seldom conducive of Fe and Mn reductive dissolution in both treatments.

Root growth and tree visual assessment

The average root dry weight extracted at different times from the ingrowth cores together with the calculated daily root growth rate are described in Figure 7. TWW-irrigated trees had consistently lower root growth in comparison with FW-irrigated trees throughout the study period, but this difference was only statistically significant (p-value < 0.05) at the July 2014 sampling. Root length and surface area were also measured and showed very close correlation to the root dry weight measurements (Figure 8); hence, the differences in length and surface area growth between treatments followed the same trend. The highest growth rate for both treatments was observed during April to July 2014, followed by the rate during the period between June 2012 and January 2013. The ingrowth-core method expresses the integrated growth of new roots into fresh soil during a long period unlike the time-sequence measurements available using minirhizotrons, for example. Despite the difference in methods, it is still clear that the periods when the most growth was recorded, spring and autumn, coincided with those described in California (Mickelbart et al. 2012) and New Zealand (Wolstenholme & Sheard 2009).

The relation between root growth and $\tau^{\mathrm{Ox}}_{<10\%}$ (the percent time with lower oxygen levels than 10%) is shown in Figure 9a and the relation to Na levels in the roots is shown in Figure 9b. The $\tau^{\mathrm{Ox}}_{<10\%}$ values were calculated for the entire period of root growth in the ingrowth cores, and Na levels are from the roots sampled in closest time-proximity to the root extraction date. The root growth rate during April–July 2014 was negatively correlated with $\tau^{\mathrm{Ox}}_{<10\%}$ values ($\alpha = 0.0042$) and with the Na levels in the roots ($\alpha = 0.0071$). A weaker link existed between the root growth rates during July–November 2014 with Na levels ($\alpha = 0.0111$), and with $\tau^{\mathrm{Ox}}_{<10\%}$ values ($\alpha = 0.2176$). The negative correlation between root growth and Na concentrations in the root concurs with findings of Bernstein et al. (2004) in avocado seedlings grown in solution culture. The negative correlation between root growth and the period of limited oxygen is in line with the well-established high sensitivity of avocado to low



Ingrowth period		06/12-01/13	01/13-05/13	05/13-09/13	09/13-01/14	01/14-04/14	04/14-07/14	07/14-11/14
Growth rate (mg/day)	FW	36.9	14.9	14.9	9.3	5.3	60.4	24.5
	TWW	20.8	8.1	8.7	7.9	1.3	31.9	14.6
prob≥|S-mean|		0.400	0.082	0.178	0.457	0.058	0.017	0.058

Figure 7. (top) Dry weight of roots extracted from ingrowth cores from FW- vs. TWW-irrigated plots; means and standard error values, the connecting lines mark the growth period (TWW marked with dotted line and FW with dashed line). (bottom) Calculated rate of growth and p-value for the difference between the two treatments according to Wilcoxon's exact signed-rank test.

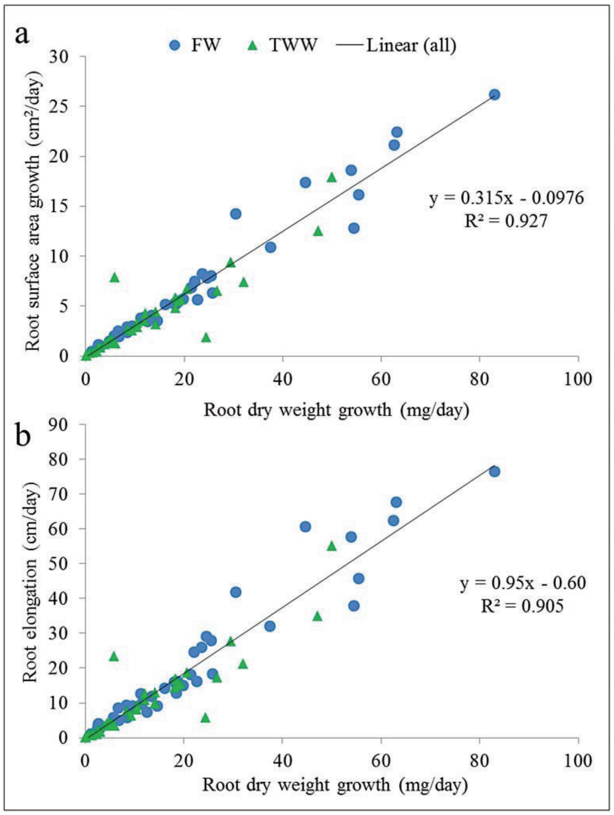


Figure 8. Relations between root dry weight growth and root surface area growth (a), and between root dry weight growth and root elongation (b) of TWW- vs. FW-irrigated plots, with linear regression equations and R^2 values.

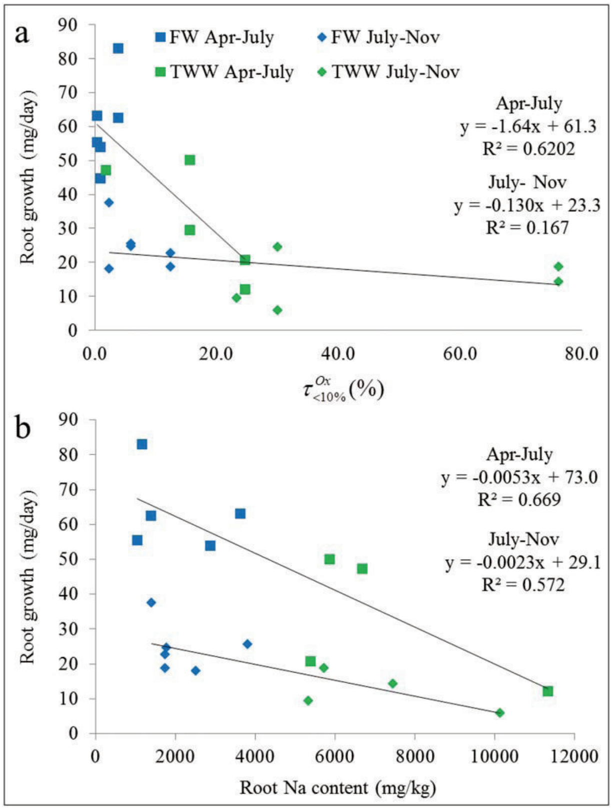


Figure 9. Relations between root dry weight growth and percent of time below 10% soil oxygen $\tau^{\mathrm{Ox}}_{<10\%}$ calculated from the data measured in the adjacent trees in each plot (a), and between root dry weight growth and root Na content sampled at the same trees (b) of TWW- vs. FW-irrigated plots, with linear regression equations and R^2 values. $\tau^{\mathrm{Ox}}_{<10\%}$ values were calculated for the period of root growth: April to July or July to November 2014, and the Na content values were taken from the sampling date closest to the root extraction date: July 2014 for the April–July data and September 2014 for the July–November data.

oxygen (Schaffer et al. 2013). Our data indicate that the effects of the high Na levels and long periods of oxygen stress are more significant in periods when root growth is greater.

TWW-irrigated trees had statistically insignificant lower volume and foliage cover compared with FW-irrigated trees and had no difference in leaf color (Table 2). Tree volume was weakly correlated with root growth ($\alpha = 0.0173$, Spearman's ρ; Figure 10). The relation to tree volume raises a question of cause and effect, whether it is the small tree volume which caused the small root growth or vice versa.

Yield and fruitlet abscission

Fruit setting during May and June 2013 was higher in the TWW-irrigated plots in comparison with FW-irrigated plants, although statistically significant differences were only measured on 24 June 2013. Later on in the summer towards the harvest time the differences between treatments were diminished, indicating greater abscission rates in the TWW-irrigated trees (Figure 11). The shown massive fruit drop is a common phenomenon in avocado orchards. The initial increased number of fruitlets in TWW-irrigated plants in relations to FW-irrigated plants may point towards a stress response of the plant.

The yield (kg/tree) of TWW-irrigated trees was significantly lower compared with FW-irrigated trees in

Table 2. Visual estimates of tree physiological conditions. Median values and *p*-value for the difference between treatments according to Fischer test.

Treatment	Tree volume	Foliage cover	Leaf color
FW	4	3.25	3
TWW	3	2.75	3
Prob>ChiSq	0.13	0.75	0.367

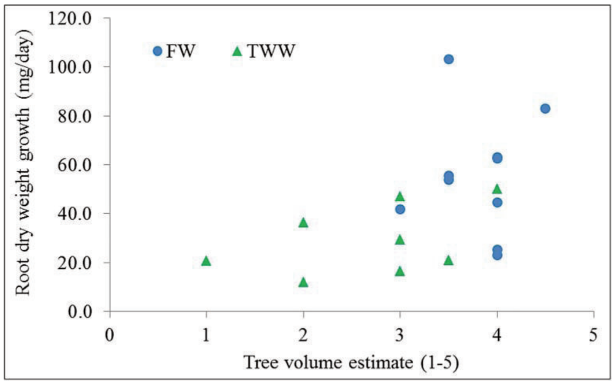


Figure 10. Dry weight growth rate during April–July 2014 by ordinal tree size estimates made at April 2014.

2014 but not so in 2013 (Figure 12). Avocado is known to have alternate bearing and indeed great differences appear between the yield of the FW trees between the two harvest times, indicating that 2014 was an "on" year. The yield gap between the two treatments was a result of smaller fruit numbers in TWW-irrigated plots supporting the idea that stress leading to greater fruit abscission is responsible for the drop in yield.

Yield was negatively correlated with the duration of low oxygen levels ($\tau^{Ox}_{<10\%}$) calculated for the period of June to September prior to harvest ($\alpha = 0.0092$; Figure 13), but not with Na levels in the roots or xylem. This strongly implies that a major factor leading to yield reduction in TWW-irrigated orchards planted in clay soils is low oxygen rather than salinity damage per se.

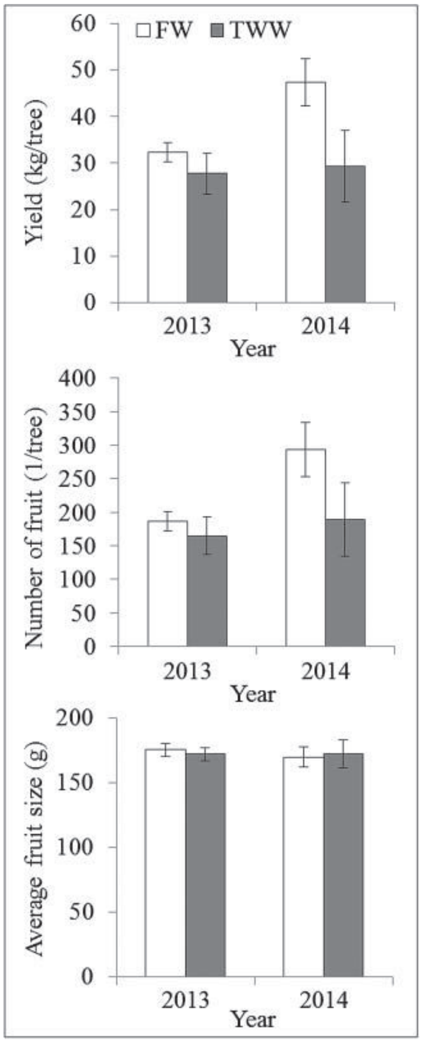


Figure 12. Yield, number of fruit and average fruit size of TWW- vs. FW-irrigated trees, mean and standard error values for 2013 and 2014 seasons ($n = 9$).

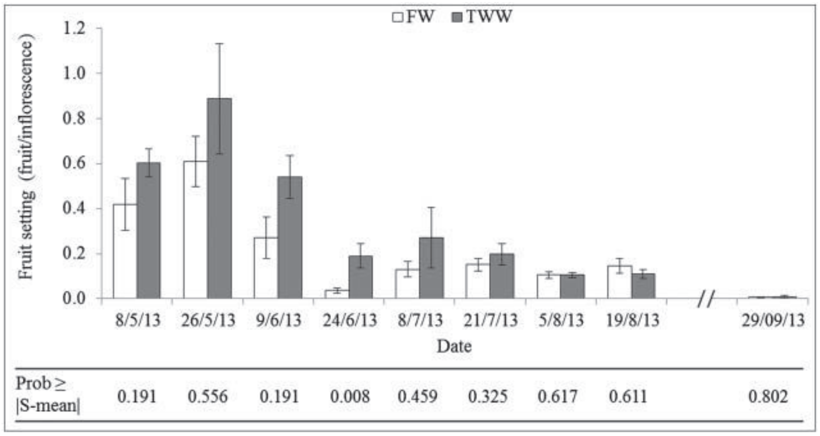


Figure 11. Remaining fruit per inflorescence on marked inflorescences of TWW- vs. FW-irrigated trees at different times during May to October 2013. Mean and standard error values with p-value for the difference between treatments at each sample date. For ease of view only biweekly values are shown.

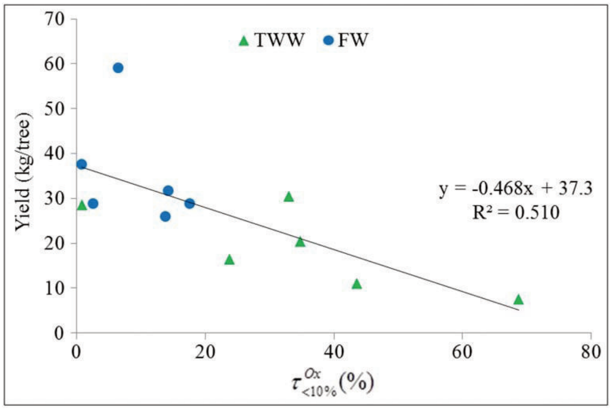


Figure 13. Yield of TWW- vs. FW-irrigated trees in the years 2013 and 2014 by $\tau^{Ox}_{<10\%}$ at 35 cm, calculated for the summer months (June–September) of each year. With linear regression line for the whole data set ($n = 12$).

Summary and conclusions

The results depicted here provide a comprehensive view regarding the effect of TWW on avocado orchards planted in clay soils, and add an important supplement to previous studies attempting to elucidate the cause of damage to TWW-irrigated orchards in clay soils (Lahav et al. 2013; Lowengart-Aycicegi et al. 2013; Assouline & Narkis 2013; Paudel et al. 2016).

Based on the assimilation of data in this report we suggest that a downward cascade occurs in TWW-irrigated orchards planted in clay soils stimulated by a negative feedback mechanism. The cascade may be instigated by the addition of Na and organic matter as components of TWW into the soil, inducing the degradation of soil hydraulic properties, and leading to limited oxygen availability to the roots. Root-impaired functioning due to the oxygen stress may result in lower water uptake and decreased selectivity against Na uptake by the roots; this in turn may hinder root growth, which may diminish the water uptake by the plant, thus leading to higher water content in the soil which further deteriorates the oxygen availability in the root zone.

Acknowledgments

We thank the staff of the Western Galilee Experiment Station and the Israel Northern R&D for their great assistance.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was financially supported by the Israel Ministry of Agriculture Chief Scientist Fund and the Israel Plants Production & Marketing Board Fruit Division.

References

Andreasson F, Gonzalez M, Augusto L, Bakker MR. 2016. Comparison of ingrowth cores and ingrowth meshes in root studies: 3 years of data on *Pinus pinaster* and its understory. Trees-Struct Funct. 30:555–570.

Armstrong W, Drew MC, 2002. Root growth and metabolism under oxygen deficiency. In: Waisel Y, Eshel A, Kafkafi U, editors. Plant roots: the hidden half. 3rd ed. New York, Basel: Marcel Dekker, Inc.; p. 729–761.

Assouline S, Narkis K. 2011. Effects of long-term irrigation with treated wastewater on the hydraulic properties of a clayey soil. Water Resour Res. 47:1–12.

Assouline S, Narkis K. 2013. Effect of long-term irrigation with treated wastewater on the root zone environment. Vadose Zo J. 12:1–10.

Assouline S, Narkis K, Gherabli R, Sposito G. 2016. Combined effect of sodicity and organic matter on soil properties under long-term irrigation with treated wastewater. Vadose Zo J. 15:150–163.

Assouline S, Russo D, Silber A, Or D. 2015. Balancing water scarcity and quality for sustainable irrigated agriculture. Water Resour Res. 51:3419–3436.

Baas-Becking LGM, Kaplan IR, Moore D. 1960. Limits of the natural environment in terms of pH and oxidation-reduction potentials. Geology. 68:243–284.

Bar Y, Apelbaum A, Kafkafi U, Goren R. 1997. Relationship between chloride and nitrate and its effect on growth and mineral composition of avocado and citrus plants. J Plant Nutr. 20:715–731.

Bardhan G, Russo D, Goldstein D, Levy GJ. 2016. Changes in the hydraulic properties of a clay soil under long-term irrigation with treated wastewater. Geoderma. 264:1–9.

Barrett-Lennard EG. 2003. The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. Plant Soil. 253:35–54.

Bernstein N. 2013. Effects of salinity on root growth. In: Eshel A, Beeckman T, editors. Plant roots: the hidden half. 4th ed. Boca Raton, FL: CRC Press; p. 36.1–36.18.

Bernstein N, Kafkafi U. 2002. Root growth under salinity stress. In: Waisel Y, Eshel A, Kafkafi U, editors. Plant roots: the hidden half. 3rd ed. New York, Basel: Marcel Dekker p. 787–805.

Bernstein N, Meiri A, Zilberstaine M. 2004. Root growth of avocado is more sensitive to salinity than shoot growth. J Am Soc Hortic Sci. 129:188–192.

Böhm W. 1979. Methods for studying root systems. Berlin: Springer.

Chinnusamy V, Jagendorf A, Zhu JK. 2005. Understanding and improving salt tolerance in plants. Crop Sci. 45:437–448.

Clay DE, Clapp CE, Molina JAE, Linden DR. 1990. Soil tillage impact on the diurnal redox-potential cycle. Soil Sci Soc Am J. 54:516–521.

Elifantz H, Kautsky L, Mor-Yosef M, Tarchitzky J, Bar-Tal A, Chen Y, Mintz D. 2011. Microbial activity and organic matter dynamics during 4 years of irrigation with treated wastewater. Microb Ecol. 62:973–81.

Fageria NK, Baligar VC, Clark RB. 2002. Micronutrients in crop production. Adv Agron. 77:185–268.

Fagerstedt KV, Blokhina OB, Pucciariello C, Perata P. 2013. Flooding tolerance mechanisms in roots. In: Eshel A, Beeckman T, editors. Plant roots: the hidden half. 4th ed. Boca Raton, FL: CRC Press; p. 32.1–32.32.

Ferreyra R, Maldonado P, Celedón J. 2008. Soil air content effects on the water status of avocado trees. Acta Hoticulturae. 792:291–296.

Ferreyra R, Selles G, Maldonado P, Celedón J, Gil P, Barrera C, Torres A. 2007. Effect of air content of soil on avocado tree water status and growth. In 4th World Avocado Congress, Viña Del Mar, Chile.

Fiedler S, Vepraskas MJ, Richardson JL. 2007. Soil redox potential: importance, field measurements, and observations. Adv Agron. 94:1–54.

Friedman SP, Naftaliev B. 2012. A survey of the aeration status of drip-irrigated orchards. Agric Water Manag. 115:132–147.

Futran V. 2013. Tackling water scarcity : Israel's wastewater recycling as a model for the world's arid lands. GWF Discussion Paper 1311. Canberra, Australia: Global Water Forum.

Gil PM, Bonomelli C, Schaffer B. 2012. Effect of soil water-to-air ratio on biomass and mineral nutrition of avocado trees. J Soil Sci Plant Nutr. 12:609–630.

Grundon NJ, Robson AD, Lambert MJ, Snowball K. 1997. Nutrient deficiency and toxicity symptoms. In: Reuter DJ, Robinson JB, editors. Plant analysis: an interpretation manual. North Clayton, Australia: Inkata Press; p. 37–51.

Haruvy N. 1997. Agricultural reuse of wastewater: nation-wide cost-benefit analysis. Agric Ecosyst Environ. 66:113–119.

Husson O. 2013. Redox potential (Eh) and pH as drivers of soil/plant/microorganism systems: a transdisciplinary overview pointing to integrative opportunities for agronomy. Plant Soil. 362:389–417.

Kadman A. 1964. The uptake and accumulation of sodium in avocado seedlings. Am Soc Hortic Sci. 85:179–182.

Kafkafi U. 2010. Chlorids in treated wastewater and their effects on plants. In: Levy GJ, Fine P, Bar-Tal A, editors. Treated wastewater in agriculture : use and impacts on the soil environment and crops. Oxford: Wiley-Blackwell; p. 234–246

Lahav E, Kadman A. 1980. Avocado fertilisation. IPI-Bulletin No. 6. Bern: Grutter Druck.

Lahav E, Kalmar D. 1977. Determination of the irrigation regimen for an avocado plantation in spring and autumn. Aust J Agric Res. 28:717–724.

Lahav E, Lowengart-Aycicegi A, Regev I, Cohen H, Israeli T, Yehieli N, Yakuti G, Zilberstein M, Naor A, Assouline S, et al. 2013. Response of avocado to irrigation with recycled water, part 2: the effect on growth and production. Alon Hanotea. 67(March):32–35. Hebrew.

Levy GJ, Assouline S. 2010. Physical aspects. In: Levy GJ, Fine P, Bar-Tal A, editors. Treated wastewater in agriculture: use and impacts on the soil environments and crops. Oxford: Wiley-Blackwell; p. 306–327.

Levy GJ, Rosenthal A, Tarchitzky J, Shainberg I, Chen Y. 1999. Soil hydraulic conductivity changes caused by irrigation with reclaimed waste water. J Environ Qual. 28:1658–1664.

Litaor MI. 1988. Review of soil solution samplers extractor. Water Resour Res. 24:727–733.

Lowengart-Aycicegi A, Lahav E, Regev I, Cohen H, Israeli T, Yehieli N, Yakuti G, Zilberstein M, Naor A, Assouline S, et al. 2013. Response of avocado to irrigation with recycled water, part 1: the effect on the soil, leaves and water potential in the tree. Alon Hanotea. 67(February):26–31. Hebrew.

Maathuis FJM, Amtmann A. 1999. K^+ nutrition and Na^+ toxicity : the basis of cellular K^+/ Na^+ Ratios. Ann Bot. 84:123–133.

Mansfeldt T. 2004. Redox potential of bulk soil and soil solution concentration of nitrate, manganese, iron, and sulfate in two Gleysols. J Plant Nutr Soil Sci. 167:7–16.

Marcar NE, Theiveyanathan T, Stevens DP. 2010. Salinity. In: Levy GJ, Fine P, Bar-Tal A, editors. Treated wastewater in agriculture : use and impacts on the soil environments and crops. Oxford: Wiley-Blackwell; p. 286–305.

Meek BD, Grass LB. 1975. Redox potential in irrigated desert soils as an indicator of aeration status. Soil Sci Soc Am J. 39:870–875.

Meek BD, MacKenzie AJ, Grass LB. 1968. Effects of organic matter, flooding time, and temperature on the dissolution of iron and manganese from soil in situ. Soil Sci Soc Am J. 32:634–638.

Meers E, Du Laing G, Unamuno VJ, Lesage E, Tack FMG, Verloo MG. 2006. Water extractability of trace metals from soils: some pitfalls. Water Air Soil Pollut. 176:21–35.

Mickelbart MV, Robinson PW, Witney G, Arpaia ML. 2012. "Hass" avocado tree growth on four rootstocks in California. II. Shoot and root growth. Sci Hortic. 143:205–210.

Netzer Y, Shenker M, Schwartz A. 2014. Effects of irrigation using treated wastewater on table grape vineyards: dynamics of sodium accumulation in soil and plant. Irrig Sci. 32:283–294.

Oliveira MR, Van Noordwijk M, Gaze SR, Brouwer G, Bona S, Mosca G, Hairiah K. 2000. Auger sampling, ingrowth cores and pin board methods. In: Smith A, Bengough AG, Engels C, Van Noordwijk M, Pellerin S, editors. Root methods: a handbook. Berlin: Springer; p. 175–210

Oppenheimer C. 1978. Subtropical fruit trees and their cultivation in Israel. Tel-Aviv: Am-Oved Tarbuth Vechinuch. Hebrew.

Patrick WH, Jugsujinda A. 1992. Sequential reduction and oxidation of inorganic nitrogen, manganese, and iron in flooded soil. Soil Sci Soc Am J. 56:1071–1073.

Paudel I, Cohen S, Shaviv A, Bar-Tal A, Bernstein N, Heuer B, Ephrath J. 2016. Impact of treated wastewater on growth, respiration and hydraulic conductivity of citrus root systems in light and heavy soils. Tree Physiol. 36:770–85.

Peters V, Conrad R. 1996. Sequential reduction processes and initiation of CH_4 production upon flooding of oxic upland soils. Soil Biol Biochem. 28:371–382.

Polomski J, Kuhn N. 2002. Root research methods. In: Waisel Y, Eshel A, Kafkafi U, editors. Plant roots: the hidden half. 3rd ed. New York, Basel: Marcel Dekker; p. 295–321.

Reddy KR, Patrick WH. 1975. Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. Soil Biol Biochem. 7:87–94.

Sato T, Qadir M, Yamamoto S, Endo T, Zahoor A. 2013. Global, regional, and country level need for data on wastewater generation, treatment, and use. Agric Water Manag. 130:1–13.

Schaffer B, Gil PM, Mickelbart MV, Whiley AW. 2013. Ecophysiology. In: Schaffer B, Wolstenholme BN, Whiley AW, editors. The avocado: botany, production and uses. Wallingford (UK): CABI; p. 168–199.

Schwab AP, Lindsay WL. 1983a. The effect of redox on the solubility and availability of manganese in a calcareous soil. Soil Sci Soc Am J. 47:217–220.

Schwab AP, Lindsay WL. 1983b. Effect of redox on the solubility and availability of iron. Soil Sci Soc Am J. 47:201–205.

Shenker M, Seitelbach S, Brand S, Haim A, Litaor MI. 2005. Redox reactions and phosphorus release in re-flooded soils of an altered wetland. Eur J Soil Sci. 56:515–525.

Shotbolt L, 2010. Pore water sampling from lake and estuary sediments using Rhizon samplers. J Paleolimnol. 44:695–700.

Sposito, G. 2008. Oxidation-reduction reactions. In: The chemistry of soils. New York: Oxford University Press; p. 144–173

Stolzy LH, Zentmyer GA, Klotz LJ, and Labanauskas CK. 1967. Oxygen diffusion, water, and *Phytophthora cinnamomi* in root decay and nutrition of avocados. Proc Am Soc Hortic Sci. 90:67–76.

Tarchitzky J, Bar-Hay M, Lowengart-Aycicegi A, Pozin Y, Sokolowsky E, Peres M, Zilberman A, Sadan D, Kenig E, Menashrov H, et al. 2006. Water and irrigation. In: National survey on treated wastewater 2003–2005. Bet-Dagan, Israel: Israel Ministry of Agriculture Extension Service.

Valoras N, Letey J, Stolzy LH, Frolich EF. 1964. The oxygen requirements for root growth of three avocado varieties. Proc Am Soc Hortic Sci. 85:172–178.

Van-Cleemput O, Samater AH. 1996. Nitrite in soils: accumulation and role in the formation of gaseous N compounds. Fertil Res. 45:81–89.

Wolstenholme BN, Sheard A. 2009. A better understanding of alternate bearing through revised growth model charts. South African Avocado Grow Assoc Avoinfo Newsl. 168(Oct 2009):17–19.

Yao H, Conrad R, Wassmann R, Neue HU. 1999. Effect of soil characteristics on sequential reduction and methane production in sixteen rice paddy soils from China, the Philippines, and Italy. Biogeochemistry. 47:269–295.

Yermiyahu U, Ben-Gal A, Keren R. 2010. Boron. In: Levy GJ, Fine P, Bar-Tal A, editors. Treated wastewater in agriculture : use and impacts on the soil environment and crops. Oxford: Wiley-Blackwell; p. 216–233.

Yu K, Böhme F, Rinklebe J, Neue HU, DeLaune RD. 2007. Major biogeochemical processes in soils – a microcosm incubation from reducing to oxidizing conditions. Soil Sci Soc Am J. 71:1406.

Zilberstaine M, Lowengart-Aycicegi A, Assouline S, Naor A, Lahav I, Keren R, Chen Y, Mintz D. 2011. Long term study on the effect of wastewater irrigation on avocado grown in different soil types. Bet-Dagan, Israel: Israel Ministry of Agriculture Extension Service. Hebrew.